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BIOECONOMY

A REVIEW OF THE IMPACTS OF CROP PRODUCTION ON THE SOIL MICROBIOME

Innovations and policy recommendations to address environmental degradation, climate change and human health

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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
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ABBREVIATIONS AND ACRONYMS

AFOLU	Agriculture, Forestry and Other Land Use
AFS	Agroforestry System
AMF	Arbuscular Mycorrhizal Fungi
AMRG	Antimicrobial Resistance Genes
AOA	Ammonia-Oxidizing Archaea
AOB	Ammonia-Oxidizing Bacteria
ARG	Antibiotic Resistance Genes
ARB	Antibiotic Resistant Bacteria
AMR	Antimicrobial Resistance
C	Carbon
CH_4	Methane
CO_2	Carbon Dioxide
CT	Conventional Tillage
Cu	Copper
CUE	Carbon Use Efficiency
ESS	Ecosystem Services
FAO	Food and Agriculture Organization of the United Nations
F:B	Fungi:Bacteria ratio
GHG	Greenhouse Gas
K	Potassium
MBC	Microbial Biomass Carbon
MBN	Microbial Biomass Nitrogen
MBP	Microbial Biomass Phosphate
MP(s)	Microplastic(s)
MWOO	Mixed Waste Organic Output
N	Nitrogen
NEA	Nitrification Enzyme Activity
NH_3	Ammonia

NH_4^+	Ammonium
NH_4NO_3	Ammonium Nitrate
NO_3^-	Nitrate
NOB	Nitrite Oxidizing Bacteria
N_2O	Nitrous Oxide
NP(s)	Nanoplastic(s)
NPK	Nitrogen Phosphorus Potassium (mineral fertilizer)
NT	No-Till, or No Tillage
OCP	Organochlorine Pesticides
OIE	World Organisation for Animal Health
OM	Organic Matter
P	Phosphorus
PAE	Phthalate Esters
PE/PET	Polyethylene/Polyethylene Terephthalate
PFLA	Phospholipid Derived Fatty Acid
POM	Particulate Organic Matter (coarse fraction organic matter)
PVC	Polyvinyl Chloride
qCO_2	Metabolic quotient
RT	Reduced Tillage
S	Sulphur
SO_4^{2-}	Sulphate
SDGs	Sustainable Development Goals of the 2030 Agenda for Sustainable Development
SOC	Soil Organic Carbon
SOM	Soil Organic Matter
TWW	Treated Wastewater
WHO	World Health Organization
Zn	Zinc

KEY TERMS AND CONCEPTS

Antibiotic resistance (AR) can develop when bacteria change in response to use of antibiotics to treat bacterial infections (WHO, 2020). **Antimicrobial resistance (AMR)** describes resistance by any microorganism (e.g. bacteria and fungi, as well as viruses and parasites) to drugs (antimicrobials) intended to treat infections (WHO, 2020). Antimicrobial resistance is expressed with **antibiotic resistance genes (ARG)**, which can be carried by bacteria, known as **antibiotic resistant bacteria (ARB)** (FAO and WHO, 2019). Pathogenic microorganisms that no longer respond to medicines pose a threat to public health (WHO, 2020).

Arbuscular mycorrhizal fungi (AMF) are soil microorganisms that form a mutualistic symbiotic relationship with plants, requiring this relationship to complete their life cycle (Smith and Read, 2008). The fungal hyphae penetrate plant roots, forming tree-like structures called arbuscules, which are the site of nutrient transfers between the plant and fungi. AMF transfer mineral nutrients to the plant (through their scavenging actions in the soil), while the plant provides the fungi with carbon in the form of sugars. Their role in providing phosphorus to plants is especially important. AMF form relationships with most plants.

Biodiversity is "the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems" (CBD, 1992). Knowledge about the relationship between species and their functions, or *functional groups*, is important to understanding the implications of biodiversity. These include the concepts of *functional diversity* and *redundance*, and **resistance** (ability of an ecosystem to resist changes when faced with disturbance) (Pimm, 1984) and **resilience** (ability of an ecosystem to absorb changes and persist) (Holling, 1973). Measurements of biodiversity are typically discussed in terms of **richness** (a count of the different species present), **relative abundance** (or evenness, which is the similarity of population size of each species present), and **diversity** (richness and relative abundance) (Hamilton, 2005).

Biological nitrogen fixation is the process by which certain soil bacteria, which form nodules in host root plants, convert nitrogen from the atmosphere (N_2) into plant-accessible forms of nitrogen (Giller and Cadisch, 1995). The bacteria receive energy from their host plant, and provide an additional source of nitrogen to the plants in return. This symbiotic relationship is best known to occur between *Rhizobia* bacteria and leguminous species.

Copiotrophs are organisms that thrive in environments with high nutrient levels (including carbon) and easily degradable material (Koch, 2001). Compared with *oligotrophs* (explained further on).

An **ecosystem** describes the interactions between living organisms and non-living components of a system (Chapin III *et al.*, 2011). Living organisms, or biotic components, include soil microorganisms, plants, and animals. Examples of non-living, or abiotic, components include climate and weather.

Ecosystem functions are a subset of natural, ecological processes and ecosystem structures (de Groot, Wilson and Boumans, 2002). They govern fluxes in energy, nutrients and organic matter in a given ecosystem. Examples include nutrient cycling and decomposition of organic matter, creation and maintenance of soil structure, and regulation of biological populations.

Ecosystem services "represent the benefits human populations derive, directly or indirectly, from ecosystem functions" (Costanza *et al.*, 1997). They can be grouped into four categories: 1) Provisioning services: the production of food, timber and fibre; 2) Regulating Services: carbon sequestration, prevention of soil erosion, climate regulation, natural flood control; 3) Supporting Services: primary production, water and nutrient cycling; and 4) Cultural services: intellectual, recreational and therapeutic activities (Millennium Ecosystem Assessment, 2005).

Functional diversity is a measure of the value and range of functional traits that exist in an ecosystem (Petchey and Gaston, 2006). This concept is critical to understand *ecosystem functioning*. An ecosystem with high functional diversity means that it features many groups of organisms responsible for different tasks; an ecosystem with high functional diversity will have diverse biological activities (Goswami *et al.*, 2017). It contrasts with *functional redundancy*.

Functional groups are a way to conceptually classify different microorganisms according to the soil function they perform. There are different models of functional groups, adapted to the purpose or context they serve. One example is to assign soil microorganisms to groups of chemical engineers, biological regulators, and ecosystem engineers (El Mujtar *et al.*, 2019). Ecological guilds are another example, defined as groups of species that exploit the same resources, or different resources in related ways (Schmidt, Mitchell and Scow, 2019).

In contrast to *functional diversity*, *functional redundancy* (or functional equivalence, or the species redundancy hypothesis) refers to the presence of different species able to carry out the same function (Goswami *et al.*, 2017). An ecosystem with high functional redundancy is more resilient to shock, because even if some species populations experience a major collapse, there are others that will continue performing their common task.

Soil ecological functions are difficult to assess directly. Instead, measurable soil properties are used as indicators of soil quality, from which soil ecological functions can be inferred. They can be categorized as *biological, chemical, and physical soil indicators* (Muñoz-Rojas, 2018). The following indicators feature in studies discussed in this paper. Biological: **microbial biomass** (mass of microorganisms, primarily bacteria and fungi, used as index for change in soil organic carbon and organic matter turnover), **microbial respiration** (process by which organisms obtain energy by aerobic or anaerobic means that results in release of carbon dioxide), **microbial community composition** (assemblage of microorganisms in a defined habitat, can be described by taxa and/or functions), **enzymatic activity** (used as a measure of microbial activity and/or functions) and **carbon use efficiency** (CUE, proportion of substrate carbon used for growth relative to other processes). Chemical: **soil organic carbon** (SOC, measure of the carbon content of organic materials, used to infer information about soil organic matter), organic nitrogen, available nutrients (e.g. phosphorus, potassium). Physical: **soil texture** (size of particles) and **soil structure** (how particles are assembled, or a measure of aggregation), **aggregate stability** (measure of soil aggregates to resist degradation), **porosity** (measure of pores, or open spaces, between soil particles).

Methanogenesis, or the production of methane, typically occurs in anaerobic conditions and is mediated principally by certain archaea (**methanogens**). **Methane oxidation** is performed by specific bacteria (**methanotrophs**) when they utilize methane as an energy source; they are the only known methane sink (Jiang *et al.*, 2011).

Multifunctionality refers to organisms performing more than one function at the same time (Delgado-Baquerizo *et al.*, 2016). For example, the soil microbiome plays a key role in maintaining multiple ecosystem functions and services simultaneously.

The production of nitrous oxide results from the **nitrification and denitrification processes** performed primarily by nitrite bacteria, nitrifying bacteria, denitrifying bacteria as well as an ammonia oxidation process driven directly by ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) (Singh *et al.*, 2010; Zeng *et al.*, 2019b). Therefore, denitrification typically results in soil nitrogen losses and nitrous oxide production.

Oligotrophs reproduce more slowly, live in nutrient-poor, carbon-scarce environments, and degrade more recalcitrant material (Koch, 2001). Classification of microorganisms under these labels is an example of functional grouping. Compared with *copiotrophs,* explained previously.

One Health is a framework that promotes synergetic, interdisciplinary collaboration from a local to global level to attain optimal health for people, animals and our environment. The World Health Organization (WHO), the Food and Agriculture Organization of the United Nations (FAO) and the World Organisation for Animal Health (OIE), for instance, work together closely using this approach (WHO, 2017). Van Bruggen *et al.*, (2019) propose a vision of One Health that encompasses soil, plant, animal and ecosystem health: the health of organisms in an ecosystem is intimately connected by the cycling of microbial communities from the environment to all organisms and back to the environment.

Planetary Health likewise provides a useful framework to understand how processes and phenomena are interconnected when conventional approaches fail (Zhu *et al.*, 2019). Simply put, the concept recognizes that human health is fundamentally linked to other planetary systems, and the health of all systems are connected to each other, albeit by complex ecosystem feedback processes.

Prokaryotes are bacteria and archaea that lack a cell nucleus, meaning that genetic material is not bound within a nucleus. **Archaea** are unicellular, like bacteria, but they are known for inhabiting extreme environments (e.g. high acidity, very hot or very cold, high salinity) and the unique ability of some taxa to produce methane. **Eukaryotes** are uni- or multi-cellular organisms that have a membrane-bound nucleus, meaning the cell DNA is contained within the nucleus. Fungi, protists, plants and animals are eukaryotes. **Protists** comprise a wide group of diverse organisms that are not animals, plants nor fungi; they may produce their own energy (autotrophs) or obtain it from other organisms (heterotrophs) and are typically unicellular. **Protozoa** is the informal term for a large and variable group of protist organisms, typically characterized by their mobility (e.g. by flagella, cilia) and predation. Amoeba, for instance are protozoa.

qCO_2 is a proxy for microbial utilization efficiency of *SOC* for metabolic activity regarding microbial growth. In other words, it is the rate of soil microbial respiration per unit of microbial biomass and indicates the capacity of soil microorganisms to utilize *soil organic matter.*

The **resistome** (or antibiotic resistome) "encompasses all types of *ARGs* (acquired and intrinsic resistance genes), their precursors, and some potential resistance mechanisms within microbial communities that require evolution or alterations in the expression context to confer resistance" (Kim and Cha, 2021). The **soil resistome** thus refers to this definition in the soil habitat. It is important to remember that these ARGs may be present in pathogenic and non-pathogenic microorganisms (Wright, 2007). Soil microorganisms are the original producers of many antimicrobial agents, therefore there is a naturally occurring resistome in the soil (Finley *et al.*, 2013).

Rhizobia (or **rhizobium**, singular) are bacteria capable of biologically fixing nitrogen. Requiring a plant host to do so, they form symbiotic relationships with legume plants, where they form nodules in the roots. These nodules are sites of biological nitrogen-fixation by bacteria, which they then transfer to plants, and where the plants provide rhizobia with carbon and other nutrients.

The **rhizosphere** is the plant root-zone in the soil. It is a specific microhabitat, characterized by plant root exudates (secretions containing nutrients and other metabolites) and the microorganisms living there (known as the root microbiome). It is known as a "hotspot" of soil microbial life. It is distinguished from the surrounding, bulk soil.

Soil microbiota are the living bacteria, archaea, fungi, algae, and protozoa inhabiting the soil. The **soil microbiome** refers to these microorganisms, as well as "their theatre of activity", meaning the microbial structures, metabolites, mobile genetic elements (e.g. viruses and phages), and relic DNA mobile genetic elements (viruses, phages), in their habitat (Berg *et al.*, 2020).

Soil organic carbon (SOC) refers to the carbon component of organic compounds in the soil. When plant roots secrete exudates (rhizodeposition), when they grow and die (plant root turnover), or when microorganisms reproduce and die (microbial necromass), their C contents are integrated into the soil through decomposition processes.

Soil organic carbon storage refers to a form of carbon held in the soil but relatively accessible to soil microorganisms. This can be distinguished from **carbon sequestration**, which results in a net removal of atmospheric carbon dioxide, the carbon being retained in the soil as stable aggregates or even more recalcitrant forms, therefore with longer residence times (Chenu *et al.*, 2019). The soil is a **carbon sink**, one of five defined carbon pools (the others being above and below ground biomass, litter and dead wood) (IPCC, 2006).

EXECUTIVE SUMMARY

Bioeconomy – a systemic approach for interdependent challenges

Humanity and the planet are grappling with extraordinary challenges. These include unsustainable production and consumption patterns, biodiversity loss, climate change, malnutrition, and a rise in diet-related, non-communicable diseases such as obesity, heart disease, and diabetes, to name just a few. Unsustainable agri-food systems are a major driver of all of these.

It is important to underline that **these challenges are interdependent**. Malnutrition includes undernutrition, micronutrient deficiencies, excess intake of dietary energy, overweight and obesity, and a range of non-communicable diseases associated with unhealthy diets. Although agri-food systems are key to fighting problems of malnutrition, the way they are managed also impacts other fundamental issues such as diminishing land availability, increasing soil degradation and biodiversity loss. Climate change, which implies shifts in ecosystems and more frequent and severe weather events, adds another layer of pressure.

In other words, the challenges mentioned above are part of different but interconnected **systems** (e.g. climate system, food production system, farm or landscape ecosystem) that have multiple **dimensions** (e.g. social, economic, cultural, political, environmental) and can be described at different **scales** (e.g. local, regional, global). Given the complexity of these challenges, **systemic approaches** are increasingly being called on to recognize and understand their intricacies and multiple layers. For instance, Planetary Health and One Health are two well-known frameworks commonly used to emphasize that different systems such as human, animal and ecosystem health are directly connected.

Another overarching framework to help address these challenges is provided by the **bioeconomy**. Bioeconomy describes a knowledge-intensive economic activity involving the use of bioscience and biotechnology in the production and management of goods, services and energy, with the aim of promoting environmental and social benefits for society. While there is no internationally agreed definition, the bioeconomy is often referred to as the production, utilization, conservation, and regeneration of biological resources – including related knowledge, science, technology, and innovation – to provide sustainable solutions within and across all economic sectors and enable a transformation to a sustainable economy (IACGB, 2020). The sustainable bioeconomy has in fact been recognized as a leading framework for agri-food systems transformation by the Scientific Group of the recently convened UN Food Systems Summit. It is against this background that the FAO takes a holistic and inclusive approach to the application of bio-based innovations – including agri-food innovations – across our entire agri-food systems. One of the game-changing solutions is Microbiome Science, Technology, and Innovation. This field is already showing potential to provide sustainable solutions that leverage the knowledge and concrete applications emerging from the fast-growing microbiome research and development field. **Agri-food systems around the world stand to gain from the enormous potential of microbiome science, technology and innovation, supported by a circular and sustainable bioeconomy framework**.

A soil microbiome perspective

Boosted by new analytical innovations, research into the soil microbiome – referring to the combined genetic material of all microorganisms living in the soil – is growing exponentially. Nevertheless, a comprehensive review of the importance of the soil microbiome, and of the effects of crop production practices on the soil microbiome and their linkages with climate change and human health, is currently missing.

It is increasingly acknowledged that, although the soil microbiome is literally microscopic, it plays a fundamental role in the relationships between healthy soils, climate and people. It is therefore critical that accurate and up-to-date knowledge in this field is made available to inform policy that addresses the challenges described above. At the global level, this includes policies that support the role that healthy soils play in achieving the Sustainable Development Goals (SDGs) of the 2030 Agenda for Sustainable Development. However, describing the complexity of the soil microbiome requires solid, scientific evidence.

Produced by the Microbiome Working Group at FAO, and strengthened by debate within the FAO-initiated International Microbiome Network, this is one of several forthcoming publications about different microbiome ecosystems and their relatively underexplored potential to alleviate global problems. Following numerous discussions with microbiome experts from industry, academia, and policymaking, a key message has emerged: FAO's unique role as an interface between science, innovation and policy at global, regional, national and local levels positions it as an important driver of change.

This study begins with a comprehensive overview of how crop production practices can impact the soil microbiome, and the ensuing consequences for human health and climate change. It then explores how that knowledge can be applied in practice, in the context of a circular and sustainable bioeconomy. Given the tension between the vast and exciting potential of soil microbial applications, on the one hand, and the technical and ethical questions related to their use and commercialization, on the other, the study concludes with a discussion of the challenges to overcome and policy recommendations to move forward.

The information and analyses in this study are based on **two literature reviews** and a **series of focus group discussions with microbiome experts**. The first review provides a general description of how different agricultural practices impact the soil microbiome. The second is a systematic review of **over 2 000 scientific publications**, describing solid, scientific evidence of strong connections between crop production practices, the soil microbiome and climate change or human health. The conclusions drawn from the literature reviews were discussed during a series of virtual conferences in July 2020 (*Microbiome: The Missing Link(s) Virtual Learning Pathway*). The participants included microbiome experts from different disciplines as well as different sectors (academic, industry, and policymaking).

The main questions explored in this study

- Why does the soil microbiome matter for a healthy planet and people? Specifically, what are the relationships between:
    - The soil microbiome and climate change?
    - The soil microbiome and human health?
- What are the impacts of crop production practices on the soil microbiome?
- Does scientific literature show any significant, causal impacts of crop production practices and the soil microbiome on climate change or human health?
- From a research perspective, which research and development issues need further investigation from a microbiome perspective?
- From a policy perspective, is there any solid evidence that can and should inform policy?

The nine crop production practices explored in the literature review

- Land use
- Tillage
- Agroecosystem diversity (plant diversity, crop rotations, cover crops)
- Crop residue management
- Plant variety selection
- Irrigation
- Fertilization
- Pest management
- Microplastics in agricultural soils

What is the soil microbiome and how is it related to healthy soils, climate and people?

What exactly is the soil microbiome? First, let's start with **soil microbiota**, which are the living bacteria, archaea, fungi, algae, and protozoa that inhabit the soil. Add to them "their theatre of activity", meaning the microbial structures, metabolites, mobile genetic elements (e.g. viruses and phages), and relic DNA found in the soil habitat, and we have the **soil microbiome.** There is no typical soil microbiome, because it varies with the soil environment. However, it is important to know that soil microbial ecosystems are likely the most **genetically diverse** communities on the planet.

This diversity is fundamental in ensuring the delivery of a wide range of **ecosystem services**, including provisioning of clean water and air, food and raw materials, recreational space and biodiversity. Furthermore, the soil microbiome is involved in the planet's **climate system**, because it is a direct driver of terrestrial greenhouse gas fluxes and soil carbon dynamics. There are strong theoretical arguments and some evidence of a direct relationship between the soil microbiome and **human health**.

Changes in the soil microbiome can cause changes in soil functions. For instance, if the type and number of microbial species are altered, the activities they perform may consequently differ. It is expected that ecosystems experience some fluctuation. However, profound, widespread changes can have significant and potentially long-term and undesirable implications. For example, how might the global decline in biodiversity – driven by human activities such as industrialization, urbanization and land-use change – affect the soil microbiome? And what might be the cascading effects of such changes to this microscopic world that underpins the functioning of so many interconnected ecosystems?

What do we know about how crop production practices impact the soil microbiome, climate change and human health?

Let's focus on potential changes in the soil microbiome caused by specific crop production practices. It is known that **different crop production practices can have a significant effect on the soil microbiome**. Here are a few examples from this study to illustrate:

- **Land-use** changes such as deforestation for crop production can affect soil microbial abundance, diversity and functional roles. In some cases, the impact of land-use change can be stronger than extreme meteorological events such as drought.
- **Excessive tillage** usually negatively affects soil microbial communities, causing changes in their composition by affecting both bacterial and fungal populations, and thereby influencing soil functioning, plant productivity and provisioning of certain ecosystem services.
- A central point regarding **agroecosystem diversification** is that plants can also influence the soil microbiome through their different biochemical compositions (e.g. nutrients and metabolites), introduced into the soil via plant litter, root exudates and rhizodeposits. Studies have reported positive or no effects of **plant diversity** on soil microorganisms; **crop rotations** seem to result in improved soil health and related ecosystem services; and **cover crops** can impact soil microorganism communities, including fungi and arbuscular mycorrhiza, with possible improved ecosystem resilience.

- The impact of **crop residues** on the soil microbiome remains inconclusive, the reviewed studies reporting positive, minor or no effects.
- **Plant variety selection** impacts the soil microbiome because plant genotypes influence root exudate composition and plant architecture, shaping the selection of rhizosphere microorganisms. Plant domestication has led to potential "missing microbes" in the root, rhizosphere and soil microbiomes.
- **Irrigation** directly changes soil abiotic properties and can indirectly modify the rhizosphere through plant rhizodeposition (i.e. root exudates and root litter). The latter effect can shape communities of microorganisms that promote crop growth and disease control. In addition, treated wastewater can carry bacteria with antimicrobial-resistant genes, causing concerns about how irrigation with such water sources impacts the soil resistome (the total assemblage of antimicrobial resistant genes, antiseptic genes and heavy metal resistant genes found in the microbial ecosystem of a given soil).
- Both inorganic and organic **fertilizers** can affect the soil microbiome directly (e.g. as a source of nutrients) and indirectly (e.g. through positive effects on plant growth and development). Organic fertilizers can contribute microorganisms (e.g. through manure or compost applications), substantial carbon and other nutrients and are therefore considered important for long-term soil fertility, soil functions and multifunctionality. However, there are concerns and unanswered questions regarding how trace element accumulation, antimicrobial-resistant genes and antibiotic residues, introduced through manure fertilizer, impact the soil resistome.
- **Pesticides** can impact the soil microbiome, significantly increasing or decreasing soil microbial communities, activities and biomass; less commonly, they can also have no detectable effect. These effects can drive selection of soil microbial species that can degrade pesticide compounds, leading to concerns about increased antimicrobial-resistant genes in the soil.
- Studies on **microplastics** in agricultural soils show mixed results, including no effects on the soil microbiome. However, they form a distinct microhabitat and can potentially impact soil microorganism communities and functioning.

Looking beyond these effects, how might crop production practices modify the soil microbiome and in turn impact climate change or human health? At present, it is difficult to predict how shifts in the soil microbiome will affect climate change. The question is very broad and involves many, interacting biotic and abiotic factors. However, there is solid, scientific evidence that demonstrates strong connections between some crop production practices, the soil microbiome, and their combined effects on greenhouse gas fluxes and soil carbon storage. The practices include tillage, fertilization, and agroecosystem crop diversification. The direct relationship between the soil microbiome and human health remains to be established, although the conceptual framework of likely connections is strong.

These findings drive home the message that the soil microbiome plays pivotal roles in ecosystem health, agroecosystems and the climate system. This must be taken into account for agroecosystem design and management to combine goals related to both food and nutrition security, and the environment.

Bringing these ideas into the context of real-life practice, two points are important to keep in mind. One is that any choice of farming practice will imply trade-offs. These need to be considered because crucial benefits may be apparent only in the long term, while remaining less so in the short term (e.g. development of good soil structure). A second is that this study explores agricultural practices individually, and therefore does not capture the complexity of interactions of crop production practices. Systemic approaches that combine specific agricultural practices, such as conservation agriculture, are beyond the scope of this study.

Ultimately, we still have so much to learn about the soil microbiome. For example, less than 1 percent of soil microbiome genetic diversity and functions have been studied so far. And there is at least as much mystery about the complex interactions between the soil microbiome, mesofauna and macrofauna, plants and abiotic aspects of the soil environment. Even so, what we do know about the soil microbiome is already of major significance, and the question is what can – and should – we do with it? And, as the United Nations agency leading global efforts to promote agri-food systems transformation, what role can FAO play?

Next steps: how to bridge the gap between science, innovation and policy?

Further research needs

FAO plays a key role in monitoring the latest scientific research, identifying knowledge gaps and informing policy on what requires further investigation. In this capacity, the Organization has identified an urgent need for research and development in the following soil microbiome-related areas:

- Soil microbiota diversity and functions
- Underexplored fields where the soil microbiome plays a decisive role (e.g. deep-soil carbon dynamics, plant breeding related to plant variety and rhizosphere microorganism associations, microplastics and nanoplastics in soils)
- Using soil microbiota to directly engineer improved soil and plant health
- Relationships between the soil microbiome and antimicrobial resistance
- Interconnections between ecosystems and human health

The soil microbiome - Innovative solutions for global problems?

Unravelling the complexities of the soil microbiome holds huge potential for innovative solutions to global problems. This field will be a major game changer in the way we manage our planet's natural resources – not only to obtain our food, but also to improve the health of people and ecosystems. These types of bio-innovations will enable us to transition into a **more sustainable bioeconomy**.

What do these innovative solutions look like in the world of crop production? There are two guiding approaches at the moment. *One approach* focuses on the choice of **crop production practices** (and their adaptation to local farming systems) designed to foster a multifunctional, healthy soil microbiome. As described in this literature review, certain practices are known to have beneficial effects on the soil microbiome and the functions they provide. *A second approach* centres on **microbial product inputs**. The private sector has already invested significantly in this field, with large agrochemical companies being key players. The market for agricultural bio-innovations built on microbiome research is growing rapidly. A 2020 report by Fortune Business Insights has projected this market to expand at a Compound Annual Growth Rate of more than 14 percent to reach a value of almost USD 11 billion by 2025. Meanwhile, the microbials segment is anticipated to dominate the agricultural biologicals market, the latter predicted to be valued at almost USD 19 billion by 2025.

It is important to state that these two microbiome-related innovation approaches are complementary rather than exclusive.

Looking beyond agricultural applications, soil microbiome-based innovations can also target multiple challenges using a systems-oriented perspective, or a One Health approach, to promote ecosystem health. A few examples include restoring degraded or polluted soils, reducing greenhouse gas emissions and increasing soil carbon storage, and implementing adaptive management strategies in the context of climate change. There is also some evidence suggesting that humans may also benefit directly from contact with the soil microbiome, the primary examples focusing on the human immune system.

While the applications of (soil) microbiome science seem very promising – be it for the commercial plant production sector or for the provision of wider public goods such as climate change mitigation or human health – there are still quite a few challenges to tackle. They range from technological and scientific issues to market and regulatory needs to public acceptance and ethical questions. These challenges can be partly addressed by policy.

Policy recommendations

Findings from this study led the authors to recommend that policy give adequate space to the critical role the soil microbiome plays in sustainable food production systems and resilient agricultural practices.

Growing knowledge of microbiome science could have significant impacts and implications for society. Scientists therefore need to work with policymakers to create an enabling environment to support this emerging science and its related innovations. Policymakers must have the adequate capacities to make informed decisions regarding related opportunities and risks, while also contributing to global research and development agendas.

The following are policy recommendations based on the review of scientific literature and complemented by the soil microbiome focus group workshops

Research, development and innovation

- Policy should channel resources into research on what constitutes a healthy microbiome, and prioritize exploring the causal relations between microbiomes and humans, plants, and animals throughout their life spans, as well as environmental health.
- Policy should encourage expanding soil microbiome research from laboratory studies to field conditions that reflect the complex interactions with other living biomass in the soil.
- Policy should further encourage national and international interdisciplinary research collaboration linking microbiome research communities such as plant, environmental, animal, marine and human research. Moreover, policy should also encourage global collaboration between developed and developing countries, and participatory approaches to include contributions from farmers and citizens.

Education and communication

- Policy should ensure training of the current and future workforce and scientists to build capacity for the field. This concerns school-aged to adult education, early-career training, as well as university curricula of bachelor, master and postgraduate programmes.
- Policy should encourage the training of consumers and citizens to ensure microbiome literacy. This should include concerted action from all responsible actors, such as the research community, food and healthcare professionals, industry, regulatory opinion leaders, the media and policymakers, to support broad efforts on microbiome literacy. It should also include the message that microbiome research can provide solutions to many global challenges. Promoting microbiome literacy will help individuals grasp the direct relevance of the microbiome to their daily lives.
- Policy should also make sure that local communities have the capacity to benefit from new microbiome innovations, including communities in developing countries. Research and industrial infrastructure to develop and provide biological solutions for the agricultural sector should also be developed in rural and coastal areas, to provide employment opportunities, including to rural youth. This could contribute to stopping rural migration and support public acceptance of these new technologies, while improving collaboration with local communities impacted by ecosystem degradation.

Commercialization of microbiome innovations and increasing the demand for microbiome practices, products and services

- Policy should particularly support the development and commercialization of those microbiome applications that are destined for the common good, such as those targeting carbon sequestration, rehabilitation of degraded or contaminated soil, plant growth promotion, and climate change resilience.
- Policy should furthermore support the competitiveness of biological applications with agrochemical alternatives, making sure that the biologicals are economically and logistically competitive in terms of price, transportation and storage. Quality regulation is also necessary to ensure efficacy of microbiome products in the field.

Framework conditions

- Policymakers should develop regulatory frameworks that require evaluation of health and environmental claims of new food or environmental microbiome-based products, legislative proposals that reward sustainable management of agricultural soils and the microbiome, and strategies that recognize the interconnectedness of different ecosystems to develop solutions for restoring soil health and functions. There is also a need for technical harmonization at the international level in order to share genetic and functional information related to biodiversity.
- To that end, it is important that policymakers, farmers, scientists, industry and citizens are all part of the conversation when it comes to highlighting the key challenges of new microbiome-based products, and identifying the steps necessary to enable innovation and mitigate risks.
- Policymakers need to allow public scrutiny of new microbiome technologies and ensure adequate safety assessment prior to any introduction, providing guidance on the use and potential misuse of new microbiome-based technologies. While it is important to highlight the opportunities of their applications, it is key that consumers are aware of the risks and protected by fit-for-purpose regulations, where necessary.

A timely endorsement

This study underscores the critical importance of soil microbial biodiversity and soil health in the provisioning of ecosystem functions and services. It also emphasizes that (microbial) ecosystems are interconnected, and traces the underlying relationship between the microbiome and healthy soils, people and the climate system. The vast and relatively underexplored diversity of soil microbial genes and functions offers exciting opportunities for innovative solutions, which could contribute to achieving the SDGs as well as a sustainable and circular bioeconomy.

FAO, with a comparative advantage as a global interlocutor in the field of food and agriculture, has a unique role to play. During the series of virtual conferences in July 2020, microbiome experts identified FAO as an appropriate intermediary between the scientific community and policymakers worldwide to identify and share innovative ideas and solutions that can bring rapid and tangible change to agri-food systems. From a microbiome science perspective, this entails investing in a dedicated, multidirectional science–policy interface, the purpose of which would be to **identify and prioritize scientific information needed for policymakers and policy priorities for research agendas**.

Leaving no one behind is a central narrative in the FAO Strategic Framework 2022–2031. The new strategy aims to achieve this vision through sustainable, inclusive and resilient agri-food systems for better production, better nutrition, a better environment and a better life. Emerging soil microbiome-based innovations in cropping systems could play a major role in better production and better environment, both of which advocate for biodiversity in agricultural systems.

The year 2021 saw the hosting of the UN Conferences on Climate Change (COP26) and Biodiversity (COP15), as well as the UN Food Systems Summit. It was also the year of the Global Symposium on Soil Biodiversity, jointly organized by the Global Soil Partnership, the Intergovernmental Technical Panel on Soils, the Global Soil Biodiversity Initiative, the UN Convention on Biological Diversity and the United Nations Convention to Combat Desertification. And, significantly, June 5 (World Environment Day) saw the launch of the UN Decade on Ecosystem Restoration, jointly led by FAO and the UN Environment Programme (UNEP). The Decade will offer major opportunities for microbiome-related innovations that bring about regenerative environmental outcomes.

This report offers a timely endorsement of the microbiome's pivotal role in supporting biodiversity, ecosystem and human health, and climate change mitigation and adaption; and outlines **FAO's potential role as a science-advisory coordinating structure to bring forward beneficial microbiome innovations.**

CHAPTER

1

SETTING THE SCENE
AGRICULTURE, THE BIODIVERSITY OF SOIL MICROORGANISMS AND THEIR LINKS TO HUMAN HEALTH AND CLIMATE CHANGE

1.1
BACKGROUND

While there is more than enough food produced today to feed everyone, between 720 and 811 million people still faced chronic hunger in 2020 (FAO, IFAD, UNICEF, WFP and WHO, 2021). Malnutrition, though, goes beyond undernourishment and hunger. It also refers to obesity and overweight as well as diet-related non-communicable diseases, which are currently taking a heavy toll across developing and developed nations alike. Globally, 13.1 percent of adults are obese and 22 percent of children under five (or 149.2 million) suffer from stunting, according to latest estimates (FAO, IFAD, UNICEF, WFP and WHO, 2021).

The 2030 Agenda for Sustainable Development offers a vision where food and agriculture are key to fighting malnutrition. Sustainable Development Goal 2 (SDG 2) aims to end hunger, achieve food security and improved nutrition, and promote sustainable agriculture. *Leaving no one behind* is a central narrative in the Food and Agriculture Organization of the United Nations (FAO) Strategic Framework 2022-2031 to achieve the SDGs. The new strategy aims to achieve this vision through sustainable, inclusive and resilient agri-food systems for better production, better nutrition, a better environment and a better life (FAO, 2021).

The above-mentioned trends that reflect malnutrition throughout the world coincide with other fundamental challenges: the diminishing availability of land, increasing soil and biodiversity degradation, and more frequent and severe weather events. The impact of climate change on agriculture compounds the situation. While agriculture is central to feeding a growing population that is expected to reach almost ten billion by 2050, it also plays a significant role in pushing the Earth system towards, or in some cases over, the boundaries of a safe operating space for humanity. Various concepts exist to describe such global environmental constraints: "carrying capacity", "sustainable consumption and production", "guardrails", "tipping points", "footprints", "safe operating space" or "planetary boundaries".

The Planetary Boundaries concept identifies nine global priorities relevant to human-induced changes to the environment, five of which are directly connected to agriculture and food systems (Campbell *et al.*, 2017; Rockström *et al.*, 2009; Steffen *et al.*, 2015). According to Campbell *at al.* (2017, p. 8), two planetary boundaries have been fully transgressed (i.e. are at high risk), biosphere integrity and biogeochemical flow; and agriculture has been the major driver of the transgression.[1] Three are in a zone of uncertainty (i.e. at increasing risk), with agriculture the major driver of two of those, land-system change and freshwater use, and a significant contributor to the third, climate change.

This clearly indicates that in order to reach the goal of zero hunger and improved nutrition (SDG 2), there is a need to simultaneously address the other sustainable development objectives. In this report, we focus on the SDGs that are especially relevant in the context of the soil microbiome – the central subject of this literature review:

- SDG 3, which aims to ensure healthy lives and promote well-being for all, at all ages;
- SDG 12, which aims to ensure sustainable consumption and production;
- SDG 13, which urges taking action to combat climate change and its impacts;
- SDG 15, which calls for sustainable forest management, combating desertification, halting and reversing land degradation, and halting biodiversity loss.

This study focuses on the smallest denominator that underpins all of the above: the microcosmos of the smallest living beings that populate the earth – the so-called microbiome. It asks the following questions as part of a series of literature reviews around different food system microbiomes (FAO, 2019a):

- Can we transform our diets and agri-food systems to solve the relentless problems of undernourishment, while at the same time tackling the emerging pandemic of obesity and diet-related non-communicable diseases?
- How will we feed 10 billion people by 2050 without destroying our natural resource base?
- Can we stop, and possibly even reverse, the loss of biodiversity, environmental degradation, and climate change?
- How can we manage soil biodiversity to achieve sustainable intensification of agroecosystems, while mitigating and adapting to climate change?

Focusing on crop production practices, this study aims to investigate scientific evidence on the importance of the soil microbiome in the context of climate change, and ecosystem and human health.

1.1.1
Healthy soils for a healthy climate

Climate change has long ceased to be a matter of scientific focus alone. The climate change movement, which is active all round the world, and the global governance agenda on climate change, have made the subject area a staple of news channels, public political disputes and private dinner conversations. Climate change is predicted to affect the climate stability (Loarie *et al.*, 2009) and, with it, our productive agroecosystems that ensures sustainable agriculture and sound nutrition over time. For these reasons, the 2030 Agenda has set Climate Action (SDG 13) as a central objective of global engagement.

More and more people perceive climate change as a real threat to their livelihoods. In parallel, law professionals such as Hammersley (2016) argue that people have the right to a stable and healthy climate, referring to several law suits in which youths from around the world are fighting against their governments. Recent climate movements such as the vocal Fridays for Future and its spokesperson Greta Thunberg are one prominent example.

Global governance mechanisms such as the Conference of the Parties (COP), the supreme decision-making body of the United Nations Framework Convention on Climate Change (UNFCCC), are attempting to address these concerns at the global policy level, albeit at a very slow pace (Newell and Taylor, 2020).

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[1] The planetary boundaries concept uses the term biosphere integrity to refer to biodiversity loss.

The Intergovernmental Panel on Climate Change (IPCC) supports the UNFCCC, by assessing the scientific, technical and socioeconomic information relevant to understanding the risks of human-induced climate change and providing possible response options. In a recent publication, the IPCC (2019) asserted that better land management can contribute to tackling climate change. For example, on the one hand, agriculture, forestry and other types of land use account for 23 percent of human greenhouse gas emissions; on the other, natural land processes absorb a quantity of carbon dioxide equivalent to almost a third of that emitted from fossil fuels and industry (IPCC, 2019). They added that land must also remain productive to maintain food security while both the population and negative impacts of climate change on terrestrial agroecosystems increase. This comprehensive approach is echoed by the United Nations Decade on Ecosystem Restoration (2021–2030): well-functioning ecosystems are the foundation of climate change mitigation and adaptation, biodiversity conservation, and ecosystem services (United Nations General Assembly, 2019). Even more precisely, a report prepared at the request of the Commission on Genetic Resources for Food and Agriculture (CGRFA) unequivocally states that "the manipulation of terrestrial ecosystems offers a potentially powerful means by which the effects of anthropogenic climate change could be mitigated" (Chatzipavlidis *et al.*, 2013). The UN Conferences of the Parties on Biodiversity (COP15) and Climate Change (COP26) in late 2021 were set to further emphasize the significance of these points. In sum, better land management is absolutely critical to fighting global climate change.

However, the devil is in the detail. Or rather, in the microscopic world around us, as the reports by the IPCC and CGRFA clearly portray. This was further substantiated in a Consensus Statement published in the renowned journal *Nature Reviews Microbiology*, in which scientists sent a warning to humanity regarding the central role of microorganisms in climate change biology (Cavicchioli *et al.*, 2019). Co-authored by 33 researchers from Australia, Europe, North America, and the United Kingdom, the statement sends a clear message to policymakers:

> *"To understand how humans and other life forms on Earth (including those we are yet to discover) can withstand anthropogenic climate change, it is vital to incorporate knowledge of the microbial 'unseen majority' " (Cavicchioli* et al.*, 2019, p. 569).*

In a nutshell, a healthy climate requires healthy soils. And healthy soils require a healthy microbiome. This relationship is explored in more detail in **Section 3.2**.

1.1.2
Healthy soils for healthy people

As already stated above, malnutrition concerns hunger, but also refers to obesity, being overweight and diet-related non-communicable diseases. Food security and adequate nutrition are key elements for SDG 2, ensuring healthy lives and promoting well-being for all (SDG 3), at every stage of life. In fact, the human right to health means that everyone has the right to the highest attainable standard of physical and mental health. This includes access to all medical services, sanitation, adequate food, decent housing, healthy working conditions, and a clean environment. Among others, the human Right to Adequate Health is protected in Article 25 of the Universal Declaration of Human Rights. Yet, global statistics show a different picture: in many parts of the world, we are failing to uphold the Right to Adequate Health, as indeed we are failing to uphold the rights of global citizens to a balanced climate.

As further explained in **Section 3.3** and a forthcoming FAO publication on the human microbiome, a key element to human health is a healthy human microbiome – that of the gut in particular – and a healthy human microbiome likely has a lot to do with healthy soils and the soil microbiome. How is that?

Let´s take a step back. In a recent review paper, Blum, Zechmeister-Boltenstern and Keiblinger (2019) asked the question: do soils contribute to the human gut microbiome? Though this question may seem somewhat odd, it does make a lot of sense. The authors tracked the relationship between the soil microbiome and the human intestinal microbiome. They hypothesized that there is a close linkage between the soil microbiome and the human intestinal microbiome, because they have co-evolved throughout history and continue to do so even today. For example, although the shift towards industrialized agriculture may seem relatively recent in the 100 000 years of human history, it has already taken a toll on the biodiversity of the soil ecosystem, including its microbiome. At the same time, a shift in agri-food processing, types of diets and food preparation and other nutrition-related variables have also impacted the human intestinal microbiome.

Ecologists and medical professionals stress the parallels between the soil microbiome and ecosystem and climate health, on the one hand, and the human gut microbiome and human health, on the other. In his paper, Ochoa-Hueso (2017) laments that knowledge generated about these parallel relationships typically occurs in isolation. He argues that discussion should rather be addressed together across disciplines, as knowledge about the microbial ecology of human and non-human ecosystems could be highly transferable.

Microbiome research has historically emerged from the field of microbial ecology, and it now offers an interdisciplinary platform for numerous fields, from agriculture to human medicine, and mathematics to bioeconomy (Berg *et al.*, 2020). Respective disciplines learning from each other and combining knowledge about the microbiomes of different ecosystems establishes an important foundation for further development. This has also been recognized by many recent global and national microbiome initiatives. Acknowledging the need for more knowledge and tools to manage this hub of microbiological biodiversity, many major initiatives have called for global action. They emphasize the need to understand how microorganisms affect health and the wider biosphere including food, agriculture and nutrition, as well as the environment and climate change. This engagement responds to the growing recognition that advances in environmental microbiome engineering can gradually replace toxic chemicals in our agroecosystems, improve our food processing, enable a more sustainable use of resources (Berg *et al.*, 2020; Hutchins *et al.*, 2019; Sessitsch *et al.*, 2018; Singh, 2017; Singh and Trivedi, 2017), help restore degraded ecosystems (Goswami *et al.*, 2018; Wubs *et al.*, 2016), and stimulate a sustainable and circular bioeconomy.

The call has been strongly voiced throughout the research community. Examples include a *Nature* paper by Dubilier *et al.* (2015), and multiple national and regional government initiatives such as the International Bioeconomy Forum (IBF), whose Microbiome Initiative was launched in 2016, or the Brazilian Microbiome Initiative. The private sector has echoed the same call, for example in the health, agriculture and chemical industries – including big players in agro-industry and specialized biopharmaceutical companies such as Second Genome. Microbial-based agricultural products are one of the fastest growing sectors in agronomy, with a compound annual growth rate (CAGR) of more than 14 percent and a predicted value of USD 11.8 billion by 2027 (Fortune Business Insights, 2020).

International conferences about R&D and business collaboration on and around the microbiome are no longer an exception and attract many participants each year. Furthermore, practitioners of organic agriculture and agroecology point to the paramount importance of plant–microbiome interactions for plant growth and the functioning of terrestrial ecosystems (Saleem, 2015); while medical doctors alert us to the importance of microorganisms, be it for digestion, immunization or weight control. In order to manage this vast amount of research – and research gaps – information technologies play a crucial role. Pooling data from scientists from around the world could encourage the integration of such information across institutions and nations.

While research consortia and national and global initiatives have started to actively address this challenge, it is now time that consumers, policymakers and the private sector alike catch up and follow suit. What are the implications of these new findings? How do innovations in the food system and research on the role of microbiome in human health and climate change translate to policy, legislation, standards and other normative instruments at national, regional and global levels? How can they inform new consumer behaviour?

1.2

WHAT IS A HEALTHY SOIL? WHICH ECOSYSTEM SERVICES DOES IT PROVIDE?

Before delving into the literature review, it is essential to understand the dynamic, living entity that is the soil. What do we mean by soil health? What are the ecosystem functions and services soil provides for the environment and human society? What are their fundamental relationships to biodiversity.

1.2.1

What is soil?

When one thinks about soil, one tends to imagine the solid ground we stand upon, in which crops grow, and on which we build houses. However, soil is far more than that. Soil Taxonomy defines soil as:

> *A natural body comprised of solids (minerals and organic matter), liquid, and gases that occurs on the land surface, occupies space, and is characterized by one or both of the following: horizons, or layers, that are distinguishable from the initial material as a result of additions, losses, transfers, and transformations of energy and matter or the ability to support rooted plants in a natural environment (1999, p. 9)*

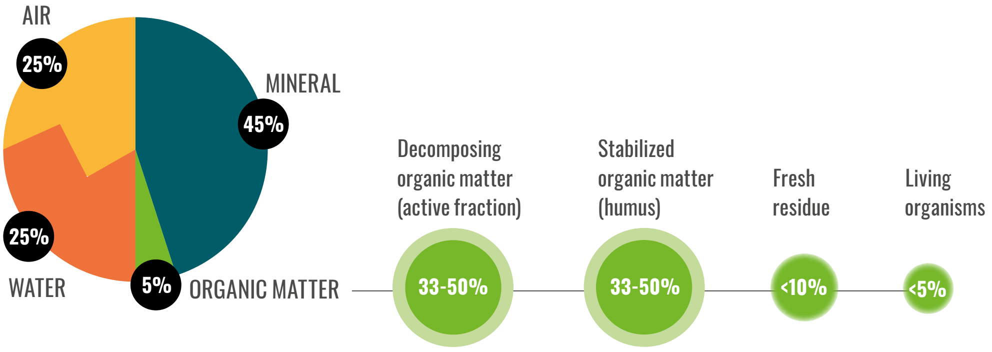
This definition implies two interesting points. Firstly, soil is not only a body of solids, but also includes gases and liquids. Secondly, the definition highlights the importance of perpetual transformations that occur in soil. Proceeding step by step will help illustrate the fascinating complexity of soils and their fundamental importance.

Soil consists of:

i A **mineral fraction**, defined by the proportion of sand, silt and clay. This proportion is called **soil texture** and influences important soil processes such as water and air availability, nutrient retention, and organic matter degradation;
ii **Organic matter (OM)**, which includes soil-dwelling organisms, plants and their residues at different levels of decomposition. Specifically, OM is comprised of living organisms, fresh residues, decomposing OM (or the active fraction) and more stable forms of OM;
iii Air; and
iv Water (**Figure 1**).

FIGURE 1.

APPROXIMATE COMPOSITION OF SOIL AND CARBON-BASED SUBSTRANCES THAT CONSTITUTE THE SOIL ORGANIC MATTER.

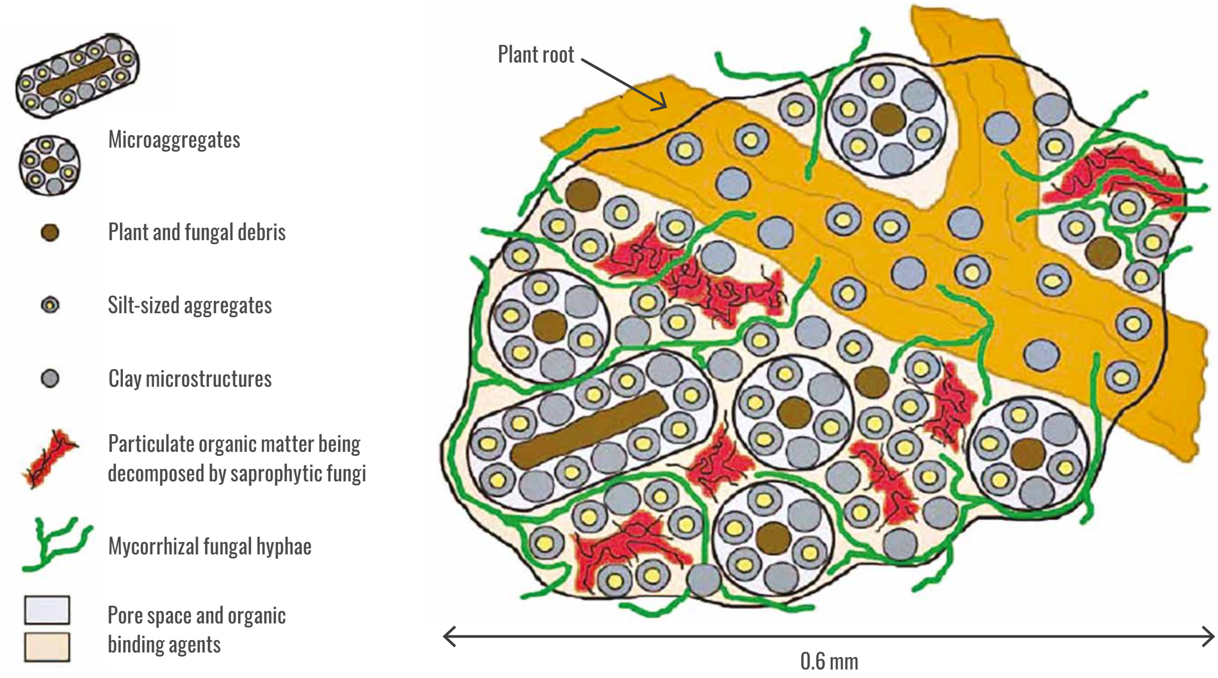


Sources: adapted from https://www.ctahr.hawaii.edu/mauisoil/a_comp.aspx and https://theconversation.com/restoring-soil-can-help-address-climate-change-121733.

Those four components can also be arranged in a different way, in units called **soil aggregates**. They are described as "groups of primary soil particles that cohere to each other more strongly than to other surrounding particles" (Encyclopaedia, 2005). In other words, **soil aggregates contain mineral fractions, OM and have pores where water and air can circulate** (**Figure 2**). Soil aggregates are classified according to their size, as microaggregates (2–250 mm) and macroaggregates (>250 mm). They can be arranged in the soil in different configurations, defined as soil structure, which regulates gaseous exchange, water movements, root penetration, OM decomposition, soil biodiversity processes and nutrient dynamics. A poor soil structure, for instance, will not allow plant roots to penetrate the substrate and take up nutrients, gases and water, resulting in negative effects on crop production. A compacted field is an example of poor soil structure.

FIGURE 2.

GRAPHICAL REPRESENTATION OF A MACROAGGREGATE.



Source: Jastrow, Amonette and Bailey, 2007

1.2.2
Soil health

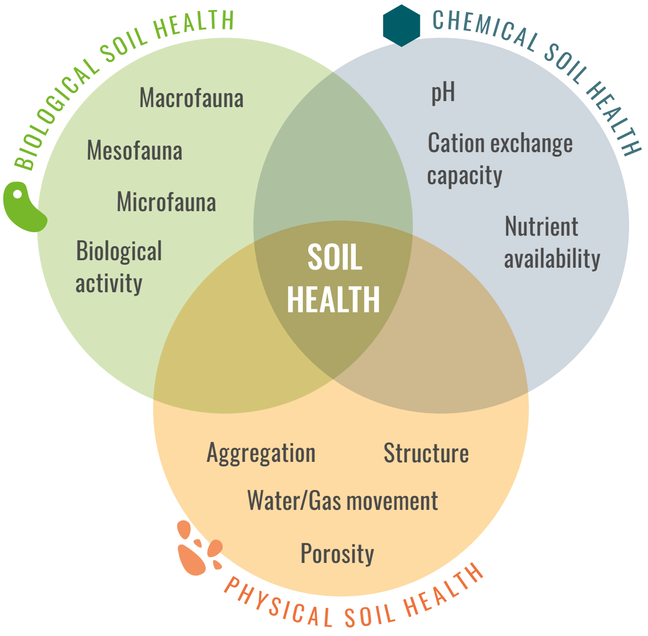
Soil health is "the ability of the soil to sustain the productivity, diversity, and environmental services of terrestrial ecosystems" (FAO, 2020).

Healthy soils maintain a diverse community of soil organisms that help to control plant disease, insect and weed pests; form beneficial symbiotic associations with plant roots; recycle essential plant nutrients; improve soil structure with positive repercussions for soil water and nutrient holding capacity; and ultimately improve crop production. A healthy soil also contributes to mitigating climate change by maintaining or increasing its carbon content (FAO, 2011).

Through the concept of soil health, soils are perceived as highly dynamic, diverse and living systems with specific processes, which guarantee their continued capacity to function. **Soil health can be divided into three categories: chemical, physical and biological soil health** (FAO, 2015). Those categories are of equal importance, and it is the interaction between them that ultimately defines soil health (**Figure 3**). A soil with a poor structure that is easily flooded will not be healthy even if endowed with adequate nutrients. This implies that soil management is sustainable only if all three dimensions of soil health are enhanced and maintained. Moreover, the concept of soil health also takes into account environmental goals such as mitigating climate change and crop production. **Soil health (or soil quality) is discussed in terms of soil quality indicators**, which are assigned to the three above mentioned categories, all of which are related key soil ecosystem functions (**Figure 3**). For instance, microbial biomass and microbial respiration are biological indicators that contribute to soil quality assessment; organic carbon (C) and nitrogen (N) and level of acidity (pH) are chemical soil quality indicators; and soil texture and structure are physical soil quality indicators. However, as individual soil properties may not be adequate indicators of soil status, there is a growing trend to use soil indexes, which are a combination of indicators (**Figure 4**).

FIGURE 3.

GRAPHICAL REPRESENTATION OF THE THREE DIMENSIONS OF SOIL HEALTH.



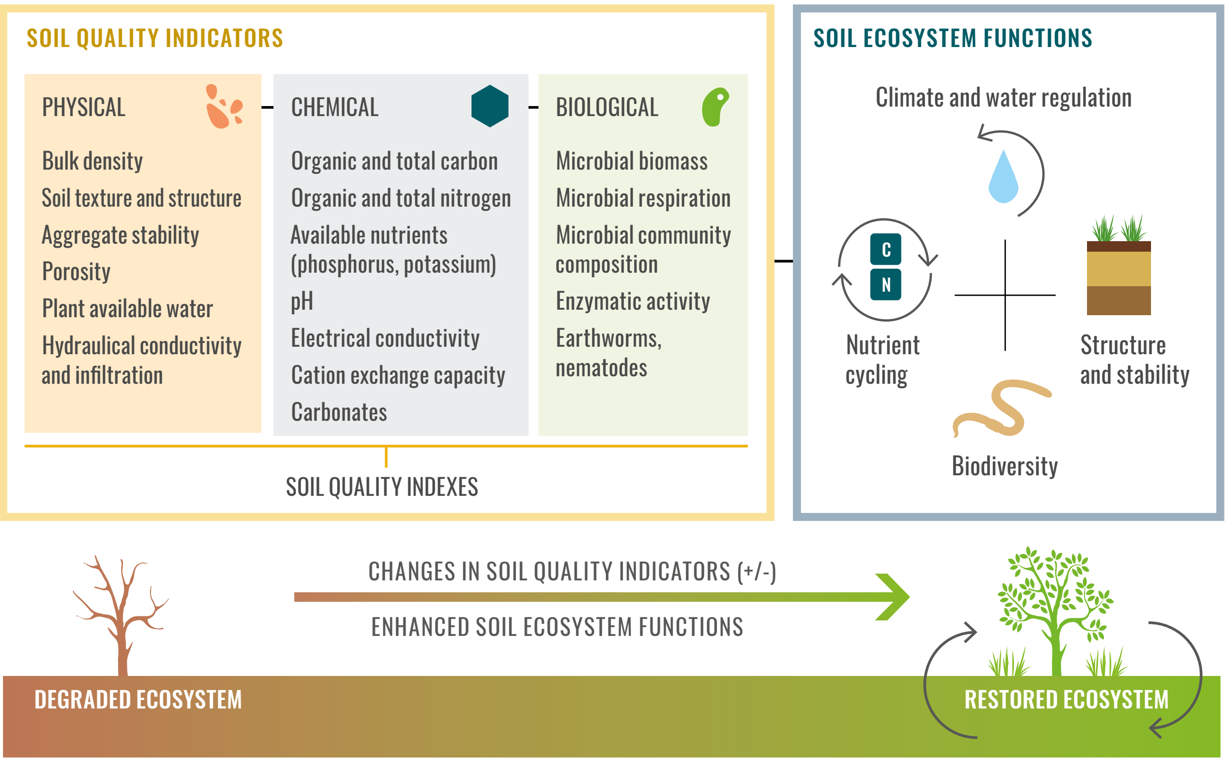
pH refers to level of acidity.

Source: Authors' own elaboration.

FIGURE 4.

SOIL QUALITY INDICATORS INCLUDE A RANGE OF SOIL PHYSICAL, CHEMICAL AND BIOLOGICAL CHARACTERISTICS.

Soil quality indexes are combinations of soil quality indicators and are used to acknowledge the multi-dimensionality of the soil environment. They are connected to key soil ecosystem functions.



Source: Muñoz-Rojas, 2018.

Determining a specific formula of indicators applicable across different biogeographical and soil composition contexts is challenging. Numerous models have been proposed (e.g. Breure *et al.*, 2004; Herrick *et al.*, 2001; Thoumazeau *et al.*, 2019; Velasquez, Lavelle and Andrade, 2007), but discussion of their relative merits is beyond the scope of this study.

Given the threefold definition of soil health, OM plays a pivotal role in sustainable soil management. Despite accounting for less than 5 percent of the soil – and much less in many soils around the world – **OM is the only resource able to potentially improve all the three spheres of soil health.** Regarding soil physical health, OM promotes soil aggregation, reduces soil bulk density, increases soil water retention, and reduces susceptibility to erosion. Concerning chemical soil health, OM is a source of plant nutrients, improves cation exchange capacity (CEC), and buffers against fluctuation in soil pH. Lastly, relevant to biological soil health, OM provides an energy substrate for soil biota and also improves habitat conditions for soil biological communities.

Furthermore, **the C mass in OM is about 50 percent** (Pribyl, 2010) **and thus represents a unique opportunity to store atmospheric C in the soils.** Generally speaking, soil C sequestration refers to the process of removing carbon dioxide (CO_2) from the atmosphere and storing it as stable soil C. The actual amount of C stored in the soil C pool is highly dependent on management, climate and soil specific factors. The recycling of OM in soils and the maintenance and increase of soil OM is altogether a key strategy to maintaining soil health and promoting sustainable crop production (IPCC, 2019).

1.2.3
Ecosystem services and soil biodiversity

Ecosystem services "are the benefits people obtain from ecosystems" (Millennium Ecosystem Assessment, 2005). They are grouped into four categories:

- **Provisioning services**: the production of food, timber and fibre;
- **Regulating services**: C sequestration, prevention of soil erosion, climate regulation, natural flood control;
- **Supporting services**: primary production, soil formation, photosynthesis and nutrient cycling; and
- **Cultural services**: intellectual, recreational aesthetic and therapeutic activities.

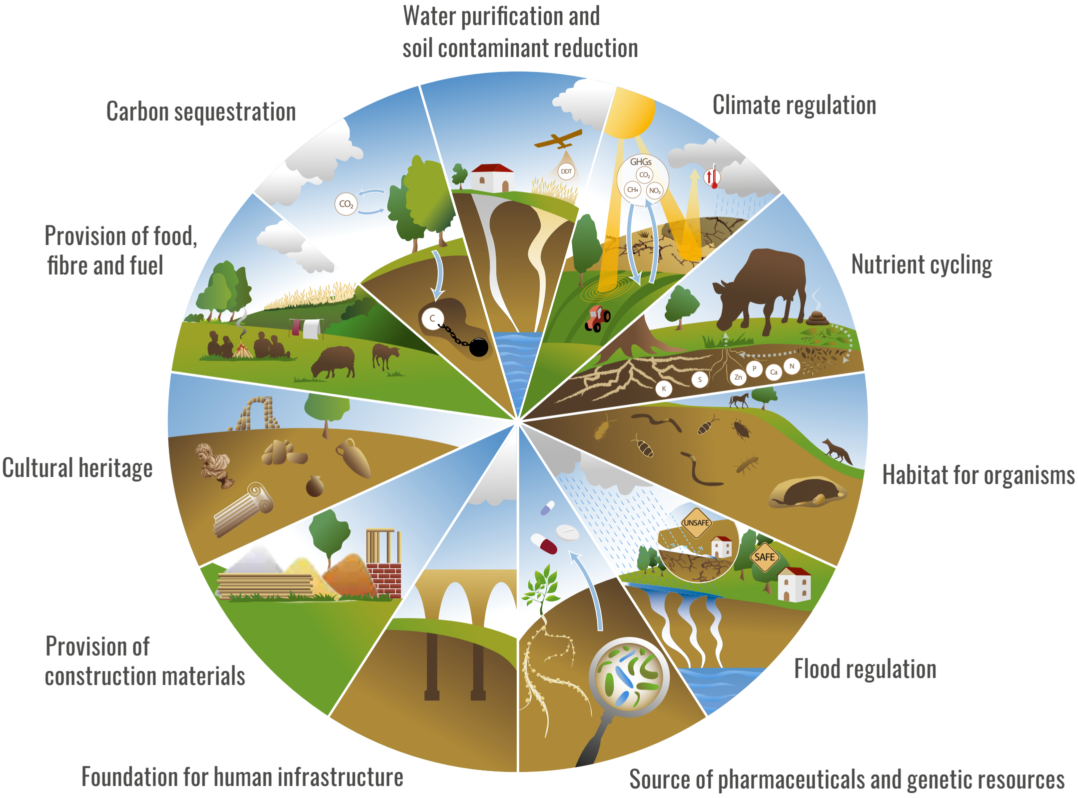
The definition of soil health already suggests that soils are not only important for crop production. Soils are at the core of each natural, urban and agroecosystem, mediating essential ESS. Soils are a vital resource that sustain life on earth, and ensure the provision of water and nutrients to crops that produce food, feed, fibres, medicines and other crucial materials (**Figure 5**). To emphasize the point, it has been estimated that about 95 percent of the food produced globally depends either directly or indirectly on soils (FAO, 2015). Soils also represent the largest C pool on earth and are therefore fundamental in mitigating and adapting to climate change (FAO, 2017). Sustainably managing soils and, in particular, restoring degraded soils, could help sequester additional C by removing CO_2 from the atmosphere, thereby contributing to climate change mitigation.

Soils furthermore host more than one-quarter of global biodiversity. Soil communities can be conceptualized in different functional groups, defined as a groups of species that perform the same function. For example, different species can all participate in the same function of decomposing labile OM, leading to their identification as a group. These functional groups of abundant and diverse mega-, macro-, meso- and microfauna and microorganisms living in soils mediate essential ecosystem services (**Figure 6**) (Barrios, 2007; FAO *et al.*, 2020). Most importantly, ecosystem services (ESS) are not relevant only to farmers, but are also essential to the well-being of human society as a whole (Orgiazzi *et al.*, 2016).

Nevertheless, soils worldwide are characterized by a rapid degradation potential, and very low formation or regeneration (Diacono and Montemurro, 2010). It has been estimated that to date, about 12 billion tonnes of soils are lost annually through erosion, compaction, salinization and unsustainable

FIGURE 5.

KEY SOIL FUNCTIONS THAT SUSTAIN LIFE ON EARTH.

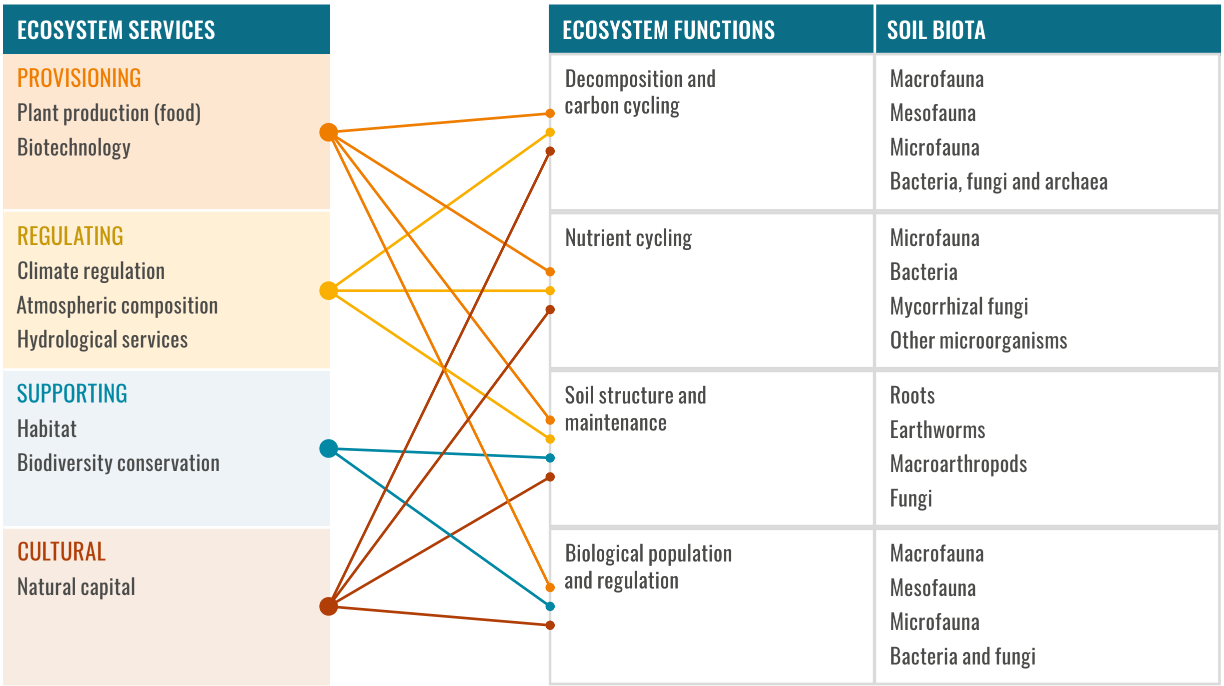


Source: adapted from https://www.fao.org/publications/card/en/c/0815e457-c6a4-47e9-ab6c-f23224279834

FIGURE 6.

SOIL-BASED ECOSYSTEM SERVICES, AND THE ECOSYSTEM FUNCTIONS AND SOIL BIOTA THAT SUPPORT THEM.

Ecosystem functions are the underlying processes that produce ecosystem benefits acquired by human society, known as ecosystem services.



ECOSYSTEM SERVICES

PROVISIONING
Plant production (food)
Biotechnology

REGULATING
Climate regulation
Atmospheric composition
Hydrological services

SUPPORTING
Habitat
Biodiversity conservation

CULTURAL
Natural capital

ECOSYSTEM FUNCTIONS	SOIL BIOTA
Decomposition and carbon cycling	Macrofauna Mesofauna Microfauna Bacteria, fungi and archaea
Nutrient cycling	Microfauna Bacteria Mycorrhizal fungi Other microorganisms
Soil structure and maintenance	Roots Earthworms Macroarthropods Fungi
Biological population and regulation	Macrofauna Mesofauna Microfauna Bacteria and fungi

Source: Orgiazzi et al., 2016.

practices. Therefore, **guaranteeing sustainable food production and ecosystem stability, and protecting natural resources for future generations begins with the decisions we make now regarding the management of our soils**. The UN Decade on Ecosystem Restoration provides a unique opportunity for us to highlight the importance of soil health and to spur action to regenerate our soils.

1.3

RESEARCH AIMS, RESEARCH QUESTIONS AND REPORT STRUCTURE

This study aims to promote debate among the scientific community, policymakers, private sector actors and consumers. It also aims to inform research on issues that need further investigation from a microbiome perspective. To this end, the research questions were:

1. Why does the soil microbiome matter for a healthy planet and ESS? Specifically, what are the relationships between the soil microbiome and climate change, and the soil microbiome and human health?
2. What are the impacts of crop production practices on the soil microbiome?
3. Does scientific literature show any significant impact of crop production practices on the soil microbiome, with subsequent, clear causal links to climate change and/or human health?
4. From a research perspective, which issues need further investigation?
5. From a policy perspective, is there any solid evidence that can and should inform policy?

To try to answer the above questions, **a literature review of recent scientific research was conducted in two parts** (see **Figure 7** for the report structure).

First, **Sections 3** and **4** present findings of an initial review by weaving studies into narratives around certain themes (referred to as the narrative review). Many studies are also summarized individually, offering the opportunity to explore a subject in more detail. Our intention is to provide clear, comprehensive messages while also communicating technical details with precision for those who wish to delve deeper into any particular subject. **Section 3** concerns why the soil microbiome matters for a healthy planet and healthy humans (research question 1), and **Section 4** addresses the impact of different crop production practices on the soil microbiome (research question 2).

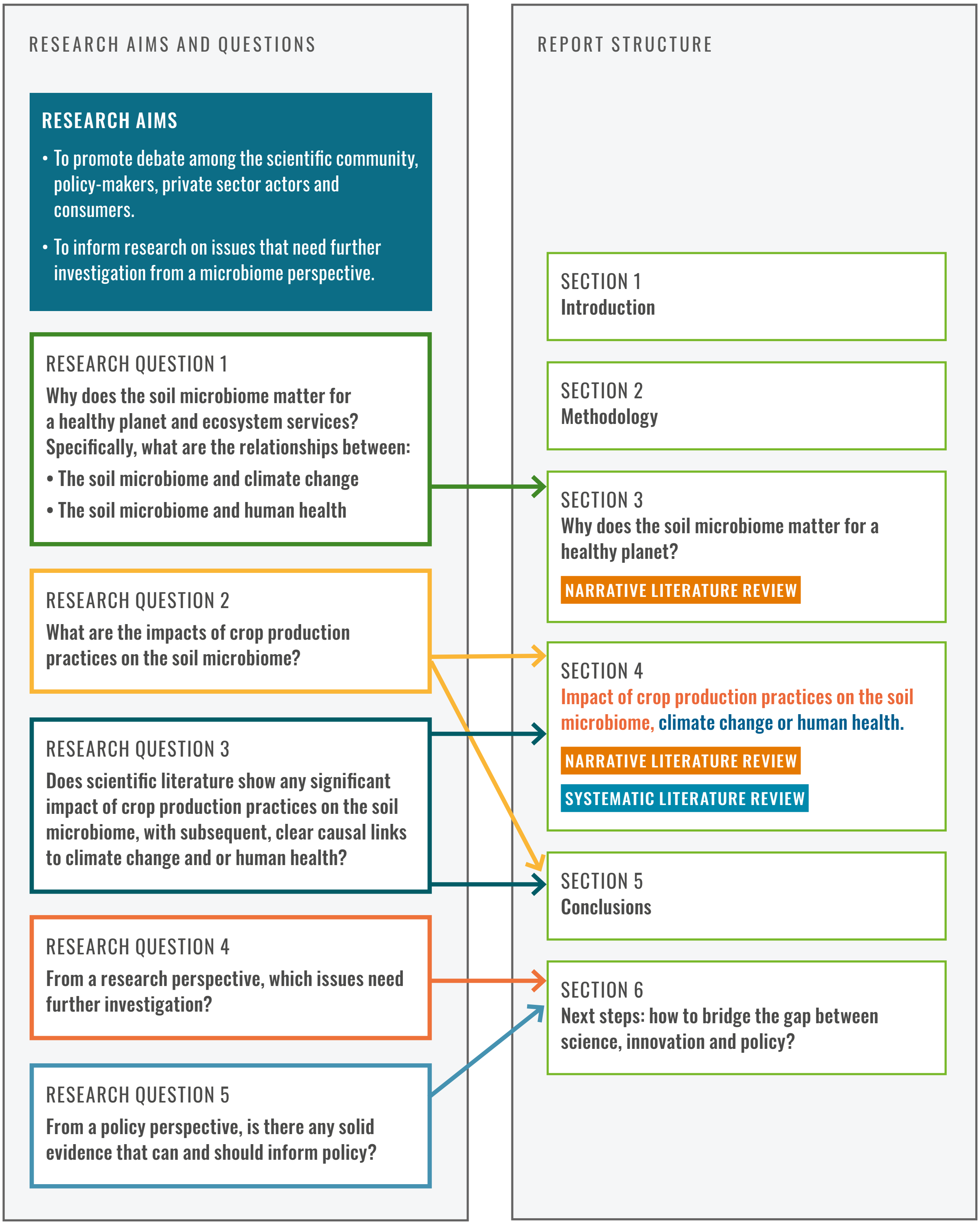
Second, selected articles from a systematic literature review with a narrower focus are discussed in greater technical detail (research question 3). They are presented in **Section 4**, under the corresponding crop production practices.

Research questions 4 and 5 provide the framework for conclusions drawn from the literature review, presented in **Section 5**.

Lastly, **Section 6** addresses how to bridge the gap between science, innovation and policy. This includes a presentation of research gaps and needs, discussion of key opportunities and challenges related to the soil microbiome and bioeconomy, and action areas and policy recommendations to address research gaps and support bio-innovations.

FIGURE 7.

REPORT STRUCTURE: RESEARCH AIMS, RESEARCH QUESTIONS AND CORRESPONDING SECTIONS.



RESEARCH AIMS AND QUESTIONS

RESEARCH AIMS

- To promote debate among the scientific community, policy-makers, private sector actors and consumers.
- To inform research on issues that need further investigation from a microbiome perspective.

RESEARCH QUESTION 1

Why does the soil microbiome matter for a healthy planet and ecosystem services? Specifically, what are the relationships between:

- The soil microbiome and climate change
- The soil microbiome and human health

RESEARCH QUESTION 2

What are the impacts of crop production practices on the soil microbiome?

RESEARCH QUESTION 3

Does scientific literature show any significant impact of crop production practices on the soil microbiome, with subsequent, clear causal links to climate change and or human health?

RESEARCH QUESTION 4

From a research perspective, which issues need further investigation?

RESEARCH QUESTION 5

From a policy perspective, is there any solid evidence that can and should inform policy?

REPORT STRUCTURE

SECTION 1

Introduction

SECTION 2

Methodology

SECTION 3

Why does the soil microbiome matter for a healthy planet?

NARRATIVE LITERATURE REVIEW

SECTION 4

Impact of crop production practices on the soil microbiome, climate change or human health.

NARRATIVE LITERATURE REVIEW

SYSTEMATIC LITERATURE REVIEW

SECTION 5

Conclusions

SECTION 6

Next steps: how to bridge the gap between science, innovation and policy?

Source: Authors' own elaboration.

CHAPTER

2

METHODOLOGY

2.1 NARRATIVE LITERATURE REVIEW

A narrative literature review was performed to explore why the soil microbiome matters for a healthy planet and ecosystem services (ESS), and the impacts of crop production practices on the soil microbiome. Selection of articles depended on what the authors considered the most pertinent scientific findings and topics. Criteria for article selection were: being a top-cited article or being particularly relevant, offering a less-explored but credible perspective, and being recently published.

Nine crop production practices were reviewed separately: land use, tillage, agroecosystem diversity (intercropping, cover cropping, agroforestry, plant diversity, crop rotation), crop residue management, plant variety selection, irrigation, fertilization, pest management, and microplastics in agricultural soils. Addressing the effects of farming systems that combine specific agricultural practices, such as conservation agriculture, were beyond the scope of this study.

2.2 SYSTEMATIC LITERATURE REVIEW

A systematic literature review was conducted in a series of searches between February 2019 and June 2020 using the Scopus database to explore whether there is solid evidence of the impact of crop production practices on the soil microbiome, with clear, causal links to climate change and/or human health. Regarding climate change, the links included positive climate feedback via greenhouse gas (GHG) emissions, soil carbon (C) storage, and biogeochemical cycling in terrestrial ecosystems.

Search term combinations were selected to simultaneously target three subjects: a crop production practice, the soil microbiome, and either climate change or human health. The crop production practices were the same as in the narrative review. Terms were searched in journal article titles, abstracts and keywords, in an unrestricted time period.

The approximately 2 000 articles were screened for relevance, and were selected only if pertinent to the three criteria described.[2] See **Table 1** for the number of results retrieved and selected for this review, per crop production practice. The search terms and combinations, dates and total number of articles retrieved and reviewed versus those selected for this study are indicated in **Annex I**.

To target the human health criteria, search terms were selected based on their common use in general and scientific literature that addresses non-communicable human diseases related to environmental factors, including conditions suspected to be influenced by microbiota. The search terms and combinations, dates and total number of articles retrieved and reviewed versus those selected for this study are also reported in **Annex I**.

2.3
FOCUS GROUP WORKSHOPS

A timely conference helped strengthen our analysis of research needs as well as inform policy recommendations. This literature review is part of a larger project within FAO that seeks to explore how microbiome disturbance in different ecosystems can affect climate change and non-communicable diseases in humans. In July

TABLE 1.

SEARCH RESULTS RETRIEVED AND INCLUDED IN THIS STUDY FOLLOWING A SCOPUS SYSTEMATIC LITERATURE RETRIEVAL.

CROP PRODUCTION PRACTICE	RESULTS RETRIEVED	RESULTS INCLUDED IN STUDY	SEARCH DATE RANGE
LAND USE	422	33	9 MARCH 2020 -12 MAY 2020
TILLAGE	125	17	20 MAY 2019 – 5 JUNE 2020
PLANT DIVERSITY	46	8	1 FEBRUARY 2019 – 12 MAY 2020
CROP ROTATION	70	7	1 FEBRUARY 2019 – 17 JUNE 2020
COVER CROP	29	5	11 FEBRUARY 2019 – 12 MAY 2020
CROP RESIDUE MANAGEMENT	87	23	27 MAY 2019 – 23 JUNE 2020
PLANT VARIETY SELECTION	95	10	1 FEBRUARY 2019 – 22 JUNE 2020
IRRIGATION	186	14	1 FEBRUARY 2019 – 3 JUNE 2020
FERTILIZER	749	96	2 MAY 2019 – 9 MARCH 2020
PEST MANAGEMENT	211	12	29 MAY 2019 – 1 JUNE 2020
MICROPLASTICS IN AGRICULTURAL SOILS	4	2	6 NOVEMBER 2019 – 23 JUNE 2020
TOTAL	2024	227	

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[2] The majority of the articles returned during searches included one of the search terms in the text, or in a figure, but not within the context of connecting impacts of crop production on the soil microbiome and climate change or human health. These articles were not selected for discussion in this review.

of 2020, it hosted the *Microbiome: The Missing Link(s) Learning Pathway*, a series of virtual presentations, discussions and workshops with international microbiome experts.[3] As part of this event, the authors of this review held three workshops focusing on the soil microbiome. The approximately 15 participants were international soil microbiome experts working in research, industry or policy.

The objectives of these soil microbiome-focused workshops were to:

- Share different expertise on the current state of knowledge regarding the soil microbiome, while building relationships for future opportunities to continue sharing knowledge that can inform policy.
- Identify and develop a common understanding of key messages related to soil microbiome knowledge gaps and research opportunities.
- Highlight opportunities and risks for engineering soil microbiomes across the full range of applications, from agricultural practices to product innovations.
- Identify existing examples of soil microbiome in policy, and discuss general recommendations that should continue, or be introduced, to inform policy.

2.4

CONCLUSIONS AND POLICY RECOMMENDATIONS

In **Section 6** we highlight which knowledge gaps or research issues merit further investigation based on solid evidence from our literature reviews as well as the focus group workshops with soil microbiome experts.

The research issues were identified based on three principal criteria: i) repeated, thematic calls in the scientific literature or by experts about the necessity and significance of the particular knowledge gap, ii) apparent, relative novelty and importance of the research issue (in other words, it may not have been a frequently addressed theme but seemed potentially influential), and iii) relevance to the concepts that frame this paper.

Overall, the work process began with our completion of the narrative and systematic reviews, followed by the presentation of highlights and conclusions of the reviews to the focus group participants. Together with the soil microbiome experts, we jointly identified the research issues, opportunities and risks of microbiome innovations, and policy recommendations presented in this study. This process was possible owing to the suggestion by participants that, given its ability to create momentum and bring together actors from different sectors from across the world, the FAO could function as a microbiome science-policy intermediary.

Contributions from the focus group workshops consisted of documented collective agreement on subjects related to objectives described above. The typical process was such that the main objective and background information were presented, followed by mini working groups of two to four people who then reported back to the main group through brief oral presentations and short written summaries. Discussion with the entire group provided feedback on different ideas, and a summary of key points related to workshop objectives was also documented. All written summaries were available to all participants on a Moodle forum (an online learning platform), including the possibility to post direct feedback, which encouraged further discussion using forum threads.

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[3] Following the recommendation by participants, *The Microbiome: The Missing Link(s) Learning Pathway* evolved into the FAO-led Microbiome Network, bringing together international microbiome experts in research, industry and policy sectors.

CHAPTER

3

WHY DOES THE SOIL MICROBIOME MATTER FOR A HEALTHY PLANET?

This section starts with a detailed introduction to the soil microbiome, emphasizing the positive relationship between soil biodiversity and the provisioning of essential ecosystem functions and ecosystem services (ESS), including contributions to food security. This is followed by a description of the direct role played by soil microorganisms in greenhouse gas (GHG) fluxes and soil carbon (C) dynamics, situated in the context of climate change adaptation and mitigation. Lastly, there is a discussion of how the soil microbiome is connected to human health.

3.1
THE SOIL MICROBIOME AND ECOSYSTEM SERVICES

HIGHLIGHT BOX 1 The soil microbiome and ecosystem services

- Soil microorganisms are the living bacteria, archaea, fungi, algae, and protozoa inhabiting the soil. The soil microbiome refers to these microorganisms, their genes, mobile genetic elements (viruses, phages), microbial structures, metabolites, and relic DNA within a given soil habitat.
- The soil microbiome performs critical soil functions, including nutrient cycling, biological regulation, and soil structure improvement. In ecology, functional diversity and redundancy are fundamental concepts that link soil microbial functions to ecosystem services.
- The soil microbiome plays a key role in simultaneously maintaining several ecosystem functions and services, referred to as multifunctionality. These include provisioning services that have impacts on food security (food availability, stability, nutrition and safety).
- The relationship between ecosystem functions and biodiversity is of key importance: enhancement and protection of soil biodiversity is essential for multifunctionality performance and sustainability of agricultural production.
- A holistic approach, compared to a reductionist approach, designs for agroecosystems that foster system multifunctionality and resilience. This is expected to improved crop production, resource use efficiency through niche differentiation, functional complementarity and functional redundancy.
- We know relatively little about the soil microbiome, yet already clearly observe its fundamental role in ecosystem functions. Therefore, pursuing knowledge in this field will be of tremendous value.

3.1.1
What is the soil microbiome?

Soil microorganisms (or soil microbiota) are the living bacteria, archaea, fungi, algae, and protozoa inhabiting the soil. The **soil microbiome** refers to these microorganisms, their genes, mobile genetic elements (viruses, phages), microbial structures, metabolites, and relic DNA found in a given soil habitat (Berg *et al.*, 2020).[4] Soil commonly consists of more than 1000 kg of microbial biomass C per hectare. Bacteria and fungi typically constitute 10^2–10^4 more biomass than protists, archaea and viruses (Fierer, 2017), though numerically, viruses are likely more abundant (Breitbart and Rohwer, 2005).

The soil microbiome is characterized by a high spatial and compositional variability due to its diversity and the multiple interactions with environment, climates and soil modifications. It is dynamic and interactive, forming micro-ecosystems that change constantly in time and scale. Furthermore, it is also integrated into macro-ecosystems – whether in eukaryotic hosts or at a field scale (Berg *et al.*, 2020). For example, overall, the soil microbiome varies more with environmental variables than with geographic distance (Bahram *et al.*, 2018). This means that **there is no typical soil microbiome**, as it can vary considerably over a scale of just centimetres.

The soil microbiome plays a critical role in fundamental soil processes such as nutrient cycling, organic matter (OM) degradation, soil structure improvement, nitrogen (N)-fixation, and biological regulation. Soil microorganisms are frequently assigned to functional groups based on genetic characterization, rather than

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[4] See Key Terms for a brief description of soil microorganisms.

associating species with specific processes in soil functions, because those direct links are difficult to identify. Studying genetic diversity provides a useful solution to understand the relationship between the soil microbiome, biodiversity and ESS.

3.1.2
Functional diversity and provisioning of ecosystem services

So what does diversity imply? **Why is soil biodiversity so important for the provisioning of ESS?** Let's start with the concept of functional diversity, which is a measure of the value and range of functional traits that exist in a given ecosystem (Petchey and Gaston, 2006). An ecosystem with high functional diversity means that it features organisms responsible for many different tasks (functions); **an ecosystem with high functional diversity will have diverse biological activities** (Goswami *et al.*, 2017). A related and equally critical concept is functional redundancy (or the species redundancy hypothesis), which refers to the presence of different species responsible for the same function (Goswami *et al.*, 2017). **An ecosystem with high functional redundancy is more resilient to shock, because even if some species populations experience a major collapse, there are others that will continue performing their common task**.

The diversity of the myriad soil organisms drives the maximum exploitation of available resources in a given habitat (Ferris and Tuomisto, 2015). However, we have studied only a tiny fraction of the soil microbiome's genetic diversity. For instance, a recent study found that out of 160 million unique genes identified in the soil, only 0.51 percent were found in published genomes and large gene catalogues (Bahram *et al.*, 2018). The massive range of unknown diversity is frequently described in terms of promising innovations, the implication being that understanding and harnessing soil microbe functions offers enormous potential for sustainable soil management. **While relatively little is known about the soil microbiome, the immense genetic variety, as well as the diverse ecosystem functions they perform, is evident.**

3.1.3
Soil microbiome functional diversity and multifunctionality

Soil microorganism biodiversity and ESS provisioning can be linked by another fundamental concept: **multifunctionality. This term describes the simultaneous maintenance of multiple ecosystem functions and services by the soil microbiome** (Delgado-Baquerizo *et al.*, 2016). Studying multiple functions offers the possibility to consider trade-offs between functions and how certain factors (e.g. biodiversity) can affect multiple functions (Allan *et al.*, 2015; Garland *et al.*, 2020). The following three studies illustrate how.

Wagg *et al.* (2014) investigated whether changes in soil biodiversity and soil microorganism community composition influenced multiple ecosystem functions.[5] The reduction in soil biodiversity correlated with a drop in plant species diversity, demonstrating that plant composition can be driven by the diversity of soil biological communities. The decrease in soil biodiversity also affected nutrient cycling, resulting in lower levels of sequestered C, recycled N, and decomposed litter. It also resulted in higher trends of nitrous oxide (N_2O) emission and phosphorus (P)-leaching. **Overall, changes in soil biodiversity affected ecosystem multifunctionality.** (**Figure 8**). Nevertheless, major changes in ecosystem functionality were only observed in highly simplified soil communities. This points to the high level of ecological functional redundancy in the soil communities studied, which resulted in ecosystem functions that were highly resilient to changes in soil biodiversity. These results clearly indicate the importance of microbial soil communities in the provisioning of ESS. **It also supports a key message: that the enhancement and protection of soil biodiversity is of utmost importance for the sustainability of agricultural production.**

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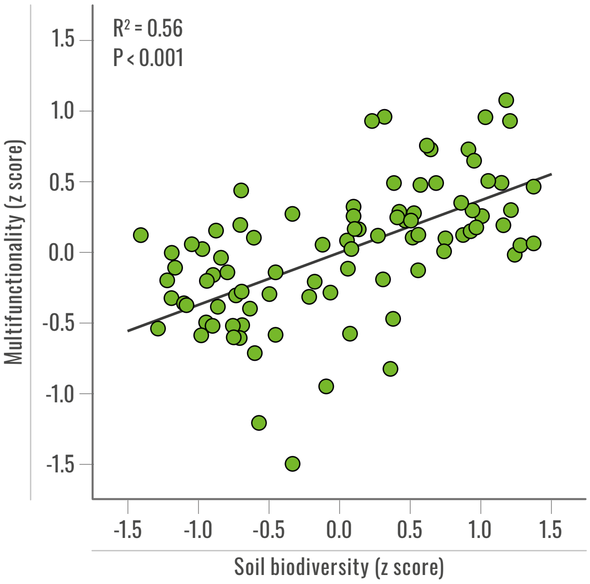
[5] The ecosystem functions studied can differ according to the design of the experiment investigating multifunctionality. Wagg *et al.* (2014), for example, considered N turnover, litter decomposition, plant diversity, plant productivity, nitrate leaching, ammonium leaching, phosphate leaching and organic P leaching.

FIGURE 8.

ECOSYSTEM MULTIFUNCTIONALITY INDEX IN RELATION TO THE SOIL BIODIVERSITY INDEX.

The dots represent data from grassland communities, and the single line shows the best fit for the different data points.



Source: Wagg et al., 2004.

Delgado-Baquerizo *et al.* (2016) explored the role of microbial diversity on multifunctionality both in isolation and including other variables such as edaphic, climatic and spatial predictors. To test these hypotheses they used two large-scale datasets: a global study including 78 drylands from all continents except Antarctica, and a national soil survey that included 179 locations in Scotland. Both datasets included diverse ecosystem types. The direct effects of microbial diversity were evident even when accounting simultaneously for other drivers such as climate, soil abiotic factors and spatial predictors. **This study provides empirical evidence that microbial diversity positively relates to multifunctionality, and that any loss in microbial diversity will likely reduce multifunctionality, with potential negative consequences on climate regulation, soil fertility, and food and fibre production.**

While the previous study described the association between losses in microbial diversity and a reduction in multifunctionality, Delgago-Baquerizo *et al.* (2020) **also provided solid evidence of the positive link between soil biodiversity and multifunctionality at the global level**. This study emphasises that understanding the link between soil biodiversity and multifunctionality is fundamental to elucidate the potential consequences of soil biodiversity loss in relation to ecosystem functions and services in the future.

3.1.4
Soil microbiome, soil biodiversity and food security

Biodiversity on the whole is essential to food and agriculture, and yet, evidence indicates that many categories of species and ecosystems are declining. **Intensive agricultural practices such as heavy use of fertilizers and pesticides impact the soil microbiome, translating into costs to the environment, food productivity** (Microbiology Society, 2015) **and food security** (Singh and Trivedi, 2017). Underlining its critical contribution in this area, the State of the World's Biodiversity for Food and Agriculture report argues that soil microorganisms are vital to food security, and that improving their management can contribute to improved food security (FAO, 2019b). Pilling, Bélanger and Hoffmann (2020), referring to biodiversity in general (and not just soil microorganisms), deliver a clear message: there is urgent need for a global policy response to augment both "the coherence and effectiveness of efforts to protect and better manage biodiversity for food and agriculture".

A recent review by El Mujtar *et al.* (2019) analysed the relationship between soil biodiversity and four dimensions of food security: food availability, food access, stability and utilization (the latter including nutrition & safety). As a starting point, they classify soil biodiversity into three broad functional groups, in which the soil microbiome plays an essential role in delivering ESS and functions that provide the foundation of food security (**Figure 9**).[6]

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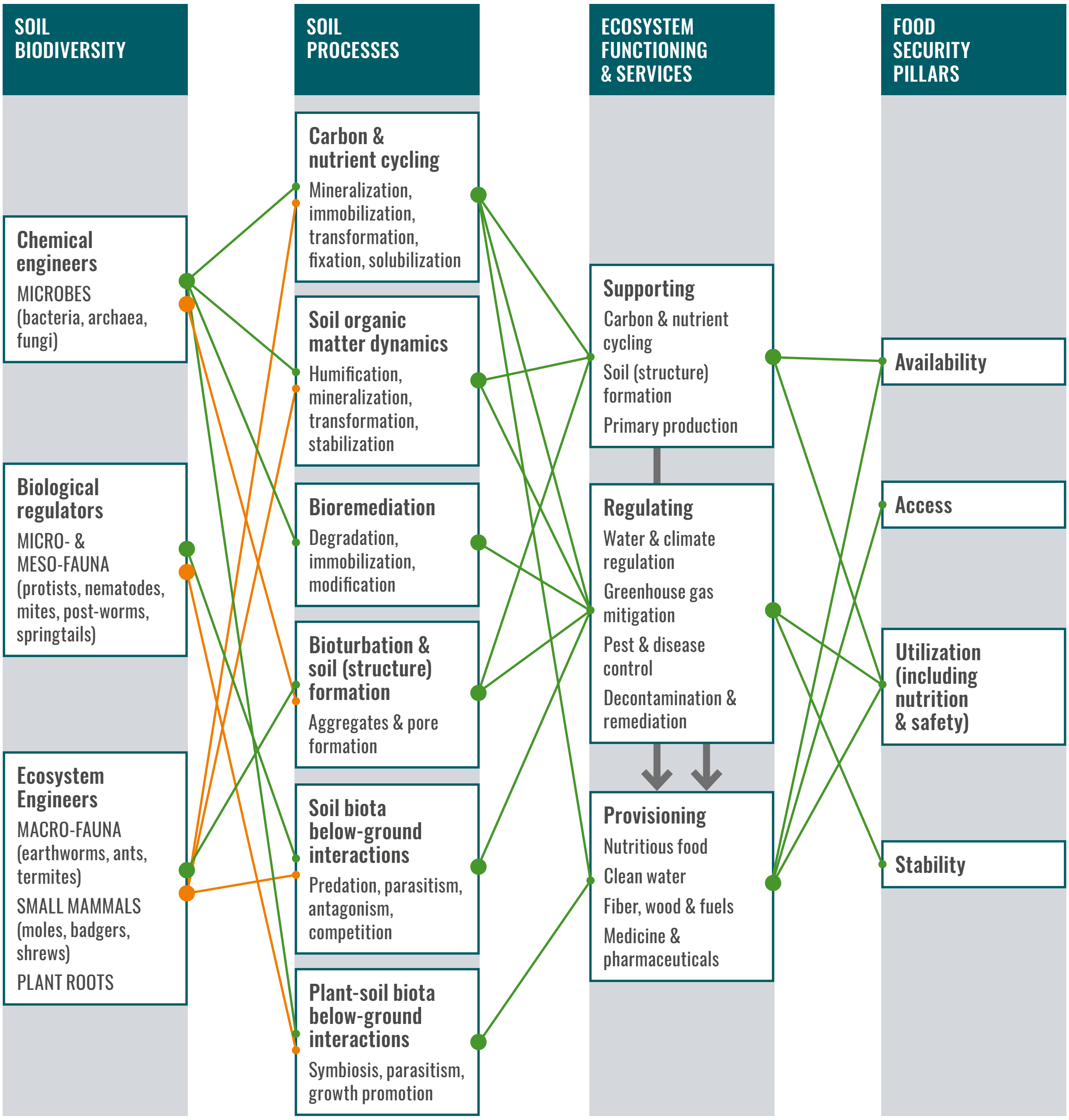
[6] Note: This is a simplification. In reality some taxa can appear in more than one functional groups.

FIGURE 9.

RELATIONSHIPS BETWEEN SOIL BIODIVERSITY AND THE FOUR FOOD SECURITY PILLARS, MEDIATED BY SOIL PROCESSES AND ECOSYSTEM FUNCTIONING AND SERVICES.

Green arrows and orange arrows indicate, respectively, major and minor roles of functional groups on soil processes. Gray arrows indicate the relationships among supporting, regulating and provisioning ecosystem services. Note that this diagram refers to soil microbes as well as other soil organisms (micro-, meso- and macrofauna).



Source: El Mujtar et al., 2019.

The authors discerned between direct and indirect impacts of soil biodiversity on agricultural productivity, and thus food availability.

a **Direct impacts** take place in the rhizosphere between soil microorganisms and plants. They can be described as biocontrol (e.g. antibiotic production) or several mechanisms collectively known as plant growth promotion (e.g. biological N fixation). There is evidence that soil biodiversity affects crop productivity.

b **Indirect impacts** include improving soil structure, forming soil organic matter (SOM), and nutrient cycling. Also, as we will discuss later in this section, soil microorganisms regulate GHG fluxes, directly implicating them in climate change mitigation and adaptation strategies, which could have indirect impacts on agricultural productivity.

The authors also found that although knowledge of these processes is fundamental, it is rarely cited while framing sustainable soil management practices. Moreover, evidence from laboratories and field studies point to desirable synergies between C sequestration, soil fertility and food security.

The review also highlighted implications for food nutrition and food safety in recent research trends. For example, soil biodiversity is critical to the extraction of nutrients from the soil, their accumulation in plants, and their subsequent availability to humans through crops. Arbuscular mycorrhizal fungi were highlighted as the most studied soil microorganism regarding nutritional value of edible plants (Kawaguchi and Minamisawa, 2010). Arbuscular mycorrhizal fungi have also been identified as being important in immobilizing trace elements in plant roots, and reducing the transference to shoots and other edible parts of the plant (Ferreira *et al.*, 2018; Gao *et al.*, 2011). Other studies have also explored soil biodiversity's role in mitigating trace element contamination and populations of human pathogens (e.g. *Salmonella enterica*) in soils and vegetables (e.g. Jeffery and Putten, 2011; Wu *et al.*, 2016). Certain rhizosphere microorganisms may also help plants produce phytonutrients, which are important in promoting human health by reducing oxidative damage and stimulating the immune system, amongst other functions (Giovannetti, Avio and Sbrana, 2013). Though more knowledge would improve understanding of these connections, there is evidence that soil biodiversity is important in ensuring food safety.

Lastly, the authors projected how trends in the literature could translate into practice. **They identified two different approaches to soil biodiversity and ESS**. One, a reductionist approach, is based on tapping the diversity of soil microorganisms and genes through biotechnology. An example could be a strategy to develop and inoculate with particular strains identified for their potential to foster beneficial soil-plant interactions, aiming to improve crop production. In such a case, however, there are significant challenges in bridging experiences between labs, where the inoculum strains are developed and tested, and the field, where the inoculum products would need to function in a real-world environment. A holistic approach, in contrast, would try to foster system multifunctionality resistance through agroecosystem design. This would be expected to lead to improved functions and services such as crop production, resource use efficiency through niche differentiation, functional complementarity, and functional redundancy.

3.2
THE SOIL MICROBIOME AND THE CLIMATE

HIGHLIGHT BOX 2 The soil microbiome and the climate

- The soil microbiome is a direct controller of biochemical cycles involved in greenhouse gas emissions and carbon storage. Its interactions with abiotic factors and biological processes are very complex. It plays a particularly important role in fluxes of nitrous oxide, carbon dioxide, methane and in soil carbon storage.
- The soil microbiome contributes to carbon dioxide fluxes mainly through processes of respiration and decomposition. This involves many different microorganisms and processes.
- Methanogens are anaerobic archaea that produce methane. Methanotrophs, microorganisms that exclusively consume methane for energy, are the only known methane sink.
- Certain bacteria and archaea are responsible for the nitrification process that produces nitrous oxide. Specific groups of microbes mediate the multi-step process of denitrification.
- Soil carbon storage and carbon sequestration differ in form and soil residence times. Microbial necromass may be an underestimated contributor to soil carbon storage and sequestration.
- Adaptation to climate change will require more knowledge of soil biodiversity and functions. This knowledge would also contribute to the engineering of agroecosystems that promote climate change-mitigating ecological processes such as increasing soil organic carbon stocks and reducing greenhouse gas emissions.
- Crop production practices may cause changes in the soil microbiome, provoking shifts in greenhouse gas emission and carbon storage. But how changes in the soil microbiome can affect global climate change is a difficult question to answer, and how to exploit positive feedbacks perhaps even more challenging.

3.2.1
Impact of climate change on land and food security

According to the recent Intergovernmental Panel on Climate Change (IPCC) assessment report on climate change (2021), global surface temperature was 1.09°C higher in 2011– 2020 than 1850–1900, with larger increases over land (1.59°C) than over the ocean (0.88°C). The average land surface air temperature increased 1.41°C between 1880 and 2018. **Looking forward, changes in variables such as temperature and precipitation, but also wind, humidity and extreme climate and weather events, are projected to negatively affect all aspects of food security at local levels, thereby leading to complex impacts at a global scale** (IPCC, 2019). Each aspect of food security (food availability, access, utilization and stability) is a multifaceted factor in itself, and all are intricately interrelated, therefore potentially compounding impacts on any single aspect.

Anthropogenic greenhouse gas emissions impact the earth through changes in climate and through the composition of GHGs themselves, carbon dioxide (CO_2) in particular. Negative effects such as new, hot climates in the tropics and increases in the frequency and intensity of extreme events like rainfall, droughts and heat waves have been widely acknowledged. It is clear that extreme weather and climates affect food security negatively. Regional reductions of crop yields are an example: a recent study demonstrated that between 18 to 43 percent of yield variance (of maize, soybeans, rice, and spring wheat) was attributable to extremes of temperature and rainfall (Vogel *et al.*, 2019).

The IPCC special report on climate change and land (2019) also outlines anticipated trends that are directly attributable to climate change. Anthropogenic global warming has already resulted in shifts of climate zones which are likely to be exacerbated in the future; these include increases in dry climate, decreases of polar climates, new hot climates in the tropics, and directional shifts in climate zones, especially in the mid to high latitudes (poleward) and in higher elevations (upward). This means that ecosystems – including the soil microbiome component – will experience temperature and rainfall extremes beyond what they are currently used to, potentially modifying their structure, composition and functioning. The effects of climate change on the abundance, diversity and functioning of soil microbiome have received attention by the scientific community in the last decades (e.g. Allison *et al.*, 2010; Classen *et al.*, 2015; Igiehon & Babalola, 2018; Dubey *et al.*, 2019; Robinson *et al.*, 2019) but the subject falls beyond the scope of this document.

Soil management practices will need to have climate change adaptation and mitigation in the forefront. How does the soil microbiome fit into this context?

3.2.2
Greenhouse gas emissions from agriculture, forestry and other land use

Agriculture, forestry, and other land use (AFOLU) contribute about 22 percent of anthropogenic CO_2, methane (CH_4) and N_2O emissions (IPCC, 2019). Their emissions and removal from the atmosphere are referred to as fluxes. **It is known that the soil microbiome is a direct controller of biochemical cycles involved in GHG emission and C storage** (Singh *et al.*, 2010). **Soil microorganism activities involved in GHG fluxes are extremely complex because they involve many different organisms, as well as their interactions with climatic, edaphic and management factors. Another challenging aspect is that so many remain unstudied or unknown**. A better understanding of soil microorganisms and their functions would inform our current understanding of the biogeochemical pathways underpinning SOC decomposition and production of GHG (Jansson and Hofmockel, 2020). One desired potential outcome, for instance, is the design and use of improved agricultural practices to prevent soil C loss.

The role of soil microorganisms in GHG fluxes is explored below, focussing on nutrient cycles and soil functions related to CO_2, CH_4, N_2O and soil C storage.

Carbon dioxide

Agriculture, forestry and other land use accounts for approximately 14 percent of all anthropogenic CO_2 emissions (IPCC, 2019).

The soil microbiome contributes directly to the net C exchange between the soil and atmosphere, primarily through processes of decomposition and respiration (**Figure 10a**). When soil microorganisms degrade biomass, or OM, their respiration incurs C-loss in the soil in the form of CO_2. Estimates have suggested that 50 percent of CO_2 released from the earth's ecosystem into the atmosphere is attributed to microbial respiration (IPCC, 2019).

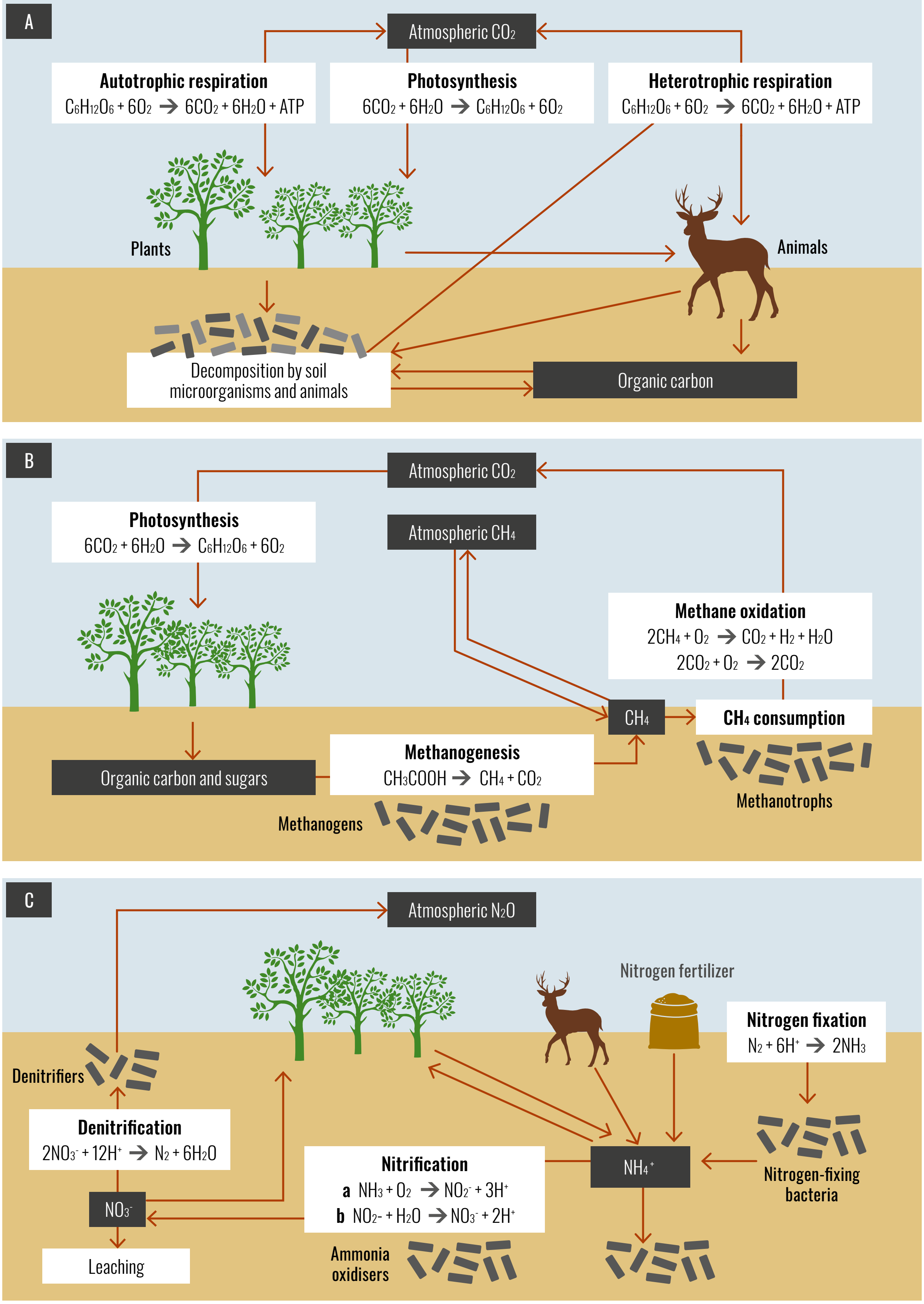
The soil microbiome also contributes to the C cycle indirectly by influencing nutrient availability and their subsequent uptake by plants. Higher plants take up the majority of atmospheric CO_2 during photosynthesis, but the soil microbiome is directly involved in the overall fluxes, thereby influencing C turnover and retention in the soil. In this way, soils act as a buffer against atmospheric CO_2 increase as well as a potential CO_2 sink (Trivedi, Anderson and Singh, 2013).

Methane

Land use-related causes are a net source of CH_4, accounting for 61 percent of anthropogenic CH_4 emission between 2005-2015, important contributors being tropical wetlands, peatlands, ruminants, and expansion of rice cultivation (IPCC, 2019). **Natural emissions are mediated by microbial methanogens, which are anaerobic archaea that produce CH_4** (**Figure 10b**). **Methanotrophic bacteria, on the other hand, consume CH_4 as a source of energy and release CO_2; methanotrophic bacteria are also the only known CH_4 sink on earth** (Jiang *et al.*, 2011; Zeng *et al.*, 2019a).

FIGURE 10.

BIOCHEMICAL TRANSFORMATIONS INVOLVED IN EMISSIONS OF (A) CO_2 (CARBON DIOXIDE), (B) CH_4 (METHANE) AND (C) N_2O (NITROUS OXIDE) EMISSIONS. A simplified depiction of the organic C (carbon) cycle is also shown in (A).



Source: Singh et al., 2010.

Nitrous Oxide

Natural and anthropogenic emissions of N_2O are directly related to nitrification and denitrification processes mediated by the soil microbiome (**Figure 10c**). **Agriculture, forestry and other land use is the main anthropogenic source of N_2O, mainly due to N-application to soils (e.g. fertilization in agriculture).** Atmospheric N-deposition contributed by fossil fuel combustion, changes in land use patterns and use of N-fertilizer (Waldrop *et al.*, 2004; Zhaohui *et al.*, 2012) may lead to imbalances of N versus P availability, as well as changes in soil microbial activity, SOM decomposition and eventual reduced ecosystem stability. Decreasing chemical fertilizer applications in farming systems where current application rates are high (and even exceed crop demands for certain moments in the growing season) are therefore predicted to affect emission reductions (IPCC, 2019).

The production of N_2O results from the nitrification and denitrification processes performed primarily by nitrite bacteria, nitrifying bacteria, denitrifying bacteria as well as an ammonia (NH_3) oxidation process driven directly by ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) (Singh *et al.*, 2010; Zeng *et al.*, 2019b). Therefore, denitrification typically results in soil N losses and N_2O production.

A brief overview of soil N-cycling, including the related microorganisms and genes (see **Table 2**), may be helpful to better understand some of the ideas presented in this study. Genes are associated with specific microbial activities responsible for soil N-cycling. The presence of these genes is frequently compared with the presence of specific compounds (e.g. N_2O), enabling identification of correlations.

TABLE 2.

FUNCTIONAL GROUPS OF SOIL MICROORGANISMS ASSOCIATED WITH SPECIFIC GENES AND SOIL NITROGEN (N)-CYCLING PROCESSES

TYPE OF SOIL MICROORGANISM	GENE	PROCESS
Denitrifying bacteria	*nirK, nirS, nosZ, norB*	Denitrification
Biological N fixing bacteria (e.g. *rhizobium*)	*nifH*	Biological N fixation
Nitrate reducing bacteria	*napA, narG*	Nitrate reduction
Ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB)	*amoA*	NH_4^+ oxidation

3.2.3
Soil carbon storage

Living organisms contribute organic C into the soil (e.g. plant root turnover, plant litter). A small share of C from degraded biomass is converted into stable OM and is retained in the soil, thus the reference to the soil as a C sink. Carbon can be stored as soil organic carbon (SOC), which is easily accessible for soil microbiota and is therefore a relatively temporary form of storage. Carbon sequestration differs from C storage because it is a process that results in a net removal of atmospheric CO_2, the C being retained in more recalcitrant forms and therefore more difficult for microbiota to access. In addition to reducing effects of atmospheric CO_2, C sequestration indirectly offers other ecosystem benefits such as water quality, soil fertility, resistance to erosion, and climate mitigation (e.g. reduced feedback to climate change) (Trivedi, Anderson and Singh, 2013).

Plant photosynthesis converts CO_2 into plant biomass, thereby storing C and transferring it to the soil through root exudates and plant litter. **Net C sequestration consequently occurs when C-input from plant photosynthesis exceeds C-losses associated with microbial respiration** (Trivedi, Anderson and Singh, 2013). Liang and Balser (2010), Lange *et al.* (2015) and Chenu *et al.* (2019) suggest that **microbial necromass could substantially contribute to soil C accumulation.** A relatively little-acknowledged but very interesting perspective, it is worth exploring in detail (**Box 1**).

BOX 1. MICROBIAL NECROMASS AND SOIL ORGANIC CARBON STORAGE

Chenu et *al*., (2019) underscored the role of agroecosystem diversity in improving SOC stocks and plant-microbe relationships. The role of microbial necromass was stressed as a critical C source.

The authors argue that that increasing the abundance and diversity of soil microbial communities shows potential to increase SOM decomposition with positive feedbacks on SOC stocks, or may favour the SOC share that has a long residence time. They reported a range of studies that illustrated how soil biodiversity can increase and stabilise SOC stock, and discussed a recent concept suggesting that long-term SOM with a residence time of decades originates mainly from microbial material. The prominent role of soil microbial communities, including necromass, was highlighted in the stabilization of organic matter.

Deep soil layers are another underexplored subject related to soil C. It has been estimated that deeper soil layers (below ~20 cm) store about 50 percent of global SOC due to the increased mass with depth (Batjes, 1996; Jobbágy and Jackson, 2000). Furthermore, the age of SOC increases sharply when descending from surface to deeper soil layers, implying that SOC is stored longer in deeper soil layers, although these SOC stocks appear vulnerable to rapid decomposition when the environmental conditions that created it change (Gross and Harrison, 2019). Loss of this substantial C stock would cause massive changes in the global C cycle that would be difficult to remediate. **Biological activity, including SOC cycling processes, often continues down to 100 cm depth** (Kramer and Gleixner, 2008; Stone and Kalisz, 1991)**, making it important to understand the role of soil microorganisms in deep-soil C cycling**. Two examples help illustrate this point. To begin with, it is known that roots initiate a rhizosphere priming effect, which stimulates rhizospheric microbiota activities, causing C mineralization. **It has been demonstrated that root penetration and exudation in deeper soil layers can stimulate mineralization of even millennia-old C through the rhizosphere priming effect** (Shahzad *et al.*, 2018). The same study furthermore showed that the release was rapid (<2 years), and that the vulnerability of deep C was comparable to that measured at the surface. The second example concerns the role of subsoil arbuscular mycorrhizal fungi (AMF) in subsoil C sequestration. Recent studies have indicated that subsoil C, presumed to exist for a long time, may actually be less stable than previously believed (Gross and Harrison, 2019; Sosa-Hernández *et al.*, 2019). Sosa-Hernández *et al.* (2019) argue that though **AMF in deep soils can increase litter decomposition in the short term, they could also help contribute to soil C stabilisation in several ways.** First, under conditions where subsoil AMF can outcompete microbial decomposers, they could reduce the loss of added or stabilised C, because AMF help protect and sequester SOC. Second, AMF in deeper soil layers can help capture nutrients that would otherwise stimulate microbial decomposer activity, including take-up of plant-produced C compounds. Lastly, there may be a great potential for long-term stabilization of C in the subsoil, where there is often a greater amount of clay minerals (which are known to promote soil attributes that protect soil C). Subsoils also accumulate root residues, which are well known to be more recalcitrant compared to aboveground crop residues.

BOX 2. SOIL CARBON STORAGE AND SOIL MICROBIOTA RESPONSES

Here are two simple examples to highlight how soil microbiota responses are variable and difficult to generalize.

Do soils richer in copiotrophs store more C compared to those richer in oligotrophs?

This example demonstrates the complexity of what may initially appear as a simple classification of microorganism activities. Copiotrophs are organisms that thrive in environments with high C levels and easily degradable material. Oligotrophs reproduce more slowly, live in nutrient-poor environments with low C levels, and degrade more recalcitrant material. One may reason that favouring oligotrophs could favour soil C storage due to a lower C turnover, and thus result in lower CO_2 emissions. However, soil dominated by copiotrophs can actually harbour more C. Copiotrophs feed on easily accessible, labile C forms, thereby leaving behind the more recalcitrant C sources which represent the bulk of the soil C (Trivedi, Anderson and Singh, 2013).

Is the fungal:bacteria (F:B) ratio a good proxy for C sequestration?

This is another example of how microorganism-soil characterizations are not necessarily predictable across different conditions, as they include a multitude of complex factors. The quality of SOM may be described by its composition of labile (easily degradable) or recalcitrant (difficult to degrade) substrates. Fungi have a higher C:N ratio and they can feed on very recalcitrant organic matter. Conversely, bacteria have a lower C:N ratio and tend to degrade more labile biomass. A high F:B ratio was found to be associated to a more C-rich soil, or to soil which could potentially sequester more C (Malik *et al.*, 2016). Other studies, though, did not find consistent effects of SOM quality on the F:B ratio. This was explained by the observation that fungi can be in low numbers but simultaneously extremely active in high quality SOM (Soares and Rousk, 2019). In addition, Fierer *et al.* (2012) reported that F:B varied considerably across climatic areas, with temperate and boreal climates showing the higher F:B ratios.

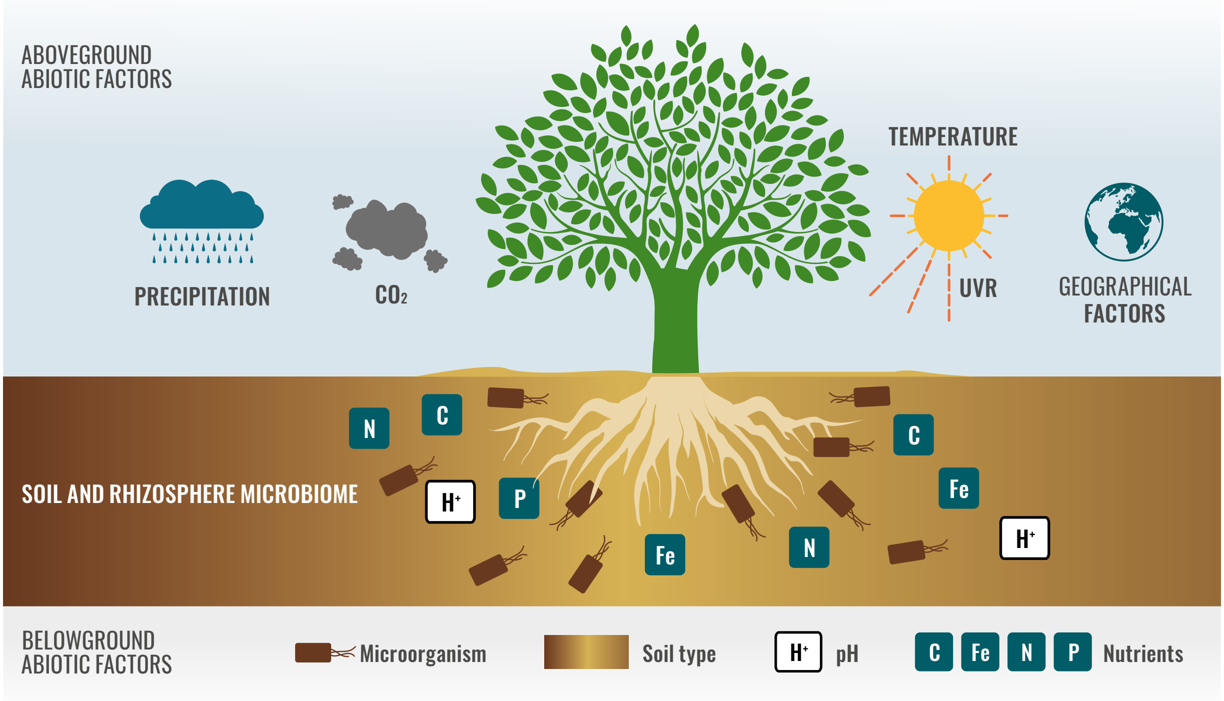
Given the key role of soil microbiota in the above-mentioned biochemical processes, changes in the soil microbiome owing to agricultural practices can potentially provoke shifts in GHG emissions and soil C storage. **Nevertheless, explicitly predicting the response of the soil microbiome to environmental changes is complex**. **For one, despite the growing body of research and literature, there is still limited knowledge about the diversity and functions of soil microorganisms. Secondly, the intricate entity that is the soil microbiome is driven by complex interactions with a large number of abiotic and biotic factors.**

Santoyo *et al.* (2017) outline specific abiotic factors that affect the soil microbiome (**Figure 11**):

- **a** Edaphic conditions: soil nutrients, SOM quantity and quality, soil type;
- **b** Climatic factors: precipitation and temperature, GHG concentration, ultraviolet rays;
- **c** Geographic factors: distance, latitude and longitude; and
- **d** Land use and management.

FIGURE 11.

ABIOTIC FACTORS THAT INFLUENCE THE SOIL MICROBIOME RESPONSE.



Source: Adapted from Santoyo et al., 2017.

Furthermore, these abiotic factors also interrelate with biological processes. For instance, enzymes are produced to respond to the quality and quantity of substrates (e.g. root exudates, dead plant matter) via changes in environmental factors such as pH, temperature, and the quality and availability of substrate (Burns *et al.*, 2013). Macro and meso fauna also interact and contribute to shaping the soil microbiome. Those interactions, processes and dynamics are complex, difficult to predict and thus exploit (Wall *et al.*, 2019). One reason is that they are difficult to classify and study separately under real-life conditions. Another is the heterogeneous nature of the soil environment, with uneven distribution of nutrients that create 'hotspots' of microbial activity and disconnected air- and water-filled pores. In other words, the soil environment is highly dynamic.

Thus, although our knowledge is growing, how changes in the soil microbiome can affect climate change remains a difficult question to be answered. How to exploit positive feedbacks may be even more challenging to answer. As posed in the review by Trivedi, Anderson and Singh (2013), **"How can we manipulate the soil microbial community to control C mobilization and storage in terrestrial ecosystems?" is indeed and yet an outstanding question.**

3.3

THE SOIL MICROBIOME AND HUMAN HEALTH

HIGHLIGHT BOX 3 **The soil microbiome and human health**

- There are strong theoretical arguments and some evidence that point to substantial connections between the soil microbiome and human health. At this point in time, however, there are many knowledge gaps to inspire further research.
- Ecological perspective: Planetary Health and One Health emphasize the interconnected health of ecosystems. The relationship between antibiotic use and antimicrobial resistance illustrates the close linkages between soil, animal and human ecosystems.
- Medical perspective:
    - Antibiotics are produced by soil microorganisms.
    - New technologies allow an additional assessment of naturally produced substances that have long been used in traditional medicines.
- Diet and lifestyle perspective: modern lifestyle changes have altered our exposure to soil microorganisms, with consequences on human health.
- Meteorological perspective: dust cloud-deposited soil microbiota and their toxins can have consequences for human health (e.g. fungal or bacterial pathogens transported by dust storms) and potentially for sensitive ecosystems in which they land.

While the links between the soil microbiome and climate change have been clearly established by the natural sciences, the links between the soil microbiome and human health are less obvious. Nevertheless, this relationship has been addressed from many perspectives, across time and cultures. Knowledge from farm practitioners and medical doctors alike, for instance, seem to have generated plenty of both hands-on and anecdotal evidence since ancient times. Knowledge about the connections between the soil microbiome and human health are part of traditional knowledge systems as well as sustainable modern agriculture approaches, such as organic and biodynamic management. More recently, research has been pursuing questions related to the soil microbiome, trace elements and antimicrobial resistance (AMR) in agricultural soils, the potential benefits for human physical and mental health by exposure to soil microorganisms, potential health risks posed by inter-continental migrations of soil microbes in dust storms, and the relationship between soil biodiversity and human health. Explored in more detail below, none of these different perspectives provide definitive answers. However, they do illustrate that the soil microbiome may play a key role in processes that can ultimately influence human health.

3.3.1
From an ecological perspective

Sir Albert Howard, for many the father of organic farming, published his book *The Soil and Health* in 1945 (Howard, 1945). In it, he laments that industrial agriculture disrupts the delicate balance of nature and steals the fertility of the soil. He then links the health crises facing crops, livestock, and humankind to the radical degradation of the soil.

In a review of Howard´s classic, Thompson (2008) describes that Howard perceived that vulnerability of unhealthy plants could be passed on to those animals or humans that consume them. Specifically, Howard argued that the inorganic N fertilizer ammonium sulphate kills mycorrhiza, thus depriving plants of an important manner of obtaining nutrients, thus making plants vulnerable to various diseases and insect pests. He believed that this mineral N-induced vulnerability of plants could be transferred up the food chain. Though decidedly unscientific, his perspective drew an early connection between the health of soil microorganisms, crops and humans.

Today, there is an ecological perspective that draws direct and indirect connections between soil biodiversity and human health (Wall, Nielsen and Six, 2015). The decline of soil biodiversity is associated with losses of ecosystem functioning and services. This impacts human health by increasing the risk of diseases caused by human pests and pathogens, by less nutritious food and a less stable food supply, and by lack of clean water and air.

Another line of reasoning suggests that if environmental microbial systems influence the microbiota and health of organisms, and there is continuous exchange of microbiota between organisms (e.g. plants, animals and humans) and ecosystems, then alterations to microbial biodiversity in one setting may well influence others (Flandroy *et al.*, 2018). This is important in light of much research that suggests that a high level of microbial diversity is linked to better human health, and that changes in microbial diversity in the human gut have potential transgenerational consequences (Flandroy *et al.*, 2018; Sonnenburg and Sonnenburg, 2014).

Summarizing these relationships, the concept of **Planetary Health** provides a useful framework to understand how processes and phenomena are interconnected when conventional approaches fail (Zhu *et al.*, 2019). Simply put, **the concept recognizes that human health is fundamentally linked to other planetary systems, and the health of all systems are connected to each other, albeit by complex ecosystem feedback processes. This notion likewise forms the foundation of the One Health framework, which promotes synergetic, interdisciplinary collaboration from a local to global level to attain optimal health for people, animals and our environment.** The FAO, for example, works together with the World Organisation for Animal Health (OIE) and the World Health Organization (WHO) to promote this approach in common, cross-cutting areas such as AMR (Lubroth, 2012). From these perspectives, we can relate the health of soils to the health of humans.

3.3.2
From a medical perspective

While ecologists and agronomists look at the opportunities that a diverse and stable soil microbiome can provide to agricultural productivity, ESS and potential benefits for consumers, **the discovery of antibiotics is probably the best known example linking the soil microbiome to human and animal health** (Chandra and Kumar, 2017). Antibiotics such as β-lactams, aminoglycosides, streptomycins, and tetracyclines and others are produced by soil bacteria and fungi. Fungal antibiotics such as penicillins, cephalosporin, fusidic acid griseofulvin, and fumagillin have been obtained through fungal species *Penicillium*, *Cephalosporium*, and *Aspergillus* – all of which live in the soil. Several *Pseudomonas* species and *Bacillus* species are among the soil bacteria exploited for the production of antibiotics like gramicidin, bacitracin, tyrothricin, pyocyanin, and pyrrolnitrin (Berdy, 1974). *Streptomyces* species, one of the soil actinomycetes, have provided the highest number of different commercial antibiotics such as tetracyclines, streptomycin, viomycin, and kanamycin.

Several other commonly used antibiotics, gentamicin and rifamycin, have been isolated from actinomycetes like *Micromonospora*, *Actinomadura*, and *Nocardia* species (Berdy, 1980).

Another, different subject relating the soil microbiome and medicine concerns **new technologies and science that enable medical professionals to assess traditional approaches to medicine from a new angle.** For instance, a product used in natural health treatments in produced by soil microorganisms stems from a humic substance called fulvic acid. Owing to its use as treatment for inflammation caused by non-communicable diseases such diabetes, it has long featured indirectly in Ayurvedic medicine, and is now widely sold as a natural health product. In their mini-review of scientific research regarding fulvic acid's ability to modulate the immune system, Winker & Gosh (2018) conclude that it may indeed modulate the immune system, influence the oxidative state of cells, and improve gastrointestinal functions; all of which contribute to prevention of chronic inflammatory diseases, including diabetes.

3.3.3
Antibiotics and antibiotic resistance

Antibiotics, however, go hand in hand with antibiotic resistance (AR). While antibiotics are indispensable in healthcare, AR and antimicrobial resistance (AMR) are currently compromising the effectiveness of antibiotics and antimicrobial drugs, thereby threatening human health, modern medicine and food security. Soil is a crucial conduit through which humans can potentially be exposed to antimicrobial resistant elements. Agricultural soils can serve as reservoirs of antibiotic resistance genes (ARGs), antibiotic residues and pathogens, particularly in mismanaged agroecosystems. Trace element accumulation, for instance, has been linked to proliferation of ARGs (Knapp *et al.*, 2011; Lin *et al.*, 2016; Wang *et al.*, 2020). **The precise mechanisms by which ARGs in the soil can impact human health still need further clarification, but the pathway has been broadly acknowledged**. Furthermore, global losses in biodiversity have raised concerns about vanishing sources of potential new antibiotics and medicines.

3.3.4
From a diet and lifestyle perspective

Recent, novel research clearly indicates that diet impacts the **human gut microbiome**, with potential consequences for human health (World Health Organization, 2020; Zmora, Suez and Elinav, 2019). What role does the soil microbiome play in this relationship, and what are the influential pathways through which humans are exposed to soil microorganisms?

There is evidence suggesting that **modern lifestyle changes have altered our exposure to soil microorganisms**. It has been demonstrated, for instance, that the diversity and richness of soil microbial communities inside homes decreased as the percentage of built area increased (Parajuli *et al.*, 2018). One of the most predominant and best-known examples of the connection between the soil and human health is probably the biodiversity and Old Friends hypothesis. It argues that urban habitat restoration can return human health benefits through exposure to green space – and associated microorganisms. It has been widely suggested that exposure to potentially pathogenic soil microorganisms during childhood is necessary to build tolerance and a functioning immune system, as well as reduce prevalence of allergic diseases (Flandroy *et al.*, 2018; Wall, Nielsen and Six, 2015). According to authors of a recent article, the loss of such exposure with urbanization and industrialization has contributed to an increase in human immune dysregulation (Mills *et al.*, 2017). The authors even go a step further and propose the Microbiome Rewilding Hypothesis, which specifically outlines that "restoring biodiverse habitats in urban green spaces can rewild the environmental microbiome to a state that enhances primary prevention of human disease" (Mills *et al.*, 2017). Lending support to this hypothesis, authors of a recent study suggest that modifying the living environment of children with microbiologically diverse natural materials (including forest floor, sod and peat blocks) could enhance immunoregulatory pathways and provide a feasible approach for decreasing the risk of immune-mediated diseases in urban populations (Roslund *et al.*, 2020).

It has also been demonstrated that there are consistent and significant **differences in gut microbiomes** between populations of hunter-gatherers (a proxy for pre-industrialized populations) and (urban) Americans (an example of an industrialized population) (Fragiadakis *et al.*, 2018; Smits *et al.*, 2017; Yatsunenko *et al.*, 2012). Across several studies, hunter-gatherer lifestyles have shown the highest richness of human gut microorganisms, while lifestyles of industrialised urban inhabitants have shown the lowest (Blum, Zechmeister-Boltenstern and Keiblinger, 2019).

A review by Blum, Zechmeister-Boltenstern and Keiblinger (2019) identified several factors suspected to contribute to the **loss of beneficial microbes in the human gut of industrialised and urbanised modern populations**: little contact with soil and faeces, hygienic measures, use of antibiotics, and low-fibre diets of processed foods. These lifestyle features contrast starkly with how humans have evolved and lived until relatively recently, as highlighted by observations of modern hunter-gatherers or isolated populations. Humans evolved in close, daily contact with soil, whether as shelter, food or water sources. This has led to questions about whether and how the soil microbiome affected the development of the human intestinal microbiome. Based on studies of modern hunter-gatherer lifestyles and archaeological evidence, it is understood that in pre-agricultural human history, root tubers were an important source of nutrition (Eaton and Konner, 1985; Kubiak-Martens, 2002; Lancaster *et al.*, 2000; Marlowe and Berbesque, 2009), and were frequently consumed with minimal or no processing (Schroeder, 1971). This implies ingestion of soil and, given the associations between certain diseases and the gut microbiome, potential positive implications for health. Indeed, the consumption of root tubers is noted to play an important role in the diets of traditional foragers in Tanzania today – populations whose gut microbiomes have demonstrated the highest biodiversity. **Results from animal studies also propose that contact with soil and its microorganisms is beneficial for gut microbiota, further supporting the positive effects of ingestion of soil microbiota.** A study by Liddicoat *et al.* (2020), for instance, found that mice exposed to a biodiversity-rich aerobiome treatment (soil microorganisms delivered by air) hosted enhanced populations of *Kineothrix alysoides*. The abundance of this soil-dwelling bacteria in mice intestines was associated with reduced anxiety-like behaviours, potentially explained by the bacteria's production of butyrate, a short-chain fatty acid known to have gut and mental health benefits. The authors proposed that biodiverse soils may be a supplementary source of butyrate-producing microorganisms for mammals, with potential implications on behaviour.

It is known that microorganisms in human gastrointestinal tract, too, can send signals to the brain, influencing human behaviour, even if the mechanisms aren't fully understood. Therefore if humans have co-evolved with microorganisms (Blum, Zechmeister-Boltenstern and Keiblinger, 2019), and we do indeed depend upon them for normal brain functions, then disrupting this relationship through a loss of microbial biodiversity or certain communities and functions in the gastrointestinal tract may translate to cognitive perturbation (Robinson and Breed, 2020).

Robinson and Breed (2020) propose a conceptual model in development: **the Lovebug Effect. It describes microbially-mediated human affinity for nature.** Their approach considers several perspectives, amongst them one where the physical and mental health of human beings is dependent on microbial communities, thus driving human affinity for nature though complex biotic processes that are not yet identified and understood. This positions microorganisms in a vital role for human health, where interaction with nature offers crucial, multiple pathways for human exposure to (soil) microbiota. This idea conceptualises human beings as holobionts, or metaorganisms, strengthening recognition of inter-connected biotic systems and processes. Applied on a broader scale, this perspective could also help encourage appreciation of planetary health and other ecosystem-based perspectives.

3.3.5

A meteorological perspective: the intercontinental migration of soil microorganisms

Finally, a somewhat underexplored connection between the soil microbiome and human health concerns the dispersion of soil microorganisms during dust storms. Topsoil from arid regions can move though the atmosphere in dust clouds, crossing continents and large geographical distances. Most of it originates in the Sahara and Sahel regions in Africa, but increasingly from Asia due to desertification and climate change. **Several observations have led some researchers to emphasize the importance of monitoring dust cloud-deposited soil microbiota and their potential toxins, particularly in sensitive environments, and including effects of land use in source regions as well as climate change-related meteorological events** (Fragiadakis *et al.*, 2018; Griffin, 2007; Powell *et al.*, 2015). To begin with, soil microorganisms from desert environments areas are used to harsh conditions. They demonstrate high resistance to environmental stresses such as high radiation, limited nutrients, desiccation, and extreme temperatures. Second, many microorganisms use aeolian means of displacement to colonize new environments, such as riding on dust particles (Weil *et al.*, 2017). Amongst them are human and plant pathogenic microorganisms (Griffin, 2007; Powell *et al.*, 2015; Weil *et al.*, 2017) that could potentially pose threats to sensitive ecosystems and public health**. Several diseases attributed to fungi or bacteria have, in fact, been associated with dust storms for decades (e.g. pulmonary and cardiovascular diseases, meningitis epidemics, coccidioidomycosis)** (Griffin, 2007; Weil *et al.*, 2017). A third point was made by Weil *et al.* (2017), who investigated the effects on microbial communities following the largest recorded Saharan dust cloud deposition in the Italian Alps. They demonstrated that not just fractions, but entire soil microorganism communities can be displaced, thereby favouring their ability to survive and colonize a new environment. For these reasons the presence of soil-dwelling, microbial pathogens and their high tolerance to environmental stress has raised concerns about the evolution of pathogenicity in new environments. A final consideration, aptly put by Griffin (2007): do modern dust clouds pose more of a threat to human health than those that blew around the planet before the era of industrialization?

In summary, whether from an ecological, medical, planetary health, dietary or meteorological perspective, there are strong theoretical arguments and some evidence that point to substantial connections between the soil microbiome and human health. At this point in time, however, there are many knowledge gaps to inspire further research.

CHAPTER

4

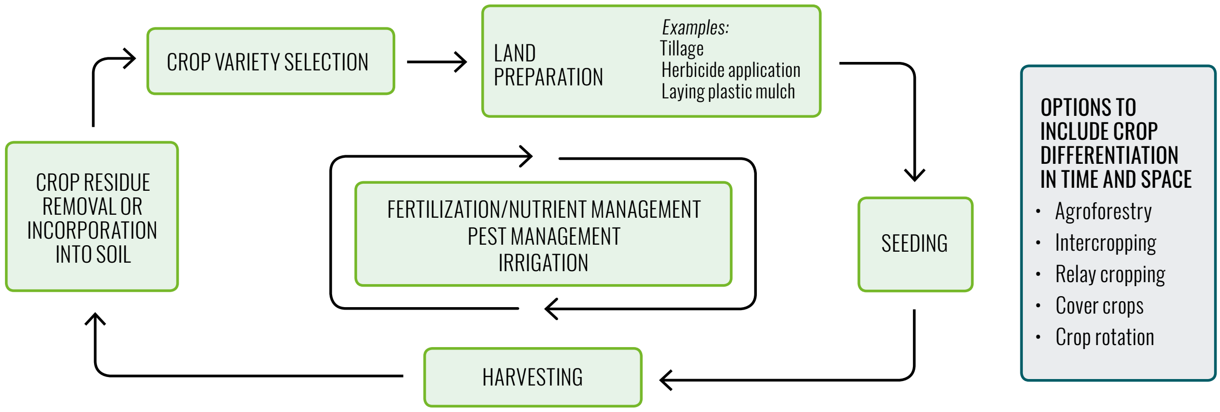
IMPACTS OF CROP PRODUCTION PRACTICES ON THE SOIL MICROBIOME, AND CAUSAL IMPACTS ON CLIMATE CHANGE OR HUMAN HEALTH

This section presents results from both literature reviews. Each of the nine subsections corresponds to a single crop production practice: land use, tillage, agroecosystem crop diversification (which includes plant diversity, crop rotations, cover crops), crop residue management, plant variety selection, irrigation, fertilization, pest management, and lastly, though not a practice, microplastics (MPs) in agricultural soils.

While the potential impacts of practices are explored separately, it should be remembered that each are just a part of larger systems with interacting components, whether at the scale of the field, farm, landscape, or region and so forth. Each practice is also just one aspect of the crop production cycle, which can be conceptualized in time as well as space (**Figure 12**). Exploring farming systems that combine practices for potential synergies, such as conservation agriculture, were beyond the scope of this study.

FIGURE 12.

TYPICAL PRACTICES IN THE CROP PRODUCTION CYCLE.



Narrative review

In each subsection, the narrative (or general) literature review is presented first. It provides an overview of how different crop production practices can impact the soil microbiome.

Systematic review

Results from the systematic review are presented second. The authors investigated whether there was solid evidence in the scientific literature demonstrating a significant impact of crop production practices on the soil microbiome, with subsequent, causal links to climate change and/or human health. In other words, **only literature that made an explicit connection between a crop practice, the soil microbiome, and either climate change or human health were included**. Depending on the subject and number of results, these publications are frequently discussed individually, offering a deeper dive into the relationship between the soil microbiome and terrestrial biogeochemical dynamics and nutrient cycling. The **Key Terms** and **Section 3** (**Why does the soil microbiome matter for a healthy planet?**) may be of help to understand foundational concepts that are discussed in detail. The responses of soil microorganisms are generally discussed in terms of their abundance (measured by biomass), activities or functions (measured through enzymes) and diversity or community structure. Specific genes are known to encode for specific enzymes, which build and break down corresponding compounds. Therefore, microbial genes are frequently used as a proxy for microbial functions.

Of all the articles selected from the systematic review, very few date prior to 2010, and about half were published within the last four years. It is possible that the relatively few articles returned addressing the risks to human health was due to inadequate identification of search terms or very narrow searches. That so many of the returned articles were published recently may be indicative of the recent, unprecedented surge in awareness and questions surrounding the soil microbiome, and the emerging, novel research that seeks to respond.

The links between **crop practices, the soil microbiome and climate change** are discussed in terms of the inter-connected processes that can collectively be described as biogeochemical cycling. Examples include different steps in nutrient cycling mediated by soil microorganisms, such as soil microbial respiration, denitrification and nitrification, and methanogenesis or CH_4 oxidation.

The studies drawing connections between **crop practices, soil microorganisms and human health** presented here are very limited. This contrasts starkly with the intensity of concerns and the knowledge gaps identified by the scientific community, namely concerning potential impacts of MPs, pesticide compounds, and AR on human health via the soil microbiome. The studies presented in this section address risks to human health by AR, pesticide use and plastic-related compounds.

4.1
LAND USE

HIGHLIGHT BOX 4 Impacts of land use on the soil microbiome, climate change and human health.

NARRATIVE REVIEW What are the impacts of different land use on the soil microbiome?

- The heterogeneous presence of soil microorganisms may be conceptualised in structured spatial scales: microscale, farm plot scale, landscape or country scale.
- Different land uses can affect the soil microbial biomass, diversity, and functional roles. The Intermediate Disturbance Hypothesis suggests that diversity tends to increase after a moderate disturbance event, and an equilibrium state harbours lower diversity. This may help explain the higher microbial diversity and functional redundancy found in agriculture and pasture sites, compared to forests where lower diversity but higher abundance of soil microorganisms was observed.
- Bacterial diversity may be able to resist land use conversion, or at least demonstrate resilience, their changes in metabolic functions reflecting strategies to survive or even thrive under such conditions.
- Land use can have a stronger effect on the soil microbiome than certain climatic events such as drought.
- Land use could be a major driver in shaping the plant phyllosphere resistome via the soil resistome.

SYSTEMATIC REVIEW What are the impacts of different land use on the soil microbiome, and their causal impacts on climate change?

- Soil microbial attributes are more sensitive and react more rapidly than soil chemical or physical properties, making them potential early indicators of changes in soil quality and ecosystem functioning, which are important to monitor in land-use changes. Soil microbial biomass carbon is a particularly sensitive indicator for soil health amongst other carbon cycling-related microbial activities.
- Land use conversion can create modifications in vegetation coverage, soil physicochemical properties, and soil microbial communities, resulting in a strong effect on soil heterotrophic respiration – measured by carbon dioxide production.
- There is generally higher soil microbial biomass carbon, soil microbial biomass, and microbial enzyme activities in forests than croplands.
- A comparatively higher metabolic quotient in croplands suggests greater carbon losses by respiration and lower carbon assimilation into microbial biomass, compared to forest and grazing lands.
- Incorporation of a perennial pasture into a cropping system can significantly affect soil microbial community composition and function, increase labile and recalcitrant soil organic carbon and thereby potential for carbon sequestration.
- Peatland drainage can cause changes in soil microbial community structure and activities,
- which can potentially cause changes in greenhouse gas emissions.
- Agricultural soils can emit more nitrous oxide than forest, linked to enhanced ammoniaoxidizing bacteria abundance.
- Natural sites tend to have the highest methane oxidation rates, forests being highest. Increasing disturbance reduces methane oxidation. These are linked to methanotroph abundance and activity. When soil disturbance is not too extreme the methanotroph community and methane oxidation rates can recover quite well with the progressive return
- of favourable soil abiotic conditions. • Organic orchards and croplands have enhanced microbial biomass and activities compared to their conventional counterparts, implying consequences on soil carbon cycling.

SYSTEMATIC REVIEW What are the impacts of different land use on the soil microbiome, and their causal impacts on human health?

- No relevant literature was found during the systematic search.

Land use describes the type of land cover, activities, and management of a given land area. The term refers to a wide range of possibilities, from natural to agricultural to urban, but here the focus is on those related to agricultural use and natural forest. This section principally refers to cropland, pasture, forest, and plantation. In the studies discussed below, these management styles are often compared with natural, or wild lands, which may experience anthropogenic influence but are not intensively managed.

While land use is frequently studied at field scale, it is useful to imagine it at the landscape, regional, country or global scale to understand impacts on the broader ecosystem and connected ecosystems.

This chapter first describes four examples that illustrate how land use can impact the soil microbiome, having direct effects on ecosystems. Specifically, the studies demonstrate impacts of land use on soil microbial biomass, diversity and function, and how land use can have a stronger effect than certain climatic events such as drought. Furthermore, it has been suggested that land use could be a major driver in shaping the plant phyllosphere resistome via the soil resistome. The second part of this chapter presents results from the systematic review, exploring the impacts of land use on the soil microbiome with consequences for climate change.

4.1.1

Overview of effects of land use on the soil microbiome

Drawing a connection between the expansive perspective of land use, on the one hand, and the miniscule world of soil microorganisms, on the other, may be conceptually challenging. **A helpful, tangible approach is to imagine the heterogeneous presence of soil microorganisms in terms of structured spatial scales**. Lemanceau *et al.* (2014) describe three levels and their defining parameters:

1. Microscale, parameters including the soil structure, porosity, and organic carbon (C) content;
2. Farm plot scale, taking into account the soil texture, pH, organic matter (OM) content, land use and plant cover;
3. Landscape or country scale, considering physical-chemical soil properties and land use.

Using data collected by the French Network for the Measurement of Soil Quality, the authors compared soil microbial biomass at a country scale, sampling a variety of forests, grasslands, and agricultural lands. Despite a high variability, they found significant differences among land uses. Vineyards and orchards, which featured the lowest plant diversity, also had the lowest soil microbial biomass. Grasslands and forests, with the highest plant diversity, had the highest soil microbial biomass. This observation is consistent with other studies that have **observed increased soil microbial biomass in forests compared to agricultural land, which appears to be linked to greater plant diversity and reduced soil disturbance** (Raiesi and Beheshti, 2015; Singh *et al.*, 2020, 2010).

Land use history can shape the soil microbial response to disturbance. Mendes *et al.* (2015), for example, reported similar results after investigating whether soil microorganisms were sensitive to environmental disturbance triggered by different land uses in the Brazilian Amazon. They compared microbial communities from a native forest, a deforested site, an agricultural site and a pasture, and looked specifically at microbial diversity and functional diversity. The two latter sites had previously been native, tropical forests, but had been cleared by slash-and-burn techniques and then converted into agricultural field or pasture. Overall, the distinct land uses had distinct microbial community structures, both in terms of taxonomy and functionality. **They found a higher microbial diversity and functional redundancy in the agriculture and pasture sites, while the forest showed lower taxonomic diversity, but a higher abundance of organisms. The Intermediate Disturbance Hypothesis suggests that diversity tends to increase after a moderate disturbance event, and an equilibrium state harbours lower diversity.** The authors suggest that according to this hypothesis, the forest with its lower taxonomic diversity but high abundance of organisms could be considered in equilibrium. After a slash-and-burn event (as in the agriculture and pasture sites), a higher diversity of species would help ensure soil ecological functions because many perform the same processes. **Therefore, they argue, both functional redundancy and biodiversity contribute to ensuring maintenance of soil ecological functions, a kind of 'ecological insurance'.**

The same study found that distinct microbial communities were correlated with soil functions (Mendes *et al.*, 2015). **Two examples illustrate how land use might influence the abundance certain bacterial phyla according to the disturbance event.** One, there was a higher abundance of Actinobacteria in the deforested site. Members of this phylum are known to play an important role in the soil decomposition process and are able to produce spores, which help them resist perturbation events such as high temperatures experienced during fires. Two, Proteobacteria were abundant in all sites, but especially so in the forest. Members of this phylum play important roles in C, nitrogen (N), and sulphur (S) cycling, functions which were indeed abundant in the forest soil.

Deforestation for oil palm cultivation is an example of a major disturbance to the soil ecosystem. A recent review found that although this conversion was associated with changes to soil biophysical conditions (increased pH, lowered C and N contents), the soil bacterial and functional diversity were unaffected or increased (Kaupper *et al.*, 2020). This was also the case for fungal abundance. The authors concluded that **the marginal changes suggest that bacterial diversity in these systems may have been able to resist land use conversion, or were at least resilient to the change more than eight years later**. They describe the shifts in microorganism functionality as strategies used to survive, or even thrive, in different conditions. The shifts were related to core metabolism functions such as cell division and secondary metabolism functions such as nutrient cycling.

Working with samples from a natural forest, a drip-irrigated organic vegetable plot, a rain-fed almond tree grove, and a semi-arid grassland abandoned forty years ago following agricultural use in a semi-arid Mediterranean ecosystem, Moreno *et al.* (2019) observed that land use and drought significantly affected the soil microbial community. Most importantly, **they found that the effect of land use was even stronger than that of drought in the ecosystems studied, strongly shaping the drought resistance of soil microbial communities.** The microbial community of rain-fed and abandoned soils were more resistant to drought than irrigated soils. The natural forest soil microbial community displayed the highest resilience to drought, and had the highest fungi:bacteria ratio compared to other samples. The authors suggest that the greater soil organic matter (SOM) content may have improved water retention, meaning there would be less water shortage in the soil that could negatively affect the microbial biomass. The higher fungi:bacteria ratio supported this hypothesis, as a potential indicator of drought resistance (Moreno *et al.*, 2019).

The following study focuses on how land use might influence antibiotic resistance genes (ARGs) found on plants and in the soil. In their experiment that compared ARGs found in the phyllosphere and soil in farmland and forest, Xiang *et al.* (2019) concluded that **land use could be a major driver in shaping the plant phyllosphere resistome via the soil resistome**. There were similar numbers of different bacterial species between farmland and forest in the phyllosphere and soil microbial communities, but the composition of bacterial communities significantly differed between phyllosphere and soil samples. These differences occurred at the phylum level. Proteobacteria, Acidobacteria, and Actinobacteria were most abundant in soil samples, and Firmicutes and Proteobacteria were most abundant in the phyllosphere. The authors explored two examples of how the soil resistome may have been shaped by land use. First, they found that of the ARGs identified in the organic fertilizer, the majority were known to confer resistance to aminoglycoside (an antibiotic). This may have explained the enriched aminoglycoside-resistance genes identified in the farmland phyllosphere and soil. Second, Firmicutes, found in greater abundance in farmland than forest, has been observed to be frequently associated with ARGs and metal resistance genes in a trace element-polluted environments. The authors suggest that the organic fertilizer application may have induced selective pressure on soil microbial communities, thereby explaining the variation in plant-associated ARGs between the different land types via the soil microbiome.

Concluding remarks

The impact of land use and land-use changes on the soil microbiome being a very broad subject, these studies provide just a few examples to illustrate that different land cover and management can impact soil microorganisms and their activities. Natural ecosystems that feature increased plant diversity and less soil disturbance appear to harbour the most soil microbial biomass. This, and other aspects of land use history, can affect the soil microbial response to disturbance such as deforestation for agricultural use. It appears that microbial functional redundancy and microbial diversity help ensure ecological functions, with shifts in functions reflecting a strategy to survive or thrive. It also seems that in some systems, land use may be more influential than climatic factors such as drought, and that it could be a driver of potentially far-reaching consequences by shaping the above-ground plant resistome via changes in the soil resistome.

4.1.2

Systematic review: Effects of land use on the soil microbiome, and their combined impact on climate change

A search using terms related to the microbiome, land use and climate change returned 402 articles (see **Annex I** for search terms). Twenty-two of those were directly relevant to this section. They address how land use types can influence terrestrial biogeochemical cycling and nutrient cycling, implying consequences for soil greenhouse gas (GHG) emissions and C storage.

In addition to land use typologies, management styles such as organic or conventional also contribute to a potential land use effect on terrestrial biogeochemical cycling and nutrient cycling. A meta-analysis of 56 studies concluded that organic systems had 32 to 84 percent greater soil microbial biomass C and N, microbial biomass, and microbial enzyme activities than conventional systems on a global scale (Lori *et al.*, 2017). The authors moreover found that differences in soil microbial biomass size and activity between organic and conventional farming systems varied as a function of land use typology. Organic cropping and orchards featured significantly increased microbial biomass C, microbial biomass and activity compared to their conventional counterparts, though there were no differences for grasslands.

Research has identified changes in soil microbial community structures, activities and correlating changes in GHG fluxes induced by land-use change. The precise mechanisms and complex dynamics of these multilateral interactions, however, remain relatively unknown. For example, conversion of natural forest to plantation can affect the soil pH and the total soil C and N, potentially impacting nutrient dynamics and soil biogeochemical cycles regulated by soil microorganisms. A recent review of literature regarding forest clearance for oil palm agriculture in Southeast Asia describes shifts in the general soil microbial community (based on 16S rRNA gene diversity), but it remains to be determined how those specialized microbe guilds that catalyse key processes in GHG fluxes respond to such changes (Kaupper *et al.*, 2020). The authors ask, for instance, how might changes in the abundance, composition and interaction amongst these microorganisms affect the processes they catalyse (e.g. methane (CH_4) production and oxidation, nitrification and denitrification)? They also suggest further investigation into the effects of tropical forest clearance on nutrient cycling performed by non-bacterial microorganisms, such as protists and fungi.

Connections between land use, nitrogen cycling and nitrous oxide emissions

Different soil management practices can alter microbial functional genes involved in the nitrification and denitrification cycles, with subsequent impacts on soil nitrous oxide (N_2O) emissions. Many studies, such as one by Morales *et al.* (2010), draw correlations between this effect and measured differences in GHG emissions, emphasizing the role of the soil microbiome in biogeochemical processes. It has also been reported, however, that despite significant differences in *nosZ* and *amoA* abundances between different land uses, there was no correlation with GHG fluxes, implying that microbial diversity and functional gene abundance were not the main drivers of GHG fluxes in the system studied (Cuer *et al.*, 2018). In other words, genes involved in GHG flux processes may be present but not necessarily active. Results from studies in this field emphasize that the underlying mechanisms in these complex relationships remain relatively unknown.

Agricultural soils can emit more N_2O than forest, underlining the importance of agroecosystem management options to mitigate soil N_2O emissions. In an incubation experiment comparing agricultural to forest soils in Canada, Lu *et al.* (2018) found higher ammonia (NH_3) oxidation activity and N_2O production in surface agricultural soils. They furthermore identified ammonia-oxidizing bacteria (AOB) as major contributors to N_2O production, rather than ammonia-oxidizing archaea (AOA), in the agricultural soils tested. Mafa-Attoye *et al.* (2020) likewise found that in comparison to soil from an undisturbed forest, a rehabilitated site, and a riparian grass buffer, soils from the agricultural site likewise had the highest abundance of AOB, rather than AOA. Furthermore, AOB dominance corresponded positively with nitrate (NO_3-) concentration and N_2O emission. The authors suggest that N_2O emission in these soils was controlled by nitrification, dominated by AOB. The study also investigated whether riparian buffer systems might be hotspots of N_2O emissions, as has been suggested due to N-loading from adjacent agricultural land (Zhao *et al.*, 2018).[7] The rehabilitated and grass buffer sites, however, had the highest abundance of *nosZ* genes, demonstrating a capacity for complete denitrification, resulting in lower measured N_2O. The authors concluded that the **N-cycling microbial community may differ among such riparian buffer zones under different land uses, thereby influencing N_2O emissions without necessarily creating hotspots of N_2O production**.

In a laboratory study investigating the impact of different land uses and land-use change on N_2O emissions, Li *et al.* (2016) reported that **following urine application, the total N_2O emissions from pine plantation soil were surprisingly more than twice those from dairy or sheep farm soils**. They had expected higher emissions from the dairy or sheep farm soils, and offered a few possible explanations for this unexpected result. One is that the AOB growth following urine application continued and exceeded those of other soils. The second is potentially enhanced biological denitrification owing to higher amounts of organic C in the soil. The third is related to pH and chemo-denitrification. In addition, they report that the AOB abundance was higher in dairy and sheep farm soils compared to pine, most likely explained by the higher N content from the N fertilizers clover or manure.

Different land uses can influence carbon dioxide emission through soil microbial respiration

Soil respiration, typically measured by carbon dioxide (CO_2) production, can be affected by land use conversion, resulting from modifications in vegetation coverage, soil physicochemical properties, and soil microbial communities. The following factors, which can differ according to different land uses, can cause variation in soil respiration: crop species and the amount of root biomass, different redistribution of precipitation and solar radiation by the vegetation canopy, soil microbial community structure, and the quality and stability of substrate.

Soil respiration is actually composed of two types of respiration, autotrophic and heterotrophic. Breaking down soil respiration into its components can help better understand this complex and critical measurement, given its role in ecosystem C balance and CO_2 emissions. Autotrophic originates from roots and the rhizosphere, primarily influenced by fine root biomass, soil temperature, nutrient availability, C allocation and age of plants or trees (Hu *et al.*, 2018). Heterotrophic, in contrast, comes from soil microbes and soil fauna that decompose SOM, and is mainly affected by soil temperature, moisture content, organic C pool size and microbial biomass (Hu *et al.*, 2018). The ratio

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[7] Riparian buffer systems are considered a best practice to intercept N surface runoff and leaching before entering aquatic ecosystems.

of autotrophic respiration to soil respiration is affected by both biotic and abiotic factors (Hu *et al.*, 2018). Hu *et al.* (2018) demonstrated that autotrophic and heterotrophic respiration responded differently to land use conversion of forest to bamboo plantation. Converting forest to bamboo resulted in increased soil and heterotrophic respiration, but not autotrophic. The inorganic fertilizer in the plantation may have accelerated SOM mineralization, thereby increasing heterotrophic respiration. It is also possible that the deep tillage in the plantation reduced the soil structure stability, increasing the exposure of soil organic carbon (SOC) to soil microbial degradation. Lastly, understory removal in the plantation may have increased the soil temperatures, encouraging SOM decomposition. The authors concluded that there was a resulting decreased potential for soil C sequestration due to increased soil CO_2 emissions under the conditions of their study.

Working in the Loess Plateau, Wang *et al.* (2018) reported that **compared to cropland, the apple orchard demonstrated higher soil respiration and a higher bacterial diversity, driven by increased soil moisture and nutrient availability.** They measured a 28 percent greater soil moisture in orchard soils. In dry and semi-dry ecosystems like the Loess Plateau, soil moisture is likely a limiting factor for physiological processes. The authors suggest that significantly higher N fertilization likely led to enhanced soil microbial activity, hypothesized to result in decreased C:N ratios, which affected soil C quality and enzyme activities. A third possible explanation for increased respiration in the orchard was the greater soil microbial population size and extracellular enzymes. Respired CO_2 is not determined by overall diversity of microbial taxa, but rather by abundance of particular taxa; the orchard soils showed lower abundance of Acidobacteria but higher abundance of Bacteroidetes and Proteobacteria. The latter phyla are associated with higher soil respiration. **Furthermore, the authors identified a lower temperature sensitivity of respiration (Q_{10}) in the orchard soils, influenced by the increased enzyme activity and possibly the soil C:N ratio**. Conversely, the lower soil moisture availability in the croplands studied might have increased the Q_{10} values by creating disconnected soil pore water, thereby inhibiting the diffusion rate of microorganism enzymes and available substrates.

Guntiñas *et al.* (2009) **unexpectedly found greater CO_2 emissions by agricultural soils under unusually high moisture conditions.** They compared CO_2 emissions from forest, grassland and agricultural soils with moisture contents at 160 and 100 percent field capacity. In forest soils, CO_2 emissions were lower at 160 percent field capacity compared to 100 percent. This was not surprising, as it is generally hypothesized that flooding creates anaerobic conditions and thereby decreases soil microorganism respiration. In contrast, grasslands showed higher emissions at 160 percent field capacity, and cropland emissions at 160 percent were similar or higher to those at 100 percent. These observations were unexpected, given the above explanation. One reason may have been that under incubation conditions, organic fertilizers applied to grass- and croplands may have created a (previously unavailable) readily metabolizable substrate used by soil microbes. Alternatively, laboratory incubation conditions may have activated populations of certain, previously inactive microorganisms such as thermophilic anaerobes, which originate from intestinal tracts of cattle, a potential source of organic fertilizer.

Land use can strongly impact soil organic carbon dynamics and soil health

Several studies in different geographic climates have demonstrated a higher soil microbial biomass, soil microbial biomass C, and microbial enzyme activities in forests than croplands (Raiesi and Beheshti, 2015; Singh *et al.*, 2020, 2010). Litter decomposition, a major type of OM turnover, is fundamental to nutrient and C cycling, and is primarily controlled by resource quality, soil properties and climate, as well as abundance composition and activity of soil microbial communities and microbe-plant interactions (Bradford *et al.*, 2016; Walter *et al.*, 2013). Ochoa-Hueso *et al.* (2019) found that in a forest ecosystem, litter decomposed two to five times faster than in grassland soils because the soil microbial communities had a greater ability to break down C-based substrates, as well as the soil N availability. Furthermore, they concluded that **land use that incurs loss or gain of forest is likely to have a more significant impact on litter decomposition rates (and soil C cycling) via the soil microbiome than global factors such as increased rainfall or increased atmospheric N deposition under the conditions of their study**.

Higher measurements of soil microbial biomass carbon (MBC), microbial enzyme activities and SOC have been associated with less-disturbed soils (Raiesi and Beheshti, 2015; Shang *et al.*, 2012; Singh *et al.*, 2020, 2015). Soil microbial biomass C can be used as an index for changes in SOC stock or turnover. A study conducted in Iran found that both soil microbial biomass and enzyme activities decreased with deforestation at two sampling depths (0-20 and 20-40 cm) (Raiesi and Beheshti, 2015). The soil enzymes urease, invertase, alkaline phosphatase, acid phosphatase and arylsulfatase were ultimately used to reveal SOM losses and soil degradation in the natural forest ecosystem subjected to deforestation. Singh *et al.* (2020), working in India, similarly found that soil microbial biomass C, CO_2 efflux, and certain enzyme activities were generally highest in forest soils, followed by fallows, then agricultural. The lower organic C, N and phosphorus (P) and cropping disturbance likely explained the lower soil microbial biomass C in the agricultural soils. The authors suggested that in the forest, litter layers act as barrier that reduce water loss by evapotranspiration while well-established root systems provide oxygen and water to microbes. The absence of these factors in fallow and agricultural soils might also have contributed to their lower microbial C and CO_2 efflux. The authors also proposed that increased enzyme activities in fallows, followed by agricultural and forest soils, indicated a higher degree of stress on soil microbes after deforestation than cropping. Also working in the dry tropics, Singh *et al.* (2015) likewise found that natural forests had the highest SOC storage and soil microbial biomass, followed by a bioenergy plantation, a degraded forest, and an agroecosystem. They furthermore found that the CO_2-C flux was highest in the agroecosystem, showing a significant negative correlation with soil microbial biomass, SOC and the macroaggregate fraction. This contrasts the general conception that this flux is regulated by biotic variables. Possible explanations offered by the authors for the relatively high soil microbial biomass and low CO_2-C flux in the forest were the presence of more stable microbial biomass, and a higher proportion of macroaggregates that protected SOM and prevented mineralization. The authors pointed out that both factors can contribute to a lower CO_2-C flux and increased soil C storage. An interesting highlight from their study is their suggestion that the CO_2-C flux was directly regulated by soil aggregate fraction by protecting SOC, rather than biotic factors such as soil microbial biomass. Indeed, ploughing is known to reduce soil structure by physical disturbance of aggregates (Nunes, Karlen and Moorman, 2020). A study comparing undisturbed natural meadow, 20-year cultivated land, and land abandoned for three and ten years after cultivation found that cultivation with ploughing reduced SOC, MBC, and light fraction organic C (Shang *et al.*, 2012). Ten years later, however, the soil C status had recovered almost to the level of the meadow content.

Certain soil microbial activities related to C cycling could be used as soil health indicators. Okolo *et al.* (2020) draw connections between land use and such soil health indicators, including MBC, water extractable C (WOC), metabolic quotient (qCO_2), substrate use efficiency, and dynamics of ^{14}C-labelled glucose added to soil.[8] **Soil fertility indicators used in the study demonstrated that for the majority of sites, impacts of land use were restricted to the topsoils**. **Although land use strongly influenced all parameters measured, MBC content was the most sensitive indicator of soil health.** Similar to other studies already discussed above, they found higher MBC contents in forests, exclosures and grazing lands in comparison to cultivated cropland soils. They proposed that this indicates that cultivated

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[8] qCO_2, or the metabolic quotient, is a proxy for microbial utilization efficiency of SOC for metabolic activity regarding microbial growth. In other words, it is the rate of soil microbial respiration per unit of microbial biomass and indicates the capacity of soil microorganisms to utilize SOM.

croplands depleted SOM stocks, which are the easily accessible substrates, in particular. Results from several types of land uses have indicated that the labile SOC fractions (or easily accessible SOC), rather than total SOC content, are highly influential in driving microbial community structure and composition and have implications for soil C storage (Ramirez *et al.*, 2019). In addition to vegetation cover, and similar to Singh *et al.* (2015), Okolo *et al.* (2020) also found that **soil texture was an important factor that controlled substrate availability and transport to subsoil microorganisms; both factors can strongly control subsoil microbial biomass and activity**. In addition, the authors found that the qCO_2 was low in exclosures, forests and grazing land, in contrast to intensively cultivated crop land. Low qCO_2 suggests high C stability and more resource-efficient use of organic substrates. They suggest that this predicts that during metabolism of SOM, croplands will experience greater C losses by respiration and lower C assimilation into microbial biomass. As a potential explanation for the high substrate use efficiencies of soil microorganisms in croplands, the authors hypothesize that the soil microorganisms were used to a high nutrient environment, which may have led to C-limited communities that used up a large proportion of the ^{14}C-labelled glucose for biomass synthesis. Consequentially, this could lead to C accumulation and stabilization from easily accessible C forms.

Peatland drainage can cause changes in soil microbial community structure and activities. Less disturbance seems to preserve a more distinctive soil microbial community structure, while increased disturbance can potentially cause changes in GHG emissions (Kanti and Sudiana, 2019; Urbanová and Bárta, 2016). Working with a spectrum of least-disturbed to very-disturbed peatland ecosystems in Indonesia, Kanti *et al.* (2019) demonstrated that fungal diversity and microbial enzyme activities of less-disturbed peatland was distinctive. The aeration of peat soils enhanced growth of aerobic fungi, the highest fungal populations being measured in degraded peat lands under agriculture (very-disturbed peatland ecosystem), and enhanced enzyme activities. While they found that drainage might increase CO_2 through increased microbial respiration, Urbanová and Bárta (2016) identified a reduction in CH_4 production potential. In their study, long-term drainage of Czech peatlands saw a strong reduction in abundance of the previously dominant methanognic archea (*Methanobacteria* and *Methanomicrobia*), which were replaced by *Thaumarchaeota*, *Marine Benthis Group A*, and *Thermoplasmata.* These shifts in bacterial and archaeal groups were consistent with the significant reduction of the CH_4 production potential. The methanotrophic bacteria also decreased (e.g. *Methylocystaceae* (*Alphaproteobacteria*) and *Methylococcaceae* (*Gammaproteobacteria*)).

Finally, **land-use changes that involve cultivation of drought-resistant deep-rooting crop species may have an impact on deep-soil C stocks**. Shahzad *et al.* (2018) suggest that higher mineralization rates of millennia-old C in deep soils can be expected with use of deep-rooting plant varieties. This does not, however, predict an overall decrease in deep-soil C stock, since contributions to new soil C formation by plant litter and other sources would need to be included in a full analysis of soil C dynamics.

Effects of grasslands, croplands, and tree plantations on carbon cycling and greenhouse gas fluxes

Transitions from grassland to cropland, and vice versa, can drive changes in soil microbial communities with potential impacts on soil C cycling and GHG fluxes (Lin *et al.*, 2020; Parmar *et al.*, 2015; Ramirez *et al.*, 2019; Tardy *et al.*, 2015). Lin *et al.* (2020) assessed the response of soil C sequestration and microbial community composition in a five-year pasture that was preceded by three years of vegetable production. They provide evidence that **incorporation of a perennial pasture into a cropping system can significantly affect soil microbial community composition and function, increase labile and recalcitrant SOC and thereby potential for C sequestration.** After five years under pasture, the SOC and SON in the top 15 cm increased 20.6 percent (approaching values of permanent pasture) and 20.1 percent, respectively, and particulate organic matter C increased 53.5 percent.[9] These effects contributed to the increase in soil microbial biomass and the associated C mineralization rates. **The potential release of CO_2 thus also increased with time under pasture.** That potential C mineralization rates increased after four years indicates that some of the stored C was relatively labile, and could therefore be lost if subjected to future land use transitions that include tillage and cropping. Lastly, the microbial community became similar after four to five years. In year five, the fungi to bacteria ratio was significantly higher, implying likely modifications to soil functions related to nutrient cycling (Riah-Anglet *et al.*, 2015).

Parmar *et al.* (2015) reported that **conversion of grassland to short-rotation forestry (separate coniferous and broadleaved tree stands) resulted in differences in soil microbial community composition and GHG production between tree species.** Compared to grassland, the CO_2 flux was lower under coniferous forest but was unchanged under broadleaved. There were no significant differences in N_2O and CH_4 fluxes between any treatments. As changes in the CO_2 flux across transitions was positively related to changes in soil pH and soil bacterial and fungal biomass, it seems that the microbial community changes may have driven the shift in soil respiration.

Finally, **even where land use does not have a significant effect on the response patterns of soil fungi and bacteria, it may still have an impact on soil C dynamics.** Tardy *et al.* (2015) found that land use stimulated populations of certain taxa, most notably, the bacteria *Burkholderia* (in grassland) and *Lysobacter* (in cropland) and the fungi *Rhizopus* (in cropland) and *Fusarium* (in grassland). Since these groups are known to be decomposers (and or plant pathogens), the authors suggest that such impacts on the soil microbial community by land use may have important consequences on soil C cycling of plant litter. Furthermore, it has been recommended that the role of fungal N_2O contribution from grasslands should be taken into account in net GHG emissions from the soil (Zhong *et al.*, 2018).

Land-use change on methane oxidation and emission

Land-use changes can influence soil CH_4 oxidation through alterations in soil properties such as soil moisture, N status, pH, thereby affecting methanogen and methanotroph community structure and functions. Soil disturbance is therefore an important factor. As argued by Tate *et al.* (2015) in their thorough review on the subject, understanding the abiotic and biotic processes that regulate soil CH_4 oxidation is instrumental in predicting which land use practices will result in high emission rates, as well as in impact assessment of net GHG emissions from land use and management changes. Focusing primarily on deforestation and afforestation processes, they reported that about 30 to 50 percent of the global soil CH_4 sink is situated in temperate latitudes, and the largest CH_4 update rates were measured in forest soils. Tree species can furthermore affect methanotroph community structure and activity; while deciduous species may have a positive effect on the community and reduce CH_4 emission, conifer species can sometimes limit CH_4 oxidation through the negative effect of toxic compounds on methanotrophs. The authors highlight that **natural sites tend to have the highest CH_4 oxidation rates, forests being highest, and increasing disturbance reducing oxidation. It seems, however, that when soil disturbance was not too extreme, the methanotroph community and CH_4 oxidation rates recovered quite well with the progressive return of favourable soil abiotic conditions.**

Tate *et al.* (2015) point to evidence that methanotroph communities appear to be very sensitive to changing CH_4 concentrations. For instance, shifts in their communities sometimes preceded changes in CH_4 emission, suggesting their regulation of soil CH_4 oxidation (Nazaries *et al.*, 2011; Singh *et al.*, 2007). Also, land use intensity that resulted in a decrease in methanotroph diversity, resulted in reduction in CH_4 oxidation rates (Levine *et al.*, 2011). In addition, some of the studies reviewed also observed a **shift in methanotroph community composition, from type I to type II dominated communities with afforestation processes and forest age** (Nazaries *et al.*, 2013; Singh *et al.*, 2009).

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[9] Particulate organic matter is a fraction of total organic matter that does not pass through a filter (filter sizes can range between 0.7 and 0.22 μm).

Type I, Gammaproteobacteria, are less dominant in most environments but are found where CH_4 is high, such as in deep soils and other anaerobic environments. Their numbers can reduce rapidly under limiting conditions, and are very responsive to substrate availability in ecosystems where disturbances are common such as arctic Tundra soil and paddies (Tate, 2015). Type II, Alphaproteobacteria, seem to be more resilient perhaps because they can remain dormant until favourable conditions return (Tate, 2015). It also appears that they are adapted to oxidize CH_4 at atmospheric concentrations (Dunfield *et al.*, 1999).

It has been shown that **change in water management when converting paddies can result in CH_4 reductions**. Liu *et al.* (2017a) found that converting paddy to orchards changed the soil from a CH_4 source to a sink. The change in land use decreased the abundance of methanogens and methanotrophs, while driving reduced diversity in the methanogen community and an increase in type II methanotrophs. Higher CH_4 emissions from paddies were owed to the increased dissolved organic C contents, more methanogens and the composition of methanogenic archaea communities.

It is clear that land-use changes can drive changes in CH_4 oxidation via shifts in the soil methanotroph community structure and activities. Nevertheless, Tate *et al.* (2015) point out that the changes in net CH_4 emissions from land use may be less important than the associated changes to N_2O emissions, which is not only a more potent GHG but also has a longer atmospheric lifetime than CH_4.

Concluding remarks

As reported by IPCC (2019), land use affects GHG and C storage differently depending on the agricultural management practices and climate. Nevertheless, different types of land use directly impact soil conditions and soil microorganisms with consequences for GHG fluxes and C storage. As land use shifts from less-disturbed to more-disturbed soils (e.g. natural forest to cropland), soil C storage potential and CH_4 oxidation can reduce while N_2O emission increases. Related to soil C storage potential, studies indicate a generally increased soil microbial biomass and microbial enzyme activities in forests compared to croplands. These effects are critical, as they can also have indirect consequences on ecosystem functioning and services, and should thus inform management choices. They also have potentially significant implications in landscape-scale prediction modelling that aims to contribute to climate change mitigation.

4.1.3

Systematic review: Effects of land use on the soil microbiome, and their combined impact on human health

Three searches linking land use, the soil microbiome and human health returned thirty articles, but none investigated causal relationships as we seek to do here (see **Annex I** for search terms).

4.2
TILLAGE

HIGHLIGHT BOX 5 Impacts of tillage on the soil microbiome, climate change and human health.

NARRATIVE REVIEW What are the impacts of tillage on the soil microbiome?

- Tillage can shape soil microbial communities and impact their activities, causing changes in their composition by affecting both bacterial and fungal populations, and thereby influencing soil functioning, plant productivity and provisioning of ecosystem services.
- Tillage tends to increase nutrient availability (owing to faster degradation of organic matter). This seems to stimulate a copiotrophic microbial lifestyle (microorganisms that feed upon abundant and labile carbon sources), while no-tillage favours oligotrophs (microorganisms that feed upon recalcitrant nutrient sources in carbon-scarce conditions).
- Tillage can negatively influence the presence of microbially produced binding agents that help form soil aggregates.
- The physical soil disturbance resulting from tillage negatively affects arbuscular mycorrhizal fungi, evidenced by reduced hyphal growth and selection for species that invest in reproduction rather than in nutrient scavenging or nutrient transfer to plant hosts.

SYSTEMATIC REVIEW What are the impacts of tillage on the soil microbiome, and their causal impacts on climate change?

- Any modification of soil structure triggered by tillage can affect soil microorganism community composition and activities, in turn impacting soil carbon storage dynamics and greenhouse gas emissions.
- Though many studies show that compared to conventional tillage, no-tillage increases denitrification (linked to increased soil nitrous oxide emission) in the short term, the positive impact on denitrification may decline in the long run.
- Tillage (and effective drainage) can increase porosity and reduce soil water content, thereby mitigating nitrous oxide emissions. Moist and well-aerated soils can favour the exchange of carbon dioxide and methane.
- Reduced tillage may increase soil organic carbon but may not necessarily result in carbon sequestration. The effect of soil depth on carbon dynamics should be taken into account as soil biotic and abiotic characteristics change with depth.
- Contrasting tillage practices alone (e.g. deep vs reduced tillage) may not influence biological functions related to soil organic carbon mineralization. However, when the same practices include crop residue incorporation they can trigger significant differences in microbial carbon use efficiency, enzyme efficiency and microbial biomass.
- Intact aggregate structures can offer protection spaces for bacteria, thereby limiting their predation by protists. Given the notable abundance of bacteria-predating protists in the soil, the carbon and nitrogen released from their prey should be taken into account when predicting nutrient dynamics.

SYSTEMATIC REVIEW What are the impacts of tillage on the soil microbiome, and their causal impacts on human health?

- No relevant literature was found during the systematic search.

In the first part of this chapter, the general review describes how different types of tillage have been shown to impact soil microbial communities and their functions in the soil, including the particular relationship between tillage and arbuscular mycorrhizal fungi (AMF). In the second part, the systematic review explores responses of the microbiome to different tillage practices, with consequences on soil greenhouse gas (GHG) fluxes and carbon (C) storage.

Tillage refers to mechanical disturbance of the soil for crop production purposes. Specific objectives include: loosening of compacted soil, seedbed preparation, incorporation of plant matter, improvement of nutrient mineralization (i.e. release of soil nutrients), improvement of soil and water conservation, and weed control (Carter and McKyes, 2005). **The practice can strongly influence the soil microbiome community composition and functions through the modification of the soil physical and chemical properties, including bulk density, soil organic C (SOC), pore structure and water availability**. Different types and degrees of tillage have been developed according to needs and constraints of the local agricultural context. These range from soil disturbance that inverts the first 25-30 cm of topsoil soil (e.g. mouldboard plough, often referred to in studies below as conventional tillage (CT)), to no soil disturbance at all (described in studies below as no-till or no-tillage (NT)). In between, there is a range of practices that employ different instruments, varying in their degree of depth and surface soil disturbance (e.g. from machine- or manually-driven tines and cutting discs to hand-held hoes).

Although minimum tillage has been promoted as improving soil quality and organic matter (OM), choosing which type of tillage, or whether to till at all, implies trade-offs between advantages and disadvantages relevant to the particular farming context (Lee and Thierfelder, 2017). For instance, tillage has been associated with decreased SOC (Haddaway *et al.*, 2017). Nevertheless, it can be necessary in low-input systems where it is important for weed control, replacing herbicide application, whether by necessity or choice. It can also be required after NT management that caused soil compaction, resulting in restricted establishment of root systems. Besides physical and chemical soil properties, how tillage affects the soil microbiome has to be taken into consideration when choosing the appropriate management system.

4.2.1
Tillage can shape the composition of soil microbial communities

The following studies illustrate how tillage can shape soil microbial communities, causing changes in their composition and functionality by affecting both bacterial and fungal populations. This, in turn, has the potential to influence soil functioning and provisioning of certain Ecosystem Services (ESS).

Xia *et al.* (2019) experimented with deep and rotary tillage, with and without straw incorporation in wheat fields. They reported that tillage significantly affected the abundance, diversity and functions of soil bacterial communities. Overall, rotary tillage samples exhibited the lowest ratio of gram positive to gram negative bacteria, high levels of this ratio being indicative of a poor nutritional soil status. The bacteria species *Mycobacterium* and *Methylibium* were sensitive to both straw returns and rotary tillage, and were proposed as candidate biomarkers for the management of the combined practices.

In an interesting study, Köhl, Oehl and Van Der Heijden (2014) confirmed the **critical role of tillage in selecting microbial communities, and in turn, potential impacts of the tillage-shaped microbiome on soil functioning related to agricultural production.** They collected soil microorganisms at two different soil depths from two fields under different, long-term agronomic management: NT and CT. They then used those soil samples to inoculate soil in which different grassland species were grown (*Lolium, Trifolium and Plantago*). Plant biomass production and plant nutrient uptake were measured to explore the effect of microbial communities from NT and CT. They found that both types of agronomic management triggered a high variation in soil microbial communities. The CT soil microbiome was associated with higher plant biomass, especially in the case of *Trifolium*, which can biologically fix nitrogen (N). In comparison, the NT soil microbiome triggered a higher phosphorus (P) uptake by plants. A likely explanation is that AMF hyphal length was twice as long in this treatment compared to CT, consequentially helping increase P uptake in plants.

Another recent, long-term experiment, this time in Germany, likewise demonstrated how tillage influenced soil microbial functions by shaping their communities (Babin *et al.*, 2019). The authors studied the effect of tillage (along with two other farming practices, N fertilization and crop rotation) on bacterial and archaeal community structure. Mouldboard plough tillage (20–30 cm depth) was compared with conservation cultivator tillage (12–15 cm depth). They found a **significant effect of tillage on community structures, and furthermore, the differences in structure reflected differences in ecological functions**. In the shallower cultivator tillage, they observed an increased abundance of Alphaproteobacteria and Actinobacteria, the latter being able to degrade complex organic compounds. Actinobacteria are also thought to be more sensitive to mechanical disturbance as they develop mycelia-like growths, which may explain their stronger presence in the reduced tillage samples. There was also a higher abundance of microorganism genes related to methane (CH_4) metabolism in the same treatment, whereas a higher number of genes involved in mismatch repair were found under the mouldboard plough system.

An experiment using perennial crops also identified significant differences in soil bacterial and fungal communities due to tillage. Chou *et al.* (2018) compared the soil microbiomes from three vintages in vineyards using different agronomic practices for weed management: glyphosate herbicide application, cultivation (weeding performed by mechanical and manual means, rototiller and hoe respectively) and natural vegetation (spontaneous grassing). **Soil fungal communities were significantly different under natural vegetation compared to both other treatments.** Similar results were obtained for bacteria, but only in two of the three vintages.

In contrast to the above literature, one study actually found only minor effects of tillage on the soil microbiome. Schlatter *et al.* (2019) investigated the effect of biosolids, fertilizers and tillage (CT and NT) on soil bacterial communities in dryland wheat farming systems. They found that just a few taxa differed between the CT and NT treatments. They accordingly concluded that tillage had only a minor effect on the bacterial communities observed. This weak effect may have been related to the relatively short timeframe of tillage treatments (four years) and the environmental constraints on microbial growth in the system studied (e.g. temperature and moisture).

4.2.2
Tillage can strongly shape functionality in soil microbial communities

The studies above highlight how tillage can change soil microbial communities, only touching on consequential functional changes. Several other studies have explored soil microbial functional shifts more explicitly from the perspectives of soil aggregation via soil microorganism activities (Cania *et al.*, 2019), soil microorganism functional profiles (Souza *et al.*, 2016), copiotrophs versus oligotrophs (Carbonetto *et al.* 2014; Degrune *et al.* 2017), and ecological guilds (Schmidt, Mitchell and Scow, 2019).

Binding agents such as polysaccharides and glycoproteins can be synthesized by soil microorganisms, helping create soil aggregates. Soil layers subjected to tillage were found to have the highest potential of binding agent production, owing to the greater abundance of bacterial communities that perform this function (Cania *et al.,* 2019). This was concluded from a long-term field experiment that compared CT to NT, which nevertheless did not demonstrate strong evidence of a tillage effect on the actual production of the binding agents (Cania *et al.*, 2019). This was surprising, but the authors suggested that it was consistent with results from other studies (Grafe *et al.*, 2018; de Vries *et al.*, 2015), implying that bacterial communities can be very stable under long-term management. In a later study, Cania *et al.* (2020) concluded that tillage intensity could affect the stability of soil aggregates in two ways. One was by influencing the abundance of genes involved in the production of two gluing agents (exopolysaccharides and lipopolysaccharides). A second was by inducing changes in the community of potential polysaccharide producers. Glomalin, a soil aggregate-stabilizing glycoprotein produced in the hyphae of arbuscular mycorrhizal fungi, has been found in lower concentrations when shifting from uncultivated to cultivated soils and from no-till to tilled soils (Bedini *et al.*, 2007; Carneiro *et al.*, 2015; Rillig *et al.*, 2003).

Looking at different soil microbial functions, Souza *et al.* (2016) **identified significantly**

reduced soil microorganism functional profiles under CT. They compared Brazilian Cerrado undisturbed soil with 23-year soybean/maize field soil under different levels of soil disturbance, CT and NT, measured at 0-10 cm depth. There were significantly less microbial biomass and enzyme activities in CT compared to both NT and Cerrado soils. Furthermore, there was more OM and nutrient contents in NT compared to CT after 23 years of cropping. The author suggest that the diminished enzyme activity was very likely related to the lower soil organic matter (SOM), as well as the addition of chemical fertilizers when the grassland was converted to cropland years before. Bacteria, archaea and viruses were more abundant in NT and CT, compared to the undisturbed soil. However, from a functional point of view, CT (the treatment with highest level of disturbance) showed higher diversity in taxonomic groups but reduced functional profiles compared to Cerrado soils. According to the authors, this implies that **soil disturbance in the system studied can result in higher taxonomic diversity. This could indicate a selection strategy that maintains many taxa under CT, thereby maintaining soil functionality even though these taxa may not be the most efficient for certain functions. Over time this could potentially lead to decreased soil quality.**

Tillage has been seen to clearly trigger changes in oligotroph and copiotroph populations.[10] Two examples, one study conducted in Argentina and the other in Belgium, serve to explain further. Comparing the effects of CT versus NT on the soil microbiome (depth 0-20 cm) in the Argentine Pampas, Carbonetto *et al.* (2014) describe how CT created an environment better suited to copiotrophs due to the increased nutrient availability (attributed to increased exposure of OM to degradation processes, which release nutrients), while NT selected for oligotrophs. The authors suggested that the high abundance of certain bacterial phyla such as *Gemmatimonadetes* and *Nitrospirae* in CT soil samples may have been due to their ability to adapt to the heterogeneous soil conditions created by tillage. In contrast, microbes better adapted to feeding upon recalcitrant compounds – oligotrophs – as well as the highest number of macro-aggregates were found in NT soils. The bacterial phyla found in this treatment (Verrucomicrobia, Plactomycetes, Actinobacteria and Chloroflexi) are indeed associated with an oligotrophic lifestyle. In Belgium, Degrune *et al.* (2017) investigated the effect of reduced tillage and CT, both with and without crop residues, on *Vicia faba* and *Triticum aestivum*.[11] They sampled soil below the seedbed, at a depth of 15-20 cm. They found that changes in soil physical and chemical characteristics triggered differences in the structure of the soil microbiome. Reduced tillage was associated with less available nutrients, water and oxygen compared to CT for two reasons. First, compaction may have affected soil pore structures, in turn affecting water flow and nutrients and root development. Second, in the treatment where crop residues were incorporated with tillage, they created more C availability. The authors suggest that the soil microbiome taxa evolved accordingly in order to adapt to these different conditions. Similar to the results in Carbonetto *et al.* (2014), CT stimulated a copiotrophic microbial lifestyle, while reduced tillage favoured oligotrophic microorganisms.

Tillage practices can influence ecological guilds, which are another way to classify microorganisms according to their function (Schmidt, Mitchell and Scow, 2019).[12] Associations of distinctly different guilds such as saprotrophic, symbiotrophic, and pathogenic fungi form larger functional communities in the soil. This study used samples at depths of 0-15 cm and 15-30 cm below the ground surface. Compared to CT, NT increased the soil N and C in the top soil layers, thereby altering the functional composition of the soil fungal community though a shift in favour of more plant symbiotrophs, rather than saprotrophs. In other words, **NT (along with cover cropping)**

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[10] Copiotrophs are organisms that feed upon easily accessible and abundant carbon sources, while oligotrophs feed upon recalcitrant matter under carbon-scarce conditions. See Key Terms for a more detailed description.

[11] Reduced tillage, in this study, was an intermediate soil disturbance tillage system where only the top ten cm of soil was disturbed.

[12] Ecological guilds describe groups of species that exploit the same resources, or that exploit different resources in comparable ways.

created a more heterogeneous soil fungal community that was also enriched in taxa that form beneficial symbioses with plants (plant-symbiont fungi). Crucial to this point about functionality, is that the diversity did not change. Under CT, symbiotrophs, saprotrophs, and pathogens all decreased in number, while all genera that increased with tillage were uniquely saprotrophs. Tillage might have caused a selection effect favouring functional groups characterized by a high growth rate, and thus higher mineralization rates. More N and nitrate (NO_3^-) were correlated with tillage, supporting this hypothesis. In addition, **it has been suggested that under CT, selection favours fungal taxa that feed upon mechanically damaged plant tissues** (Sharma-Poudyal *et al.*, 2017). The taxa that were more abundant under NT in this study are those known to either prefer an intact root environment or to form extensive hyphae and therefore more likely to be disturbed by tillage. Lastly, it is worth highlighting that in this study, tillage had a stronger effect than cover crops on fungi; approximately 45 percent of the guild-assigned fungi responded to tillage, compared to only 10 percent with cover crops.

4.2.3

Tillage can impact the structure of soil fungal communities

Tillage has been seen to affect soil fungi, as already mentioned in this section (Köhl, Oehl and Van Der Heijden, 2014; Chou *et al.* 2018; Schmidt, Mitchell and Scow 2019). Indeed, they are known to be more sensitive to mechanical disturbance due to its negative effect on fungal hyphae (Miller and Lodge, 2007). Root and bulk soil environments host dissimilar microbiomes, which can be differently affected by agricultural management practices. Furthermore, the timespan of an agricultural practice may also influence the contrasting characteristics of these microhabitats (Hartman *et al.*, 2018). **The effects of agricultural practices such as tillage may alter the bulk soil microbiome in the short-term, but the effects can decline over time because they only occur at specific moments during the cropping season. Since crops are typically sown following tillage, however, the root microbiome recruits microorganisms in this environment and the ensuing colonization tends to be more stable over time.** Hartman *et al.* (2018) suggest that may explain why root and bulk soil bacterial and fungal communities were affected differently in their experiment comparing CT and NT under organic and conventional management. Bulk soil was collected at a depth of 10–20 cm, and whole root systems were collected at a rooting depth of about 10 cm. In the bulk soil, bacterial communities were strongly determined by tillage, while fungi communities were determined by both tillage and the type of farm management. Conversely, in the root soil, the structure of fungal communities was primarily influenced by tillage, while those of bacteria more so by the type of farm management.

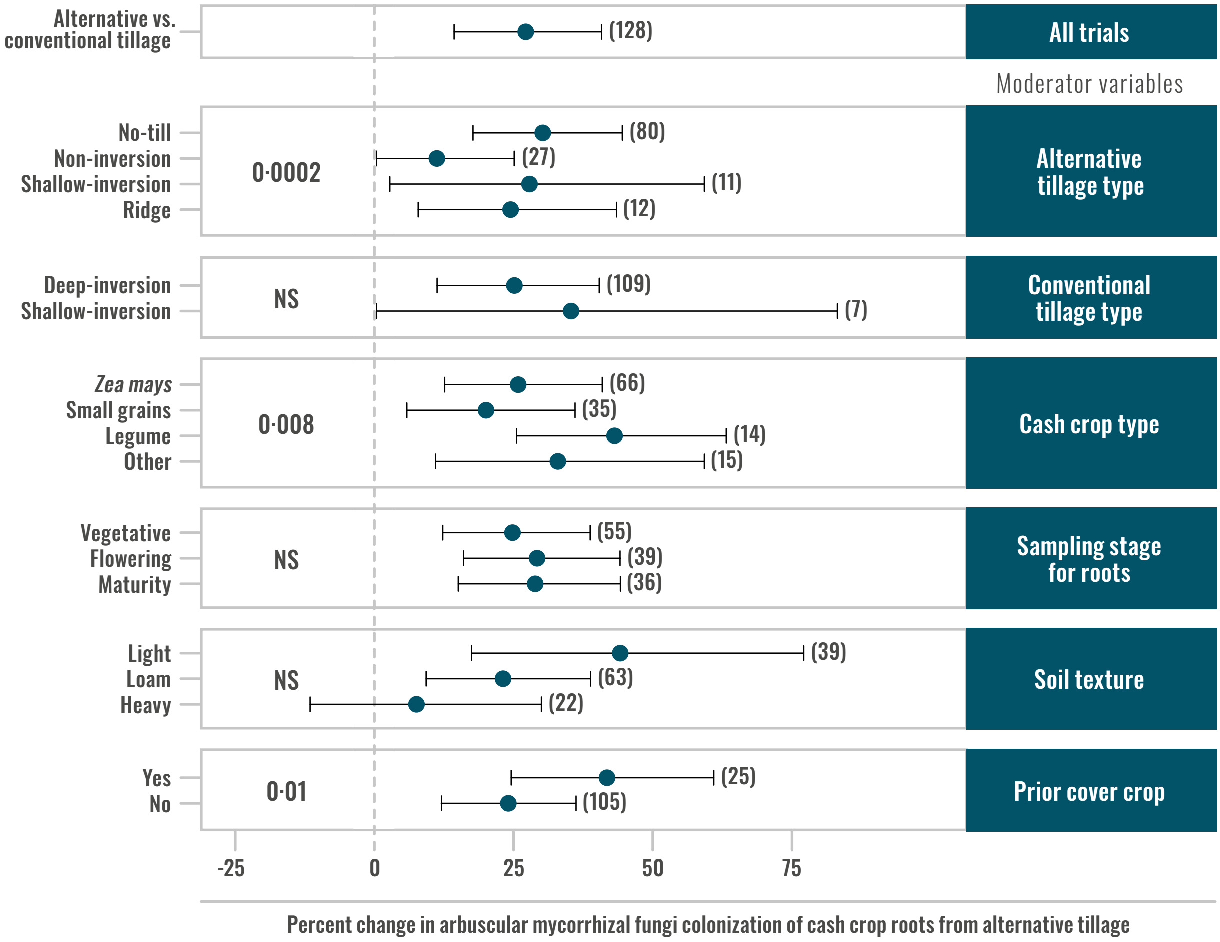
Arbuscular mycorrhizal fungi are a specific type of fungi which penetrate plant roots with their hyphae, forming mutually beneficial relationships with plants. Like other fungi, they can be very sensitive to soil disturbance, as demonstrated above where AMF hyphal length was significantly longer under NT compared to tillage (Köhl, Oehl and Van Der Heijden, 2014). **Different AMF species have different traits, and soil disturbance may select for species that invest more in reproduction, rather than nutrient scavenging or nutrient transfer to their plant hosts** (Oehl *et al.*, 2003). As AMF play several crucial roles in soil functioning, including increased plant nutrient access and uptake, it is especially important to understand how tillage practices can be managed to the benefit of both AMF and their host crops. In particular, their role in helping plants access P takes on an additional dimension of importance looking towards the future: global P stocks are expected to continue to diminish (Cordell, Drangert and White, 2009). Purposeful manipulation of beneficial soil microbial communities such as AMF, may be a way to enhance P uptake from the soils. For example, NT may be helpful to encourage AMF hyphal growth to increase P uptake in a P-deficient field (Köhl, Oehl and Van Der Heijden, 2014).

A comprehensive meta-analysis on the effects of tillage and cover cropping on AMF that included 54 studies from five different continents, demonstrated the effect of tillage on AFM plant-root colonization with two particular points (Bowles *et al.*, 2017).[13] First, **the number of different AMF species increased by 11 percent under alternative tillage compared to CT. This was in line with the frequent observation that reduced root soil disturbance accommodates for taxa adapted to intact roots or taxa that grow extensive mycelia**. Second, they also found **an approximately 25 percent increase in root colonization of AMF under alternative tillage practices compared to CT** (**Figure 13**). The variability associated with those data was also investigated by taking into account the type of alternative or CT practice, cash crop type, sampling stage of roots, soil texture and prior cover crop. The type of alternative tillage practice, the cash crop type and the prior cover crop were significant, demonstrating the effect of tillage on AMF colonization.

FIGURE 13.

META-ANALYSIS RESULTS OF THE CHANGE IN ARBUSCULAR MYCORRHIZAL FUNGI COLONIZATION OF CASH CROP ROOTS IN RESPONSE TO ALTERNATIVE TILLAGE FROM FIELD EXPERIMENTS IN FIVE CONTINENTS.

The vertical dotted line represents no effect. Statistical significance is indicated to the left of the dotted line; NS means no significance. The horizontal lines are error bars, which represent 95 percent confidence intervals, and the single dot is the mean value. The numbers in parentheses are the number of observation in each category.



Source: Bowles et al., 2017.

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[13] In this meta-analysis, alternative tillage categories were based on the level of soil disturbance. They included no-till, non-inversion (e.g. chisel), shallow inversion (e.g. shallow disking) or ridge tillage. Conventional tillage category consisted of either deep inversion (mouldboard plough, which represented the majority of conventional tillage treatments) or shallow inversion.

Finally, an experiment in Austria also found that the combination of reduced tillage (and use of cover crops) can increase AMF abundance, with subsequent consequences for crop yields (Rosner *et al.*, 2018). The sampling depth was 15 cm. In particular, tillage affected AMF colonization in winter wheat, but not in cover crops. Reduced tillage resulted in increased root colonization, perhaps because plant roots could follow previous root channels thereby encountering more AMF propagules, as suggested by Kabir (2005). There were less AMF spores found in the CT treatment.

Concluding remarks

In conclusion, tillage can shape soil bacterial and fungal communities and impact their activities, thereby influencing soil functioning, plant productivity and provisioning of certain ESS. Different degrees of soil disturbance alter soil microbial community functionality in different ways. Tillage compared to NT, for example can result in reduced microbial functionality or select for copiotrophs rather than oligotrophs. Arbuscular mycorrhizal fungi are particularly sensitive to tillage. Compared to CT, alternative or reduced tillage practices encourage AMF species diversity and colonization.

4.2.4

Systematic review: Effects of tillage on the soil microbiome, and their combined impact on climate change

Searches relevant to the effect of tillage on the soil microbiome and, subsequently, climate change returned 122 publications. Sixteen were selected as relevant (see **Annex I** for search terms).

Tillage management strongly shapes the soil structure, and soil structure affects microbial activity. Given the soil microbiome's key role in GHG cycling and soil C storage, tillage thus has a close connection with climate change-related factors. Conventional tillage causes the most soil physical disturbance, inverting the soil at depths of about 15-20 cm. Conservation tillage seeks to reduce or even eliminate soil disturbance altogether.

Effects of no-till vs tillage techniques on soil nitrous oxide emission

According to the literature reviewed in this section, there is no general consensus on whether NT or contrasting CT results in nitrous oxide (N_2O) emission increase or decrease. While NT can lead to increased emission, the opposite may be true, or indeed there may be no difference at all. These disparities may be due to differences in soil types, other management practices (such as N mineral fertilization, crop residue mulching or incorporation), climatic conditions, or the length of the experiment (Badagliacca *et al.*, 2018).

Conservation agriculture is perhaps the most widely known no-till, or reduced tillage, systems. Wang *et al.* (2019b) compared CT, chisel plough tillage (a type of reduced tillage), and NT management in a 7-year field experiment at a depth of 0-20 cm. In this study, NT and chisel plough tillage were referred to as conservation tillage practices. They found that compared to CT, chisel plough and NT significantly reduced cumulative soil N_2O emissions by about 46 and 74 percent, respectively. **The abundance of genes involved in N-cycling was significantly affected, there being a significant positive correlation between N_2O emission flux and archaea *amoA*, *nirS* and *nosZ* genes**. Archaea *amoA*, *nirK* and *nirS* genes were less abundant under conservation tillage than CT. The *nosZ* gene was more abundant under conservation tillage than CT, implying the potential for more complete denitrification (reduction of N_2O to N_2). The abundance of bacteria *amoA* was relatively lower under tillage practices, compared to archaea *amoA*. While this correlation offers some information, it does not explain the mechanisms responsible for the increased N_2O emission under CT. The authors suggest two complementary explanations. First, although soil under CT may have a soft structure, it has been observed to hold a low C stock and oxygen concentrations. Soil aggregates with low oxygen conditions provide a favourable growth environment for anaerobic denitrifying bacteria. Second, there were more N_2O precursors such as NO_2- in soil under CT.

Other studies have also reported reduced N_2O emissions under conservation tillage, such as one by Lal *et al.* (2019) that measured a 20 percent reduction of this GHG emission under NT, compared to CT (sampling depths 0-15 cm, and 15-30 cm).

Seasonal changes can also influence nitrifier and denitrifier community structure in conjunction with tillage management. Smith *et al.* (2010) report that in their field experiment with measurements down to 10 cm soil depth, NT plots had significantly lower cumulative N_2O emissions compared to those under CT, mainly due to the **lower spring flux of N_2O. This was attributed to a lower nutrient availability under NT owing to a lower degree of soil freezing, caused by the insulating effect of crop residues.** Their study also suggests that there were significant differences in the composition of denitrifier and nitrifier communities under the two tillage treatments, particularly following a thaw in early spring. Another field study also found that **CT vs NT during the growing season also created different denitrifier community structures throughout two winter seasons**, **but did not affect the active, cold-adapted denitrifier community structure** (sampling depth 0-15 cm) (Tatti *et al.*, 2015). Rather, the N_2O surges observed in winter were attributed to soil environmental factors (temperature and volumetric water content). The authors furthermore suggest that active members of *nirK*, *nirS* and *nosZ* communities may continuously adapt to evolving winter conditions, evidenced by the significantly different community structures of these genes between sampling times. They argue that for a complete analysis of soil functional communities, the contribution of eukaryotic microorganisms such as fungal denitrifiers should probably be included.

In contrast to the literature above, a recent meta-analysis of 57 studies from around the world (tillage of varying depths, when the data were available), concluded that NT rather increased soil denitrification compared to CT, seeming to lead to increased N_2O emission, related to changes in soil microbial communities and soil environment (Wang and Zou, 2020). The increased soil denitrification was associated with the increase in size and activity of the denitrifying community (potential denitrification activity, number of denitrifiers and abundance of denitrifying genes were increased in NT by 66, 116 and 14-70 percent, respectively). Also, NT tends to increase water-filled pore space due to greater soil moisture and bulk density, contributing to enhanced populations and activities of heterotrophic denitrification and/or nitrifier denitrification (Badagliacca *et al.*, 2018; Wang and Zou, 2020). Moreover, this effect seems to be stronger in dry than humid climates. Nevertheless, Wang and Zou (2020) point out that their metanalysis had far more short-term studies than long-term. This may well have impacted their conclusions, given that the positive impact of NT on soil denitrification tends to decline in the long run.

An additional study, not included in the above meta-analysis, also reported higher N losses by denitrification under NT compared to CT. In their experiment in Argentina, Palma *et al.* (1997) found that CT created greater oxidation and therefore approximately twice less denitrification. They also observed that under NT, the consumption of C by heterotrophic organisms, including denitrifiers, led to an increase in denitrification with an increase in C availability. They sampled the 0-10 cm soil layer.

Ball (2013) compiled an interesting review, linking soil structure to GHG emissions by analyzing twenty years of experimentation from Scotland, Japan and New Zealand. The study included the influence of tillage, compaction and animal trampling. **Tillage and any other practice influencing OM distribution and creation of soil pores influence N_2O emission, because the GHG is released around accumulation of OM (which fuels increased microbial activity), within aggregates, and inside aggregates with restricted diffusivity**.

The study by Jahangir *et al.* (2011), sampling the 0-10 cm soil layer, provides a good example to illustrate these factors. Their study found that **cumulative N_2O emissions were 4.5 and 2.5 times higher in reduced tillage (harrowing down to 10 cm) compared to NT and CT.** This may have been due to several reasons. One might have been related to the higher total soil C content in reduced tillage. The mineralizable C might have driven denitrifier activity, as already described. Another may have been caused by the larger soil aggregates in this treatment. Larger aggregates create more internal anaerobic conditions (likely dominated by denitrification), and the larger pore spaces can promote the rapid escape of N_2O. They suggest that reduced-tillage in the silt loam soils of their study is more likely to form soil aggregates because the long-term compaction common to NT may result in deformed soil aggregates, and the mechanical disruption caused by CT deteriorates soil structure. A last potential explanation for lower N_2O emission under NT is its associated higher bulk density, which may have decreased N_2O diffusivity, providing an opportunity for the N_2O to be reduced to N_2 before reaching the surface. The authors also found that tillage affected spatial variability in soil conditions, microbial community structure, and resulting N_2O emission – and that these occur at a microscale rather that plot-scale. This happens because **tillage affects aggregate formation, as described above, as well as nutrient pools according to the different distribution of inputs with different tillage systems**. Lastly, **there was a significant positive correlation between N_2O emissions and fungi:bacteria ratio, indicating that higher fungal abundance, rather than bacterial, is an important factor in explaining emission of this GHG**. Indeed, it has been suggested that most fungi lack the N_2O reductase enzyme. The authors also suggest that fungal growth was inversely influenced by soil bulk density. In sum, Jahangir *et al.* (2011) describe how different tillage systems impacted soil conditions in their study. These were, namely, aggregate formation and associated pore space, bulk density, and spatial variability of nutrients and GHG emissions driven by differently stimulated soil microbial communities. Thus, reducing soil N_2O release to the atmosphere involves increasing soil porosity as well as a reduction in soil water content through decompaction and drainage, effectively providing escape routes for the GHG into deeper soil layers, thereby playing a smaller role in surface GHG fluxes (Ball, 2013).

Effect of no-tillage and different tillage techniques on soil carbon storage and carbon dioxide emission

Chenu *et al.* (2019) stress the difference between C sequestration and soil organic C (SOC) storage, which are sometimes incorrectly used as interchangeable terms. **C sequestration describes a net removal of atmospheric carbon dioxide (CO_2)**, and is retained in the soil in the form of stable aggregates. It can be for short-term (e.g. 20 years) to long-term (e.g. millennia). **Soil organic C storage typically describes processes that are linked to the labile, or active, pool of OM. This type of C storage refers to an increase in C in the soil, but it does not imply stable removal of atmospheric CO_2**. C sequestration is not always the primary objective, though, as trophic organisms – which collectively perform multiple soil functions – consume labile C for energy. Rather, both SOC storage and C sequestration have their respective interests. In their review, Chenu *et al.* (2019) describe that NT management practices can increase SOC stocks in upper soil layers, but it has low to no effect on SOC stocks below 30 cm. Conversely, tillage increases SOC mineralization because the mechanical disturbance breaks up soil aggregates, creating more exposure to microbial degradation processes. **So, reducing tillage may increase SOC, but does not necessarily result in C sequestration.** This seems to be the case particularly in humid and temperate conditions, compared to drier climates where substantial benefits with NT compared to CT have been recorded. The authors also note that studies in the tropics on NT systems suggest that in order to increase SOC storage, additional inputs such as cover crops and intercropping, rather than reducing losses through tillage reduction, are more effective.

CO_2 is produced by several microbial process, and its emission results from both decomposition processes and heterotrophic respiration. Fast CO_2 releases have often been observed one or two days following ploughing (Ball, 2013; Xiao *et al.*, 2019). In contrast, the review by Ball (2013) found that CO_2 emissions were not affected by compaction or poor soil structure, while **CO_2 emission was higher in**

well-structure soils where the loose, well-aggregated structure provided good aeration and optimal conditions for the soil microbial communities. This effect can be explained by the flush of microbial-produced CO_2, released through the large spaces created by tillage. Frequent tillage may thus increase microbial metabolic activity, stimulating short-term CO_2 emissions through changes in soil aggregates, SOC, DOC and MBC (Xiao *et al.*, 2019).

In a study comparing ten years of conservation tillage in north eastern China, Jia *et al.* (2016) concluded that **ridge tillage increased soil microbial respiration, SOC concentration and soil microbial biomass at 0-5 cm depth, but did not influence annual CO_2 emissions compared to CT.** No-tillage significantly reduced CO_2 emissions compared to CT. Also, while both NT and ridge tillage significantly increased SOC concentrations in the superficial soil layer 0-5 cm, NT significantly decreased SOC in other soil layers. Leaving crop residues on the soil surface under NT resulted in a lower soil C input because some residues were blown away, and also because they were less exposed to microbial decomposition (compared to tillage which incorporates them into the soil, thereby increasing exposure). Altogether, they suggest that ridge tillage may therefore be an appropriate practice to promote soil microbes and SOC sequestration in the types of soils studied.

Similarly, Dong *et al.* (2009), also working in China, also found that after five years, **CT (mouldboard and rotary) had the highest annual CO_2 flux compared to NT. This may have been caused by reduced microbial immobilization of soil C under long-term, intensive tillage.** The experiment used samples from 0-30 cm soil depth. Furthermore, the CO_2 fluxes correlated with the ratio of dissolved organic C (DOC) to microbial biomass C (MBC). A higher immobilization of C per unit of microbial biomass tended to decrease CO_2 losses. Therefore, that the lower MBC under mouldboard ploughing was associated with higher CO_2 emission signals that there was little conversion of plant residue C into MBC. This study also included treatments with residues. Consequentially, almost all tillage management choices increased SOC. No-tillage, nonetheless, produced the least crop biomass, suspected to result in less soil C storage, but also the lowest CO_2 flux. The authors therefore suggest that tillage management choices offer an opportunity to balance residue inputs and the resulting CO_2 emissions. Another study that also looked at tillage, residues and microbially-mediated C dynamics concluded that **changes in soil and microbial properties induced by five years of contrasting tillage practices** (CT vs reduced) **did not influence the biological functions of SOC mineralization in the 0-5 cm soil layer** (Sauvadet *et al.*, 2018). However, when straw was added to the soil samples, the different tillage practices trigged important differences in terms of microbial CUE, enzyme efficiency and microbial biomass.

Effects of no-tillage and soil methane emission

Undisturbed soils are understood to act as methane (CH_4) sinks, because mechanical soil disturbance creates a less favourable environment for methanotrophic organisms. Working with NT grass and legume-based cover crop systems in southern Brazil, Bayer *et al.* (2012) found that after about twenty years, NT indeed had an effect of slightly decreasing the soil CH_4 emission while having a significant effect on soil fertility. This was particularly the case in systems with high production of plant biomass, which had a positive impact on soil quality. In the NT soil there were, nonetheless, fluxes resulting in net consumption or emission depending on the period of year and cropping system. The authors further suggest that the potential of the soil in this system to uptake CH_4 was off-set by two factors. One was a negative effect of biologically fixed N from legumes on methanotrophic microbiota. The second was an increase in methanogenesis resulting from dioxygen (O_2) depletion in niches with high biological activity in the uppermost soil layer of the NT treatments. They sampled soil in the 0-30 cm layer.

Soil structure influences carbon and nitrogen mineralization caused by amoeba predation of microorganisms

Protist predators influence soil C and N dynamics when they release nutrients from their prey. Amoebae, specifically, may be of particular importance as they are the most abundant soil protozoa. Zhan *et al.* (2016) argued that **bacterial predation by protists plays a key role in soil C and N dynamics and, furthermore, this dynamic is strongly influenced by aggregate structure and temperature.** When aggregates were crushed, as can occur during tillage, the predation-induced C mineralization was statistically higher at 25°C, but not at 15°C. This demonstrated that under high temperatures, amoebal predation is very much influenced by physical barriers (e.g. aggregates). It is thought that amoebae may be better able to access bacteria in protective spaces than bacterial predators. However, the authors found that intact aggregate structures limited the predation-caused respiration. In other words, bacteria were better protected by aggregate soil structures. These results indicate the relevance of (i) soil structure and the role of temperature in mediating the predation-induced increase in C and N mineralization, and (ii) the need to account for these interactions when predicting soil C and N dynamics under climate change scenarios, especially in regions where significant warming is expected. Soil samples in this study were from the 0-5 cm layer.

Concluding remarks

Tillage directly impacts soil structure – pores, aggregates, OM distribution – which, along with soil moisture, regulates soil microbial activities of GHG production and release from soils. Well-aerated soils with good drainage are recommended to reduce surface N_2O fluxes, although anaerobic soils do not necessarily enhance N_2O emission owing to the possibility of microbial adaptation and consumption under these conditions. Well-aerated, moist soil conditions favour CH_4 oxidation by methanotrophs and CO_2 exchange, while water-blocked soil pores hinder the escape of CO_2 to the surface. In view of these dynamics, it is beneficial to study multiple GHGs simultaneously, at the pore-scale, in order to better identify physical and biological processes that could help improve soil health and reduce GHG soil emissions. Many studies tend to focus on the top soil layer where tillage occurs. The effect of soil depth, however, on C dynamics should also be taken into account as soil biotic and abiotic characteristics change with depth.

Many studies show that NT increases denitrification in the short term, seemingly linked to increased N_2O emissions. However, the duration of this practice should be considered because its positive impact on denitrification may decline in the long run. In addition, data are hard to find in places where NT is practiced widely, which could also impact conclusions of meta-analyses such as that by Wang and Zou (2020). There is likewise a need for long-term research connecting CO_2 and CH_4 exchange to C sequestration in the context of tillage management choices.

Finally, the effects of tillage-shaped soil structure on soil microorganisms other than bacteria, archaea and fungi, such as protists, should also be considered.

4.2.5
Systematic review: Effects of tillage on the soil microbiome, and their combined impact on human health

Three different searches for articles linking tillage, the soil microbiome and human health returned three articles, none of which were pertinent (see **Annex I** for search terms).

4.3
AGROECOSYSTEM CROP DIVERSIFICATION

4.3.1
Plant diversity

HIGHLIGHT BOX 6 Impacts of plant diversity on the soil microbiome, climate change and human health

NARRATIVE REVIEW What are the impacts of plant diversity on the soil microbiome?

- Plants can influence the soil microbiome through their different biochemical compositions, introduced into the soil via plant litter, root exudates and rhizodeposits. Their effects can continue even after the plant has gone.
- Though some studies have reported no effects of plant diversity on soil microorganisms, many have reported positive effects.
- Those positive effects have been attributed to higher soil carbon input, enhancing soil microbial biomass, respiration and community diversity. These factors in turn imply consequences for soil functions such as soil organic carbon storage, including in the form of microbial necromass.
- The effects of plant diversity on the soil microbiome offer an opportunity for adaptive management of agroecosystems to climate stress.
- Owing to the soil ameliorating effects of trees, agroforestry systems have been associated with increased soil microbial biomass and activities, including increased diversity and or abundance of mycorrhizal fungi.
- Legume intercropping can enhance microbial activity or biomass, often due to interactions between specific plant and bacterial species. It can also facilitate soil organic matter decomposition rates and promote arbuscular mycorrhizal colonization. It has been observed to promote phosphorus storage in the form of soil microbial biomass, thereby influencing soil phosphorus cycling according to specific plant and soil microbial interactions.

SYSTEMATIC REVIEW What are the impacts of plant diversity on the soil microbiome, and their causal impacts on climate change?

Intercropping:

- Intercropping can result in a soil microbial community that processes and transforms nitrogen more effectively than monocrops. This may or may not result in significant reductions in soil nitrous oxide emission.
- Shifts in plant–microbe and microbe–microbe interactions contributed to a higher potential nitrous oxide emission rate in grass roots intercropped with the legumes compared to single crops.
- Plant legacy refers to the species-specific effects a plant may have on the soil environment after it has disappeared. Due to this effect, intercropping can provide more stability for soil microbial activities regarding soil carbon and nitrogen dynamics when subjected to heat stress.

Agroforestry:

- Tree cover might affect microbial processes that consume greenhouse gases in Mediterranean oak tree forests
- Trees intercropped with grasses may increase soil carbon sequestration potential.

SYSTEMATIC REVIEW What are the impacts of plant diversity on the soil microbiome, and their causal impacts on human health?

- No relevant literature was found during the systematic search.

Agroecosystem plant diversity can be promoted through different farming techniques, including intercropping, crop rotations, cover cropping, and agroforestry systems (AFS). Changes in plant diversity are known to affect above-ground ecosystem functioning, but less attention has been paid to effects below-ground. Plants, with their different biochemical compositions, can influence the soil microbiome through their root exudates, rhizodeposits and residues. Microorganisms use plant matter and products as resources in various processes, resulting in nutrient cycling. Therefore **the choice of plant species can have consequences on the soil microbiome via their interaction with plant exudates and litter** (Eisenhauer *et al.*, 2010).

Furthermore, these plant-specific rhizodeposits can continue to influence their soil environment, even after the plant has gone. De Oliveira *et al.* (2020) suggest that plant legacy effects could contribute to designing adaptive strategies to heat stress-related climate change in Mediterranean soils. Compared to the monocrop treatments in their study, intercropping provided more stability for soil microbial activities related to carbon (C) and nitrogen (N) soil dynamics. They suggest that further research address whether prolonged plant presence could have a more persistent effect on soils, as this information could help design agroecological cropping systems that include intercropping (and/or crop rotations).

Numerous studies have, nevertheless, reported either positive or no effect of plant diversity on soil microorganisms (Eisenhauer *et al.*, 2010). Gastine *et al.* (2003), for instance, reported no significant effect of plant species and functional group diversity on microbial respiration.[14] Habekost *et al.* (2008) similarly observed no effect of the plant functional group on microbial respiration and biomass, while plant species diversity did affect microbial biomass. The positive effects of plant diversity have been attributed to higher soil C input, causing higher soil microbial biomass, respiration and community diversity. This is demonstrated in the two following studies. They were both part of the Jena Experiment, a long-term grassland biodiversity experiment in Germany.

Based on nine years of experimentation in grassland in the Jena Experiment, Lange *et al.* (2015) showed that **higher plant diversity increased C inputs into the soil and created a more favourable microclimate for microorganisms. These factors were responsible for creating a more abundant, active and diverse soil microbial community that, in turn, increased the soil C storage through a higher turnover rate of root litter and exudates.** The increased C inputs came primarily from increased plant root exudates and fine root litter. Soil C storage is known to be related to root inputs, and it influences the activity and community composition of the root-soil microbiome, as observed in this study. Reduced soil water evaporation, caused by denser vegetation with higher plant diversity created a more favourable microclimate for microorganism growth and activity. Several specific results illustrate these relationships: the increase in soil organic matter (SOM) throughout the study period was strongly correlated with sown plant species richness (**Figure 14**); the increase in C stored was directly related with the higher microbial activity (**Figure 15**); and indeed, C uptake by the soil microbiota in the rhizosphere was increased. A final point that explains the increase in soil organic C (SOC) associated with increased plant diversity, is that **as microorganisms put energy towards growth and reproduction there was an increase and accumulation of necromass over time. This microbial necromass, in combination with the products of microbial activities, accumulated in the slow-cycling SOM pools.** Other studies have also shown that microbial necromass can significantly contribute to recalcitrant soil C through the integration of microbial biomass residues (e.g. Liang and Balser, 2010; Zhu *et al.*, 2020).

Another, earlier study from the Jena Experiment found that **plant diversity is very important for soil microbial community functions** (Eisenhauer *et al.*, 2010). **The increase in microbial biomass or respiration was due to plant species diversity and species complementarity, rather than due to certain key or increased plant functional groups**. The soil microbial biomass, respiration, and C use efficiency (CUE) were affected by the increase in plant species richness; and the quality of the rhizodeposits was more important than the quantity or plant productivity in changing the soil microbiome. Whether the plants were grasses or legumes, however, did not trigger any major shift in soil microbial communities. Another observation is that the soil microbial community response to plant diversity took about two to four years to be visible. Possible reasons are that time is needed for plant material and exudates to accumulate in the soil; and microorganisms become more energy efficient with time.

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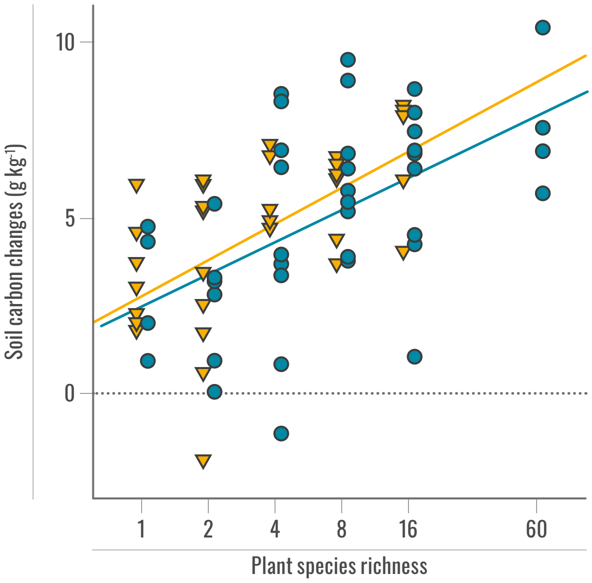
[14] A plant functional group describes plants with similar characteristics (e.g. similar responses to their environmental conditions, use similar resources, have similar functions in an ecosystem).

FIGURE 14.

THE RELATIONSHIP BETWEEN PLANT DIVERSITY AND SOIL ORGANIC CARBON STORAGE.

Plant species richness refers to the total count of different plant species in a given sample. The soil carbon changes were measured for the period between 2002 and 2011. Orange triangles are data points for samples that included legumes, whereas the blue circles represent data points for samples that did not include any legumes. The orange and blue lines show the best fit for the data, samples with and without legumes, respectively. The dotted horizontal line represents no change in soil carbon; the values below this line therefore represent samples for which decreases in soil carbon were measured.



Source: *Lange et al.*, 2015.

Impact of agroforestry plant diversity on the soil microbiome under drought and heavy rainfall

The following articles address the impact of plant diversity on soil microorganisms in the context of heavy rainfall or extended droughts. Meteorological events such as these are associated with climate change, and are anticipated to increase in frequency and intensity. A better understanding of the relationships between plant diversity and the soil microbiome may help adapt agroecosystems to these challenges.

Agroforestry can be defined as "a form of multi-cropping which involves combining at least one woody-perennial species with a crop which results in ecological and economic interactions" between them (Palma *et al.*, 2007). Trees can modify the soil environment by contributing soil cover, intercepting rainfall, taking up water through the root systems, and providing a steady flow of organic material. These processes influence the soil biotic and abiotic properties (e.g. moisture, temperature, nutrient content). **It has been proposed that AFS can help mitigate risks to agroecosystem stability and productivity by increasing ecological resistance and resilience** (Barrios *et al.*, 2012).

The soil enhancing effects of trees (e.g. organic matter (OM) inputs, differences in litter quantity and quality, root exudates) have been observed to increase the soil microbial biomass and activities in AFS (Gomez, Bisaro and Conti, 2000; Myers *et al.*, 2001; Sørensen and Sessitsch, 2007). In addition, compared to monocropping, increased plant diversity in an AFS has been associated with increased diversity and or abundance of mycorrhizal fungi (Cardoso and Kuyper, 2006).

A study conducted in a Mediterranean climate found that the AFS studied resulted in higher microbial biomass and mineralization activity near the tree rows (Guillot *et al.*, 2019). There was also higher SOM content near tree rows. However, despite those observations, the legacy effect of the tree row did not lead to higher ecological stability, measured by soil microbial biomass resistance and resilience, under the drought, and drought+heat stress treatments.

Illustrating the connection between climate stress and interactions between plant species and soil microbial communities, Sun *et al.* (2016) highlight that **different mixed plant associations in an AFS resulted in different soil food-web structures under extreme rainfall, leading to differences in biochemical cycling, soil functioning, and nutrient availability.**

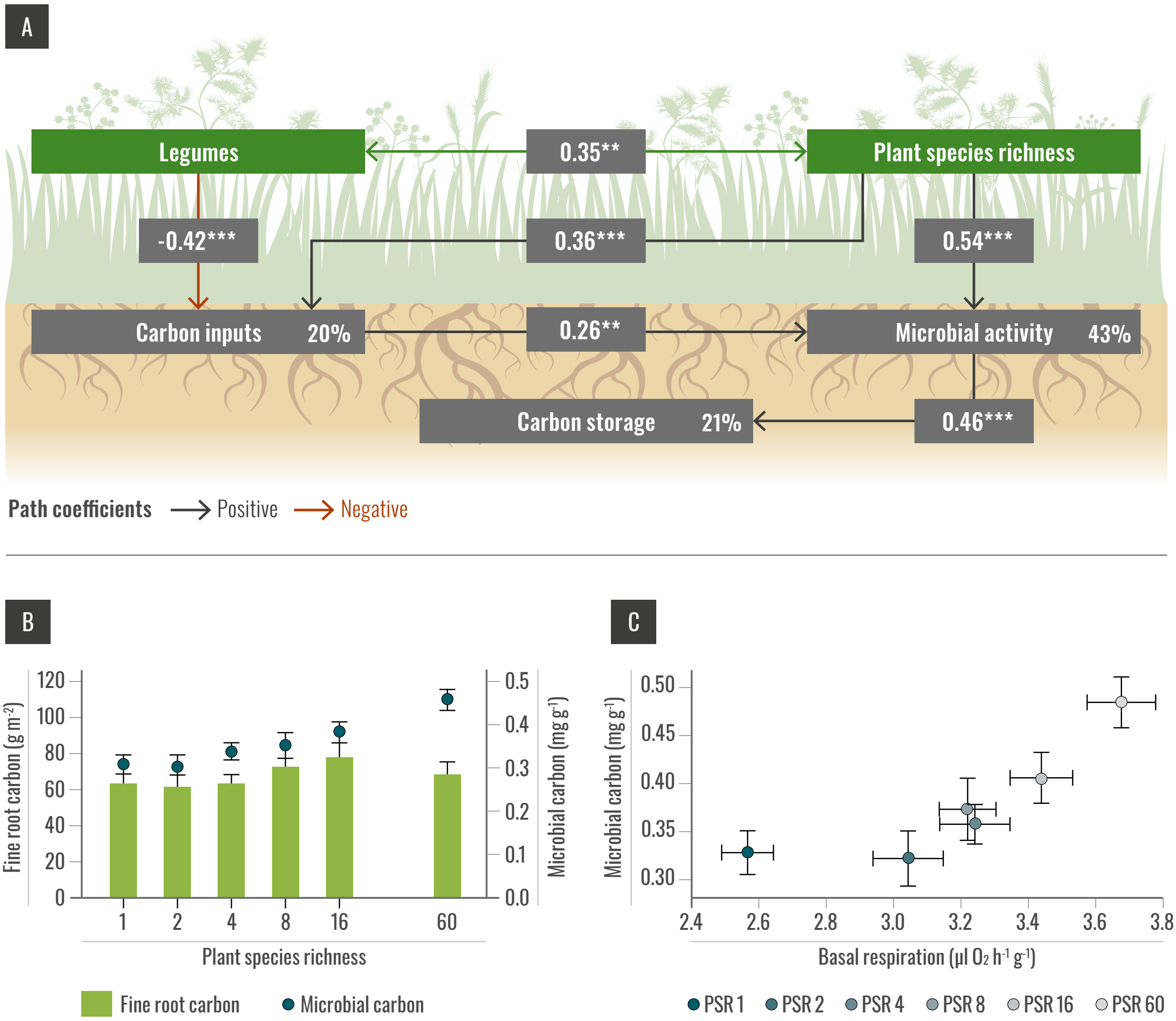
FIGURE 15.

MECHANISMS OF SOIL CARBON STORAGE.

(A) A predictive model explaining the underlying mechanisms of the positive relationship between plant diversity and soil organic carbon storage (otherwise known as a most parsimonious path model). The percentages next to the variables refer to the percentage of the variance explained by the variable. Numbers in line with the arrows indicate the effect (standardized path coefficients), and asterisks mark their significance (**, <0.01; or ***, <0.001).

(B) Impact of plant species richness on mean fine root carbon and mean soil microbial biomass. Lines with end points are error bars, which indicate the variability of data.

(C) Relationship between mean metabolic activity (measured as basal respiration) and mean biomass of the microbial community (measured as microbial carbon) at plant species richness levels. Lines with endpoints are error bars.



Source: Lange et al., 2015.

Working in Sichuan province, China, Sun *et al.* (2016) assessed whether an AFS would help stabilize the soil microbial food web under extreme rainfall conditions, thereby increasing the available N uptake of the crop. Results showed a marked decline in microbial biomass that corresponded with nutrient loss during extreme rainfall, suggesting that nutrient leaching is most likely related to the decrease in microbial biomass. The authors suggest that the leaf N contents of the principal crop species had a significant positive correlation with total microbial resistance, bacterial resistance, and net mineralization rate, and a negative correlation with total nematode resistance and bacterivore resistance. Therefore, they propose that **maintaining stability of the lower trophic level (i.e. soil microorganisms) is more important than maintaining stability of higher levels (i.e. nematodes)**.

Legume intercropping can impact soil microbial abundance, diversity and community structure

Legume intercropping enables plant diversity in space by alternating crop rows with a legume species, the residues of which may be removed or retained in the field. The ability of leguminous plants to biologically fix N offers the possibility to reduce input of mineral fertilizers. It is also a useful practice to increase on-farm diversity while also establishing a complementary relationship between crops, resulting in positive effects on pest control, soil health, and crop productivity. The concepts of complementarity and facilitation are particularly relevant to keep in mind while considering effects of legume intercropping on the soil microbiome and between plant species. When multiple species use a given resource differently it can result in complementarity, in this context defined as "a decrease in competition through resource partitioning between the intercropped species" (Tang *et al.*, 2014). Facilitation occurs when "one species enhances the growth or survival of another through direct or indirect mechanisms" (Tang *et al.*, 2014), resulting in net positive interactions.

A recent review on legume intercropping summarised the microbial dimension of this association (Duchene, Vian and Celette, 2017), highlights of which are discussed in the following paragraphs. **Legume crop rotations and intercropping have been reported to have an effect on the structure and diversity of the soil microbiome, as well as improve microbial activity and/or biomass** (Duchene, Vian and Celette, 2017). For example, legume synthesis and exudation of:

i lectins positively affect plant growth promoting rhizobacteria,

ii flavonoids seem to facilitate the lateral root colonization by *Azospirillum brasilense* (a root-colonizing, N-fixing bacteria),

iii isoflavones attract *Bradyrhizobium japonicum* (another root-colonizing, N-fixing bacteria; this example is specific to soy bean roots),

iv molecules similar to N-acyl homoserine lactone influence certain bacterial activities (e.g. quorum sensing and bacterial coordinated activity) (Duchene, Vian and Celette, 2017).

The authors report that many of these associations appear to be interactions between specific plant and specific bacterial species, and that legume intercropping seems to facilitate increased SOM decomposition rates, probably by stimulating specific soil microorganism communities with the addition of fresh OM. The enhanced mineralization of this OM could then benefit both intercropped species (Duchene, Vian and Celette, 2017). The same study highlighted similar results obtained in grasslands, where plant diversity and root functional traits triggered changes in the soil microbiome. The authors highlighted the **role of complementarity between different plant groups, including legumes, that promote better use of resources, leading to improved plant production (including increased root biomass), which stimulates microbial activity**.

Duchene, Vian and Celette (2017) also addressed the relationship between plant diversity and AMF, as the two are known to be linked. **In addition to attracting certain rhizobium, legumes also seem to promote arbuscular mycorrhizal fungi (AMF) colonization, especially in low-input farming systems.** AMF seem to be attracted by flavonoid secretions exuded by legumes, involving them both in a tridimensional symbiosis between the legume, the rhizobium, and the AMF. For example, one particular experiment using *Medicago* species as a model legume found that the legume increased the diversity and abundance of mycorrhizae (Pivato *et al.*, 2007). This was attributed to the legumes' dependence upon mycorrhiza for efficient phosphorus (P) uptake. Tang *et al.* (2014) similarly found that **intercropping with legumes may promote P storage in the form of microbial biomass. They suggest that because microbial biomass P (MBP) can be released as simple inorganic P during microbial turnover, it may be a more efficient P source (than mineral P), being released over time rather than in a single application**.

Finally, a recent study by Liu *et al.* (2019) **found that legume intercropping with rubber trees in an AFS showed minor but positive effect on bacterial diversity compared to rubber tree monocultures, particularly in mature tree stands**. Soil microbiome dynamics in AFS are necessary to evaluate their role in ecosystem functions such as C sequestering and nutrient cycling. This is especially relevant in the context of large-scale tree monocultures. This conversion is understood to be a major cause of biodiversity loss, although studies do not show consistent, negative effects on bacterial diversity (Berkelmann *et al.*, 2018; Wang *et al.*, 2017a). Nevertheless, in this study, AFS consistently demonstrated a large number of copiotrophs (specifically, Proteobacteria and Bacteriodetes at 0-30 cm depth) which was most likely due to positive changes in soil quality. The oligotrophic bacteria Acidobacteria and Chloroflexi were either negatively or not affected by AFS. Regarding the stand age, the young AFS showed a decrease in bacterial abundance while maintaining levels of bacterial diversity, and the mature AFS actually promoted bacterial diversity while maintaining abundance.

Concluding remarks

In conclusion, plant diversity influences the soil and soil microbiome through plant species-specific biochemical profiles. The plant's legacy can continue to exercise an effect. There are numerous examples demonstrating that increased plant diversity can result in increased C input to the soil, thereby stimulating growth and activities of soil microorganisms. This effect can consequentially increases the SOC storage, the soil microbial necromass being a potentially major deposit. Agroforestry systems may be able to contribute soil ecosystem stability under certain climate stress conditions via the soil ameliorating effects of trees. Intercropping – in AFS, arable crops or grasslands – can affect the structure and diversity of soil microbial communities by promoting AMF colonization, soil microbial P storage and release, as well as increasing soil bacterial biomass.

4.3.1.1 Systematic review: Effects of plant diversity on the soil microbiome, and their combined impact on climate change

Searches for the systematic review returned 43 total articles for this subsection, 23 for searches related to intercropping and 21 related to AFS. Of these, seven publications were relevant and are discussed below.

The effect of intercropping on nitrogen and carbon cycling and nitrous oxide emission

Intercropping, or the growing of multiple species at the same time in the same field, is widely acknowledged to have multiple benefits for crop production as well as the broader agroecosystem. A more efficient use of resources, such as nutrients and water, is one such advantage. Less N wastage, for example, could lead to reduced nitrous oxide (N_2O) emissions. An incubation study by Bichel *et al.* (2017), found that **maize-soybean intercropped soils impacted soil microbial diversity and activity, resulting in a community that processed and transformed N more effectively than sole-crop soils.** This did not, however, result in any significant differences in N_2O emissions between soils from intercropped and monocrop treatments. In contrast, Chen *et al.* (2019d) performed a field experiment and found that **intercropped maize-soybean soils demonstrated decreased N_2O emissions compared to monocrop treatments**. **The intercropped treatments resulted in a higher per-unit farmland productivity and lower land use intensity, as well as the lowest Greenhouse Warming Potential N_2o (GWP_{N_2o})**

Based on the increased abundance of ammonia (NH_3) oxidizers and denitrifiers (*amoA* and *nirS*, respectively), the authors suggested that intercropping improved the soil microorganism communities. They also reported that intercropping increased the ammonifying and nitrifying capacities to increase soil N, while decreasing NH_3 volatilization and N_2O emissions. Finally, intercropping demonstrated that the increased N-use efficiency was due to improved N utilization efficiency, rather than N uptake efficiency.

Soil N dynamics clearly involve a multitude of complex biophysical interactions that remain poorly understood. Soil N_2O emission can be difficult to predict because there are crucial knowledge gaps about the underlying processes that influence its production. One area of research focuses on identifying and characterizing soil bacterial communities that perform processes in the N cycle. It was observed, for instance, that **shifts in plant-microbe and microbe-microbe interactions contributed to a higher potential N_2O emission rate in grass roots intercropped with the legume lucerne** (Graf *et al.*, 2019). This coincided with a lower abundance of *nosZ* clade II bacteria. The authors suggested that a potential increase in N inputs (from N_2 fixation and N-rhizodeposition) could decrease the C:N ratio, potentially explaining the decreased abundances of *nosZ* clade II N_2O reducers. A large fraction of these bacteria carry N_2O reductase, produced by the *nosZ* gene, and thus they are recognized as the only known biological N_2O sink (Hallin *et al.*, 2018). Also, in the same treatment, there was a shift in the composition of *nosZ* clade I bacterial communities towards incomplete denitrifiers, which results in N_2O production. **In addition, there were several other ideas worth highlighting regarding potential plant-microbe interactions.** First, lucerne is known to produce secondary metabolites with antimicrobial effects, which could have negatively impacted denitrification activities. Second, how N-fixing bacteria in lucerne nodules might affect net N_2O emissions remains unclear. Lastly, increased above ground biomass supported belowground interactions, potentially contributing to a higher N_2O emission potential in the intercropped treatment.

How plant species may select for specific groups of microorganisms may help improve understanding of some of the underlying mechanisms that influence N and C cycling as well as other soil nutrient dynamics. De Oliveira *et al.* (2020) demonstrated a short-term legacy effect where, compared to monocropping, intercropping provided more stability for soil microbial activities regarding soil C and N dynamics when subjected to heat stress.

Relationship between agroforestry and biogeochemical cycling

Many studies concerning AFS likewise point out how much there is to explore in order to understand the complex interactions between plants, soil conditions, soil microorganisms and nutrient cycling. The two studies described below were found during the literature search, and offer just a glimpse of the wide range of questions.

Shvaleva *et al.* (2015) explored how tree cover might affect microbial processes that consume greenhouse gases (GHGs) in Mediterranean (cork) oak tree forests. They found that oak tree vegetation, compared to open areas without oak trees, affected soil properties, key enzyme activities, and the abundance of CH_4 and N_2O-oxidizing soil microorganisms. Those complex interactions consequently impacted net CH_4 and N_2O exchanges**. Overall, authors suggested that oak tree vegetation did not change the soil CH_4 uptake, but did significantly increase the N_2O fluxes**, neutralizing soil non-CO_2 GHG uptake in these particular forest ecosystems. They suggested that these dynamics can even alter the soil non-CO_2 GHG balance from negative to positive when compared to non-oak-vegetated areas.

A study conducted in Germany demonstrated that **C sequestration potential was highest for an AFS system of willow and grass alleys** (8.8 t Ct ha^{-1}) **compared to two tree monocultures** (willow 3.4 t Ct ha^{-1}, popular 5 t Ct ha^{-1}) (Tariq, Gunina and Lamersdorf, 2018). However, changes in total soil C weren't obvious after three years of plantation, prompting their call for more work to provide evidence of significant changes in C sequestration in similar systems.

Concluding remarks

On the one hand, intercropping can improve resource use of N via mediation by the soil microbiome, possibly leading to reductions in soil N_2O emission. On the other, intercropping systems have also been observed to contribute to higher potential N_2O emission rates. To generalize these findings requires additional field experiments with different soil types and crop combinations, amongst other factors, as well as a much more precise understanding of the microbe-microbe and plant-microbe responses to those management choices. Likewise, while it seems clear that intercropping does have a short-term legacy effect on soil microbial responses regarding soil C and N dynamics, it could be valuable to test longer cropping periods to investigate potentially more persistent – and beneficial – effects. This may be particularly useful in considering adaptive strategies for extreme conditions induced by climate change.

Agroforestry is a broad term that encompasses diverse systems. The few studies presented here address disparate subjects, making it difficult to offer a general summary. Nevertheless, there is evidently much to explore regarding AFS and soil microbiome interactions and consequences for GHG emissions and C storage.

4.3.1.2 Systematic review: Effects of plant diversity on the soil microbiome, and their combined impact on human health

Three different searches were performed for studies linking plant diversity, the soil microbiome and human health. They returned three articles, none of which were relevant for this section (see **Annex I** for search terms).

4.3.2

Crop rotations

HIGHLIGHT BOX 7 Impacts of crop rotations on the soil microbiome, climate change and human health

NARRATIVE REVIEW What are the impacts of crop rotations on the soil microbiome?

- Biochemical differences in root exudates, rhizodeposits, and crop residues can shape the community structure, diversity and functions of the soil microbiome, with consequences for subsequent crops and long-term soil health.
- Crop rotations can stimulate bacteria that have a positive effect on soil nutrient content and soil organic matter through their role as decomposers.
- Crop rotations may be associated with higher microbial diversity and specific community compositions correlated with disease resistance.
- More diverse rotations may result in higher soil organic matter quality, indicated by ratios of cellulose and lignin degrading enzymes, which influence soil microbiome composition and activities.
- Crop rotations seem to result in overall improved soil health and related ecosystem services.

SYSTEMATIC REVIEW What are the impacts of crop rotations on the soil microbiome, and their causal impacts on climate change?

- Crop rotations can impact soil microbial decomposition rates thereby impacting soil organic matter quality, as well as cycling of carbon and nitrogen, thereby impacting soil carbon storage and denitrifying activities.
- The practice generally seems to promote soil microbial abundance and activity, thereby maintaining or improving soil health and related ecosystem services.
- A crop rotation that incorporated five years of pasture into a cropping system can significantly affect community composition and function, increase soil organic carbon and therefore potential for carbon sequestration. Long-term effects regarding the persistence of increase in soil carbon storage upon subsequent tillage and cropping cycles in this system, however, remain an open question.
- Monoculture systems likely feature substrate limitation, suppressed microbial activity, and potentially higher nitrous oxide losses due to multiple factors (e.g. pH, selection of nitrifiers).
- Legumes in a crop rotation may contribute to higher soil microbial nitrogen as well as soil organic carbon via increased soil microbial activities and biomass.

SYSTEMATIC REVIEW What are the impacts of crop rotations on the soil microbiome, and their causal impacts on human health?

- Manipulating crop rotations may offer a strategy to reduce aflatoxin severity (caused by *Aspergillus flavus*).

Crop rotation is the practice of growing different crops in succession, thereby increasing plant diversity over space and time. It can be contrasted with monocropping, which can lead to problems such soil nutrient availability and disease intensification. Crop rotations are considered a useful strategy to improve the soil nutrient status, reduce pests, and enhance overall soil functioning. Much of those beneficial functions are performed by the soil microbiome, indicating many positive effects of crop rotations on soil microorganism communities and activities.

Crop rotations can have positive effects on soil microbial communities and activities

Multiple studies have demonstrated positive effects of crop rotations on the soil microbiome in different farming systems. This is particularly important in agroecosystems that produce the most-grown grain crops in the world, such as maize and rice. Continuous maize cropping, for instance, can cause nutrient deficiency in the soil, a lack of nutrient uniformity in the field, and exacerbate disease. In contrast, Zhang *et al.* (2019c) found that **a soybean-maize crop rotation provoked a shift in the bacterial**

soil community by stimulating gram-positive bacteria, which had a positive effect on soil nutrient content (specifically, phosphorus (P) and nitrogen (N)) and soil organic matter (SOM) through their role as decomposers. In addition, the rotation also provoked changes in gram-negative bacteria, fungi, and the fungi to bacteria ratio. **Paddy-upland are rain-fed, rice-producing fields found in tropical regions. They are likewise considered important for meeting food requirements. Rotations in these systems can help manage biodiversity and disease**. Hou *et al.* (2018), studying a paddy-upland system, observed that higher microbial diversity and specific composition correlated with disease resistance. Identified in the rice-rice-vegetables rotation, Burkholderiales and *Streptomyces* are known to promote plant growth and antifungal activity, while certain subgroups of Acidobacteria (found in greater abundance in the same crop rotation), play important roles against pathogens. In addition, Burkholderiales have the capacity to form effective symbioses with legumes (Chen *et al.*, 2005).

An interesting trial on the effect of long-term crop rotations on crop residue decomposition **demonstrated that crop rotation can improve soil health and related ecosystem services (ESS) through its effects on the soil microbiome** (McDaniel *et al.*, 2014)**.** In contrast, the monoculture system resulted in substrate limitation and suppressed microbial activity. Soils from more diverse cropping systems decomposed residues faster, clearly demonstrating a crop rotation effect on microbial activity. This is because crop rotations caused changes in interactions between soil microbes and SOM related to carbon (C) and N availability. Those changes then affected how new residue inputs were processed by soil microorganisms. Furthermore, results suggested that there was a higher SOM quality in the most diverse crop rotations, indicated by ratios of cellulose- and lignin-degrading enzymes. **The increased N-retention in microbial biomass involved in degrading high-quality residues, the faster decomposition of low-quality residues, and the higher microbial activity indicate very strong and close relationships between crops, soil and microbes associated in diverse cropping systems**.

Lastly, a global-scale meta-analysis that included 56 studies and 149 paired comparisons concluded that the inclusion of legumes in rotations seems to enhance the microbial biomass N and thereby create differences in microbial community abundance (Lori *et al.*, 2017). The study aimed to quantify the possible differences in key indicators for soil microbial abundance and activity between organic and conventional farming systems.

Crop rotations can have a stronger effect on the soil microbiome than other practices

Two of the studies already described above furthermore suggest that crop rotations can have a greater effect on the soil microbiome than other factors. Hou *et al.* (2018) demonstrated that **crop rotations in their study played a significantly stronger role in shaping the microbiome than the compared fertilizer treatments**. In their meta-analysis of key indicators of soil microbial abundance in organic farming systems, Lori *et al.* (2017) observed **that plant species may have a stronger effect than the farming system (organic vs conventional) on the soil microbial populations**.

Plants can create a soil memory effect

Despite the literature presented here, there is still relatively little known about specific mechanisms by which crop rotations affect biogeochemical processes. The idea of a soil 'memory effect', proposed by Babin *et al.* (2019), might be helpful in conceptualizing these dynamic and complex interactions. Similar to the notion of plant legacy (see 4.3.1 Plant Diversity) , the concept suggests that effects from a crop can leave an imprint on the soil microbiome due to plant-specific root exudates and rhizodeposits that select for certain soil microorganisms, with potential impacts on subsequent crop(s) and long-term soil quality. For example, in their long-term study conducted in Germany, the authors argue that **plants secrete different exudates and rhizodeposits in order to select beneficial microorganisms. They propose that this consequentially shapes overall microbiome structures, diversity and functions, hence the soil 'memory effect'.** Also, by nature of their tissues that vary in composition, different crop residues may likewise select for certain microorganisms. Rapeseed, for instance, has a more recalcitrant cellular wall composition compared to maize. They found more *Bacteroidetes* when rapeseed, rather than maize, was the preceding crop. As *Bacteroidetes* are capable of degrading cellulose and other complex organic compounds, it seems that the quality of rapeseed litter thus influenced the composition of the related soil microbiome.

Concluding remarks

Crop rotations can shape the soil microbiome community structure and activities via a soil memory effect, whereby the biochemical specificities of a plant can select beneficial soil microorganisms. Broadly speaking, crop rotations have been seen to improve soil health and ESS, including soil nutrient status, SOM, disease resistance, and increased soil microbial biomass.

BOX 3. CONVERSION OF GRASSLAND TO ARABLE LAND VIA A LEGUME CROP ROTATION: EFFECTS ON THE SOIL BACTERIAL COMMUNITY

Pressure on land to produce enough food for the world population is expected to increase conversion of marginal lands into arable land. It is therefore important to identify practices to help conduct the transition process in a stable manner, including sustainable management of nutrient balances and soil microbial community structure. The following study focused on changes in the soil bacterial community following a legume crop rotation that provided a transitional phase during conversion of grassland to arable land.

Obermeier *et al*. (2020) investigated the impacts on soil properties and bacterial community structure in response to different stages throughout the transition: grassland removal, tillage, intercropping with faba bean (*Vicia faba*) and its later incorporation. They sampled the topsoil (0-20 cm depth) throughout the experiment, which was conducted in Germany.

The combination of several processes greatly increased the nitrate-N contents of the soil (from 4 to almost 50 µg N g-1 *dw*), a sufficient quantity for future cropping on arable land but also created a potential risk of leaching. First, incorporation of the grassland green residue caused mineralization. Subsequently, the faba bean crop contributed N to the soil through biological N-fixing activities. Finally, when the crop itself was incorporated into the soil it contributed yet more N.

The bacterial community structure remained stable at the phylum level (Proteobacteria, Actinobacteria, Acidobacteria, Chloroflexi, and Bacteroidetes). **There were, however, some changes to the community structure at lower levels**. Overall they were attributed to enriched nitrate N, the changing C:N ratio, and other effects of the decomposition processes. **Most notably, at the final stage of conversion (after having incorporated the faba beans), the authors observed increasing abundances of the genera *Massilia* and *Lysobacter***, both of which are in the phylum Proteobacteria. *Massilia* has been associated with the rhizosphere and plant growth-promoting rhizobacteria in leguminous plants, and have been observed to increase during the early decomposition phases of plant material. In addition, they can reduce nitrate, which also explains their increased presence with the highest level of nitrate-N in the final converted field soil. **Lastly, although the bacterial species richness did not change throughout the experiment, a diversity index showed a highly diverse bacterial community that had slightly decreased by the end of the conversion**. The family Gaiellaceae and order Myxococcales, for example, were associated with the decrease of C:N ratio during the conversion.

Overall, results showed a relatively stable soil bacterial community at the phylum level throughout the crop changes. The enriched nitrate-N, lowered organic C:N ratio and effects from the decomposition process were the primary drivers of community changes in this system-in-transition.

4.3.2.1 Systematic review: Effects of crop rotations on the soil microbiome, and their combined impact on climate change

Searches from the systematic review returned a total of 70 articles, seven of which were relevant and are discussed below.

Crop rotations offer a strategy to increase plant diversity, in both space and time, at a field and farm scale. Given that different plant species have varied chemical properties, their inputs through above or belowground litter, root exudates, or even as residues can thereby influence soil properties and soil microorganism communities and activities, in turn impacting nutrient cycling, greenhouse gas (GHG) fluxes and soil C storage.

The effect of crop rotations on soil carbon and nitrogen cycling

In an interesting trial on the effect of crop rotation on crop residue decomposition, for instance, McDaniel *et al.* (2014), incubated soils from different long-term crop rotations with four residue types that had different chemical characteristics. **Soils from more diverse cropping systems decomposed residues faster, clearly demonstrating a crop rotation effect on microbial activity.** This was attributed to crop rotations causing changes in interactions between soil microbes and SOM related to C and N availability. Those changes then affected how new residue inputs were processed by soil microorganisms. Furthermore, results suggested that **there was a higher SOM quality in the most diverse crop rotations, indicated by ratios of cellulose- and lignin-degrading enzymes.** The study demonstrated that the increased N-retention in microbial biomass involved in degrading high-quality residues, the faster decomposition of low-quality residues, and the higher microbial activity indicate very strong and close relationships between crops, soil and microbes in diverse cropping systems. Consequentially, the enhanced microbial activity and resulting increase in microbe-available SOM is believed to likely promote improved crop yields and positive changes in long-term soil C dynamics. Their results strongly suggest that monoculture systems, in contrast, can result in substrate limitation and suppressed microbial activity.

Incorporating a short-term pasture phase in crop rotations can offer multiple benefits such as reduced weed and pathogen pressure. In addition, **a crop rotation that incorporated five years of pasture into a vegetable cropping system significantly affected the soil microbial community composition and function, increased soil organic C (SOC) (in labile and recalcitrant pools) and therefore potential for C sequestration** (Lin *et al.*, 2020). For example, the authors observed that after five years of pasture, SOC and N in the top 15 cm of soil increased 20.6 percent and 20.1 percent, respectively. The microbial community also increased with time; both total biomass and all microbial taxonomic groups increased throughout the five years. C mineralization and particulate organic matter (POM) were the key factors that contributed to the increase in microbial biomass and associated C mineralization rates. **Lastly, the potential release of carbon dioxide (CO_2) increased over time, and was correlated to increased fungal abundance.** The authors suggested that the increase in microbial community and activity very likely improves the overall sustainability of a cropping system. However, that potential C mineralization rates increased after four years under pasture rotation strongly indicates that some of the stored C was relatively labile. This indicates that some of this C could be lost during subsequent cycles of tillage and cropping.

Effect of crop rotations on nitrification, denitrification and nitrous oxide emission

How might variations in systems of long-term crop rotations of monocultures impact N-cycling and nitrous oxide (N_2O) emission? Behnke *et al.* (2020) investigated the soil N cycle after about 20 years of monoculture maize and soybean rotations, finding that crop rotations with more maize years increased SOM, reduced soil pH, reduced ammonia-oxidizing archaea (AOA), and increased ammonia-oxidizing bacteria (AOB) and fungal abundance. In addition, more maize years in the rotation multiplied the amount of N needed to sustain yield levels. In parallel, continuous maize selected for microorganisms that were adapted to conditions of increased inorganic N. **Altogether these factors intensified the system's N cycle, which could lead not only to acidification and enhanced bacterial nitrification, but could also create an environment primed for N losses and increased N_2O emissions.**

Other studies have likewise found that crop rotations impact the soil microbial denitrifier community. In farming systems that included combinations of maize, soy, wheat, and legumes in their crop rotations, Maul *et al.* (2019) report that *16S*, *nirK* and *nosZ* gene abundances changed significantly in response to the phase of crop rotation. *nirK* was particularly sensitive, with an abundance two orders of magnitude greater in soybean than in wheat. Their observations also imply that **denitrifying microbial communities are dynamic, their abundance fluctuating throughout the season and different crop rotations.** Nevertheless, the relationship between gene abundance, enzyme activity, and N_2O production requires further elucidation through techniques that permit better quantification and detection. In their meta-analysis of 47 field studies across the world, Ouyang *et al.* (2018) also observed that crop rotation and soil pH were influential factors in the response of N-cycling genes. They found that crop rotations increased the abundance of AOB genes compared to monocultures, though no differences were observed for AOA. However, when the soil pH was greater than six, both AOB and AOA levels increased. Lastly, denitrification genes *nirK*, *nirS* and *nosZ* were higher under crop rotations than monocultures.

The inclusion of legumes in crop rotations can impact soil microbial carbon and nitrogen

In contrast to the study by Lin *et al.* (2020) above**, a study involving three different crop rotations** (grains – soybean, maize, wheat, lupin and oat) in southern Brazil, **found that neither microbial biomass C or N, nor soluble C and N levels were affected by crop rotation** (Franchini *et al.*, 2007). However, the soil C and N stocks did vary with crop rotation systems, primarily influenced by soil tillage and the ratio of legumes to non-legumes in the rotations.

A meta-analysis of 56 studies and 149 paired comparisons reported a significant influence of legumes in crop rotations on soil microorganisms (Lori *et al.*, 2017). The authors found that, inclusion of legumes in crop rotations enhanced microbial biomass N (MBN) whether in the form of green manure, cover crops or cash crops. They furthermore compared organic and conventional systems, and specified that in the absence of legumes, there were no differences in MBN in system. **With legumes, though, organic systems showed higher microbial N than conventional**. Based on comparisons of soil microbial enzyme activities, **they suggest it possible that the plant species had a stronger effect than the farming** system. This echoes findings by other studies showing that including crop rotations can have positive effects on microbial biomass C (MBC) and MBN (e.g. McDaniel *et al.*, 2014), and that diverse crop rotations can have positive effects on microbial richness and diversity (e.g. Venter, Jacobs and Hawkins, 2016). Furthermore, organic farming systems, which typically feature relatively diverse crop rotations including legumes, have demonstrated similar or higher SOC stocks than conventional systems despite the same organic C inputs and even more frequent tillage (Chenu *et al.*, 2019). This may be partially attributed to a higher C use efficiency (CUE) and microbial growth rate, which results in higher microbial necromass, in turn contributing to SOC stocks and stabilized long-term SOM.

Concluding remarks

Crop rotations can indeed impact nutrient cycling of C and N via soil microbial activities. These processes are integral aspects of those that govern soil C storage and soil GHG emissions. A more thorough understanding of these dynamics, as influenced by crop rotations, can help better predict these cycles and inform management strategies.

4.3.2.2 Systematic review: Effects of crop rotations on the soil microbiome, and their combined impact on human health

Three different searches were performed for studies linking crop rotations, the soil microbiome and human health. No articles were returned, though one relevant study was found in a search for crop rotations and emissions (see **Annex I** for search terms).

The fungus *Aspergillus flavus* can cause aflatoxin contamination and poisoning. Jaime-Garcia and Cotty (2010) studied the fungus propagules and its morphotype S strain in South Texas agricultural fields, concluding that **manipulating crop rotations may offer a strategy to reduce aflatoxin severity**. They found that the previous crop influenced the quantity of both the propagules and the S morphotype. Maize favoured a higher soil population of *A. flavus* compared to cotton and sorghum, while cotton and sorghum favoured S strain incidence compared to maize.

4.3.3
Cover crops

HIGHLIGHT BOX 8 Impacts of cover crops on the soil microbiome, climate change and human health.

NARRATIVE REVIEW What are the impacts of cover crops on the soil microbiome?

- Soil microorganism activities can respond strongly throughout cover crop decomposition.
- Cover cropping can increase fungal species diversity, possibly leading to improved ecosystem resilience.
- The combination of reduced soil disturbance (reduced or no-tillage) and cover cropping can increase the diversity of the symbiotrophic fungal community (i.e. fungi which derive nutrients through mutually beneficial relationships with other organisms), potentially increasing crop access to limiting nutrient resources.
- Cover cropping can be associated with positive effects on arbuscular mycorrhizal fungi:
  - Winter cover cropping, as opposed to winter fallows, can increase arbuscular mycorrhizal fungi colonization of the following summer cash crop.
  - Continuity of arbuscular mycorrhizal fungi plant-root association may be as important as decreased soil disturbance.
  - Even non-arbuscular mycorrhiza hosting cover crop species, such as those in the Brassicaceae family have been associated with increased or no effect on arbuscular mycorrhizal fungi colonization.
  - Legumes are known to be arbuscular mycorrhiza hosts, and have been observed to increase arbuscular mycorrhizal fungi colonization when grown as cover crops.

SYSTEMATIC REVIEW What are the impacts of cover crops on the soil microbiome, and their causal impacts on climate change?

- Residues from mixed cover crop species might reduce greenhouse gas emissions because the different chemical compositions of plant species can stimulate the soil microbial community to use nutrients more efficiently as a result of increased carbon use efficiency.
- Fungi have been observed to contribute significantly to nitrous oxide emission in a no-till cover crop system.
- Subsoil arbuscular mycorrhizal fungi can potentially improve fertilization efficiency, carbon sequestration, and reduce greenhouse gas emissions. Cover crops may be an appropriate strategy to increase arbuscular mycorrhiza fungal inoculum.

SYSTEMATIC REVIEW What are the impacts of cover crops on the soil microbiome, and their causal impacts on human health?

- No relevant literature was found during the systematic search.

Cover cropping – the practice of growing vegetation between (cash) crop plantings – increases agrobiodiversity and crop diversification in time and space. The agronomic and soil benefits of cover crops have been widely studied and reported. They can reduce erosion caused by wind and water, improve pest regulation, and provide organic matter (OM) amendments, thereby increasing soil organic carbon (SOC) and likely increasing nutrient use efficiency by mitigating nitrogen (N) losses (Nevins, Nakatsu and Armstrong, 2018). These functions contribute to overall maintenance or even improvement of soil quality, which in turn affects crop productivity and other ecosystem services (ESS). Cover crops are therefore recommended as a sustainable practice to improve soil health (FAO, 2017).

Despite many studies on soil benefits of cover crops for agroecosystems, their specific impact on soil microbiome has not received the same attention by the scientific community. Some research has nevertheless demonstrated that cover crops can significantly influence soil microorganism communities, including bacteria, fungi and arbuscular mycorrhizal fungi (AMF). They are discussed below.

In a study involving 155 cereal fields across a 3,000 km north-south gradient in Europe, Garland *et al.* (2021) found that while crop diversity had a relatively minor effect, the length of time of a cover crop had a significant and positive effect on soil bacterial diversity, soil multifunctionality and crop yields. The authors suggested that increasing cover crop time, rather than crop diversity within rotations, could enhance yields and soil functioning while creating a favourable habitat for soil microorganisms in the systems studied.

Aiming to understand more about how the cover crop decomposition process affects nutrient availability for the subsequent crop, Nevins, Nakatsu and Armstrong (2018) **described the evolution of the soil microbial response throughout cover crop decomposition, and how their progressive activities synchronised with nutrient needs of the subsequent cash crop the soil microbiome.** With time, the microbial communities became more homogeneous, speculated to be the result of environmental filtering, increased competition and selection for microorganisms most efficient in decomposing the cover crops. **Time after the conclusion of the cover crop practice appeared to be the most important factor driving differences in soil microorganism communities across treatments.** In the early decomposition period, selection favoured microbiota that could produce β-glucosidase (and perhaps other enzymes) that could obtain simple sugars from the cover crop residue. The least variation in soil microbiome activities was observed at the mid-point of the cover crop decomposition period. From 39 to 109 days following cover crop practice termination, the soil microbiome also differed significantly according to cover crop treatments. After 109 days their effect on the soil microbiome was less evident, because 90 percent of the cover crop had been decomposed and was no longer influential in selecting microorganisms.

While cover crops can provide additional, varying types of organic carbon (C) that soil fungi use as a nutrient, fungi are also known to be very sensitive to mechanical disturbance. A reduced tillage regime combined with cover crops might therefore develop a more diverse and plant-beneficial soil fungal community. To test this hypothesis, Schmidt, Mitchell and Scow (2019) monitored soil fungal communities under different tillage regimes and cover crop treatments in clay loam soil in Mediterranean climates. They concluded that **cover cropping increased fungal species diversity, which could lead to improved ecosystem resilience. Moreover, the authors reported that the combination of reduced soil disturbance and cover cropping can increase the diversity of the symbiotrophic-enriched fungal community, which might provide increased crop access to critical, limiting nutrient resources.** Their results indicated that there was no effect on fungal functional composition, in that cover cropping did not change the relative abundance of saprotrophs, symbiotrophs, or pathotrophs. Overall, though, the fungal community was more impacted by tillage than cover crops. On average 45 percent and 10 percent of the guild-assigned fungi responded to tillage and cover crops, respectively.[15] In particular, no-till (NT) favoured the shift from symbiotroph:saprotroph ratio towards symbiotrophs, while cover crops increased species diversity. It has been suggested that cover crops can create favourable conditions for fungal plant pathogens, including providing refuge in plant litter on soil surface, and protection from high temperatures, limited water availability, and physical disturbance. However, there were no observations of significant differences in plant-feeding fungal populations between any treatments.

Effects of cover cropping vs winter fallows on arbuscular mycorrhizal fungi

Arbuscular mycorrhizal fungi form intimate relationships with plant roots, and are known to help improve access to soil nutrients for plant uptake through soil exploration with their hyphae. In return, they feed on C (and other metabolites) exuded from plant roots. **A fallow season, such as experienced in Europe between the summer crop harvest and sowing of the next crop in spring, creates a major reduction of active crop roots in the soil. The lack of substantial root habitats may reduce the abundance of AMF, thereby reducing**

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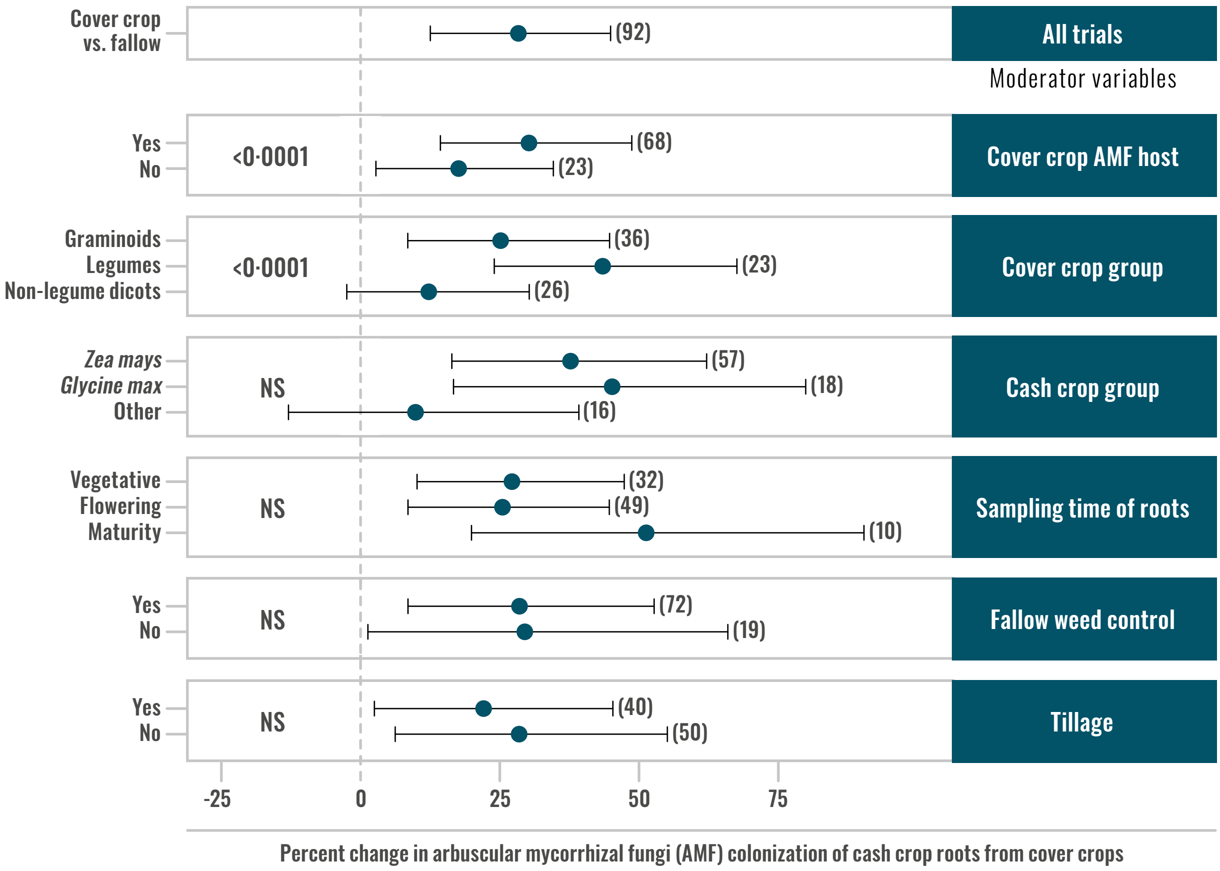
[15] A guild refers to a group of species that share similarities in the resource they use, or their manner of using it.

their beneficial functions to the subsequent crop. Sosa-Hernández *et al.* (2019), focusing on management practices to encourage subsoil AMF abundance, thus recommend using cover crops in order to increase AM colonization of the next crop. **In their excellent metanalysis,** Bowles *et al.* (2017) **confirmed that compared to winter fallows, winter cover cropping increased AMF colonization of summer cash crop roots by an average of 30 percent and up to 65 percent with a legume cover crop** (Figure 16). They also included tillage effects in their analysis, finding that cover crops increased arbuscular mycorrhiza (AM) formation whether tillage was used or not. This suggests that the continuity of AMF-root association is as important for AM formation as decreased soil disturbance. This is important to recognize in systems that rely on AMF services, such as organic farming or low-input farming, which is practiced by a majority of farmers in the world. Since such low-input systems frequently rely on tillage as weed control, using a legume cover crop may help AMF withstand such soil disturbance. The authors also looked at how cover crops might affect the diversity and community composition of AMF, and concluded that the literature did not report consistent results.

FIGURE 16.

META-ANALYSIS RESULTS OF THE CHANGE IN ARBUSCULAR MYCORRHIZAL FUNGI COLONIZATION OF CASH CROP ROOTS IN RESPONSE TO FALL/WINTER COVER CROPPING FROM FIELD EXPERIMENTS IN FIVE CONTINENTS.

The vertical dotted line represents no effect. Statistical significance is indicated to the left of the dotted line; NS means no significance. The horizontal lines are error bars, which represent 95 percent confidence intervals, and the single dot is the mean value. The numbers in parentheses are the number of observation in each category.



Source: Bowles et al., 2017.

Effects of Brassica cover crops on arbuscular mycorrhizal fungi

Plants may be described as hosts or non-hosts of AMF, leading to questions about how non-host cover crops can effect AMF colonization in subsequent crops. In their study, Bowles *et al.* (2017) reported that they could have expected reduction in AM formation after Brassica (cruciferous vegetables) cover crops, which produce compounds that inhibit fungi. The results were inconsistent, but **even non-AM hosting cover crop species (including buckwheat (*Fagopyrum esculentum*) and some from the *Brassicaceae* family) showed significant increases in AMF colonization in the subsequent crop**. This may have been due to several factors, including weeds that were AMF hosts, differences in soil moisture and temperature patterns between fallow and cover crop fields (which could affect AMF spores), or that additional OM made the soil physical properties more conducive to hyphal growth and therefore subsequent crop colonization. Rosner *et al.* (2018) also investigated Brassica cover crop effects on AMF colonization in a wheat-sunflower rotation, expecting a significant drop in colonization. **They did not, however, identify any such negative effects, as the Brassica exudates did not have the toxic effect they may have anticipated**. Legumes are known to host AMF. In the same study, soils cover-cropped with legumes showed AMF colonization up to 45 percent. Overall, reduced tillage in conjunction with cover cropping increased the AMF abundance and positively affected crop yield of the following crop.

Concluding remarks

In conclusion, the use of cover crops can support provisioning of multiple ecosystem functions and services. When the residues are left on the soil, their decomposition can cause strong responses by soil microbes, potentially synchronising with nutrient needs of the subsequent cash crop. The practice has been seen to increase fungal species diversity, including within the symbiotrophic-enriched fungal community, which could respectively contribute to improved ecosystem resilience and access to crucial, limiting nutrient resources. Arbuscular mycorrhizal fungi, in particular, may benefit from winter cover cropping by proving a habitat to overwinter, and subsequently colonize and offer benefits to the proceeding summer cash crop. Even non-AM hosting cover crop species, such as those in the *Brassicaceae* family, may still encourage significant increases in AMF colonization in the subsequent crop.

4.3.3.1 Systematic review: Effects of cover crops on the soil microbiome, and their combined impact on climate change

Searches returned 29 results, only two of which drew a direct connection between use of cover crops, the soil microbiome, and subsequent impacts on climate change. They are discussed below along with two other studies, which did not focus on these links explicitly but provide examples of possible future research directions.

Cover cropping is used for a number of reasons, one being use of cover crop residues as green manure prior to introducing cash crops. Cover cropping, tillage, and crop residue management are therefore closely linked crop production practices. How might cover crop rhizodepositions or decomposing residues interact with soil microbiota and influence greenhouse gas (GHG) emissions? Zhaorigetu *et al.* (2008) suggested that **fungi made a significant contribution to nitrous oxide (N_2O) emissions in cover-cropped soils under NT**. In their field experiment conducted in Japan, they compared cereal rye and hairy vetch cover crop systems with different tillage systems. No-till with a rye cover crop significantly increased the soil fungal biomass but not bacterial populations in the first 10 cm soil depth. N_2O flux was positively correlated with fungal biomass, but not with denitrifying or ammonia-oxidizing bacteria (AOB).

If decomposing cover crop residues are associated with GHG emissions, how might their management contribute to GHG emission reduction? It is possible that **residues from mixed cover crop species might reduce GHG emissions; the different chemical compositions of plant species can stimulate the soil microbial community to use nutrients**

more efficiently as a result of increased C use efficiency (CUE). Testing this hypothesis using a microcosm experiment, Drost *et al.* (2020) found that mixtures of cover crop residues did stimulate microbial functional diversity, evidenced by a higher ability to degrade substrates. Like Zhaorigetu *et al.* (2008), they found that fungal biomass increased, but in this case for all treatments, with no significant differences between mixtures and monocultured cover crop residues. The mixtures also showed reduced N_2O and carbon dioxide (CO_2) emissions compared to monoculture residues at the start of the experiment, but not over the whole incubation period. Nevertheless, the C:N ratio of cover crop residues were an important variable in explaining dynamics of N_2O and CO_2 emissions. Residues with highest N (from vetch, which is N-fixing) had the highest cumulative N_2O emissions, which underscored the influence of plant material nutrient content on soil GHG fluxes and microbial activity. In comparison, the correlation of C to CO_2 was weaker, mostly likely because the quality (recalcitrance), and not just the quantity of C, is an important factor. Testing these dynamics under field conditions would be valuable in observing the response of soil microbial diversity over time.

In their study describing nitrifier assemblages in the rhizosphere of cultivated olives on a regional scale Caliz *et al.* (2015) found an effect, albeit minor, of cover crop practices on nitrifier assemblages. The authors studied the nitrifying assemblage of AOB, ammonia-oxidizing archaea (AOA), and nitrite-oxidizing bacteria (NOB) present in the rhizosphere of 96 olive orchards in south of Spain, under different management practices. Results demonstrated that the olive variety and soil texture affected both the structure of AOA and their abundance. In contrast, soil management (cover crops vs bare soils) and olive variety affected only the abundance of AOB, not its structure. Though N_2O emissions were not investigated in this study, it makes a connection to the critical roles played by bacteria and archaea in soil N dynamics.

Concluding remarks

Only a few studies returned during the literature search directly investigated links between cover crops, the soil microbiome and GHG fluxes. However, it may be that because cover crops are frequently used as green manure, or as part of a rotation, other searches may return more relevant studies. Nevertheless, how to reduce GHG emissions while using cover crops remains a question. Drost *et al.* (2020) suggest that cover crop mixtures can improve microbial ability to degrade substrates, potentially resulting in reduced GHG emissions. Selecting for subsoil AMF may be another strategy. In their review, Sosa-Hernández *et al.* (2019) argue that subsoil AMF should be considered key partners to help improve fertilization efficiency, improve C sequestration, and reduce GHG emissions in agriculture. Citing multiple studies that identified using cover crops as a strategy to increase AM fungal inoculum, and another concluding that deep rooting and mycorrhizal plants can increase AM fungal abundance, the authors suggest using cover crops as a practice to foster subsoil AMF.

4.3.3.2 Systematic review: Effects of cover crops on the soil microbiome, and their combined impact on human health

Three different searches were performed for studies linking cover crops, the soil microbiome and human health. No articles were returned (see **Annex I** for search terms).

4.4
CROP RESIDUE MANAGEMENT

HIGHLIGHT BOX 9 Impacts of crop residue management on the soil microbiome, climate change and human health.

NARRATIVE REVIEW What are the impacts of crop residue management on the soil microbiome?

- Crop residues may be removed or retained in the field, according to the farming context and management decisions. Removal can lead to loss of soil organic matter and nutrients, affecting soil structure, plant production, nutrient cycling and other ecosystem services.
- The effect of crop residues on the soil microbiome remains inconclusive. Studies have demonstrated positive effects of straw returning on bacterial communities, only minor effects of crop residue retention on soil bacterial and fungal diversity, and no positive effects of crop residue incorporation on bacterial communities.
- Soils from more diverse cropping systems can decompose residues faster than less diverse systems, owing to the enhanced nutrient-cycling activities of soil microorganisms.
- Fire and burning of crop residues in the field can influence competitive relationships within the bacterial community and cause significant initial shifts in the soil microorganism communities.

SYSTEMATIC REVIEW What are the impacts of crop residue management on the soil microbiome, and their causal impacts on climate change?

- Surface application and/or incorporation of crop residues or straw can differently influence soil microbe-mediated decomposition processes, thereby impacting soil greenhouse gas emissions and soil carbon balances. While incorporation of residues may increase carbon dioxide emission, it can also increase carbon retention and create a soil environment that is more resistant to prolonged dry conditions.
- Mixed residues can result in reduced greenhouse gas emissions and positive long-term soil carbon dynamics, owing to increased nutrient-use efficiency by a more diverse microbial community.
- Retaining or removing crop residues can affect soil conditions, thereby impacting field-scale nitrous oxide emissions. Both removal and retention of residues have been observed to enhance soil nitrous oxide emissions.
- A chemistry-based approach (stoichiometric), balancing crop residue carbon content with inorganic fertilizers, can increase the soil carbon sequestration potential.
- Despite higher nitrous oxide emission, biochar may contribute to a decrease in Global Warming Potential by no increase of carbon dioxide or methane emissions as well as an increase in soil carbon storage compared to rice straw amendment.
- Residues with relatively higher amounts of easily accessible carbon can stimulate microbial activity more strongly, impacting nutrient cycling and greenhouse gas production.
- Applications of 25 to 50 percent of rice straw yield to saline soils were not sufficient to significantly counteract salinity effects, as only minor responses were observed in the soil microbial community.

SYSTEMATIC REVIEW What are the impacts of crop residue management on the soil microbiome, and their causal impacts on human health?

- No relevant literature was found during the systematic search.

Crop residues are the above-ground plant biomass, such as stalks and leaves, which remain after the crop has been harvested. They may be kept in the field as mulch, be burned or be removed to serve as animal feed, fuel, or mulch elsewhere. These different uses can cause competition for crop residues in contexts where production of plant biomass is limited (e.g. tropical, low-input farming systems) (Valbuena *et al.*, 2012). Residues left in the field may remain on the soil surface or be incorporated into the soil using a range of tillage techniques. **Closing nutrient cycles by returning crop residues to fields can help maintain long-term, agricultural soil fertility and soil functioning** (Sandén *et al.*, 2019). **They are nutrient-rich and can thereby contribute to a reduction in fertilizer use and to formation of soil structure and water-stable aggregates** (Xia *et al.* 2019). **In contrast, consistent removal of crop residues, along with other practices characteristic of intensive agriculture, can lead to soil organic matter (SOM) depletion** (Lehman *et al.*, 2014; Sandén *et al.*, 2019)**, resulting in poor soil nutrient status and structure and ultimately negatively affecting plant production and other ecosystem services (ESS).** A quantitative review found that removing crop residues from the field resulted in lower average soil organic carbon (SOC) contents in both tropical (18 percent) and temperate climates (12 percent) (Warren Raffa, Bogdanski and Tittonell, 2015). The authors emphasized the need for site-specific strategies to manage crop residues in order to reduce negative impacts on soil fertility and crop yields in tropical climates, and to increase SOC in temperate regions.

4.4.1

Effects of crop residues on the soil microbiome show varied results

Although retention or deposition of crop residues in agricultural fields is a widespread practice, studies exploring how crop residues shape the soil microbiome show varying results, pointing to a yet unclear consensus on their effect. **Research has demonstrated positive effects of straw returning on soil bacterial communities** (Valbuena *et al.*, 2012), **only minor effects of crop residue retention on soil bacterial and fungal diversity** (Degrune *et al.*, 2017)**, and no positive effects on soil bacterial communities with crop residue incorporation** (Sandén *et al.* 2019). The following three studies offer more detailed examples.

Xia *et al.*, (2019), for instance, studied the influence of straw returning in combination with different tillage depths on soil bacterial community structure and activities. **They found that straw returning positively influenced the bacterial community, even in the short-term. This can be explained by the rich content of carbon (C), nitrogen (N), phosphorus (P) and other nutrients in straw which** stimulated soil microorganism activities. In particular, soils from plots with the combination of shallow tillage and straw return resulted in the highest bacterial diversity and lowest ratio of gram-positive to gram-negative bacteria. A high percentage of gram-positive bacteria is typically read as an indicator of a poor soil nutritional state, as they use C sources from more recalcitrant organic matter (OM). Another study observed that in response to straw application, the total diversity of soil microorganisms decreased while copiotrophic populations temporarily dominated (Tardy *et al.*, 2015)**.** Furthermore, fungi and bacteria responded at the same time but the resilience of fungal diversity lasted longer. **This is a reminder of the temporal dimension of the soil microbiome response; the involvement of each community in OM decomposition evolves throughout the process.** Furthermore, it is clear that microbial biomass and nutrient cycling can be influenced by the previous effects of crop rotation in conjunction with residue management. McDaniel *et al.* (2014) illustrated this effect by demonstrating that **soils from more diverse cropping systems decomposed residues faster. Crop rotations caused changes in interactions between soil microbes and SOM related to C and N availability, which then affected how new residue inputs were processed by soil microorganisms.**

Lehman *et al.* (2014) also concluded that the structure of soil microbial communities can be impacted by continued residue removal, but that certain conditions such as no-till (NT) may improve resistance of the soil microbial community to such changes.

The long-term study carried out by Sandén *et al.* (2019) on maize was carried out in Italy where the soil characteristics, nutrient and bacterial communities affected by organic fertilization were assessed down to a depth of one meter. The experiment involved the following treatments: crop residue removal, crop residue incorporation, crop residue removal and cattle slurry, crop residue removal and manure. **Concerning crop residue incorporation alone,** in contrast to the study by Xia *et al.* (2019), **the data did not show positive effects on bacterial abundance or diversity.**

4.4.2
Effects of fire on the soil microbiome

Burning of crop residues is a widespread practice in many parts of the world. It is a fast way to clear them from the field and prepare a clean seedbed. In addition, burning residues serves as weed or pest management, and is sometimes perceived to enhance fertility (Erenstein, 2002). Nevertheless, it is increasingly discouraged as it causes air pollution and deprives the soil of potential SOM input. Fire alters abiotic soil conditions. For example, it causes an increase in short-term availability of some nutrients while leading to a loss of other nutrients (including N) and OM (Kumar *et al.*, 2019). However, how it affects the soil microbiome remains unclear. **It has nonetheless been observed that fire tends to favour phylogenetic lineages (evolutionary development) with heat-resistance capacities** (Pérez-Valera, Goberna and Verdú, 2019). These include species featuring the ability to produce resistance structures such as spores, endospores (dormant spores) and akinetes (thick-walled, dormant cells), and/or that have potential fast growth strategies. **Fire also influences competitive relationships within the bacterial community by altering the availability of resources such as OM, nutrients and water** (Pérez-Valera, Goberna and Verdú, 2019). **It seems, though, that soil microbial communities or functions can recover, depending on the parameters observed, following fire events.** The following two studies provide specific examples.

Kumar *et al.* (2019) demonstrated that paddy-straw burning resulted in significant reductions in populations of bacteria, fungi, and actinomycetes, including some microorganisms with specific roles regarding nutrient cycling (P and K) and cellulose degradation. **However, abundance of these groups returned to normal 30–60 days following the burning event. The microbial population and enzymes involved specifically in biomass recycling did not recover, however, even after the latest sampling time at 60 days**. The authors suggest that observation might be explained by a lack of energy substrates available to those microorganisms.

Another recent study carried out by Pérez-Valera, Goberna and Verdú (2019) did not specifically target residue burning, but contributes all the same to better understanding the soil microbiome response to fire. The authors studied the post-fire succession of the phylogenetic composition of soil bacteria before and during one year following an experimental fire in a Mediterranean ecosystem. Changes in microbiome were observed immediately, from the first day after the fire, favouring bacteria with heat resistance traits. This response was likely due to the high temperature which stimulated spore production. In addition, there was a temporary, flush of ammonium-N, which is a direct product of combustion. It is likely that certain bacteria therefore likely took advantage of the post-fire mineral N-availability. Afterwards, another clade of organisms (a group with a common ancestor) took over due to their competitive ability to use high levels of oxidizable C in soils. Yet, one year after the fire, most of the soil ecological functions had recovered even though the bacterial community structure remained altered. **This suggests that fire stimulated the development of different communities, but which were functionally equivalent.**

Concluding remarks

In conclusion, the response of the soil microbiome to crop residues appears to be quite variable, influenced by multiple factors including the type of residue (and therefore nutrient contents), the temporal aspect of the decomposition process, the status of the soil microbiome into which the residues are introduced, and the type of incorporation (or lack of) employed. There is quite some attention given to how crop residues impact those taxa and activities within soil microbiome that are involved in greenhouse gas (GHG) fluxes and soil C stabilization. This subject is explored in detail in **Chapter 5**. The practice of burning residues in the field also incurs short-term and long-term changes to the composition and activities of the soil microbiome.

4.4.3

Systematic review: Effects of crop residue management on the soil microbiome, and their combined impact on climate change

This section addresses studies involving crop residues and/or straw, their effects on the soil microbiome, and subsequent relevance to climate change. Of the 84 articles returned from searches, 22 were selected for their relevance and are discussed below. Several articles are also discussed in **Section 4.7** about fertilization (Yuan *et al.*, 2012; Zhang *et al.*, 2007; Zhao *et al.*, 2015; Zheng *et al.*, 2008).

Plant nutrient material is a major driver of microbial activity, GHG fluxes and soil C storage during the decomposition process, in which soil microorganisms play a major role. **N from crop residues, for example, has been demonstrated to be a main source of microbial nitrous oxide (N_2O) production, resulting from both fungal and bacterial denitrification** (Yamamoto *et al.*, 2017). **Land use and soil management history can influence the diversity of decomposers, which can in turn impact the decomposition of plant residues and have implications on soil C balances** (Tardy *et al.*, 2015). In their study investigating fungal and bacterial responses to wheat straw, Tardy *et al.* (2015) observed that there were clear changes in those microbial communities as decomposition progressed. Immediately following straw incorporation, copiotrophic populations temporarily dominated, and thus diversity decreased. The fungal and bacterial response occurred at the same time, but the resilience of fungal diversity lasted longer. Zheng *et al.* (2018) found that **earthworms influenced soil microbial C:N acquisition, indicating that soil macrofauna activities involved in residue decomposition can also influence the soil microbiome.** The C to N-degrading enzyme activity ratio of soil microorganisms was significantly increased by earthworm presence in a mixture of low lignin residues (from clover, maize stover and wheat straw), while the opposite was observed with high lignin residues.

Effects of surface or incorporated residues on soil carbon dynamics

Incorporating crop residues by tillage, rather than leaving them on the soil surface as mulch, increases residue contact with soil microorganisms. This **proximity can thereby strongly shape distinctly different community structures and influence decomposition and nutrient cycling processes. For example, while both practices can increase soil C** (Feiziene *et al.*, 2015)**, surface mulching can result in retention of about 50 percent or less C in the soil** (Helgason *et al.*, 2014). Carranca *et al.* (2009) found that soil disturbance led to faster residue composition of both oat (cereal) and lupin (legume) residues, and particularly so for the legume. Feiziene *et al.* (2015) found that in comparison to no-till and leaving residues on the soil surface, tillage with residues created a soil environment better resistant to prolonged dry conditions through parameters such as increased SOC and enzyme activity. Long-term incorporation of residues under tillage, however, deteriorated the soil physical quality (e.g. field capacity, plant available water content, permanent wilting point). Compared to those of incorporated residues, **surface mulch C dynamics are subject to higher site-specificity due to different climates** (e.g. arid vs humid), which may help explain the high variability in soil C accumulation found in NT systems across different environments (Helgason *et al.*, 2014).

Patiño-Zuniga *et al.* (2009) found that **incorporation of residues with tillage increased carbon dioxide (CO_2) emission by 1.2 times and N_2O by 2.3 times**, compared to when residues were left on the soil surface.

Effects of mixed species residues on greenhouse gas emissions and soil carbon dynamics

The following study found that **mixed residues can result in reduced GHG emissions owing to increased nutrient-use efficiency by a more diverse microbial community**. Drost *et al.* (2020) report that mixtures of cover crops showed reduced N_2O and carbon dioxide (CO_2) emissions compared to monocultures at the start of experiment, but not over whole incubation period of 50 days. The C:N ratio of the cover crop residues were an influential variable in explaining the dynamics of these GHG emissions. Residues with the highest N content (from vetch, a legume) created the highest cumulative emissions. Furthermore, though the mixed residues saw a more gradual increase in microbial biomass than the monocrop residues, they stimulated the microbial functional diversity, evidenced by a higher ability to degrade substrates. Of note, the authors report that this higher ability to degrade substrates neither increased GHG emissions nor nutrient availability compared to monoculture residues.

Another incubation study also found that more diverse residues (from intercropped maize-soy) similarly impacted soil microbial diversity and activity, resulting in a community that processed and transformed N more effectively compared to monocrop residues (either maize or soy), therefore reducing N_2O emissions (Bichel, Oelbermann and Echarte, 2017). **However, the differences in N_2O emissions between soils from intercropped and monocrop treatments were not significant**.

The diversity of crops in a rotation has been seen to induce changes in interactions between soil microbes and SOM (McDaniel *et al.*, 2014). **Residues from more diverse crop rotations led to increased N-retention in soil microbial biomass involved in degrading high-quality residues, a higher microbial activity, and a faster decomposition of low-quality residues. These effects thus enhanced residue decomposition and likely promoted positive changes in long-term soil C dynamics.**

Effects of residue retention vs removal on nitrous oxide and carbon dioxide emissions

Returning plant residues to the soil can result in increased GHG emissions resulting from microbial degradation processes. At the same time, however, it is an important practice that can improve soil biotic and abiotic properties, resulting in better soil fertility and crop production (Chen *et al.*, 2015). Comparing with no residue application, Lal *et al.* (2019) observed that residue mulching enhanced soil CO_2-emissions, as well as N_2O emissions by about 50 percent and 65 percent under tillage and NT management, respectively. However, covering the soil with residues resulted in improved yield, energy output and carbon efficiency. Patiño-Zuniga *et al.* (2009) likewise found that non-tilled raised beds with retained residues were associated with CO_2 emissions 1.2 times larger and nitrate (NO_3^-) production 1.8 times larger compared to when they were removed. The benefits, however, were increased organic C, soil microbial biomass, total N and water holding capacity. The authors point out that in comparison to soils subjected to conventional tillage (CT), crop residue retention with NT decreased N_2O and CO_2 emissions.

In contrast, Bent *et al.* (2016) reported that **two years of corn/soybean residue removal resulted in significantly higher levels of N_2O emission**, and that the community structure of bacterial nitrification and denitrification genes was significantly different between treatments. Specific taxonomic clusters were observed to drive community shifts in both fields, some being positively or negatively correlated with levels of N_2O flux. Anayses revealed positive relationships between (i) the extent of variation of *nirK nirS* or *nosZ* and the extent of variation of ammonium levels in the soils with residues and (ii) *amo*A, *nirK* or *nosZ* and DOC or water content in soils without residues. They concluded that **retaining or removing residues can affect soil moisture, temperature, and atmospheric conditions, thereby influencing the composition of nitrifying and denitrifying genes, in turn impacting field-scale N_2O emission**.

Stoichiometric balancing of crop residues can increase carbon sequestration

Adding other nutrients such as inorganic N, P, and sulphur (S) along with crop residues, which are rich in C, may help increase C sequestration (Zhao *et al.*, 2015). Zhao *et al.* (2015) observed a significant, positive correlation between the increased abundance of bacteria with SOC content with crop residues and NPK fertilizer, compared to NPK application alone. They suggest that balanced fertilization greatly improves crop growth, resulting in increased C input either by direct input from the growing plant or as compost. The authors also attributed this effect to soil microbial stimulation by N and P fertilization, given that they are the primary limiting nutrients in rice growth and microbial use of SOC. In another study comparing effects of inorganic and organic fertilizers, crop residue retention with inorganic NPK fertilizer resulted in the highest abundance of bacterial cbbL genes, accounting for 1.5 times the amount found without any fertilization (Yuan *et al.*, 2012).[16]

Delving deeper into these dynamics, Kirkby *et al.* (2016) demonstrated that stoichiometrically balancing crop residues increased C sequestration by changing nutrient cycle dynamics related to humification. Specifically, they observed a significant increase in the fine fraction carbon (FF-C) concentration when residues were coupled with inorganic fertilizer (NPS), with a net increase in organic C stock of 5.5 tonnes per ha in five years. **However, significance differences did not occur at all soil depths, and half of the depths that showed an increase in FF-C concentration were below 1 m**. No statistical differences were found during the five years when residues were applied without nutrients. **The authors hypothesize three explanations for the accumulation of fine fraction soil organic matter (FF-SOM) at a depth below that of residue incorporation**. First, the FF-SOM may have been formed near the surface and then precipitated in a dissolved or fine particulate form. Second, the FF-SOM may have derived from crop roots and rhizodepositions and their subsequent turnover by microbial communities, especially in treatments where nutrients were added. And third, the FF-SOM may have been formed *in situ* by microbial transformation; enzymatic degradation of crop residues near the soil surface created soluble SOM and inorganic nutrients, which may have diffused downwards through the soil. They suggest that results from this study may explain the why C sequestration is sometimes low under many long-term crop residue retention systems. In other words, management practices may not have calculated C input from residues in the overall nutrient balance. They argue that current fertilization strategies may actually limit the formation of new FF-SOM by only taking into account crop nutrient uptake, rather than a comprehensive stoichiometric approach. In addition, they add that it is possible that unbalanced residue C input could cause a priming effect, therefore increasing mineralisation and thereby actually creating loss of FF-SOM.

Effects of biochar vs crop residues on greenhouse gas emissions

Biochar has been proposed as a tool to improve soil fertility and mitigate climate change owing to its potential to sequester C and reduce GHG emissions (Laird, 2008; Sohi *et al.*, 2010). It has been observed to increase soil pH and cation exchange capacity, two abiotic factors that have a major influence on microbial activity (Liang *et al.*, 2006; Van Zwieten *et al.*, 2010). Multiple studies have reported that total porosity and aeration in soil also changed following biochar application to soils (Lehmann *et al.*, 2011; Mukherjee and Zimmerman, 2013), influencing microbial activity by providing microhabitats (Thies and Rillig, 2009).

Yoo *et al.* (2016) found that despite the higher N_2O emissions, soil amended with biochar significantly decreased the Global Warming Potential by about 1600 g C m^{-2} in comparison to rice straw application. In their study, biochar increased neither CO_2 nor methane (CH_4) emissions, and C storage increased substantially (+84 -119 percent) compared to the control and straw treatments. The authors attributed this effect to the change that biochar triggered in

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[16] cbbL is a gene responsible for coding Rubisco enzymes, which are crucial in the process of carbon fixation.

the soil microbial communities. For biochar pyrolyzed at high temperatures, in particular, results showed a reduction in microbial biomass C (MBC) as well as maintained or increased enzymatic activities, indicating an increased microbial C use efficiency (CUE).

The effect of carbon quality from different residue types on microbial activities, nutrient cycling and greenhouse gas production

Residues with relatively higher amounts of easily accessible C can stimulate microbial activity more strongly, impacting nutrient cycling and GHG production. Carranca *et al.* (2009) compared decomposition rates of legume (lupine) and cereal (oat) residues in the field, finding that the potential contribution of cereal residue-amended soil to CO_2 concentration was about double that of the legume-amended soil. However, in the cereal residue-amended soils, there was an approximate two percent accumulation of organic C after six months, contributing more than lupine.

A study comparing simple and complex C sources (glucose, red clover and barley straw residues) found that the addition of glucose and red clover increased microbial activity, leading to NH_3^- depletion and an increased consumption of N_2O (Miller *et al.*, 2008). Barley straw, however, did not create enough stimulation of microbial activity to induce a measurable increase in emissions, resulting in a higher N_2O molar ratio. **The authors suggest that the available C increased microbial activity and dioxide (O_2) consumption, leading to favourable denitrification conditions.** This effect was evidenced by the significant relationship between respiration and cumulative denitrification, and occurred for both simple and complex C sources. The C source did not significantly affect the total bacterial community or *nosZ* numbers. As were no significant correlations between denitrifier community densities and cumulative denitrification or N_2O emissions, the authors propose that the denitrification activity was decoupled from the denitrifier community abundance in their experiment. Similarly, in a comparable study, the *nirS* and *nosZ* communities responded differently to glucose and plant residue amendments, while the denitrifier communities measured had similar responses to different plant residues despite changes in the quality of organic C and the different C:N ratios (Henderson *et al.*, 2010).

The root and shoot litter of the same plant can also vary in composition, resulting in selection of distinct soil bacterial community compositions and different N_2O emissions. Comparing maize root and shoot litter, Rummet *et al.* (2020) **identified a significant correlation between the total CO_2 and N_2O emissions, the soil bacterial community composition, and litter level, thereby demonstrating a clear separation between root and shoot samples**. The more easily degradable C from maize shoot litter favoured fast-growing C-cycling and N-reducing bacteria (of the phyla Actinobacteria, Chloroflexi, Firmicutes, and Proteobacteria), strongly increasing CO_2 and N_2O emissions in hotspots when both litter types were added. Emissions stimulated by root litter remained low, owing to the more slowly degradable C compounds and lower concentrations of water-soluble N.

Long-term input of NPK fertilizer combined with residues (rich in C) were found to stimulate rice paddy soil microorganisms, the methanotroph community structure, in particular, experiencing significant changes that led to increased CH_4 emissions (Zheng *et al.*, 2008). These included changes to the abundance and composition of both type I and II methanotrophs.

Concluding remarks

Crop residue management can affect soil environmental conditions, influencing soil microbial community structures and activities that are directly related to OM degradation and other soil biogeochemical processes. Some areas of enquiry seem quite clear, such as the enhanced microbial functional diversity or activity induced by mixed crop residues compared to single crop residues. It is also clear that soil microbial biomass and nutrient cycling are both influenced by the previous effects of rotation as well as interaction with current residue management. Lastly, studies offer strong evidence that adding

C residue inputs with other nutrients promotes soil C stabilization. Other subjects remain inconclusive, including whether retaining or removing residues from the soil surface result in net increases of GHG gases. The consequential increase in microbe-available SOM being likely to promote improved crop yields and positive changes in long-term soil C dynamics is another area that requires confirmation.

4.4.4

Systematic review: Effects of crop residue management on the soil microbiome, and their combined impact on human health

Three separate searches linking crop residue management, the soil microbiome and human health returned a total of three searches, but none were relevant for this section (see **Annex I** for search terms).

4.5

PLANT VARIETY SELECTION

HIGHLIGHT BOX 10 Impacts of plant variety selection on the soil microbiome, climate change and human health.

NARRATIVE REVIEW What are the impacts of plant variety selection on the soil microbiome?

- Plant genotypes are primarily responsible for the composition of root exudates, which in turn strongly influence the selection of soil rhizosphere microorganisms.
- The rhizosphere microbiome is highly dynamic and changes with the plant developmental stage. The seed-borne microbiota can be transferred to the next generation (vertical transmission) and can be considered another source of diversity. Seed-borne microbiota can potentially influence the soil microbiome recruited by plant exudates (horizontal transmission).
- Morphological traits, such as plant root architecture can likewise impact the rhizosphere physical environment and selection of soil microorganisms.
- Rhizospheric microorganisms can also influence plant health, growth and production through output of their own respective metabolites.
- Genotypic changes that have occurred during plant domestication have led to changes in the root microbiome. "Missing plant microbes" and indigenous crop microbiomes could play a critical role in plant breeding programmes, helping global agriculture develop solutions to challenges related to environmental changes.
- Transgenic plants can have direct, indirect and pleiotropic effects on the rhizosphere microbiome. Whether their effects are significantly different to those of conventional varieties, however, remains to be determined.

SYSTEMATIC REVIEW What are the impacts of plant variety selection on the soil microbiome, and their causal impacts on climate change?

- Root traits (morphophysiology, exudates, and rhizodepositions) of rice cultivars can differently shape rhizosphere communities of methanogens, methanotrophs and other taxa involved in upstream processes related to methane metabolites, thereby impacting soil methane emissions.
- High yielding cultivars, including hybrids, are a means to increase rice yields while mitigating methane paddy field emissions, though other factors such as input requirements need to be factored into Global Warming Potential calculations.
- Higher mineralization rates of millennia-old carbon in deep soils can be expected with use of deep-rooting plant varieties. This does not, however, predict an overall decrease in deepsoil carbon stock, since plant litter also contributes to the formation of new soil carbon.

SYSTEMATIC REVIEW What are the impacts of plant variety selection on the soil microbiome, and their causal impacts on human health?

- No relevant literature was found during the systematic search.

Plant varieties are subgroups of a plant species that share a common set of characteristics. They may arise as a result of natural evolution, as well as through direct human intervention. This includes techniques ranging from selection (a multi-generational process of selecting and growing seeds from plants with desired traits) to molecular breeding (such as marker-assisted selection).

4.5.1
Root exudates and root architecture as drivers for soil microbiome differentiation

It has been estimated that about 40 percent of plant photosynthetic products are released into the soil, and that those products are key influencers of the composition of microorganism communities in the rhizosphere (Bais *et al.*, 2006).[17]

On the one hand, Patel *et al.* (2015) reported that **genotypes are primarily responsible for the composition of root exudates, which in turn strongly shape the selection of soil microorganisms**.[18] The compositionality of the root exudates is so dependent on the plant genotype that different individuals belonging to the same plant species often have a unique rhizodeposition profile. In turn, this is often linked to a specific rhizosphere microbial community composition. In practice, the alteration of even a single plant gene can have an impact on the assembly of the rhizosphere microbiome. **Attention has also been called to the interaction between the seed-borne microbiota, which can be transmitted from one generation to the next, and the soil microbiome recruited by plant exudates** (Santos *et al.*, 2021). **Furthermore, morphological traits such as plant root architecture can also impact the rhizosphere physical environment, and thus the selection of soil microorganisms** (Saleem *et al.*, 2018). Architectural traits include length, diameter, number, and branch angle, as well as the interior and exterior root cell types. Not only do the interior and exterior of roots feature great variation in cellular structure, anatomy, and biochemical profiles, but any single root system also comprises different types of roots (e.g. fine root hairs, thicker tap roots, lateral root formation, root hair density), making for diverse environmental niche that can select for different soil microorganisms. Understanding more about how plant root architecture – in general and amongst varieties – influence the rhizosphere microbiome, could inform management decisions aiming to maintain or enhance agroecosystem functions.

On the other hand, the rhizosphere microbiome can also influence plant growth and development. For example, it can mitigate the effect of biotic and abiotic stresses on plant physiology, influencing flowering time and even enhancing the nutritional value of edible plant parts (Jain *et al.*, 2014; Sahni *et al.*, 2008; Singh *et al.*, 2014). How different plants shape the rhizosphere microbiome and the effect of different rhizosphere microbial communities on plant growth and development remain an active area of research (Patel *et al.*, 2015). Nonetheless, research studies show that plants can "communicate" with the rhizosphere microbiome by altering their root exudation profile in response to a certain abiotic or biotic stress to attract specific beneficial microorganisms (Lemanceau *et al.*, 2017). It is also known that rhizospheric microorganisms can help plants produce defense metabolites, thereby improving plant performance (Mhlongo *et al.*, 2018). **Overall, the association between plant variety and the soil microbiome is of pivotal importance for plant health, growth and development, but breeding programmes still fail to consider this interaction** (Patel *et al.*, 2015).

Mahoney, Yin and Hulbert (2017) came to a similar conclusion about the importance of plant-microbe interactions, but they also state that the soil and rhizosphere microbiome could be manipulated through plant species selection and breeding. They carried out a comprehensive study on the rhizosphere-associated bacterial community of different winter wheat cultivars (cultivars are varieties cultivated by humans rather than found in the wild, and do not cast fertile seeds). **They demonstrated that the host-plant genotype has a minor but significant influence on the bacterial diversification in the rhizosphere, and that the presence of certain bacteria can be manipulated by choosing specific wheat cultivars.**

Similar results were found by Sharaf *et al.* (2019) in their study on the effect of different cultivars and two different water regimes on the soil bacterial community in soybean nodules.

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[17] See related concepts of plant legacy and a soil memory effect in **Section 4.3 Agroecosystem Crop Diversification**

[18] Genotypes are the genetic makeup of an organism. Different plant varieties within a species have their own unique genetic identity.

The rhizosphere microbiome is regulated by a complex and interconnected number of plant genes. This currently impedes breeders from including the rhizosphere microbiome in their breeding schemes. Thus, finding the genetic basis of a certain rhizosphere microbiome composition is challenging and dependent on the specific environmental conditions in which the plant is growing. Nonetheless, recent advances in Genome-Wide Association Studies (GWAS) have allowed researchers to explore the link between specific plant genes and certain microbial species. These studies represent the first step towards targeted plant breeding schemes for microbiome traits.

4.5.2
Effects of plant varieties, developed by domestication or genetic modification, on the rhizosphere microbiome

Plant domestication is a process that has enabled human civilization as we know it today. In this long process of 13,000 years, humankind has slowly changed wild plants into domesticated ones. We now rely upon these domesticated varieties as our main food sources. The first, major consequences of plant domestication are often a reduction of genetic diversity within a given plant species and the strong dependency of the crops on human intervention for their successful reproduction.

Several studies have suggested that the genotypic changes caused by plant domestication had consequences for the root-associated microbial community. They were conducted with different species such as beet (Zachow *et al.*, 2014), barley (Bulgarelli *et al.*, 2015), and lettuce (Cardinale *et al.*, 2015). Another study also indicated that domestication had a deleterious effect on arbuscular mycorrhizal fungi (AMF) and rhizobia (Pérez-Jaramillo, Mendes and Raaijmakers, 2016). In their very interesting meta-analysis, Pérez-Jaramillo *et al.* (2018), investigated how plant domestication might have affected the composition of the root-associated microbiome. **They demonstrated that plant domestication has indeed resulted in a taxonomic change of the prokaryotic community.** Specifically, modern accessions showed a reduction in the Bacteroidetes phylum and an increase in the phyla Actinobacteria and Proteobacteria.[19] The authors suggest that because Bacteroidetes often produce a set of enzymes that can digest complex biopolymers, it is possible that their prevalence on wild type plant roots was a response to such biopolymer exudation in the rhizosphere. Moreover, it has been observed that under stress conditions such as drought, modern wheat cultivars exuded simple sugars (fructose, maltose, glucose) in higher abundance than wild wheat cultivars; this may be related to the modern cultivar's inability to control sugar release (Shaposhnikov *et al.*, 2016). Whether the increased presence of these easily digestible sugars is partially responsible for the higher abundance of Proteobacteria and Actinobacteria in modern cultivars remain unclear (Pérez-Jaramillo *et al.*, 2018). In another research study, Jaramillo *et al.,* (2017) noted that **differences in root architecture, namely longer root depth and lower root density in wild varieties of common bean, were correlated with a higher abundance of Bacteroidetes**. These root traits are associated with a higher efficiency to search for water and nutrients in low-fertility soils (Comas *et al.*, 2013; Toro, Tohme and Debouck, 1990). Most recently, Chen *et al.* (2021) highlighted the potential benefits and current challenges of using **indigenous crop microbiomes to help reduce agrochemical inputs and increase crop resistance to biotic and abiotic stresses**. To conclude, **missing plant microbes and indigenous crop microbiomes could play a critical role in plant breeding programmes, helping global agriculture develop solutions for challenges related to environmental changes.**

If plant domestication resulted in modifications to the rhizosphere, one may also wonder how genetically modified plants, or transgenic plants, might influence the soil microbiome. In their review on the subject, Turrini, Sbrana and Giovannetti (2015) identified potential direct, indirect and pleiotropic effects of transgenic plants on the

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[19] An accession is a distinct and unique sample of plant material representing a cultivar or a breeding line.

microbiome, especially on the rhizosphere.[20] For instance, they suggest that the rhizosphere microbiome could be affected directly or indirectly owing to genotype influence on root exudates and pleiotropy alteration of crop chemistry and metabolism. They also addressed effects of varieties with improved traits for the benefit of the food industry and consumers. Several crops have modified sugar types and contents in order to suit certain needs, such as potatoes that have more starch and less sugars and tomatoes that have delayed ripening to prolong shelf-life. Among the few studies conducted on these varieties, some showed no effects (Gschwendtner *et al.*, 2011) while several showed that altered starch-content potatoes did affect rhizosphere bacteria (Dias *et al.*, 2013; Milling *et al.*, 2005) and mycorrhizal fungi (Hannula *et al.*, 2012). **However, an outstanding question is whether the effects of transgenic crops are more extreme or go beyond differences than would be found with a range of conventional varieties.**

Concluding remarks

Plant varieties can differently shape their rhizosphere microbiomes through the varying chemical compositions of their exudates and root architecture. Domestication of wild cultivars, and the ensuing genetic and root architectural modifications, has visibly led to alterations in their associated rhizosphere microbiomes. Given that this relationship features many complex interactions and feedback responses, the ability of cultivars to differently shape the rhizosphere-dwelling microorganism community may well have impacts on plant health and performance. For these reasons, it is opportune to explore the roles of root-associated microorganisms, and especially those that seem to have disappeared with modern cultivars, in plant breeding programmes.

4.5.3

Systematic review: Effects of plant variety selection on the soil microbiome, and their combined effects on climate change

Searches for this section returned 93 articles. We selected nine based on their relevance. The majority concern rice cultivars, their effects on soil microbes, and soil methane (CH_4) emissions. One study explores the impact of deep rooting plant varieties on stable carbon (C) in deep soils.

Deep-rooting varieties can stimulate higher mineralization rates of millennia-old carbon in deep soils

The stability of deep soil C (>20 cm) in the context of climate change is of great concern. The large pool of C in deep soils could cause massive changes to the global C cycle if the deep-soil microbial activity is stimulated. Breeding for deep-rooting plants has been proposed as an agroecological strategy to fix and sequester deep-soil carbon dioxide (CO_2), the idea being that roots contribute to soil C through litter turnover (Carter and Gregorich, 2010; Kell, 2011; Lorenz and Lal, 2005).

However, it is also known that roots initiate a rhizosphere priming effect (RPE), which causes C mineralization (Cheng *et al.*, 2014; Dijkstra and Cheng, 2007). This occurs when enzymes, released by soil microorganisms feeding upon plant root exudates and litter, decompose organic matter (OM) in the surrounding soil. In other words, root exudation can potentially decrease soil C content through stimulation of soil microorganism activity. A full calculation of soil C balance should nonetheless also include the formation of soil C through the process of humification of plant material. Therefore, it is important to determine whether root penetration and exudation in deep soil layers can stimulate the mineralization of millennia-old C through the rhizosphere priming effect, as investigated by Shahzad *et al.* (2018). Using *Festuca arundinacea* (a deep rooting grass species), dual labeled CO_2 (^{13}C and ^{14}C), and soil columns of 0-80 cm depth, the authors confirmed that root penetration of deep soils induces mineralization of millennia-old C. Furthermore, the release was rapid (<2 years),

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[20] Pleiotropy occurs when an inserted, foreign gene causes development of unexpected, seemingly unrelated traits.

and they found that the vulnerability of deep C was comparable to the process measured at the surface. They also point out that plant presence, even more than soil depth, influenced the microbial community composition along with the soil depth profile. Although the structure of rhizospheric microbial communities changed along with the soil depth profile, their capacity to mineralize C remained consistent, indicating that soil organic C turnover was primarily driven by roots. Lastly, their study suggested that a group of saprophytic fungi play a dominant role in RPE. In conclusion, **this study suggests that higher mineralization rates of millennia-old C in deep soils can be expected with the use of deep-rooting plant varieties. This does not, however, predict an overall decrease in deep-soil C stock, since plant litter also contributes to the formation of new soil C**.

Impacts of different rice cultivars on methane paddy soil emission

Rice paddies are a significant source of agricultural release of methane into the atmosphere. Root characteristics appear to be related to the amount of CH_4 released (Kim *et al.*, 2018). Some estimates suggest that 80 to 90 percent of total CH_4 released from rice paddies are attributed to the root systems (Das and Baruah, 2008). While it is known that rhizosphere activities strongly influence CH_4 emissions from rice paddies, the responsible mechanisms are not well understood. The interactive effects of rice root morpho-physiological traits, root exudates, soil characteristics and rhizosphere microorganisms on CH_4 cycling are predictably complex and feature several unresolved questions. Nevertheless, **studies strongly suggest that different rice cultivars, or genotypes, can influence CH_4 paddy soil fluxes, mediated by archaeal methanogens and bacterial methanotrophs.**

One of the challenges in elucidating these dynamics are the techniques and methodologies used to study rhizosphere microorganisms. For instance, Lüke *et al.* (2011) described the methanotrophic communities in the rhizospheres of 18 different rice cultivars as highly diverse and dominated by type II and ib methanotrophs (CH_4-oxidizing bacteria). They furthermore identified a rice cultivar effect on the microbial community composition, which was affiliated to the plant genotype. Numerous other studies, described below, support this conclusion. Inubushi *et al.* (2011), for example, found that **microbial biomass C, which was positively correlated with CH_4 oxidation, also varied amongst rice cultivars**.

How does a genotype influence rhizosphere microbial communities? Chen *et al.* (2019e) found that **root traits** (rather than above-ground plant traits) **were responsible for significant differences in CH_4 emissions among three rice cultivars.** Root morphological and physiological traits such as root length, dry weight, and oxidation activity were negatively correlated with CH_4 fluxes. The interaction between stronger root systems, higher oxygen delivery capacity, and suitable root exudates established a favourable habitat for soil microbial populations, leading to reduced CH_4 emissions from rice paddies during the mid-growing period.

Some research investigated soil microbe-mediated CH_4 fluxes according to specific types of differences between rice cultivars: wild vs cultivated, high yielding vs conventional, transgenic vs non-transgenic, and hybrid vs non-hybrid.

As elegantly demonstrated by Shenton *et al.* (2016), certain desirable traits may have been lost in the transition from wild to modern rice cultivars, resulting in so-called missing microbes. Indeed, certain methanotrophs were overrepresented in the earliest diverged species of the *Oryza* genus, suggesting that plant domestication influenced methanotroph community composition. There were, for example, higher counts of methanotrophs of both type I (obligate) and II (facultative) in the families Methylococcaceae and Methylocystaceae (Shenton *et al.*, 2016).

Growing high-yielding rice cultivars is one of the potential means to reach the increasing global rice demand. However, there have been concerns about the possibility that high-yielding cultivars cause higher CH_4 emissions due to increased soil C contributions through root exudates and the subsequent stimulation of methanogenic archaea. Jiang *et al.* (2017) used three experiments and one meta-analysis to explore the effects of high-yielding rice cultivars on CH_4 emissions related to soil C availability. Results suggested that **high-yielding cultivars increased substrates for methanogens, but only when soil C content was low**. In contrast, **when soil C content was high, high-yielding cultivars enabled CH_4 oxidation by creating conditions favourable for methanotrophs.** This second observation was attributed to increased root biomass and root porosity facilitating O_2 transport into the rhizosphere, which encourages CH_4 oxidation through methanotroph activities.

Hybrid rice cultivars can feature high-yielding varieties. Results from a comparison of a hybrid rice cultivar (Xieyou 9308) against two non-hybrids (*Indica* and *Japonica*) suggest that the hybrid variety stimulated the growth of methanotrophs in the rice rhizosphere, enhancing CH_4 oxidation and therefore attenuating CH_4 emissions (Ma, Qiu and Lu, 2009).[21] Furthermore, the hybrid rice cultivar produced 50 to 60 percent more shoot biomass than the two non-hybrid cultivars. However, the use of hybrid cultivars is often associated with high input applications, which may significantly increase the GWP of those systems when assessed with a comprehensive methodology (e.g. life cycle assessment).

Transgenic Bt rice cultivars are yet another proposition to increase rice production owing to their increased resistance to insect pests. A comparison between a non-transgenic cultivar (Minghui 63) and a transgenic Bt cultivar (TT51, derived from the parent Minghui 63) found that the **Bt cultivar reduced *in situ* CH_4 soil emissions, and also influenced the methanogenic archaeal and methanotrophic bacterial community abundance and diversity in the rhizosphere** (Han *et al.*, 2013). The authors suggested that the reduced emissions observed with the Bt cultivar were likely explained by the lower methanogenic archaeal community abundance and diversity in this treatment – and therefore lower CH_4 production – combined with higher methanotrophic activity. They also suggested that differences in root exudates may have been key factors that shaped microbial communities. First, the Bt cultivar may have provided less C sources through rhizodepositions, therefore offering less substrate for methanogenesis. Second, the Bt cultivar may also have released more O_2 than the non-hybrid cultivars into the rhizosphere, which could then have been be available for CH_4 oxidation processes.

All studies mentioned so far have focused on methanogens and methanotrophs. However, there is recent evidence that **other microbial taxa influence soil CH_4 fluxes during upstream processes related to CH_4 metabolites** (Liechty *et al.*, 2020). In a comparison between one high and low CH_4 emitter rice cultivar (cultivars Sabine and CLXL745, respectively), Liechty *et al.*, (2020) found that the rhizosphere microbiome of the Sabine cultivar not only hosted a higher abundance of methanogens but also a higher number of taxa responsible for the production of methanogenesis precursors (acetate, CO_2, and H_2), as well as sulphate-reducing and iron-reducing taxa (processes necessary to lower soil oxidation-reduction potential before methanogenesis can occur).

Concluding remarks

Most of the studies returned for this subject were related to how different rice cultivars impact CH_4 paddy soil emissions. It should be noted that most of these experiments were conducted during a specific time point of the rice growing season. Furthermore, there are many knowledge gaps regarding the precise relationships studied, particularly considering the multilateral, complex interactions in the field. Nevertheless, it seems that CH_4 soil emissions from paddy fields could potentially be altered by manipulating rice plant genotypes.

4.5.4
Systematic review: Effects of plant variety selection on the soil microbiome, and their combined impact on human health

Three different searches were performed for studies linking plant variety selection, the soil microbiome and human health. Two articles were returned, but they were not relevant for our purposes (see **Annex I** for search terms).

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[21] This, of course, does not take into account the mineral fertilizer requirements (and consequences for GHG emissions) for such varieties. Such hidden costs should be included in GWP calculations.

4.6
IRRIGATION

HIGHLIGHT BOX 11 Impacts of irrigation on the soil microbiome, climate change and human health.

NARRATIVE REVIEW What are the impacts of irrigation on the soil microbiome?

- Irrigation directly changes soil abiotic properties and can indirectly modify the rhizosphere through plant rhizodepositions (i.e. root exudates and root litter). The latter effect can shape communities of microorganisms that promote crop growth and disease control.
- Irrigation with treated wastewater, which varies tremendously in sources and composition, can also have direct and indirect effects on soil microorganisms depending on the abiotic and biotic composition of the wastewater.
- Introducing dissolved organic matter and nutrients to the soil via irrigation with treated wastewater can i) help select for copiotrophs, which thrive in environments with high carbon levels and easily degradable organic material (direct influence), and ii) affect plants and rhizodepositions or the rhizosphere properties (indirect influence).
- Treated wastewater can contain antimicrobial resistant genes, introducing them into agricultural soils with irrigation, thereby creating a potential pathway for antibiotic resistance genes migration into the food chain.
- The pathway mentioned in the above point has many knowledge gaps. First, the role of horizontal gene transfer between introduced, antimicrobial resistant gene-carrying bacteria and indigenous plant and soil bacteria is not clear. Second, whether and how antimicrobial resistant genes migrate into the food chain is inconclusive, although there is enough evidence to raise concern and incentivize further research. Third, it is unknown whether even low abundance of transmission can eventually cause a general decline in health conditions as a result of long-term colonization.
- Literature reviewed here indicates that: 1) Treated wastewater irrigation does not seem to have strong effects on antimicrobial resistance levels in the soil microbiome; 2) Residual antibiotic concentrations do not induce propagation of antimicrobial resistant genes in treated wastewater irrigated soils; 3) The antimicrobial resistant bacteria and antimicrobial resistant genes from treated wastewater do not persist in the soils; and 4) Antimicrobial resistant genes present in soils do not necessarily transfer to edible parts of vegetable crops. However, considering its importance and complexity, this subject merits further research.

SYSTEMATIC REVIEW What are the impacts of irrigation on the soil microbiome, and their causal impacts on climate change?

- Denitrifying genes can respond differently to different irrigation regimes
- The multiple consequences of different irrigation practices should be considered in the context of a particular farming system when determining best irrigation practices.
- Treated wastewater varies tremendously in quality.
- Under certain conditions, water quality may be more influential than water quantity in selecting for soil microbial communities, and may offer a strategy to promote resilience.
- There is evidence that decreased water quantity can result in reduced soil nitrous oxide fluxes, the explanation being related to soil properties that create favourable conditions for soil microorganisms involved in nitrous oxide production.
- Aerated irrigation appears to slightly increase nitrous oxide emission.
- The time of irrigation (e.g. night or day) can significantly influence greenhouse gas emissions.
- Intermittent irrigation in rice paddies can reduce methane emission, while simultaneously increasing nitrous oxide emission.

SYSTEMATIC REVIEW What are the impacts of irrigation on the soil microbiome, and their causal impacts on human health?

- Irrigation water can carry pathogenic bacteria such as Salmonella. Weather events that cause water stress, either drought or heavy rain, can impact crop internalization of pathogens, depending on the crop species. The mechanisms to explain these observations are not yet clear.

▶▶

- It is uncertain whether irrigation with treated wastewater causes a rise of antibiotic resistance levels in the soil microbiome, what any such impacts may be in the short and long term, and whether the occurrence of horizontal gene transfer between introduced (antibiotic resistance gene-carrying) bacteria and established soil or plant microorganisms plays a significant role in augmenting the risks for human consumption of produce.
- Even with the current limited knowledge, it is reasonable to believe that antibiotic resistant bacteria or antibiotic resistance genes in agricultural soil do pose a potential risk to human health, and even more so if humans have high exposure to places where antibiotic-resistant bacteria are present (e.g. vegetable crops irrigated with treated wastewater).

Crops are grown with a wide variety of irrigation methods. The purpose of irrigation is to deliver water to the soil, bringing it into contact with all soil life. Depending on the water source, however, it can also contribute nutrients and potentially toxic compounds, pathogens or antibiotic resistance genes (ARGs).

This section begin with a brief description of how soil moisture affects soil microorganisms, then gives a few examples of how irrigation can directly or indirectly affect the soil microbiome. It continues with a discussion about impacts of irrigating with treated wastewater, including concerns about pathogenic bacteria and antibiotic resistance. The last two sections (results from systematic literature review) address links between irrigation, the soil microbiome and biogeochemical cycling, and discuss two additional studies related to pathogenic bacteria and antibiotic resistance with treated wastewater.

4.6.1
Overview of interactions between water and soil microorganisms

Water in the soil is essential for microbial life. It affects soil microbial dynamics through water potential, as a solvent for nutrients, and as a means of transportation (Schimel, 2018). Soil water potential is primarily controlled by the physical soil matrix and by solutes, and is fundamental in the survival and function of soil microbes. For example, as soil dries out and the water potential drops, microbial cells must choose between matching their internal solute potential with that of the surrounding soil to avoid dehydration, or forming impermeable cell walls but no longer be able take up other resources (Schimel, 2018). The soil aqueous phase is dynamic, undergoing constant change in time and space. Wetting and drying cycles replenish and reduce water in the soil, respectively connecting and fragmenting these aqueous microhabitats. Both theoretical and experimental evidence suggest that the size and connectivity of these microhabitats shape nutrient diffusion pathways and the dispersion of microorganisms, providing opportunities for the microbial community to self-organize according to favourable habitats (e.g. anaerobic, aerobic) and resources distributed along the soil profile (Tecon and Or, 2017). It is widely recognized that soil drying and rewetting can cause large pulses in nutrient mineralization and soil respiration. Rewetting causes microbial biomass and activity – including respiration – to increase sharply, rapidly decomposing soil organic matter (SOM) to meet their needs for energy and nutrients (Song *et al.*, 2018).

4.6.2
Direct and indirect effects of irrigation on the soil microbiome

Irrigation can impact the community structure and activities of the soil microbiome either directly or indirectly. The following are just a few examples to illustrate how this may occur.

Irrigation can directly impact the rhizosphere microbiome, which may have consequences for interactions with plant growth or health. While studying the response of the wheat rhizosphere microbiome, Mavrodi *et al.* (2018) observed that the **soil water status (as determined by irrigation) drove the development of beneficial antibiotic-producing Phz+**

rhizobacteria, which contribute to natural suppression of soil-borne diseases of cereal crops.[22] Irrigation had a negative effect on Phz+ rhizobacteria. The authors speculated that increased soil moisture may have perturbed interactions within the rhizosphere microbiome as well as altered rhizodepositions and soil properties. Sharaf *et al.* (2019) studied the effect of irrigation and no-irrigation on the microbiome within soybean nodules.[23] The water treatment was partially responsible for functional differences among different nodule microbiomes, seen for example, by the differing amino acid profiles among nodules.[24] This highlights the potential **impact of irrigation in shaping microbial diversity and functions in nodules.** Moreover, the Nitrogen (N)-fixing rhizobia appeared to be the most sensitive microbial group of the nodule habitat, confirming that these communities are very sensitive to soil moisture and plant water status. **This enforces the idea that the process of biological N-fixation is also sensitive to soil water status.**

In research performed in grassland soils, Zhang *et al.* (2018a) **found that the abundance and composition of fungal phyla and classes shifted significantly under different N and water addition rates, and that water addition modified the effects of N addition on the soil fungal community.** As those changes were mainly attributed to the genera *Mortierella* and *Geastrum* and family Entolomataceae, these three taxa were proposed as indicator species to monitor changes in soil fungal community structures in the grassland soils studied.

Working with Scots pine trees in the European Alps, Hartmann *et al.* (2017) report that irrigation affected trees positively through increased biomass, increased litter fall and greater root biomass. **They hypothesized that increased availability of plant matter (litter) and root exudates consequently stimulated soil microbial activity, and moreover, induced a community shift from oligotrophs to copiotrophs** (i.e. shift to favour taxa that thrive in high carbon (C) environments). It is also known that increased soil moisture correlates with increased microbial activity (Manzoni, Schimel and Porporato, 2012), unless the soil is waterlogged. Hartmann *et al.* (2017) suggest that enhanced microbial activity (decomposition) could mean that mineralization increased, resulting in increased C respired from soils. However, C capture from primary production (i.e. tree growth and biomass production) probably increased as well, potentially resulting in similar levels of soil C. **The authors emphasise that temporal scales are crucial to understand different ecosystem responses as some processes and feedback mechanisms may take years to occur or be detected**.

4.6.3

Irrigation with treated wastewater can impact the soil microbiome

Using reclaimed water is a necessary solution in arid regions with lengthy dry periods, and a desirable solution in semi-arid regions, in order to reduce stress on traditional freshwater sources (Tran, Schwabe and Jassby, 2016). Furthermore, it could also be argued that rather than discharging wastewater effluent into freshwater ecosystems where the introduced nutrients can cause ecosystem damage through algal blooms and eutrophication, it would be more appropriate to apply the water on agricultural soils where the introduced nutrients could contribute to improved crop production. Vinasse, for example, is an aqueous effluent produced by sugarcane or sugar beet biorefineries. It contains high quantities of organic matter and mineral elements. When properly used as fertirrigation, results have shown that it can contribute to improvements in soil quality and agricultural productivity (Prado, Caione and Campos, 2013). Indeed, many farmers in water-scarce developing countries irrigate with wastewater because it is the only consistently available water

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[22] Phz+ refers to the ability to produce the antibiotic phenazine-1-carboxylic acid (PCA).

[23] Soybean nodules are found on the roots. They are specialized organs found on the roots of legume species. The nodules host bacteria known as rhizobia, which are capable of fixing atmospheric N. In this symbiotic partnership, the legume supplies nutrients to rhizobia, and rhizobia supplies N to the legume.

[24] Amino acid profiling gives an indication of plant-microbe interactions based on metabolism processes.

source throughout the year, and its rich nutrient content reduces the need to purchase and apply fertilizers (Qadir and Sato, 2016). However, management of the collection, treatment and use of wastewater varies widely between developing and developed countries, as well as low- and high-income countries (Qadir and Sato, 2016). **The use of treated wastewater (TWW) is therefore not that straightforward, as it can also carry some biological and chemical hazardous agents**. The consequences of these disturbances to indigenous soil microbial communities are not very well understood, as demonstrated by a review of literature that found contradictory results (Lopes *et al.*, 2015). Nevertheless, **there is enough evidence to state that TWW can impact the soil microbiome from multiple perspectives.** It is extremely important to bear in mind that the composition of TWW varies vastly, and the effects are dependent on the nutrients and dissolved compounds and agents it carries. In other words, **TWW is a single term that refers to potentially very different water qualities.**

Irrigation with TWW can potentially influence specific parameters related to the structure or composition of soil, including salinity, organic matter (OM) content, pH, available phosphorus (P) and N content, and metal and micropollutant concentrations, including antibiotics. All of these parameters can directly or indirectly affect the soil microbiome.

Salinity varies spatially and temporally in soils, and can affect soil microorganisms through changes in water potential. For instance, it can induce alterations of water flow into or out of cells as they adjust their internal conditions in response to extracellular osmotic pressure. These rapid and drastic changes in cell hydration status are consequences of what is called hypo- or hyperosmotic stress. For example, following water infiltration, bacteria may experience hypoosmostic stress owing to their release of intracellular solutes. Following fertilizer application or dry periods they may experience the opposite, hyperosmotic stress. **Irrigation can contribute to increased soil salinity in the long-term** (Brouwer, Goffeau and Heibloem, 1985). This can occur with use of brackish waters when freshwater supplies are limited, and/or with improperly managed irrigation that does not provide adequate drainage for salts to leach out. This increased soil salinity implies detrimental effects on soil microbial diversity, biomass and activity (Rietz and Haynes, 2003; Yan *et al.*, 2015), thereby reducing soil fertility and productivity. Zolti *et al.* (2019), for instance, observed **increased salinity and alterations to the soil microbiome with TWW irrigation compared to freshwater, though they suggest that the effect could be reversed after events such as a washout during a rainy season.**

Dissolved OM in TWW contains nutrients, which can cause an increase in soil microbial biomass and activity as the microorganisms feed upon these substrates. In their three-year experiment, Frenk *et al.* (2014), observed that **OM in TWW induced a temporal shift in soil bacterial abundance and community composition, but that the community populations returned to a baseline at the end of each rainy season**. The authors suggest that the pattern of returning to a baseline population after each rainy season might be an expression of natural resilience by communities accustomed to wet-dry climates such as those experienced in Mediterranean soils.

Krause *et al.* (2020) concluded that soil organic C **(SOC) content and soil texture were significant in the response of soil bacteria and archaea to TWW irrigation**. All irrigation treatments corresponded with a decline in bacteria and archaea diversity. However, TWW-induced changes to bacterial communities were less pronounced in silt loam, pointing to the role of SOC and clay in the buffering effect. As an energy source, SOC content can clearly shape soil bacterial communities while also influencing the surface properties of soil particles, which can also shape bacterial community composition. It has been observed that soil bacteria may have preferences for soil particle size fractions (e.g. fine or coarse textures), resulting in differing soil bacterial responses to nutrient inputs (Hemkemeyer *et al.*, 2015).

4.6.4
Pathogenic bacteria and antibiotic resistance in treated wastewater: potential risks to human health via the soil microbiome?

Van Bruggen *et al.*, (2019) propose a **vision of One Health that encompasses soil, plant, animal and ecosystem health: the health of organisms in an ecosystem are intimately connected by the cycling of microbial communities from the environment to all organisms and back to the environment.** They emphasise the importance of the soil microbiome, as it influences that of terrestrial animals and plants. This vision offers a framework to conceptualise the potential risks to human health by ARGs and pathogens introduced by TWW irrigation pathways.

Treated wastewater as well as freshwater irrigation sources can potentially carry pathogenic bacteria. The following study by Allard *et al.* (2019) identified no particular risk to human consumption under the experimental conditions, but nevertheless **provides an example of a potential pathway for pathogen contamination of vegetable produce.** Using creek water confirmed to have *Escherichia coli* levels consistently above the Food Safety Modernization Act Produce Safety Rule for agricultural water (stipulated by the USA Food and Drug Administration), as well as consistent presence of *S. enterica* and *L. monocytogenes*, the authors explored microbial contamination risks to humans via irrigated vegetable consumption. The study focused on two crops, kale and radish, irrigated through drip irrigation. They monitored *E. coli*, total coliforms, *Salmonella enterica* and *Listeria monocytogenes*.[25] Total coliforms levels were significantly influenced by irrigation in soil. *Escherichia coli* increased but no significant differences were detected. Radish soil samples revealed that bacterial community structure and composition changes due to irrigation were larger than those in kale soils. In this study, the harvested crops did not highlight any particular risk for humans through consumption, so it was therefore unlikely that bacterial pathogens were transferred to the field via drip irrigation.

In addition to pathogenic bacteria, **there is also increasing worry about the transmission of ARGs and antibiotic resistant bacteria (ARBs) to the soil resistome through use of TWW, particularly in fresh food crops such as vegetables, which could ultimately impact consumer health** (Cerqueira *et al.*, 2019; Christou *et al.*, 2017; FAO and WHO, 2019; Gatica and Cytryn, 2013; Holvoet *et al.*, 2013).[26] It has been observed that wastewater treatment plants do not succeed in removing all antibiotics, ARBs, and ARGs. Antimicrobial resistant genes have indeed been collected in wastewater samples, and other studies have observed that TWW irrigation resulted in increased levels of antibiotics in soils (Gatica and Cytryn, 2013; Lopes *et al.*, 2015). **It is important to note that ARBs and ARGs are naturally present in microbial communities in any given natural environment because they are the original source of many clinical antibiotics** (Finley *et al.*, 2013)**.** It might therefore be said that the soil is the largest natural environmental reservoir of antibiotics, and that the continuous addition of TWW, biosolids and manure to agricultural soils thereby contributes to its enrichment. See **Figure 17** for a depiction of how increased anthropogenic activity such as agriculture intensifies the dispersal of ARGs in the soil environment.

Concern has been voiced about risks to human (and animal) health, the overarching questions being: first, whether and how ARGs present in agricultural soils might transfer resistance to human-pathogenic bacteria; second, whether and how those bacteria or ARGs might find their way to humans through the agri-food chain; and third, the consequences of their presence in the human body. Although there are studies that seek to elucidate the complex mechanisms involved, there remain many unanswered questions. In the following paragraphs, we explore both what is known and unknown according to recent studies.

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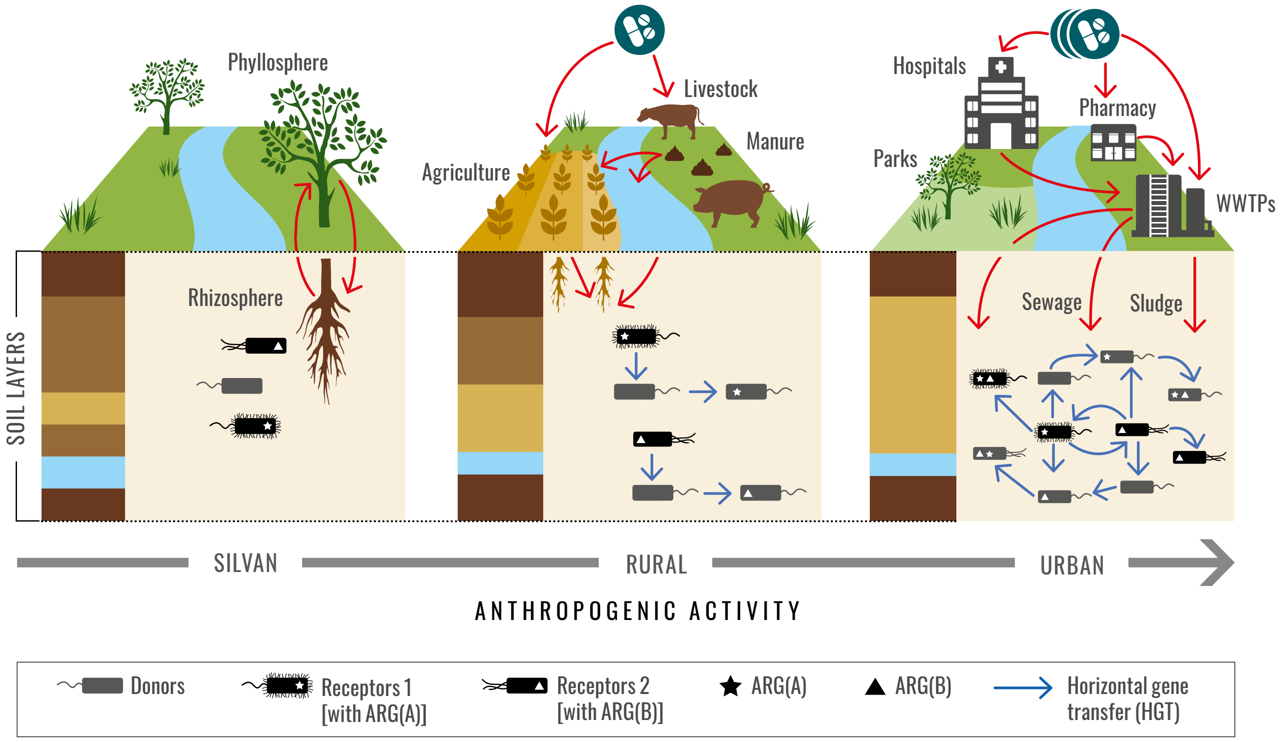
[25] Coliforms are organisms that indicate presence of potentially harmful bacteria (i.e. pathogens).

[26] **Section 4.7** on Fertilizers explores similar questions that arise with use of farmyard manure as organic fertilizer.

FIGURE 17.

THE EFFECTS OF ANTHROPOGENIC ACTIVITY ON ANTIMICROBIAL RESISTANCE IN THE SOIL ECOSYSTEM.

Urbanization intensifies the dispersal of resistance genes in the soil environment and beyond, therefore threatening planetary health.



Source: Zhu Y et al., 2019.

First, it is still not clear whether the occurrence of horizontal gene transfer between introduced, ARG-carrying bacteria and established soil or plant microorganisms plays a significant role in augmenting the risks for human consumption. Christou *et al.* (2017) argued that a better understanding of these dynamics should address not only biotic interactions, but also the abiotic factors that influence retention of antibiotics in the soil. These include the physiochemical properties of antibiotics and chemistry of soil pore-water (pH, mineral concentration, SOM, structure). They identified three processes that strongly influence the fate of antibiotics in TWW-irrigated agricultural soils: i) sorption (which is highly influenced by soil pore water pH), ii) transport, and iii) transformation (which includes abiotic and biotic factors). They suggested that the effects of antibiotics on soil microorganisms therefore depend on their bioavailability and thus on soil properties, and not only biotic factors.

Secondly, whether and how those bacteria and or ARGs might find their way to humans through the agri-food chain is likewise unclear, though there is enough evidence to incite concern and investigation. The very presence of ARBs and ARGs in TWW-irrigated agricultural fields, for instance, is controversial. On the one hand, multiple studies have found little impact on antimicrobial resistance (AMR) levels in the soil microbiome, suggesting that antibiotic resistant elements are not able to compete and survive in the soil environment, and therefore do not contribute significant ARGs to pre-established soil bacteria (Christou *et al.*, 2017; Gatica and Cytryn, 2013; Negreanu *et al.*, 2012). Gatica and Cytryn (2013), for example, concluded that TWW irrigation does not seem to affect AMR levels in the soil resistome. According to their results and analysis, it seems that residual antibiotic concentrations do not induce propagation of ARGs in TWW irrigated soils, and moreover that the antibiotic-resistant bacteria (ARB) and ARGs from TWW do not persist in the soils. Furthermore, freshwater soils actually had identical or higher abundance of resistance isolates compared to TWW-irrigated soils, despite the significant loads of ARBs and ARGs in the TTW. The researchers concluded that the antimicrobial-resistant bacteria that entered soil microbiome with TWW were not able to compete and or survive. They suggest that the high levels of ARBs and ARGs measured were therefore explained by the native soil resistome. They caution, though, that their conclusion should be considered carefully, and encourage further research on antibiotic resistance gene transfer from TWW to soil and crops.

On the other hand, **studies have noted a higher diversity and increased abundance of ARGs in agricultural soil irrigated with TWW** (e.g. Cerqueira *et al.*, 2019), **an increased concentration of antibiotics in soils irrigated with TWW than in the irrigation water itself** (e.g. Calderón-Preciado *et al.*, 2011) and, though the subject is beyond the scope of this paper, **an increase of antibiotics in urban park soils irrigated with TWW** (Wang *et al.*, 2014). While it is not well understood whether irrigation with TWW causes a rise of antibiotic resistance levels in the soil microbiome, and what the short- and long-terms impacts of any such impacts may be, contradicting observations do show that ARB and ARG dynamics are very complex, depending on a myriad of abiotic and biotic factors. Christou *et al.* (2017) point out that they may also be due to practical and methodological limitations. First, bacteria tend to live as aggregates, therefore quantification of ARGs may be difficult due to heterogeneous samples. Second, a minority of bacteria host ARGs, which means even if ARG concentrations increase they may still be difficult to detect. Nevertheless, inconsistent conclusions from different studies does not equate an absence of risk.

Even with the current, limited knowledge, it cannot be excluded that ARB or ARGs in the environment pose a potential risk to human health, and even more so if humans have high exposure to places where ARBs are present (e.g. vegetable crops irrigated with TWW). This would be particularly worrying if ARB are able to colonize humans (and, even worse, if those bacteria exhibit virulence factors) and or if ARGs transfer to human-pathogenic bacteria already present in the human body. In response to these concerns, research has investigated how irrigation of vegetables with TWW can potentially impact ARGs found in the soil and vegetables themselves. **Overall, though the studies we discuss here have demonstrated a low risk of ARG transfer to humans via vegetable consumption, they did clearly identified it as a possible pathway. Indeed, as mentioned above, TWW composition differs and therefore effects are likely to vary accordingly.**

In their field study, Cerqueira *et al.* (2019) investigated the response of the native resistome in soils and on *Vicia faba* (fava beans) grown under different irrigation regimes in three peri-urban plots around Barcelona, Spain. They irrigated with groundwater, a water channel where about 92 percent of water flow emanated from wastewater treatment plants, and river water with about 18 percent water flow from wastewater treatment plants. **There was a higher ARG diversity and prevalence in soils due to irrigation, while beans and leaves actually had very poor ARB diversity and had very small ARG loads. The authors suggested that human consumption of those beans therefore carried only a small risk of ARG exposure.** Of note, the leaf microbiome mostly consisted of *Rhizobacteria*, which the authors suspect may have displaced other bacterial groups, including those carrying ARGs. In sum, the ARGs were prevalent in the soil but not on consumed plant parts. **The authors concluded that the legal irrigation standard should therefore take into account crop physiology, in addition to the existing regulations in some countries that ban or limit TWW application on leafy vegetables that are typically consumed raw.**

In another study, this time in Belgium, Holvoet *et al.* (2013) examined soil, irrigation water, and lettuce leaves for *Escherichia coli* and AR. This study did not use TWW, but is included as it explores the potential ARG pathway from vegetables to humans. They tested 473 isolates of *E. coli* for resistance to 14 antimicrobials. They found that 11.4 percent isolates were resistant to one or more antimicrobials, the highest resistance rates being for ampicillin (7 percent), followed by cephalothin, amoxicillin-clavulanic acid, tetracycline, trimethoprim, and streptomycin (within a range of 4.4-3.6 percent). No resistance to amikacin, ciprofloxacin, gentamicin, or kanamycin was observed. Of the 37 *E. coli* isolates that showed multiresistance, ampicillin and cephalothin showed highest rates. The authors note that the results in this study were overall similar to other, comparable studies. Two interesting points of discussion were that, first, *E. coli* isolates from lettuce leaf samples showed higher antimicrobial resistance rates than samples from soil or irrigation water, though the reasons were not clear. Second, according to comparisons with other Belgian data on antimicrobial resistance, it seems that cattle were the main source of resistant *E. coli* contamination in their study. **Most importantly, this study showed that vegetable produce can serve as a reservoir and vector of AMR. Produce such as lettuce, which is consumed raw without prior treatment, may thereby contribute to direct human exposure to AMR bacteria.** Indeed, other studies have also demonstrated presence of an AMR pool in food-borne commensal bacteria in many ready-to-eat food products (e.g. Ruimy *et al.*, 2010; Schwaiger *et al.*, 2011).

The conclusions from the last paper bring us to the third major question regarding consequences of ARB or ARG introduction into human bodies though consumption of produce**. It is clear that a potential pathway of ARB or ARG transfer to humans exists via consumption of vegetables. Could even a low abundance of transmission eventually cause a general decline in health conditions as a result of long-term colonization?** Christou *et al.* (2017) again aptly summarised why this point is difficult to assess. First, there are difficulties in detection and quantification of ARBs and ARGs (as described above). Second, the number of ARBs required to start successful colonization in human body is unknown. Third, there is very limited information regarding dissemination paths and transmission from environment to humans, including those that involve the soil microbiome.

Concluding remarks

Delivery of water to the soil can shape the soil microbiome. This might happen directly, for example, by influencing the process of biological N fixation in legume nodules, by delivering OM carried in the water, or by an increase in water availability and less gas present in the soil environment. This effect may also be indirect, such as water availability inducing plant growth that provides more litter and exudates, thereby providing more nutrient sources for soil microorganisms.

Global use of TWW is irreplaceable from a perspective of sustainable use of resources. However, TWW sources and composition vary, as do soil microbial community compositions and responses in different soil types and climate ecosystems. Therefore, its potential impacts via interactions with the soil microbiome on the following areas need to be carefully monitored: i) soil health and long-term soil fertility, ii) plant health, yield and quality through the introduction of phytopathogens, and iii) risks to human and animal health owing to contamination of produce by pathogens or AMR microorganisms.

4.6.5
Systematic review: Effects of irrigation on the soil microbiome, and their combined impact on climate change

Searches for publications linking irrigation, the soil microbiome and climate change returned 167 articles, twelve of which made direct links between these three subjects and are discussed below. They explore variations in irrigation techniques such as water quality, water quantity, frequency, timing, and water-saving practices in rice paddies, and their potential consequences on greenhouse gas (GHG) emission as well as crop yield. Climate change is addressed through biogeochemical nutrient cycling, including C cycling, and the production of nitrous oxide (N_2O), methane (CH_4) and carbon dioxide (CO_2).

Higher soil moisture generally correlates with higher soil activity (Manzoni, Schimel and Porporato, 2012), unless the soil is waterlogged. Irrigation delivers water to the soil, affecting soil moisture and soil microbial processes involved in nutrient cycling, including C and N soil dynamics. Consequently, the by-products, intermediary or end products of those processes result in soil gas fluxes, including CH_4, CO_2, N_2O, nitric oxide (NO) and ammonia (NH_3) (Kim *et al.*, 2012). For example, the more pore spaces in the soil are filled with water, the less dioxide (O_2) (and other gases) can circulate. Anoxic conditions favour methanogens, whereas environments with more O_2 favour methanotrophs, which use O_2 to oxidize CH_4 as an energy source.

Soil moisture also influences nitrification (Placella and Firestone, 2013) **and denitrification** (Di *et al.*, 2014) **processes, two of the most common pathways that can result in production of N_2O in the soil.** Nitrification, or the oxidation of NH_3 into nitrogen dioxide (NO_2^-), is driven directly by ammoniza-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB). Denitrification is performed primarily by bacteria. These processes are typically studied by monitoring genes understood to be responsible for producing particular enzymes that catalyse certain pathways. **Denitrifying genes** include *nirK*, *nirS*, *nosZ*, and *nor*B. Though they are involved in the same process, they **can respond differently to different irrigation regimes**. Yang *et al.* (2018) found that different, long-term irrigation regimes significantly altered the abundance, diversity, and community structure of *nirS* and *nosZ*, but only minimally affected the community structure of *nirK*. The changes in *nirS* and *nosZ* were significantly correlated with variations in soil properties: increased soil moisture increased the gene abundances, as did a decreased pH resulting from the alkaline water used in the experiment.

Effects of quality and quantity of irrigation water on the soil microbiome

Bastida *et al.* (2017) asked the following question about irrigation strategies: to promote the resilience of biogeochemical processes, which is better, a reduced amount of high quality water, or an optimal quantity of reclaimed water? The answer seems to depend on the situation. The authors found that the higher quality water, transferred from the river under a reduced deficit regime, sustained higher soil microbial enzyme activity and biomass as well as soil organic C (SOC). It might therefore have promoted soil fertility and a certain soil microbial community structure. The reclaimed water applied at an optimal quantities, however, appeared to promote a more resilient community, mediated by copiotrophic microorganisms. The reduced water quantity indeed had a negative effect on soil microbial biomass and enzyme activities. However, promoting copiotrophic soil microorganisms in the phyla Proteobacteria and Bacteroidetes resulted in better resilience several months later when water conditions were optimal. **In harsher conditions where fresh water is restricted, using reclaimed water under a reduce deficit irrigation regime may thus be an appropriate strategy to promote development and activity of microbial communities**. This study was conducted in a Mediterranean grapefruit orchard, and results moreover showed that the yield was not affected under this second irrigation regime. Though this study did not address GHG emissions directly, the findings are pertinent to understanding how irrigation can impact soil microbial community structure and enzymes, both of which directly influence biogeochemical processes.

There is evidence that **decreased water quantity can result in reduced soil N_2O fluxes, explained by effects on soil properties and soil microorganisms involved in N_2O production** (Chen *et al.*, 2019a; Ye *et al.*, 2018). Ye *et al.* (2018) reported that subsoil and drip irrigation, compared to furrow irrigation, could reduce soil N_2O emissions in greenhouse. Furrow irrigation delivered the most water, thereby creating alternating wet and dry conditions that likely induced necromass production. Mineralization of this source of N and C likely induced higher microbial abundance and activity, which was furthermore more prone to denitrification. Chen *et al.* (2019a) found that the association between greater water quantity, greater N_2O emission, and higher abundance of denitrifiers shed light on potential explanatory mechanisms. N_2O fluxes were most influenced by water-filled pore spaces in the soil, temperature and denitrifier abundance. The authors also found that aerated irrigation slightly increased N_2O emission, as well as the soil nitrifier abundance and urease activity, but decreased the soil denitrifier abundance.

Effects of treated wastewater on biogeochemical cycling and greenhouse gas emissions

Treated wastewater, or reclaimed water, encompasses a broad category of water that can vary tremendously in quality. Depending on the upstream sources that feed into the treatment process, the exiting water may have vastly different levels of dissolved OM or trace elements – to name just two of the common variables. It is important to keep in mind that the term is all-encompassing when considering effects of so-called TWW or reclaimed water.

Li *et al.* (2020c) report that **both treated and untreated domestic sewage decreased CH_4 emission in a Chinese rice paddy field with straw-return.** The mitigating effect of the treated sewage was greater than that of the untreated sewage. The treated domestic sewage was characterized by nitrate (NO_3^-), while the untreated by NH_3. CH_4 correlated with the abundance of soil methanogens and methanotrophs. They also investigated N_2O fluxes, finding that the emission increased with both types of sewage, although the N input was the same for all treatments. Furthermore, there was no significant correlation between N_2O emission and denitrification functional genes (includes active and inactive soil microorganisms). Overall, considering both GHGs**, irrigation with treated domestic sewage in this experimental set-up reduced the global warming potential (GWP) by 66.7 percent, while the untreated water exerted no obvious influence on GWP**.

The study by Mkhinini *et al.* (2020) did not investigate GHG fluxes directly, but did show that in their experimental site in Tunisia, **20 years of irrigation with TWW increased the metabolic activities of soil microorganisms**. They measured microbial biomass C, as it relates to respiration and therefore biogeochemical cycling of C and other nutrients. Though the initial response to irrigation was a decrease in enzymatic activities in soils (specifically, arylsulfatase, acid and alkaline phosphatase), there was a major increase in enzymatic activities and soil microbial biomass over the long-term. The responsible mechanisms were not investigated, but the OM and minerals introduced with the water, as well as residues from different cropping systems are likely to have been influential. The changes in soil microbial enzyme activities have many potential influences (biotic and abiotic) that were not specifically investigated in this study. Nevertheless, the increase in soil microbial biomass over time is favourable, particularly when recalling the potential soil C storage in the form of microbial necromass (Chenu *et al.*, 2019).

Effects of time and frequency of irrigation on the soil microbiome

Franco-Luesma *et al.* (2019) found that **the time of irrigation (night or day) had a stronger effect than frequency on GHG emissions.** Specifically, they found no effect on CH_4 emission, concluding that the soil acted as a CH_4 sink. This may have been due to the low WFPS levels, which created better air porosity and circulation of soil gases, creating conditions favourable for CH_4 consumption by methanotrophs. CO_2 emission was influenced by time, frequency, and the sampling date. The fluxes tended to increase with day time irrigation. A possible explanation is that night irrigation resulted in lower water losses by evaporation, creating higher water filled pore space levels that resulted in less gas diffusivity. Night irrigation increased N_2O emission by 29 percent, while the frequency had no effect. Nevertheless, night irrigation resulted in an 11 percent increase in maize grain yield. So when the cumulative N_2O emissions were scaled per grain yield and N uptake, the authors concluded that there was no effect on N_2O emission. Considering this point, in Mediterranean conditions similar to those in this study, night time irrigation could optimize grain yield without a GHG trade off per unit of grain yield.

Effects of continuous vs intermittent flooding and ground cover on the soil microbiome in rice paddy fields

Intermittent irrigation in rice paddies can reduce CH_4 emission, while simultaneously increasing N_2O emission (Jiao *et al.*, 2006; Yue *et al.*, 2005; Zeng *et al.*, 2019b). Jiao *et al.* (2006) and Yue *et al.* (2005) observed that the decrease in CH_4 production and increase in N_2O production both occurred during the drainage period, that CH_4 emission was closely related to methanogen populations, and that there were positive correlations between nitrifier and denitrifier populations and N_2O emissions. This last point suggests that the changes in populations may have stimulated processes of nitrification and denitrification.

Jiao *et al.* (2006) found, however, that the integrated GWP of both GHGs was reduced significantly while the rice yield remained unaffected. The authors suggested that drainage created conditions of increased soil O_2, which methanotrophs use to oxidize CH_4. During the mid-season drainage, for instance, the methanotroph population increased while the methanogen population decreased. This strongly suggests that there was a respective increased consumption and decreased formation of CH_4, resulting in in reduced emission.

Zeng *et al.* (2019b) compared conventional, thin-shallow-wet-dry and alternate wetting and drying irrigation, finding that N_2O emissions with alternate wetting and drying were much higher than in the conventional treatment. The alternate wetting and drying enhanced populations of AOB and nitrifying bacteria, but reduced denitrifying bacteria. Similarly, in comparison to the control, the thin-shallow-wet-dry treatment increased the population of nitrifying bacteria while decreasing the population of denitrifying bacteria. N_2O fluxes had a significant positive correlation with nitrifying bacteria, thus leading the authors to conclude they had a critical effect on N_2O emissions in this experiment, even if the exact mechanisms remain unclear.

A ground cover rice production system reduces irrigation thanks to use of a plastic film that covers the soil. **With decreasing soil water during the growing season,** Chen *et al.* (2018b) observed **an increase of N mineralization, a reduction in biological N fixation, and increased nitrification and denitrification – in other words, net soil N losses resulting in increased net N_2O emission.** This study provides a microbial-oriented mechanistic understanding of N-cycling based on gene expression patterns, which were further supported by measuring *in situ* N_2O emissions. First, an increase in soil O_2 availability increased the gene expression levels for mineralization, resulting in a strong increase in N mineralization. Second, overall higher ratios of *nirS*:*nosZ* and *qnor*B:*nosZ* were found for covered soils compared to conventional open paddies. This suggests that the increased N_2O production was not balanced by a similar increase in gross N_2O consumption, resulting in increased net N_2O emissions. Also, the oxidation-reduction potential of soils were responsible for shifts in the compositions of denitrifying and N_2-fixing communities, indicating that soil water content indeed had a strong influence.

Concluding remarks

Overall, these studies demonstrate factors to consider when selecting irrigation practices that aim to reduce GHG emissions or GWP, while maintaining crop yield. In summary, these include timing (night rather than day), delivery systems (drip or subsurface vs furrow, or aerated vs nonaerated), reduced quantities of water and water quality. Furthermore, multiple consequences of these different practices should be considered in the context of the farming system to determine the best irrigation practice. It seems, for instance, that drip irrigation and subsurface irrigation could both save water and reduce N_2O emission in a greenhouse setting. Also, there are potential ecological benefits (e.g. reuse of water resources, increased soil microbiome resilience) to using reclaimed water under restrictive conditions, which are likely to continue featuring in climate change scenarios. While ground cover rice production results in higher net N_2O emission, exploring other practices that could help mitigate N soil losses would complement its water-saving advantage. Intermittent irrigation in rice paddies (wet-dry rice production) may result in an overall reduced GWP, associated with changes in populations of soil microbes directly involved with soil GHG fluxes (methanogens, methanotrophs, nitrifier and denitrifier populations). Finally, though these studies primarily convey correlations rather than elucidation of specific mechanisms, it is clear that there are strong relationships between soil microbial community structures and enzyme activities that influence soil biogeochemical cycling.

4.6.6

Systematic review: Effects of irrigation on the soil microbiome, and their combined impact on human health

Three separate searches linking irrigation, the soil microbiome and human health returned a total of 19 studies, one of which we discuss below. The remaining studies were not pertinent. Another study of relevance to human health was found when searching for studies concerning climate change (above), and it is presented here (see Ge, Lee and Lee, 2012). These two studies complement the discussion framed by One Health in 4.6.4, about pathogens and ARGs posing potential risks to human health.

Impact of water stress on vegetable internalization of pathogenic soil bacteria

Irrigation with contaminated water can deposit pathogenic bacteria such as *Escherichia coli*, *Salmonella enterica* and *Listeria monocytogenes* in agricultural soils. This is of particular concern in soils planted with leafy vegetables that are typically consumed raw, as the potential soil-plant pathway is believed to increase the risk of foodborne illnesses.

Climate change is anticipated to increase the frequency of extreme events that cause stress on plants, such as drought and heavy rain. Ge, Lee and Lee (2012) **investigated whether plant stress affected levels of pathogenic bacterial internalization by vegetables.** Specifically, they tested the response of *Salmonella Typhimurium* internalization in lettuce and green onion under three levels of *Salmonella* inoculation and under drought, optimal, and storm water conditions. They found that overall, **neither drought nor heavy rain seemed to affect *Salmonella* populations in the rhizosphere soil in their experiment**. On the whole, **both lettuce and green onion showed *Salmonella* internalization under all irrigation conditions**, and furthermore, a high concentration of *Salmonella* soil inoculation facilitated the internalization level in both crops overall. When exposed to high levels of contamination, green onion samples showed a much higher rate of internalization than lettuce. Under both drought and storm conditions the high concentrations of *Salmonella* internalization increased significantly in lettuce compared to the treatment receiving optimal irrigation. Green onion did not demonstrate any patterns regarding water stress on levels of *Salmonella* internalization. Internalization in lettuce was found only in leafy parts, a possible explanation being the presence of increased nutrients in the leaves due to photosynthesis, thereby benefitting bacterial growth and multiplication. Internalization in green onion was significantly higher in roots than leafy parts except the lowest concentration of inoculation under drought. The authors note that *Salmonella* levels in plant parts might actually have been higher than reported due to techniques used in their study. **It is possible that a high concentration of human pathogens in the rhizosphere soil might cause stress on plant roots, or that plant types have varying concentration thresholds (which are further influenced by growth conditions) required for pathogen internalization. The facilitating mechanisms are not yet clear. Some ideas summarized by the authors that could explain uptake from rhizosphere soil to plant tissue nevertheless include i) passive uptake through root system, ii) that the rhizosphere has a higher concentration of microbes than bulk soil, and that iii) sugars in plant root exudates attract *Salmonella* to the rhizosphere.**

What risks does irrigation with treated wastewater pose to human health: antibiotic resistance genes?

Treated wastewater varies widely in composition and sources. In some cases, it can carry ARGs, antibiotic residues and trace elements. The potential ARG pathway of water-soil-plants has incited worry about risks to human (and animal) health implied by consuming such crops. Nevertheless, with many unanswered questions, the subject remains open for debate.

First, though it is widely acknowledged that TWW used for irrigation can carry ARGs, the presence of ARBs and ARGs in agricultural soils irrigated with such water is controversial. The review by Christou *et al.* (2017) offers a good overview on the issue.[27] On the one hand, some studies show little impact on AMR levels in the soil microbiome, suggesting that antibiotic resistant elements are not able to compete and survive in the soil environment, and do not contribute significant ARGs to soil bacteria. On the other hand, some studies have observed a higher diversity and increased abundance of ARGs in urban park soils irrigated with TWW. These contradicting studies show that ARB and ARG dynamics are very complex, depending on a myriad of abiotic and biotic factors. The review points out that they may also be due to practical and methodological limitations. First, bacteria tend to live as aggregates, therefore quantification of ARGs may be difficult due to heterogeneous samples. Second, a minority of bacteria host ARGs, which means even if ARG concentrations increase they may still be difficult to detect. The authors emphasize that even with the current limited knowledge, it is reasonable to believe that ARB or ARGs in the environment do pose a potential risk, and even more so if humans have high exposure to places where ARBs are present (e.g. vegetable crops irrigated with TWW). This would be particularly concerning if ARB are able to colonize humans, and, even worse, if those bacteria exhibit virulence factors.

Concluding remarks

It is uncertain whether irrigation with TWW causes a rise of antibiotic resistance levels in the soil microbiome, what the short- and long-terms impacts of any such impacts may be, and whether the occurrence of horizontal gene transfer between introduced (ARG-carrying) bacteria and established soil or plant microorganisms plays a significant role in augmenting the risks for human consumption of produce. These issues are difficult to assess because i) there are difficulties in detection and quantification of ARBs and ARGs, ii) the number of ARBs required to start successful colonization in human body is unknown, and iii) there is very limited information regarding dissemination paths and transmission from environment to humans (Christou *et al.*, 2017).

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[27] The review by Christou *et al.* (2017) is also discussed in **Section 4.6.4**.

4.7
FERTILIZATION

HIGHLIGHT BOX 12 Impacts of fertilization on the soil microbiome, climate change and human health.

NARRATIVE REVIEW What are the impacts of fertilization on the soil microbiome?

- Both inorganic and organic fertilizers can affect the soil microbiome directly (e.g. by altering abiotic and biotic environmental factors) and indirectly (e.g. through positive effects on plant growth and development). Inorganic nitrogen fertilization:
    - Can affect the abundance of soil microbiome genes involved in nitrification and denitrification processes, and therefore impact the functional community composition.
    - Can improve resistance and resilience of soil microbial gene functions to stress under drying-wetting cycles.
    - Can cause changes to soil microbial communities that consequently influence microbiota living in soil fauna.
    - May have a stronger (negative) effect on protists than bacteria or fungi.
- Organic fertilization:
    - Contribute microorganisms, substantial carbon and other nutrients and are therefore considered important for long-term soil fertility, soil functions and multifunctionality.
    - Can enhance the soil microbiome and ecosystem functioning, likely increasing crop yields.
    - Can cause larger shifts in the soil microbiome than inorganic fertilizers, such as changes in community composition and functional roles (e.g. carbon and nitrogen cycling, pathogenic or beneficial microbial communities).
    - Can positively affect the bacterial community diversity down to 1 m depth.
- Biochar may enhance soil microbial activity related to soil carbon cycling, prompting interest in its soil carbon storage potential.
- Soil microorganisms can act as biofertilizers themselves. Examples include plant inoculation with arbuscular mycorrhizal fungi, seed inoculation with beneficial bacteria that can be transmitted vertically (microorganisms passing from parent generation to offspring).
- Cattle and swine treated with antibiotics excrete antibiotic residues in manure and urine. Manure applications have indeed been observed to increase the abundance and diversity of antibiotic resistance genes in the soil. However, antibiotic resistance gene-carrying bacteria that originate from livestock intestines do not seem to survive in the soil environment.
- Microorganisms also inhabit above-ground plant parts, known as the phyllosphere. There may be cross-talk between soil and phyllosphere resistomes (totality of antibiotic resistance genes), and the soil resistome may be a reservoir of antibiotic resistance genes that influences the phyllosphere.
- There is concern that antibiotic resistance genes may migrate into the food chain. This is of particular concern when produce is consumed raw.

SYSTEMATIC REVIEW What are the impacts of fertilization on the soil microbiome, and their causal impacts on climate change?

- The type of nutrients, application rate, and nitrogen-form of fertilizers, as well as the nitrogen-fertilization history of soils, soil type, particle size fraction, and seasonal variations can differently and significantly impact the abundance and activities of soil microorganisms involved cycling of critical nutrients, with consequences on soil carbon storage and greenhouse gas emissions.
- Agricultural management choices exist that promote fungal-based soil food webs and plant–microbial linkages, thereby reducing nitrogen losses and impacting crop yields. However, their trade-offs should be taken into account when considering their application.
- Regarding inorganic nitrogen fertilization:
    - Soil microbial biomass can decline about 15 percent on average under nitrogen fertilization, and more substantially under heavier nitrogen loads and longer fertilization durations, with a corresponding decline in carbon dioxide emissions. However, the effect of nitrogen fertilization on other greenhouse gases should be considered during assessment of the overall effect of nitrogen input on greenhouse gas emissions.

▶▶

  - Nitrogen fertilization can increased amoA gene abundance from both archaea and bacteria, but ammonia-oxidizing bacteria are typically far more responsive, and produce a higher yield of nitrous oxide. Increasing rates of nitrogen fertilizer are thus frequently accompanied by increases in soil nitrous oxide emissions.
  - However, enhanced greenhouse gas emissions (nitrous oxide and carbon dioxide) may be a result of enhanced soil bacterial community composition and functioning, which can be a sign of improved soil fertility following nitrogen fertilization.
- Fertilization may increase or at least maintain soil organic carbon, but organic fertilizers (e.g. manure, or inorganic combined with straw) further improve soil organic carbon stocks, enhancing soil fertility and ecological factors compared to inorganic fertilizers. One aspect is that nitrogen mineralization from natural degradation processes fosters low reactive nitrogen and thus the potential to reduce nitrous oxide emissions through coupling of carbon and nitrogen. A second, related, aspect is the enhancement of abundance and diversity of soil microorganisms, whose activities influence soil quality and biogeochemical cycling. Organic fertilization may thus result in increased or decreased nitrous oxide emission.
- Organic fertilizer in the form of manure or compost may result in increases or decreases of methane.
- Biochar has the potential to offer benefits to soil functioning and ecosystem services such as stimulating soil carbon storage, increasing the pH of acidic soils, and thereby reduce soil greenhouse gas emissions via shifts in soil microbial communities and activities. These effects, however, may vary according to the physical and chemical properties of biochar, as well as the soil environment to which they are applied.
- Slow-release fertilizers, amended with nitrification inhibitors, can reduce soil nitrous oxide emissions.
- Inorganic nitrogen fertilization combined with certain other practices such as tillage or intercropping can have a significant effect on greenhouse gas emissions and soil organic carbon conservation. The effect is significant and variable both in terms of intensity and direction.
- N supply in rice paddies has been seen to inhibit or even stimulate methane oxidation via methanotrophs. It might decrease or increase soil methane emission, depending on the response of soil methanotrophs and methanogens as well as their interaction with rice plant response to nitrogen inputs.

Long-term manure fertilization in rice paddies, even in combination with mineral fertilizer, can substantially enhance the soil microbial abundance and richness and activity, producing positive effects on soil organic matter and soil carbon storage.

SYSTEMATIC REVIEW What are the impacts of fertilization on the soil microbiome, and their causal impacts on human health?

- No relevant literature was found during the systematic search.

The addition of nutrients to the soil by means of either mineral or organic fertilizer can cause shifts in the soil microbiome through multiple processes.[28] The subject being vast, this chapter is organised according to different themes. Beginning with the general review, the themes include how the soil microbiome can be impacted through the use of mineral and organic fertilization, its responses to fertilization in deeper soil layers, how fertilization can indirectly affect soil fauna and protists via soil microorganisms, and using soil microorganisms as different types of biofertilizers. There is also a substantial subsection about organic fertilizers and antimicrobial resistance (AMR). Several boxes offer complementary details or focus on additional subjects. The systematic review discusses fertilization and relationship with biogeochemical cycling with potential implications for soil greenhouse gas (GHG) fluxes and nutrient cycling.

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[28] The terms *mineral* and *inorganic* fertilizers are used interchangeably in this section. Elsewhere they are also referred to as *synthetic* or *chemical* fertilizers.

4.7.1
Inorganic nitrogen fertilizers can influence soil microbial communities involved in nitrogen-cycling

Inorganic nitrogen (N)-fertilization can cause shifts in soil microbial communities, most notably through changes in community composition and abundance of genes involved in N-cycling functions. Fierer *et al.* (2011) provide an example of a general shift in community composition. The authors reported that N fertilization provoked a marked increase in copiotrophic species with a simultaneous decrease in oligotrophic species, without influencing the bacterial diversity. In other words, there was a significant increase in taxa that thrive in nutrient-rich environments. N fertilization has also been observed to increase the abundance of bacterial genes involved in nitrification and denitrification processes (Babin *et al.*, 2019; Ouyang *et al.*, 2018). In their long-term experiment in Germany, Babin *et al.* (2019) reported that while the effect of fertilization on soil bacterial community structure was minimal, it increased the abundance of bacterial *amoA* genes. These genes are involved in ammonia (NH_3) oxidation, which plays an important role in the nitrification process; this was interpreted by the authors as a response to the high concentration of urea-N and ammonium-nitrate present in the fertilizer. A recent meta-analysis aimed to quantify the N input effects on the abundance of N-cycling genes in bacteria (Ouyang *et al.*, 2018). The study took into account the following marker genes: *nifH* (encodes a nitrogenase subunit, a key enzyme for N_2-fixation), *amoA* (encodes ammonia monooxygenase, a key enzyme for nitrification), *nirK* and *nirS* (encode nitrite reductase, a key enzyme for denitrification) and *nosZ* (encodes nitrous oxide reductase, a key enzyme for denitrification) (**Figure 18**). They found that N fertilization likewise increased the abundance of genes involved in nitrification and denitrification, which were furthermore significantly correlated with each other. It did not, however, affect the abundance of *nifH*, the gene related to biological N-fixation. In addition, the duration of the fertilization, as well as soil pH and crop rotation, were important factors in determining the response of N-cycling genes following N fertilization.

Luo *et al.* (2019) found that **not only did inorganic N fertilization contribute to crop nutrient needs, but it also impacted the resistance and resilience of soil microbial functions, which can affect crop production as well as long-term soil functioning.** Withstanding harsh drying-wetting cycles requires soil microorganisms to invest heavily in resource-intense processes, such as reassembling from spores. Under these conditions in a wheat-rice rotation, Luo *et al.* (2019) report that N inputs increased the resistance and resilience of soil microbial gene functions to stress under drying-wettingcycling. This, in turn, contributed positively to crop productivity. The authors considered the resistance and resilience of soil enzyme activities as having a greater impact on crop yields than functional gene abundance, as enzyme synthesis can be similar even with different soil microbial communities.

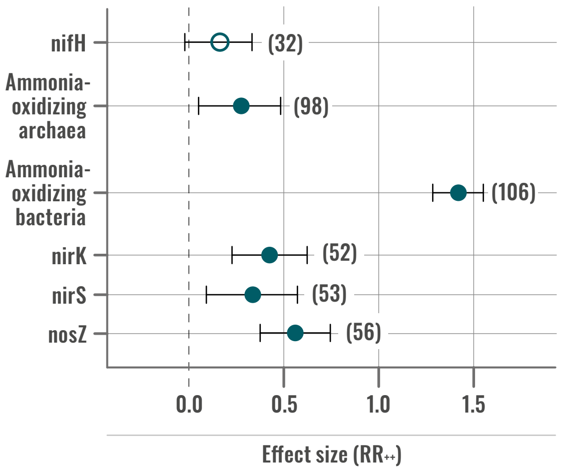
4.7.2
Comparing soil microbiome responses to organic and mineral fertilizers

While inorganic fertilization (also known as mineral, chemical, synthetic fertilization) has positive effects on plant growth and development, it can also create negative effects such as run-off and over-application, both of which can cause environmental pollution. Furthermore, application of inorganic fertilisers (e.g. ammonium (NH^+_4), N, phosphate (P)) can impair the activity of a range of beneficial microbes, such as N_2-fixing bacteria and arbuscular mycorrhizal fungi (AMF) (Akter *et al.*, 2018; Albizua *et al.*, 2015; Camenzind *et al.*, 2014; Saito *et al.*, 2014; Treseder and Allen, 2002). **Organic fertilizer can likewise positively affects plants, but in addition introduces microorganisms (e.g. present in manure), substantial carbon (C) (e.g. with plant residues) and other diverse nutrients** (Chen *et al.*, 2019c). Nutrient cycling by using organic fertilizer is thus considered important for long-term soil fertility, soil functions, and reduction in the use of external synthetic fertilizers (FAO, 2017).

FIGURE 18.

EFFECT OF NITROGEN (N) FERTILIZATION ON N-CYCLING GENE ABUNDANCE.

The dashed line represents no effect. Data points to the right of the dashed line indicate that N fertilization increases the effect of the selected parameter. Conversely, data points to the left of the dashed line indicate that N fertilization decreases the effect. The filled circles indicate that the N fertilization effect is significant ($p<0.05$), and the lines with end points represent 95 percent confidence intervals.



Adapted from Ouyang et al., 2018.

When comparing the effects of organic vs mineral fertilization, the temporal dimensions of studies are important to take into account. Short- and long-term soil responses can be very different, the latter associated with potentially more persistent impacts as they give time for a soil ecosystem to reach an equilibrium state. Significant changes in the short-term are not always observed in mineral and organic fertilization (Francioli *et al.*, 2016).

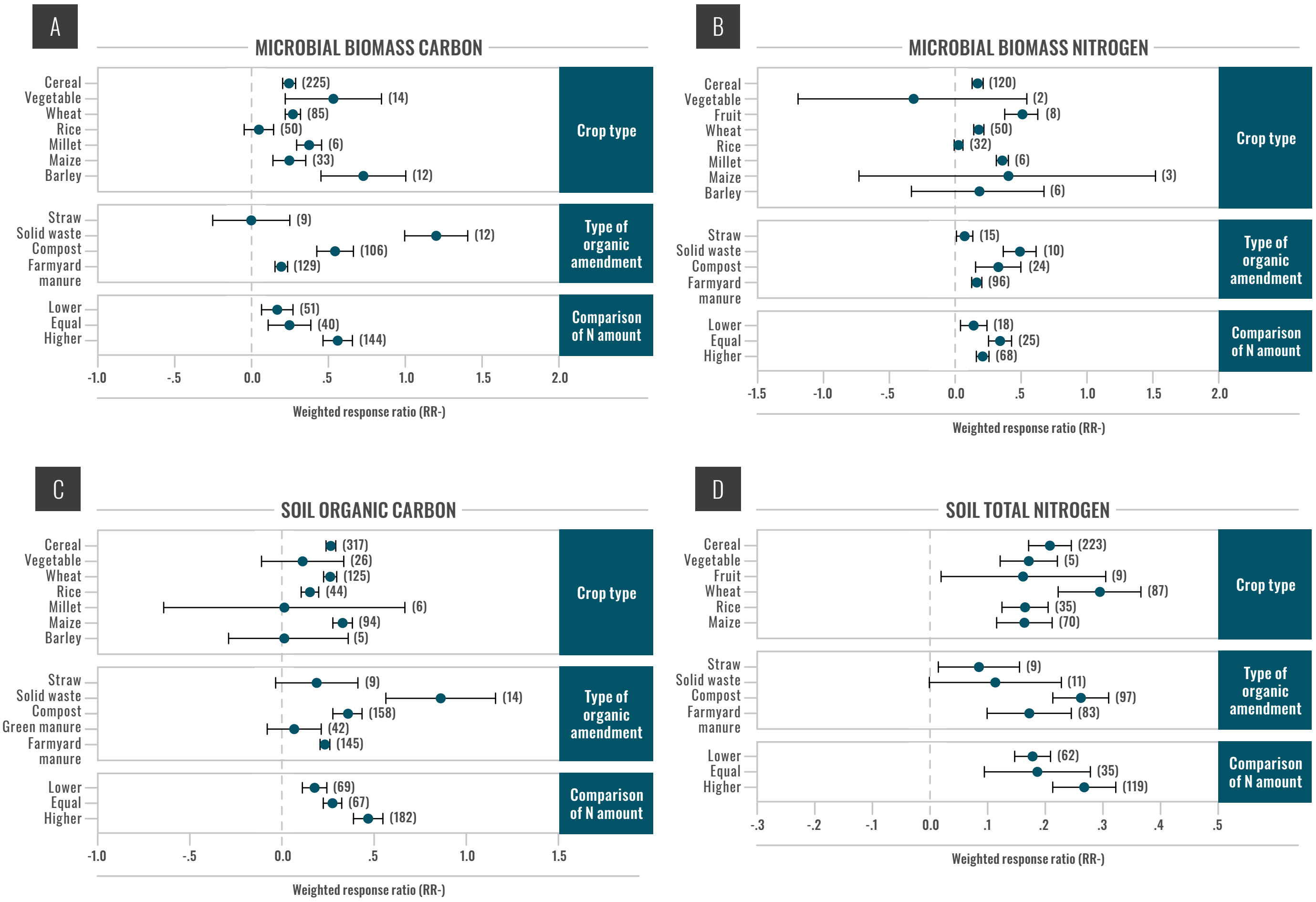
A global-scale meta-analysis found that organic fertilizers improve the soil microbiome and ecosystem functioning when compared to mineral fertilization, and that this likely increases crop yield (Luo *et al.*, 2018). The authors looked at a total of 106 studies, accounting for 690 experiments. Overall, organic amendments increased crop yield (+27 percent). Manure showed the highest effects (+49 percent), especially in wheat (+40 percent). Increases in soil organic C (SOC) (+38 percent) and total N (+20 percent), microbial biomass C (MBC) (+51 percent) and microbial biomass N (MBN) (+24 percent) were reported for the organic fertilization treatment, as compared to the mineral fertilizer-only treatment. Moreover, organic fertilizers also increased the soil microbiome enzyme activity, specifically, the hydrolytic C acquisition (+39 percent), N acquisition (+22 percent) and P acquisition (+48 percent) and oxidative decomposition (+58 percent) (**Figure 19**). The authors suggest that the last indicators likely explain the increased crop production observed under organic treatments.

In a long-term experiment (>20 years) under organic management, Hartmann *et al.* (2015) **found that organic fertilizers caused a significant shift in the soil microbiome.** The addition of a rich substrate like farmyard manure increased richness by promoting copiotrophic organisms, and therefore decreased the evenness in the different microorganism populations. **Furthermore, the differences in changes of the soil microbiome were smaller in the case of integrated management, which uses a combination of organic and synthetic nutrient amendments. The authors highlighted that differences in the soil microbiome were very much related to the quality of the organic fertilizers.** A similar study was conducted by Chen *et al.* (2019c). The authors investigated the impact of organic and inorganic fertilization for 28 years on 15 variables commonly used as proxies for ecosystem services (ESS) provided by agroecosystems. Plant productivity, nutrient transformation, organic matter (OM) decomposition, soil C and nutrient status were all positively influenced by long-term fertilization. However, the effect significantly differed between organic and inorganic fertilizers. Overall, organic fertilization increased some microbial species and genes involved in C and N metabolism and plant growth promotion**. The authors mainly attributed differences in the provision of different ESS to the changes induced by organic fertilizers to the soil microbiome. As a result, the organic fertilizers significantly increased the multifunctionality index (Figure 20).**

FIGURE 19.

THE EFFECT OF ORGANIC AMENDMENTS AND MINERAL-ONLY FERTILIZATION ON: (A) MICROBIAL BIOMASS CARBON (MBC); (B) MICROBIAL BIOMASS NITROGEN (MBN); (C) SOIL ORGANIC CARBON (SOC); AND (D) SOIL TOTAL NITROGEN (TN).

The dashed line represents no effect. Data points to the right of the dashed line indicate a larger effect of organic amendments than mineral-only fertilization on the crop yields. The horizontal lines with end points represent the 95 percent confidence interval, and the numbers in parentheses are the corresponding sample size. 'Lower', 'Equal', and 'Higher' denote the amount of N in organic relative to mineral-only fertilization.



Adapted from: Luo et al., 2018.

One last point to make from the study concerns N availability. **Long-term organic fertilization greatly promoted soil microbial functional potential associated with the decomposition of complex organics. Organic fertilizer may have contained the same quantity of N as inorganic fertilizer, but in organic only a small part of N is available immediately. This may be considered an advantage because it results in less N-leaching loss and fluctuation in available N in the long-term**. The authors only found increases in the N reductase *napA* gene in inorganic, NPK fertilized soil; these genes are indicators of inefficient N-use due to higher risks of N-leaching and denitrification.

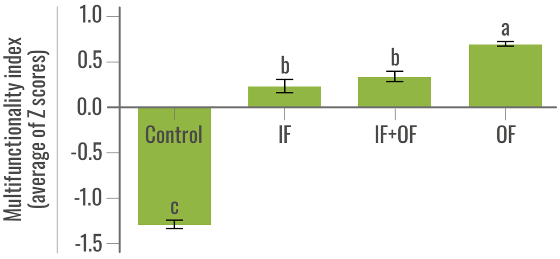
The longest trial on this topic was carried out by Francioli *et al.* (2016). They studied the effects of mineral vs organic fertilizers on soil microbial community structure, activity and abundance of agriculturally relevant microbes from a long-term experiment established in 1902. Similarly to Chen *et al.*(2019c), they found that **fertilization increased soil OM content, nutrient concentration and MBC**. Organic fertilizers furthermore triggered a larger, significant effect compared to mineral fertilizers. **Concerning the soil microbiome,**

manure fertilization increased bacterial and fungal diversity (Figure 21) and fostered copiotroph communities. Conversely, oligotroph communities were found in plots that did not receive organic manure. Concerning pathogenic or beneficial microbial communities the authors found that the fertilization strategy affected specific microbial communities. For example, *Verticillium* species (plant pathogens responsible for wilt disease) increased in the field fertilized by chemical input (only, or in combination with manure). In contrast, plant pathogenic fungi were more diffuse in unfertilized soil. The beneficial organism *Mortierella*, was positively related with farmyard manure and mineral fertilization, while *Streptomyces* (fungi responsible for biocontrol agents) had higher representation in fertilized vs unfertilized soils. The authors concluded that fertilization strategy affected the soil microbiome, and that particular fertilization treatments can have positive or negative effects on certain fungal or bacterial groups which feature beneficial or detrimental organisms. The study showed that organic fertilization could encourage some beneficial taxa while reducing certain harmful organisms. Moreover, confirming the meta-analysis results by Luo *et al.* (2018), the combined application of manure with synthetic fertilizers has often been reported as a successful strategy to increase plant biomass production. **The findings of this study suggest that such an increase in plant biomass might not only be a result of the OM and nutrient input, but also a response of the beneficial microorganisms and modified microbial activity triggered by organic fertilization. Lastly, the authors argued that as the treatments continued for so many years, their study shows stable microbial community responses.** This is valuable because many soil parameters are known to change slowly.

FIGURE 20.

MULTIFUNCTIONALITY INDEX IN RESPONSE TO 28-YEAR APPLICATION OF DIFFERENT FERTILIZATION TREATMENTS.

The control was an unfertilized treatment. Different letters indicate significant differences at the level of $\alpha = 0.05$, and the vertical lines with end points are error bars.



IF = Inorganic fertilizer – OF = Organic fertilizer
IF + OF = Inorganic and organic fertilizers

Adapted from Chen et al., 2019c.

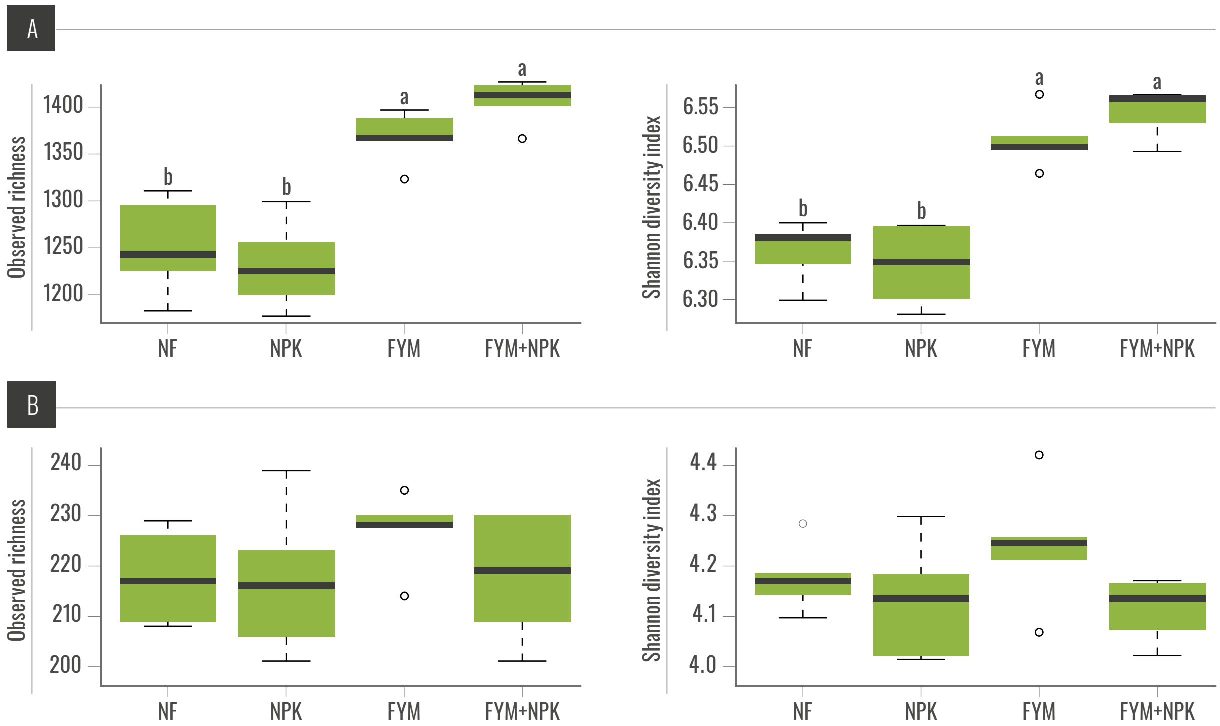
BOX 4. COULD BIOCHAR AMENDMENTS ENHANCE CARBON STORAGE IN SOILS?

Biochar is the result of biomass pyrolyzed at temperatures up to 500°C. It is considered a universal sorbent due to its ability to retain organic and inorganic contaminants. Different types of biochar have different properties (e.g. microporosity, surface area). It has been proposed as a potential amendment to store C in soils and thus positively contribute to mitigation and adaptation to climate change. Converting fresh organic material into biochar results in a product composed of organic material (it is C-rich), but that decomposes more slowly. Therefore, in theory, it could increase the stability of organic matter, or in other words, remove CO_2 from the atmosphere for long periods of time. Xu *et al.* (2015) studied the response of soil microbe C metabolism to different mineral fertilizations with and without biochar in a poplar plantation on the coast of Northern China. They experimented with the following treatments: control group, NPK fertilizers, biochar+NPK fertilizers and a high level of biochar. **The treatment with a high level of biochar showed significantly higher soil microbial metabolic C activity, suggesting that biochar may have been responsible for significantly enhancing soil microbial activity**. While biochar also provoked a shift in microbial diversity, it did not affect functional diversity. Another study by Ali *et al.* (2019) also found that biochar applications to soils grown with vegetables increased the abundance of certain bacteria, attributed to their capacity to degrade recalcitrant C compounds.

FIGURE 21.

BOX PLOTS ILLUSTRATING THE OBSERVED RICHNESS AND SHANNON'S DIVERSITY INDEX OF BACTERIAL (A) AND FUNGAL (B) COMMUNITY IN FOUR TREATMENTS.

Observed richness is the count of different species in a sample. Shannon's diversity index is a measure of species diversity in a sample that takes into account the abundance (number of species in a sample) and evenness (closeness in numbers between different species in a sample) of the species present. The different letters indicate significant differences based on Tukey's HSD test p<0.05. The vertical dashed lines with endpoints are error bars. The bold horizontal lines indicate the median values, and the dots are outlier values.



NF = No fertilization
NP = Mineral fertilizer
FYM = Farmyard manure
FYM+NPK = Farmyard manure + mineral fertilizer

Adapted from Francioli et al., 2016.

BOX 5. USING ORGANIC FERTILIZER TO STIMULATE THE SOIL MICROBIOME IN TROPICAL, ACID SOILS

Tropical soils are frequently characterized as highly-weathered acid soils, and often associated with high aluminium contents. Lime application is a known method to increase soil pH, while also ameliorating toxic effects of aluminium. It can, moreover, increase soil biological activity, and long-term applications have exhibited increased microbial biomass and soil respiration. However, organic fertilizer has also proved useful in remediation of these types of soil because it adds organic matter. This is important in soils that often do not have enough, because high temperatures and humidity increase the soil organic matter (SOM) mineralization rate.

Organic fertilization improved soil microbial activities and plant biomass in banana production in acid soils in a study by Zhang *et al.* (2019a). Their study in China compared a control with two treatments: lime and organic fertilizers. Both treatments reduced aluminium toxicity due to the increase in soil nutrient availability and pH. Organic fertilization resulted in an increase in SOC, enzyme activities, root length density, plant biomass and nutrient uptake as compared to both control and lime treatments. The number of several plant-promoting rhizobacteria (e.g. *Bradyrhizobium*, *Mesorhizobium*, *Lysobacter*, *Azospirillum*) increased following organic fertilization. Conversely, the relative abundance of pathogenic *Fusarium* decreased in the organic treatment, probably as a consequence of pathogenic communities present in the organic fertilizer. The study concluded that heavy liming is not enough to improve banana production, and that rather, organic fertilizers can play a crucial role. Not only do organic fertilizers contribute nutrients to the soil, but also influence soil microbiome activities through a priming effect.

BOX 6. FOCUS ON FERTILIZERS AND SOIL MICROORGANISMS IN RICE PADDY SOILS

Silicate (Si) fertilizers are used in rice paddies. They have been observed to improve crop productivity, alleviate soil acidification, stabilize trace elements in contaminated soils, and help alleviate abiotic and biotic stresses (e.g. salinity, toxic metals, stem borer, blast). They also play a role in rigidifying plant tissues, counteracting stress and increasing photosynthesis. Das *et al.* (2019a) reported that the Si fertilizer input increased soil pH, photosynthesis rate, nutrient availability (Si, Fe, P) and crop production, but also decreased N availability, (methane) CH_4 and (nitrous oxide) N_2O emissions. Those collectively drove changes in the soil microorganism communities. **Si input affected soil microbiome composition and abundance by increasing functional genes responsible for C degradation, C and N fixation, P utilization, CH_4 oxidation and metal detoxification. CH_4 production and denitrification genes were diminished**. Overall, their study contributed to a better understanding of Si fertilization effects on soil microorganisms, drawing attention to the potential of microbial mitigation of GHG emissions.

The effect of P fertilizers on bacterial community structure and functions in paddy soil was investigated in a long-term study in the Republic of Korea, established in 1967 (Samaddar *et al.*, 2019). The effect of P availability on the soil microbiome has not yet been widely explored. Results indicated that P fertilizers clearly triggered changes in the soil microbiome. Specifically, unfertilized plots showed a higher presence of bacteria genera known to be P-solubilizers and P-transporters. Overall, **P limitation triggered an increase in the abundance and diversity of P-cycling responsible bacteria.**

Zhan *et al.* (2018) carried out a very interesting study exploring the relationship between fertilization (inorganic, organic) and straw degradation in paddies. **They found that only a small share of community ecotypes (bacteria) actively degraded straw**, in both treatments. Organic amendments increased copiotroph bacteria due to increased fertility and organic substrate, earning them the role of "keystone species" as they were critical to the local ecosystem functions. They were associated with accelerated anoxic straw degradation in paddy soils, as observed in inorganic fertilization treatments. In summary, **the study demonstrated how under anoxic conditions typically found in paddy soils, fertilization can increase the degradation rate of straw**.

4.7.3
Impact of fertilization on soil microorganisms in deep soil layers: responses and opportunities

Most studies regarding effects of fertilization focus on the topsoil, which typically corresponds to tilling depth. **Less attention has been paid to the effects of organic fertilization in deeper soil layers, where crop roots are less present. These deeper soils matter because they hold dissolved organic C, root products and transported particulates from the topsoils** (Rumpel and Kögel-Knabner, 2011). **Deeper soils layers (below ~ 20 cm) have been estimated to contribute over 50 percent of global SOC stock due to the increased mass** (Batjes, 1996; Jobbágy and Jackson, 2000). **Biological activity frequently continues down to at least 100 cm depth** (Kramer and Gleixner, 2008; Stone and Kalisz, 1991), **and the microorganisms inhabiting those layers still play a role in soil functions**. Sandén *et al.* (2019), for example, assessed soil characteristics, nutrients and bacterial communities down to a depth of 1 meter in a long-term study with treatments of crop residues, bovine slurry, and farmyard manure. As expected, the bacterial biomass generally decreased significantly with depth, more than SOC or labile C. Nevertheless, the soil microorganisms in the deep layers were present and potentially active, as they observed that the potential N mineralization per unit of microbial biomass increased with depth. Compared to mineral fertilizer treatments, the authors observed that only **organic N fertilization affected the size and diversity of the bacterial community down to 1 m depth**.

In their review, Sosa-Hernández *et al.* (2019) also addressed deeper soils layers, arguing that **subsoil AMF should be considered**

key partners to help improve fertilization efficiency by mitigating emissions related to fertilizer application. They have the potential to do so by increasing plant nutrient uptake (of P, especially) and decreasing nutrient leaching by capturing nutrients as they descend through soil layers. The authors argue that both functions are particularly valuable in regions where fertilizer access may be irregular and limited, and under drought conditions. They also add that increasing fertilizer prices and their predicted scarcity in the future are yet another reason to optimize fertilization management with ecological approaches.

4.7.4

Effects of fertilization on soil fauna and protists via soil microorganisms

Shifts in the soil microbiome triggered by fertilization practices also affect soil fauna, which play an important role in soil and ecosystem functions (e.g. decomposition, nutrient cycling). **Soil fauna are influenced by their own gut microbiome, which is involved in the degradation of recalcitrant substances and controlling disease vectors. Soil fauna microbiomes are partly determined by the soil microbiome of their soil habitats.** Ding *et al.* (2019) demonstrated that both inorganic and organic fertilizers can provoke changes in the soil and collembola microbiomes.[29] However, they found that inorganic fertilizer application caused the most significant shifts in bacteria associated with collembola, this being a consequence of shifts in the soil bacteria microbiome. The authors explain that differences in the fertilizers likely affected the soil microbiome differently: the higher NH^+_4 found in inorganic fertilizer may have disturbed collembolan gut microbiota, known to be sensitive to NH^+_4. Soil pH, total N and total C were also different between fertilizer treatments. **The authors suggest it possible that a change in bacterial communities associated with collembola can trigger further differences in soil organic matter (SOM) degradation.**

It has also been reported that soil protists are more sensitive to N fertilization than bacteria and fungi (Zhao *et al.*, 2019).[30] Protists are an important component of the soil microbiome that have received relatively little attention in the scientific literature. It has been recommended that protistology be incorporated along with studies of soil bacteria, fungi and animals (Geisen *et al.*, 2018). **They play key roles in microbial food-webs; they are consumers of bacteria, fungi and other small eukaryotes.** They may be plant, animal, or larger protist parasites, in this way regulating populations and shaping communities. Pathogenic species can play important roles in public health issues as human parasites or as agricultural pests. Predatory soil protists can release nutrients that enhance plant growth. Some may also serve as bioindicators of soil quality. Zhao *et al.* (2019) found that N fertilizers increased the network complexity of the soil microbiome, indicating that fertilization tightened interactions between soil microorganisms. Moreover, **the study demonstrated that fertilization caused changes in the abiotic soil properties and shifts in bacterial and fungal communities, which then influenced protist diversity. The authors described this as a “bottom-up” dynamic.** As protists exhibited the strongest seasonal dynamics, the authors concluded that protists could be used as bioindicators of soil changes and in microbiome engineering that aims to reduce synthetic fertilizer and pesticide input. Lastly, they warn that reduction in protist diversity as a result of N fertilizer use may have a long-term, detrimental effect on agricultural soil ecosystem functioning.

4.7.5

Soil microorganisms as biofertilizers

Inoculating plants with beneficial, plant tissue- or rhizosphere-dwelling microorganisms could be a suitable strategy

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[29] Collembolans, also known as springtails, are arthropods. They are amongst the most numerous of macroscopic animals. They consume mainly fungi and bacteria, and play an important role in decomposition processes.

[30] Protists are unicellular eukaryotes that are not plants, fungi or animals. Amoeba, for example, are protists.

to respond to the global climate change trends and the simultaneous demand for sustainable food production (Uzoh and Babalola, 2018). Biofertilizers are products containing live, microorganisms that are expected to promote plant growth and development, offering a source of renewable, eco-friendly plant nutrients (Mącik, Gryta and Frąc, 2020). Although a number of such microorganisms have been identified (e.g. AMF, *Rhizobia*), relatively few have been developed into biofertilizers. Nevertheless, it is a promising field, as these microorganisms provide a range of potential benefits, including enhanced nutrient uptake, improved soil fertility, increased crop yields, pathogen suppression, detoxification of below-ground pollutants, and mitigation of biotic and abiotic plant stress (Mącik, Gryta and Frąc, 2020).

Bernardo *et al.* (2019) conducted a study aiming to identify metabolite responses in durum and bread wheat cultivars following inoculation by mycorrhizal species *Funneliformis mosseae* under full irrigation and water deficit regimes. **They confirmed that AMF colonization triggered changes in wheat root metabolic processes, those enhancing plant response to water stress, in particular**. Their study highlighted the importance of the three-way interaction between AMF, plants and the environmental stress of water deficit. **A meta-analysis of 290 field and greenhouse trials found that overall, mycorrhiza inoculation has been shown to increase AMF colonization with positive effects on plant biomass and shoot P** (Lekberg and Koide, 2005)**.** Nevertheless, the authors found that those effects were negligible when soil P availability and soil AMF inoculum potential were high, regardless of the farming practice applied.

Inoculating soils with *Rhizobia* to promote biological N fixation in legume cropping systems is another example where soil microorganisms can be employed as biofertilizer. In practice, effective application of the technology is predictably challenging owing to factors such as crop variety, bacterial strain compatibility and availability of limiting soil nutrients (Giller, 2001).

A third, different example of biofertilizer use involves inoculation of seeds rather than crop soils. The seed microbiome is thought to be implicated in promoting plant growth and the development of succeeding plant generations (Sergaki *et al.*, 2018). **Vertical transmission (i.e. microorganisms passed from parent generation to offspring) of fungi or bacteria has been observed; it helps transfer beneficial microbiota that assist in protection against pathogens and viruses, herbivore biocontrol, or abiotic stress mitigation** (Bragina *et al.*, 2013; Hodgson *et al.*, 2014). The bacterium *Azospirillum brasilense* is used as a plant-growth-promoting biofertilizer worldwide. It is known to inhabit the rhizosphere, providing some additional N to the plant through biological N fixation but primarily by secreting hormones that promote plant root growth and development. It is typically used by inoculating seeds, and has wide variability in success, measured, for example, by crop yields. Malinich and Bauer (2018) demonstrated that this bacteria can indeed be vertically transmitted in the common bean (*Phaseolus vulgaris* spp). Furthermore, they found that beans inoculated by vertical transmission had better *Azospirillum brasilense* colonization of plant roots than surface-inoculated sterilized seeds. The exact mechanisms to explain this phenomenon remain unknown. The authors ask what might cause the bacteria to migrate (e.g. chemicals emitted by flowers?). This study not only shows that inoculum with *Azospirillum* can be effective, but also underscores that microbe-plant associations are more complex than currently understood.

4.7.6

Organic fertilizer, the soil resistome, and antibiotic resistance

There is concern about the potential of certain agricultural practices to increase antibiotic (AR) in the soils, in conjunction with concerns about resistance worldwide to clinically relevant antibiotics. Irrigation (see Section 4.6) and application of organic fertilizers are two such practices, both of which imply potential introduction of microorganisms, ARGs, antibiotic residues, and trace elements to agricultural soils. Organic fertilizers are employed as cost-effective and sustainable fertilizers owing to their OM and mineral content, as well as their contribution to nutrient-cycling. However, **livestock farms are considered "hotspots" of ARGs that originate from veterinary antibiotics. Manure can be a rich source of bacteria that carry ARGs, and when applied as fertilizer, such manure can influence the soil ARG dynamics** (Lima, Domingues and Da Silva, 2020; Yang *et al.*, 2020). Xie *et al.* (2018), for instance, observed that in their 25-year field experiment comparing different fertilization regimes (mineral fertilizers, mineral fertilizers with straw return, pig manure, and pig manure with mineral fertilizers), the addition of pig manure introduced additional ARGs and also boosted the indigenous soil ARG members. Similarly, Chen *et al.* (2019f), also working in China, investigated the effects of swine and dairy cattle manures on ARG and bacterial communities in pasture and maize fields, compared to adjacent grassland and golf course. They likewise found that manure-amended soil, whether in grassland or maize field, generally showed increased ARG diversity. Moreover, the maize soil that frequently received raw swine manure had the greatest ARG abundance. The study by Amador *et al.* (2018) in Portugal provides one last example. Their experiment aimed to characterize soil contaminated with antibiotic-resistant bacteria, introduced by chicken and cattle manure. Ampicillin-resistant *Enterobacteriaceae* isolates from manure and manured-soil samples were tested for their resistance profile to 13 antibiotics as well as the presence of tetracycline and sulphonamide resistance genes.[31] They found higher frequencies of AR phenotypes in isolates from manured soil rather than in manure itself, and that there was higher resistance from poultry than dairy cattle manure. In addition, both manure and manured-soil showed multidrug-resistant *Enterobacteriaceae* rates of approximately 70 percent. Overall, the authors concluded that there was a risk of soil contamination caused by exposure to antibiotics introduced by manure fertilizer, and that high rates of multidrug-resistant *Enterobacteriaceae* might present a worrying public health matter due to their potential environmental spread.

One estimate quoted that worldwide use of antibiotics in livestock is expected to increase from 60,000 tons in 2010 to more than 105,000 tonnes by 2030 (Van Boeckel *et al.*, 2015). This is troubling, given that other estimates suggest that up to 90 percent of antibiotics administered to livestock are excreted either as the parent compound or a metabolite (Wei *et al.*, 2019a). The following study offers a concrete example of veterinary antibiotic transfer from livestock to soils, and the ensuing potential human health risks. Wei *et al.* (2019a) conducted a study in four Chinese provinces to investigate residue levels and distribution patterns of sulfonamides, macrolides, amphenicols, quinolones, and tetracyclines (all compounds found in known drugs administered in livestock farms) in soils from manure-fertilized vegetable farms. The authors also aimed to assess the potential ecological risk of selected veterinary antibiotics and typical resistant bacteria and ARGs (including tetracycline, plasmid-mediated quinolone, macrolide, amphenicol and sulfamethoxazole resistance). They found that 96 percent of the 53 soil samples were contaminated by the selected antibiotics, sulfonamides being highest (present in about 77 percent of samples) followed by quinolones (identified in 75 percent of samples). That antibiotic residues were higher in vegetable farm soils than in the soils of livestock farm was explained by long-term and frequent application of animal manure, leading to their accumulation. Regarding their risk assessment, the authors found that in the soils sampled, there were severe ecological risks posed by the antibiotics oxytetracycline, chlortetracycline, enrofloxacin and ciprofloxacin. They were able to isolate resistant strains in 30 samples, *Escherichia coli* (50 percent) and *Klebsiella pneumonia* (23.3 percent) being the predominant bacterial hosts. The ARGs tetA, tetB, qnrS, oqxA, sul2, ermA, and floR were detected in strains resistant to tetracyclines, quinolones, sulphonamides, macrolides, and amphenicols, respectively. The authors suggest that these strains may pose a potential threat to animal and human health via crop consumption.

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[31] Ampicillin is a penicillin antibiotic. *Enterobacteriaceae* are a large family of bacteria that include many harmless taxa as well as human pathogens such as *Salmonella* and *Escherichia coli*.

4.7.7
Complex factors challenge the prediction of soil microbiome and antimicrobial resistant gene responses to manure fertilization

Though the studies just described demonstrated that manure fertilizer can induce changes to the soil resistome, accurately predicting such impacts is difficult because of complex, interacting biotic and abiotic factors. A few examples serve to illustrate.

Lopatto *et al.* (2019) investigated the impact of swine manure (from a concentrated animal feeding operation) on the soil microbiome, looking specifically at the abundance of select ARGs and mobile genetic elements. They found that the soil microbial manure community was indeed significantly different from that of the soil samples, but that the ARG dynamics in the soil after manure application varied according to the resistance gene**. In other words, it was not possible to generalize abundance patterns across ARGs. Their results also suggested that bacteria from manure did not survive well in the soil, as similarly observed by** Chen *et al.* (2019g) **and** Liu *et al.* (2017c). In the latter study, also using pig manure, the abundance of aminoglycoside, sulfonamide and tetracycline resistance genes initially increased before gradually returning to previous levels. The authors suggested that this was due to die-off of ARG-carrying bacteria that originated in pig intestines, because they could not survive in the new soil environment. **The authors therefore concluded that changes in the soil resistome were driven by changes in bacterial community composition, rather than horizontal gene transfer.**

Though the mechanisms may not be clear, **studies have observed correlations between trace elements and AMR or ARG proliferation** (Berg, Tom-Petersen and Nybroe, 2005; Chen *et al.*, 2019b; Hu *et al.*, 2017; Knapp *et al.*, 2011). This relationship was suspected to have influenced a soil ARG bloom in a study by Lin *et al.* (2016), where a high dose of chicken manure contributed notable quantities of zinc (Zn) and copper (Cu), likely the cause of a noticeable reduction in soil microbial activity. Nevertheless, the authors stress that changes to the soil resistome did not necessarily affect the overall microbial community, because bacteria-carrying ARGs represent a relatively small proportion of the overall soil microbiome.

4.7.8
Cross-talk between soil and phyllosphere resistomes

The global, total surface of the plant phyllosphere may constitute one of the largest microhabitats on earth, some estimates suggesting twice that of the land surface (Lindow and Brandl, 2003). It seems that ARGs, transported by bacteria, can travel from the soil into the plant phyllosphere via xylem and aerosols, and furthermore, that those bacteria can colonize their new environment (Xiang *et al.*, 2020). In other words, **the soil resistome may act as a crucial reservoir of ARGs found in the plant phyllosphere. Above-ground plant biomass can also come into direct contact with ARGs during aerial spreading of manure. Both pathways suggest that ARGs may enter the food chain through consumption of crops, thereby posing a potential public health risk.** Produce typically consumed raw raises particular concern. The exact mechanisms for how this might occur, however, have not been clearly established. Several studies help explore this subject.

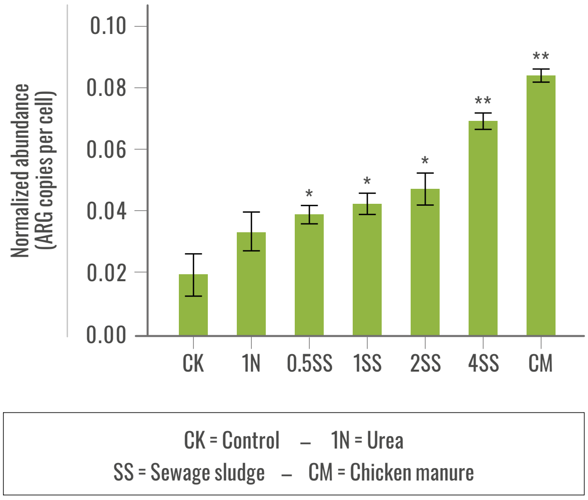
A long-term study initiated in 2006 in China assessed the effect of organic fertilizers on the antibiotic resistome in the maize phyllosphere (Chen *et al.*, 2018a). Treatments involved different levels of sewage sludge and chicken manure. **The application of sewage sludge and chicken manure increased the abundance and diversity of ARGs in the phyllosphere (Figure 22). The abundance of ARGs showed similar trends in both the soil and phyllosphere. This led the authors to suggest that soil resistome could serve as a reservoir of ARGs that can shape the phyllosphere resistome through potential 'cross-talk' between below- and above-ground plant parts. In other words, the soil resistome can influence the plant-associated microbial communities (Figure 23).**

Another study in China by Xiang *et al.* (2020) came to similar conclusions. The authors compared ARGs found in the phyllosphere and soils of farmland and forest sites, both in peri-urban settings. While the forest had experienced minimal anthropogenic disturbance, the farmland had historically received applications of human and livestock manure, the majority being pig manure (>80 percent). They found that ARG diversity in soil was significantly greater than in phyllosphere samples. Furthermore, all ARGs found in the phyllosphere were also found in soil samples, and 43 unique ARGS were detected only in soil (mainly encoding resistance to Aminoglycoside, Macrolides, Lincosamides, and Streptogramin B (MLSB) and Vancomycin, representing about 14 percent of total ARB abundance in soil). The ARGs investigated almost all recognized major classes of antibiotics commonly administered to humans and animals. The authors also thus inferred that soil resistome may represent a crucial reservoir for phyllosphere resistance. **Put simply, plant variation in ARGs was driven by the transportation of ARG-carrying soil bacteria to plants.**

FIGURE 22.

THE NORMALIZED ABUNDANCE OF ANTIBIOTIC RESISTANCE GENES (ARGS) IN THE LEAF PHYLLOSPHERE OF MAIZE UNDER SEVEN DIFFERENT FERTILIZATION TREATMENTS.

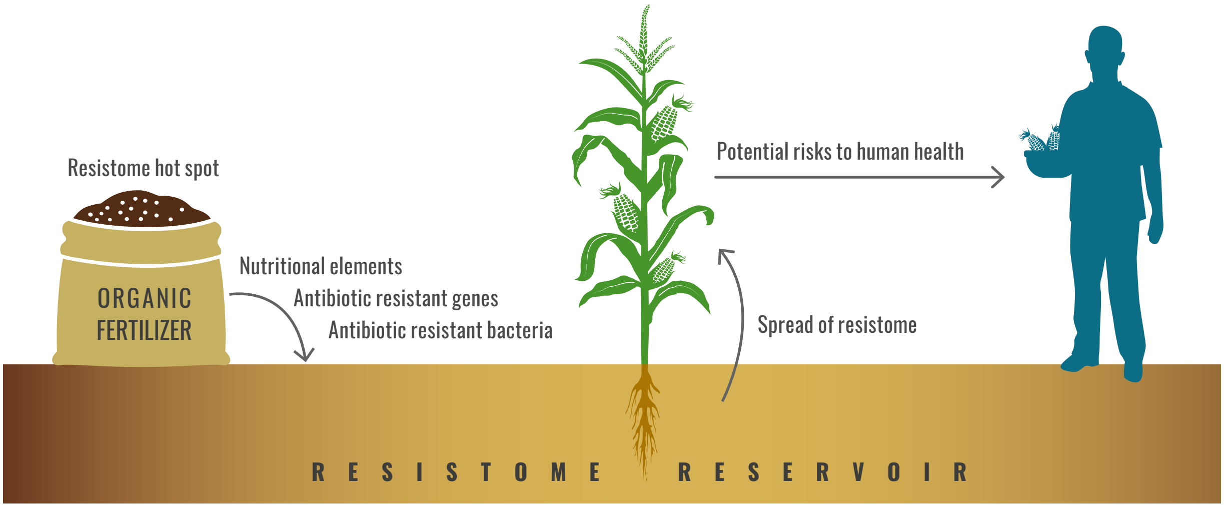
The vertical lines with end points are error bars. **(P<0.01) and *(P<0.05) on the error bars indicate that fertilization significantly increased the abundance of ARGs.



Adapted from Chen et al., 2018a.

FIGURE 23.

GRAPHICAL REPRESENTATION OF POTENTIAL RISKS ASSOCIATED WITH SOIL AS A RESISTOME RESERVOIR.



Adapted from Chen et al., 2018a.

Another study in China also explored the phyllosphere of rice and wheat grown under pig manure fertilization to ascertain the presence of ARGs (Zhou *et al.*, 2019). Four treatments were applied, namely: unfertilized control, mineral fertilizer, clean (reduced antibiotic practice) and dirty (current antibiotic practice) pig manure. Both clean and dirty pig manure increased presence of ARGs, and dirty manure more than clean. Among crops, wheat showed higher ARG presence than rice. The reasons are likely due to multiple factors, but the authors highlighted several possibilities. One is that different bacterial phyllosphere communities exist between crops. Secondly, there was a lower bacterial community diversity in the rice phyllosphere, perhaps due to the high soil water content in rice paddies. And thirdly, the high water content in rice paddies may have reduced the contact-pathway from manure-amended soils to the phyllosphere. **The study draws attention to the influential role of pig manure and ARGs, which can accumulate in soil, pass to above-ground parts of staple crops and therefore potentially cause health problems for consumers.**

This issue is even more worrisome when dealing with leafy vegetables that may be consumed raw. A recent study on the roots and phyllosphere of radish and lettuce was implemented with raw manure from antibiotic-treated cows, composted manure from antibiotic-treated cows, composted manure from antibiotic-free cows, and an inorganic chemical fertilizer control (Fogler *et al.*, 2019). **The greatest number of ARG classes were found on lettuce in the antibiotic raw manure treatment. Therefore, the authors concluded that using raw manure from antibiotic-treated cattle may affect the microbiota associated with the leaf surface of lettuce.** Composting manure was suggested by the authors as a strategy to decrease ARGs (as by other studies, e.g. Marti *et al.*, 2013). In addition, since the resistomes between radish and lettuce differed, the authors suggest that the extent of soil contact with leaves should also be considered as a strategy to reduce ARGs on fresh produce.

Guron *et al.* (2019) explored the effects of manure-based composts and other organic amendments on ARGs, also using lettuce (leaves) and radish (roots). Composts were based on manure from antibiotic-treated dairy cattle and manure from antibiotic-free dairy cattle. **Results demonstrated that the vegetable type had a strong effect on the resistomes and the diversity of microbiota**. Radishes had higher load and diversity of ARGs than lettuce. Overall, the results indicate that the resistome of the crop grown using organic fertilizers was significantly different from the control, and suggest that composting alters the resistomes. In addition, this study reported higher species richness in the phyllosphere microbiome for both crops when amended with organic fertilizers.

Concluding remarks

Several points are worth revisiting in order to conclude the broad subject of how fertilizers can affect the soil microbiome. First, organic and inorganic fertilizer application can influence the composition and activities of soil microorganisms. Second, one must consider the temporal dimension, as it may take time for stable effects to become evident. Third, organic fertilizer, compared to inorganic, can further enhance the soil microbiome, resulting in improved ecosystem functioning and ESS provision. This can occur, for instance, by provoking an increase in bacterial and fungal species diversity, including groups that degrade complex organic compounds. This is also related to the observation that organic fertilizers can increase microbial enzyme activities that promote nutrition acquisition. Beneficial and pathogenic soil bacteria have been observed to respond strongly to organic fertilizer; thus, the observed increases in plant biomass may be a combined result of organic fertilization and the positive response of beneficial organisms, not just fertilizer alone. Furthermore, the quality of organic fertilizer has a relationship with the effect on the soil microbiome. **On the subject of organic fertilizer: while manure fertilizer returns precious nutrients to the soil, it can also carry ARGs, provoking concerns about the risk of antimicrobial resistance (AMR) entering the agri-food system.** Potential cross-talk between the soil and phyllosphere resistomes is another aspect to explore in to ARG risk assessment, particularly for produce typically consumed raw. Effects are difficult to predict, owing to the multiple and complex biotic and abiotic interactions in the soil.

A fourth point concerns the great potential of biofertilizers from an agro-ecological perspective: microorganisms can be used as fertilizers, themselves. Examples include inoculation of seeds and crop soils.

Lastly, some less studied areas merit more attention. One is how fertilization can affect the microbiome communities and activities in soil layers beneath the topsoil. This is important because there may be opportunities to reduce nutrient leaching and promote soil C storage. **A second is soil microbiome interaction with gut microbiomes of soil fauna, which may influence OM decomposition processes. A third concerns protistology.** Given their abundance, their key roles in food web interactions, and their sensitivity to N fertilization, the response of protists to fertilization practices also deserves more attention.

4.7.9

Systematic review: Effects of fertilization on the soil microbiome, and their combined effect on climate change

We selected 86 of 696 studies returned during the searches for this section, according to their relevance.

A fertilizer's type of nutrients, the quantity, and N-form can directly or indirectly impact the soil microbiome, thereby influencing biogeochemical cycling. It is a substantial subject because not only is nutrient-returning a critical aspect of agricultural systems, but also because the variety of fertilizers and cropping systems are wide.

This chapter starts with an overview of plant-microbial linkages that demonstrate potential to decrease N-losses from agriculture. Next it outlines some fundamental relationships before the rest of the chapter delves into more technical descriptions of biogeochemical cycling.[32] This is followed by an introduction to which soil microorganisms are impacted by typical fertilizer forms, why and how they respond, and the potential implications for soil GHG fluxes and nutrient cycling. These dynamics are subsequently discussed in more detail according to: (i) the different rates and forms of inorganic N fertilizer and (ii) organic fertilization, which includes use of manure, anaerobic digestates, thermophilic digested sewage sludge, biofertilizers, and biochar, (iii) use of slow-release fertilizers, (iv) a brief exploration of fertilization in combination with other agricultural practices (tillage and intercropping), and finally, (v) inorganic and organic fertilization in rice paddies.

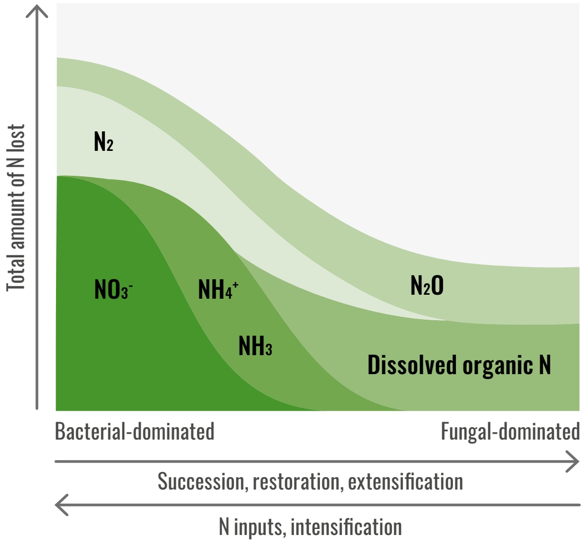
Nitrogen cycling responses to plant-microbial relationships

The review by De Vries and Bardgett (2012) **concludes that systems with tight plant and soil microbial linkages, and with fungal-dominant food webs demonstrate the greatest potential to decrease N-losses, including in the form of N_2O.** They reported that the **intensification of N inputs influenced soil bacterial communities, which in turn altered the N-loss pathways** (Figure 24).

FIGURE 24.

ABSOLUTE AMOUNTS AND DOMINANT PATHWAYS OF NITROGEN (N) LOSS ALONG A GRADIENT FROM N-RICH TO N-POOR ECOSYSTEMS.

N is nitrogen, N_2 is dinitrogen (nitrogen gas), NO_3^- is nitrate, NH_4^+ is ammonium, NH_3 is ammonia, N_2O is nitrous oxide, and DON is dissolved organic nitrogen.



Adapted from de Vries and Bardgett, 2012.

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[32] Please refer to **Section 3.2.2** for a discussion of key nutrient cycles.

Factors that influence soil microbial responses and related biogeochemical processes to nitrogen fertilization

Numerous factors influence the soil microbial response to fertilization, with potential consequences on soil N_2O emission. These include the rate and supply of fertilizer, N form, soil pH, soil type, and seasonal variation.

It has been known for some time that maintaining higher rates of soil N than organic C over a long period promotes the development of microbial communities capable of producing more N_2O under conducive conditions (Clark *et al.*, 2012). Such soils are home to denitrifier communities that feature fewer copies of *nosZ* genes, implying a diminished ability to reduce N_2O to N_2. Moreover, mineral N fertilizers provide a source of energy for NH_3 oxidizers, which produce N_2O as a result of several metabolic processes carried out by ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) and complete ammonia oxidizers (commamox bacteria). The N_2O yield of AOB is highest of the three (Hink *et al.*, 2018; Prosser *et al.*, 2020).

Ammonia-oxidizing archaea and AOB seem to have niche differentiation in terms of environmental conditions. Low soil pH favours AOA, and low rates of ammonium supply (like slow release fertilizers) favour AOA, while a high supply (like high concentrations of inorganic ammonium or urea) favour AOB (Akiyama *et al.*, 2013; Soares *et al.*, 2016; Wang *et al.*, 2017b). Many studies have shown that fertilization stimulated AOB abundance more than AOA, thereby attributing increased N_2O emissions primarily to the former (Bao *et al.*, 2012; Chen *et al.*, 2020; Lourenço *et al.*, 2018; Ouyang *et al.*, 2018; Wakelin *et al.*, 2007). However, some studies have found that with N fertilization, AOA had a stronger correlation with N_2O production and were more abundant than AOB (Inselsbacher *et al.*, 2011). It was also suggested that AOA, rather than AOB, were the active contributors to nitrification during a winter experiment (Tatti *et al.*, 2014). **Understanding more about the microbial nitrifying and denitrifying communities and their activities could help improve both fertilizer efficiency as well as N_2O emission mitigation** (Hink *et al.*, 2018; Prosser *et al.*, 2020).

Soil microbial nitrifiers and denitrifiers can respond differently according to the soil type (Chen *et al.*, 2020; Inselsbacher *et al.*, 2011; Wang *et al.*, 2015) **as well as the N form** (Castellano-Hinojosa, González-López and Bedmar, 2018; Wang *et al.*, 2017b). Chen *et al.* (2020), for instance, described how excessive urea N fertilization was more effective in inhibiting N_2O emissions in an anthrosol than a fluvo-aquci soil. The authors suggested that the acidic anthrosol may have limited soil nitrification activities, pH being a known, critical factor that determines the both the distribution and activities of NH_3 oxidizers. Wang *et al.* (2015) similarly found that five different paddy soil types fertilized with urea resulted in increased but varied N_2O emissions. Another study reported that ammonium fertilizer increased the AOB abundance and *nirK* genes in alluvial soil, but reduced abundances of AOA and *nirK* and *nosZ* in a red soil, and that NH_3^- fertilizer had a negative effect on AOA abundance in a red soil, only (Wang *et al.*, 2017b). Furthermore, different fertilization forms appeared to affect the biodegradation of water extractable OM in one study, owing to the latter's inherent traits as well as soil solution properties, thus shaping the soil microbial community structure and their activities (Hui *et al.*, 2019).

Ammonia oxidizers are not the only soil microorganisms influenced by fertilization. **Fungal and bacterial populations can also be affected, with consequences for GHG fluxes and soil C cycling.** Working with forest soils, Silva-Sánchez *et al.* (2019) observed that when fungal growth was enhanced (as a competitive response to inhibition of bacteria by mineral N or low pH), it stimulated detrital C-use, creating a reduced CUE. When litter was combined with increased N it caused a shift towards fungal dominance. Adding more litter stimulated both bacteria and fungi, but when the pH increased with litter, the ratio shifted in favour of bacteria, thus bacterial growth was enhanced by increased pH and reduced by mineral N, and fungal growth was unaffected by both factors. This is consistent with other studies that observed shifts in fungal communities in response to N fertilizer and crop residues (e.g. Wakelin *et al.*, 2007).

There are yet other factors that influence the dynamics between fertilization, the response of the soil microbiome and subsequent soil nutrient cycling. The microbial response in the rhizosphere vs bulk soil can have different consequences on N_2O and N_2 emissions (Senbayram *et al.*, 2020). **The N fertilization history of soils, and not just oxygen availability, can drive different contributions of nitrification and denitrification to soil N_2O emissions** (Castellano-Hinojosa *et al.*, 2020). **Where in the soil particle size fractions these processes occur is another point to consider.** A study comparing inorganic and organic fertilizers determined that the fertilizer applications caused a stronger change in the bacterium and methanogen activities in the coarse fractions, where CH_4 was predominantly produced (Zhang *et al.*, 2007). There was, however, only a minor effect on the methanogenic archaeal community in the particle size fractions. The authors also suggested that higher production of CO_2 and CH_4 in the coarse fractions may have contributed to storage of labile organic C within those fractions. **Seasonal variations are another factor to consider, given that even in winter, fields can emit N_2O.** Tatti *et al.* (2014) found that winter nitrifier and denitrifier communities showed different compositions depending on the N source – mineral N fertilizer, cattle or poultry manure – which had been applied the previous spring. They did not, however, find any correlations between nitrifier and denitrifier community abundance or composition and N_2O and denitrification.

Finally, before exploring further, **it is important to take into account multiple, interactive factors and their potential trade-offs when weighing the value of agricultural management practices.** For example, a study comparing dry-seeding and puddle-planted rice and different fertilizer forms, found that under their experimental conditions, dry seeded rice had an approximately 75 percent reduction in CH_4 emissions but emitted significantly higher N_2O and CO_2 (Mohanty *et al.*, 2017). Nevertheless, the Global Warming Potential (GWP) of dry-seeded rice was lower than puddle-planted. When the lower grain yield was taken into account, however, the Greenhouse Gas Intensity (GHGI) of both was comparable.[33] The authors suggest that soil moisture conditions influenced the soil microbial enzyme activities and populations, and thereby impacted how they differently processed the varying N fertilizer forms. The ammonium oxidizers, for instance, use ammonium-nitrogen (NH_4^+-N) in their aerobic oxidation process – therefore their populations were likely influenced by both the availability of ammonium and the aeration status of the soil (Mohanty *et al.*, 2017).

Effects of different rates and forms of inorganic nitrogen fertilizer on soil microorganisms and greenhouse gas fluxes

In their meta-analysis of 82 field studies, Treseder *et al.* (2008) describe that **soil microbial biomass declined about 15 percent on average under N fertilization, and more substantially under heavier N loads and longer fertilization durations**. Fungi, in particular, decreased with these two factors. Across studies that focused on fungi, however, the authors observed no significant decline with N fertilization, owing to a small number of short-term studies that recorded fungal increases with N fertilization. Furthermore, there was a significant correlation between soil CO_2 emission and the soil microbial biomass response to N fertilization. There was no significant effect of N fertilizer form. Treseder *et al.* (2008) therefore concluded that N fertilization enrichment could reduce soil microbial biomass in different ecosystems, with corresponding declines in CO_2 emissions.

Another, more recent meta-analysis of 47 field studies investigated the response of N-cycling genes to N fertilization. Ouyang *et al.* (2018) determined that **overall, N fertilization did not affect the abundance of *nifH*, the gene related to N fixation, but did increase the *amoA* gene abundance from both archaea and bacteria.** AOB was, however, nine times more responsive than AOA to N fertilization. They explain that this effect may have been due to their physiological responses to ammonium availability, implying that they may be a more important target group

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[33] The Greenhouse Gas Intensity (GHGI) was calculated by dividing the global warming potential (GWP) by grain yield.

for N management, as suggested by other studies already mentioned above (e.g. Chen *et al.*, 2020; Wakelin *et al.*, 2007). The authors found that N fertilization also increased abundance of denitrifier groups (either *nirK*, *nirS* or *nosZ* genes), which were significantly correlated with each other. They suggested that *nifH* probably did not respond because the genes were carried primarily by symbiotic N-fixers, which live in plant-controlled environments and were therefore less likely to respond to environmental changes such as fertilization. The authors furthermore identified that the duration of fertilization was one important factor in determining the response of N-cycling genes following N fertilization. Genes *nifH* and *nosZ* showed the greatest increases when fertilization duration was less than five years and between 10 to 20 years, respectively, while AOB showed a stronger change when the duration was longer than 20 years.

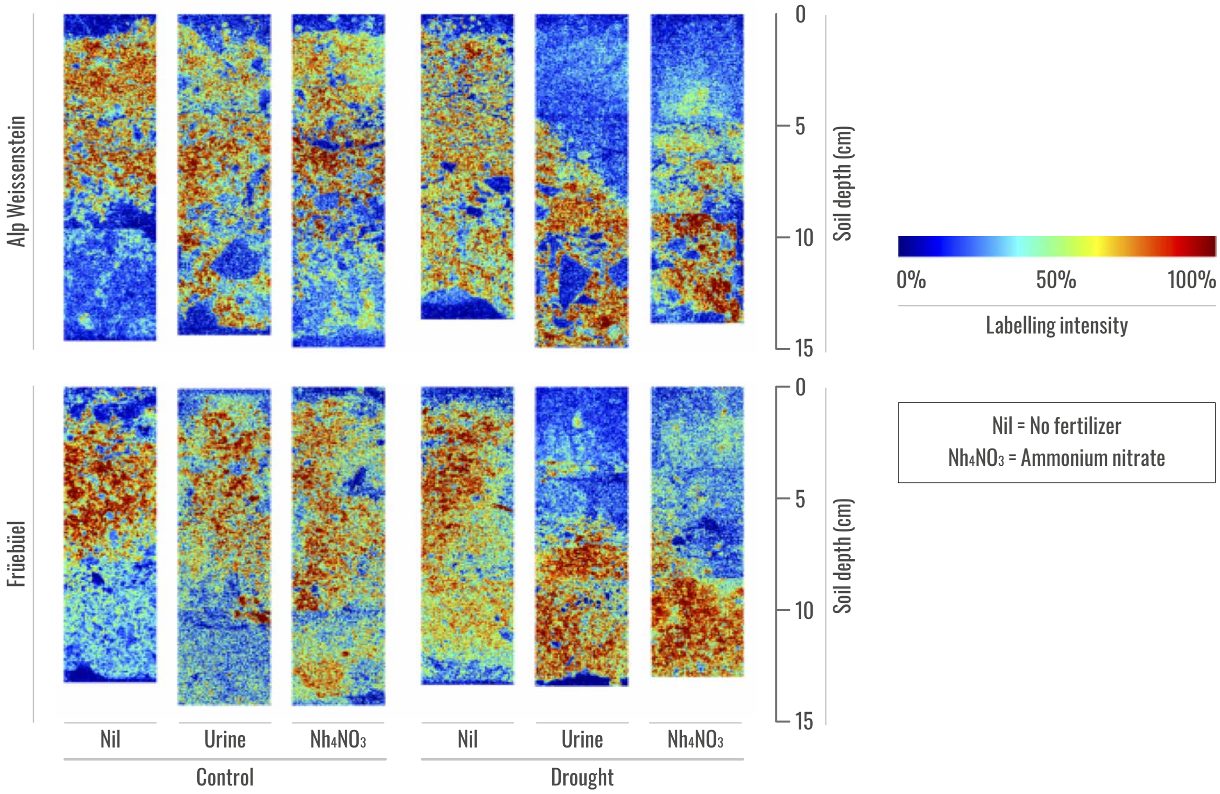
Several studies have identified an increase in N_2O emission in response to increasing N fertilizer rates (Avrahami and Bohannan, 2009; Qiu *et al.*, 2020; Yang, Hamel and Gan, 2015). Yang, Hamel and Gan (2015) found that increasing N fertilizer rates in canola fields increased the abundance of nitrite reductase-carrying denitrifiers more than N_2O reductase-carrying denitrifiers. This suggests potential to increase the rate of transformation of NO_3 to N_2O, rather than N_2O to N_2 in such high N input cropping systems, thereby increasing the risk of higher N_2O emissions. Other factors such as soil moisture and temperature may also have an interactive effect on fertilization, as reported by Avrahami and Bohannan (2009) in their experiment with soils amended with low or moderate N fertilizer. They found that N_2O emission rates decreased sharply with increasing soil moisture and temperature. The soil moisture influenced the rates directly, but also indirectly though AOB community structure, albeit less so. In contrast, when soils were amended with high amounts of N fertilizer, the N_2O emission rates were rather influenced by an interaction between soil moisture and temperature. The authors believed that the main pathway by which NH_3 influenced N_2O nitrification rates was indirect, seemingly through the abundance of one particular phylogenetic group (AOB ‘cluster 10’). They suggested that given the high emission rates recorded at the lowest soil moistures, bacterial nitrifiers may use denitrification as a stress response. Another study found that while N fertilization significantly increased N_2O and CO_2 emissions, it also increased SOC as well as soil bacterial diversity and abundance of N-cycling functional groups (Qiu *et al.*, 2020). **N fertilizer applied to these dryland soils improved the soil fertility and enhanced the soil bacterial community composition and functioning, which likely explaind the increased GHG emissions** (Qiu *et al.*, 2020). In the Loess Plateau, where soils are known to have low N content, Wang *et al.* (2019a) concluded that N fertilizer seemed suitable to sustain crop yields, increase SOC storage and minimize CO_2 emission. The five rates of N fertilizer all increased soil respiration, which was positively correlated with grain yield. Changes in bacterial abundance at the phyla level were related to the enhanced respiration: Acidobacteria showed a positive correlation to soil respiration, while Proteobacteria and Bacteroidetes showed a negative correlation. The authors point out that despite the elevated respiration, results imply that this soil microbial activity did not reduce SOC levels as might have been expected. Other research in cropland and grass meadows found that long-term mineral fertilization in croplands also altered the soil microbial community, increasing SOC stocks significantly, though they did not reach those measured for meadow soils (Li *et al.*, 2020b). The authors also suggested that macroaggregates may be a key driver of changes in bacterial community and SOC; they were important in providing microbial community habitat as well as physical protection for SOC in the study.

While Wang *et al.* (2019a) found that N fertilization in the Loess Plateau reduced the annual temperature sensitivity of CO_2 emission (soil respiration), Song *et al.* (2018) report that **soil amendment with NH_4NO_3 enhanced the temperature sensitivity of N_2O emission**. This was associated with faster N mineralization, an increased proportion of nitrification-N_2O emission, and faster growth of AOB at higher temperatures. Denitrification also factored in the higher temperature sensitivity of N_2O with NH_4NO_3, but due to an increase in substrate availability rather than a shift in the soil microbial community.

Stiehl-Braun *et al.* (2011) investigated the interactive effects of drought and N fertilization on the spatial distribution of methanotrophic bacteria in Swiss grasslands. The N fertilizer treatments were a control, NH_4NO_3, or cattle urine. The combination of autoradiographic methods with CH_4 flux shed light on the mechanisms related to CH_4 uptake. **Drought clearly increased the soil CH_4 sink by facilitating gas diffusion and pushed the main area of CH_4 assimilation to deeper depths** (Figure 25). **Concerning fertilizers, the results were more complex. First, a decreased level of CH_4 assimilation was found under NH_4NO_3.** Cattle urine also saw a decrease, but only when applied under drought conditions and probably because NH_4^+ was not taken up by plants or nitrified under those conditions. **Second, the effect of fertilizers strongly depended on the soil layer, there being an inhibitory effect in the top soil.** This was influenced by the soil structure and thus soil hydraulic characteristics, which determine the portion of soils reached by fertilizers. **Third, CH_4 oxidation in deeper layers could compensate for the reduction in methanotrophic activity in the top soil, but only when soils are dry and diffusion through those layers is sufficient.** This suggests that top soil samples should not be used to infer CH_4 sinks at a larger scale of analysis. The authors warned that any quantification of biomass and activity of methanotrophic microbes is particularly weak if the spatial organisation of soils is not taken into account. **Other studies have also highlighted the shifts in soil microbial abundance and activities with soil depth**. For example, one factor explaining variations in N gas, N_2O and N_2 emissions down to a 20 cm depth was the soil depth-related dissolved oxygen content, where nitrification production of N_2O dominated in the 0-10 cm soil horizon and denitrification production of N gas in the 10-20 cm horizon (Castellano-Hinojosa, González-López and Bedmar, 2018). In the same study, AOB and AOA abundance decreased with soil depth, while denitrification gene abundance increased with depth to reach maximal values in the deepest soil layer.

FIGURE 25

AUTORADIOGRAPHS SHOWING THE RADIOCARBON (^{14}C) DISTRIBUTION IN SECTIONS OF RADIOCARBON METHANE ($^{14}CH_4$)-LABELLED SOIL CORES, ACCORDING TO TWO SITES (FRÜEBÜEL OR ALP WEISSENSTEIN), THE SIMULATED DROUGHT, AND NITROGEN (N) FERTILIZATION.



Source: Stiehl-Braun et al., 2011.

To conclude, inorganic N fertilizer generally appears to reduce soil microbial biomass, particularly with heavier loads and longer durations. Fungi appear to be particularly sensitive to N fertilizer. It also seems to increase *amoA* gene abundance from both AOB and AOA, the former being notably more responsive. Shifts in NH_3-oxidizing microbial populations is likely related to the observation that increasing rates of N fertilization result in increased N_2O emissions. Similarly, the improved soil fertility, caused by multiple effects of N input, can enhance the overall soil bacterial community composition and functioning resulting in increased microbial production of N_2O and CO_2. However, in the case of inorganic N input, this enhanced microbial activity can increase the decomposition of low-quality organic inputs and SOM. This can lead to a continuing decline of SOM content, resulting in a corresponding decline in soil structure and the detrimental consequences on its ability to retain water, air and nutrients (Beed *et al.*, 2011). Lastly, soil depth also needs to be taken into account in analyses of fertilizer effects on biogeochemical cycling, because microbial communities and activities vary in different soil layers. For instance, there appears to be an inhibitory effect of fertilizers on CH_4 assimilation in the top soil, while CH_4 oxidation could be significant in deeper layers under conducive soil conditions.

Effects of phosphorus, potassium and zinc fertilizers on soil microorganisms involved in soil GHG fluxes

In contrast to the literature above that focuses on addition of nutrients, it appears that a lack of soil P and K can cause changes in plant growth states and soil properties, driving fluctuations in the population dynamics of methanogens and methanotrophs (Sheng *et al.*, 2016). Compared to soils with NPK input, soil without P input had significantly reduced CH_4 flux rates, whereas soils without K input did not. Sheng *et al.* (2016) suggest that P-deficiency induced changes in the soil physiochemical properties, which may have constrained methanogen activity, while methanotrophs may have been better adapted. The authors observed less methanogens and methanotrophs in K-deficient soils.

It appears that applying synthetic Zn chalates can help reduce yield-scaled GHG emissions while enhancing Nz biofortification for human nutrition (Montoya *et al.*, 2018). Bacterial *amoA*, *nirK*, *nirS* and *norB* gene abundances, as well as the extractable Cu content, decreased with Zn chalate application, suggesting that enzymes of metal co-factors of Cu were chelated (Montoya *et al.*, 2018).

Effects of organic fertilization and soil carbon cycling

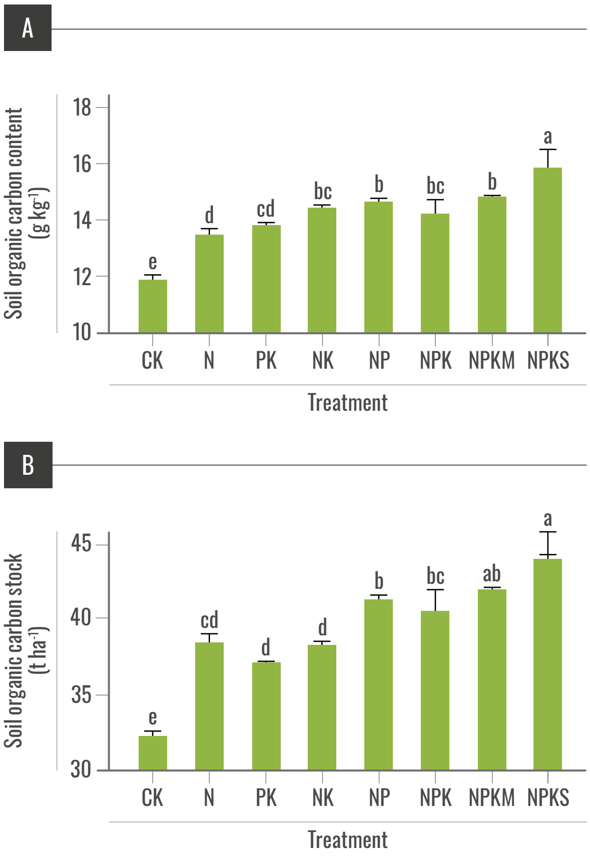
It has been observed that in most cases, fertilization may increase or at least maintain SOC, but fertilization with organic fertilizers further enhanced SOC. In their 12-year experiment in a rice-wheat rotation system, Zhao *et al.* (2015) confirmed that treatments with mineral plus organic input (pig manure and rice straw), compared to mineral fertilizer alone, showed the highest organic C content and stock (**Figure 26**), amounts of culturable bacteria, actinomycetes and fungi (**Figure 27**). NPK plus organic amendments also affected the functional community structure of the anaerobic bacteria. Specifically, those treatments increased the population of anaerobic cellulolytic bacteria, anaerobic fermentative bacteria, hydrogen-producing acetogen, methanogenic bacteria, denitrifying bacteria, and sulphate-reducing bacteria than treatments with the NPK fertilization only or no fertilization. Lastly, bacteria, Actinomycetes and fungi correlated with SOC content.

CO_2-fixing bacteria are an ally in the mitigation of climate change. These bacteria assimilate CO_2 and have displayed great adaptability to extreme conditions (e.g. volcanic sediments and lake wetlands), and can respond to both organic and inorganic fertilization. Yuan *et al.* (2012) studied the impact of chemical and organic fertilizers on CO_2-fixing bacteria in a long-term paddy experiment in China, observing a significant effect of fertilization treatments on the abundance and community structure of the CO_2-fixing bacteria. Fertilization generally increased cbbL gene diversity, with the NPK treatments showing the highest diversity.[34] However, treatments with crop residues revealed the highest abundance of bacterial cbbL genes, accounting for 1.5 times the amount compared to the control.

FIGURE 26.

SOIL ORGANIC CARBON CONTENT AND STOCK IN THE PLOUGH LAYER OF PURPLE PADDY SOIL AS AFFECTED BY LONG-TERM FERTILIZATIONS.

Treatments describe the type of chemical fertilizer applied. Vertical bars with end points are error bars. Different lower case letters in the same set indicate significant differences at P<0.05 level.



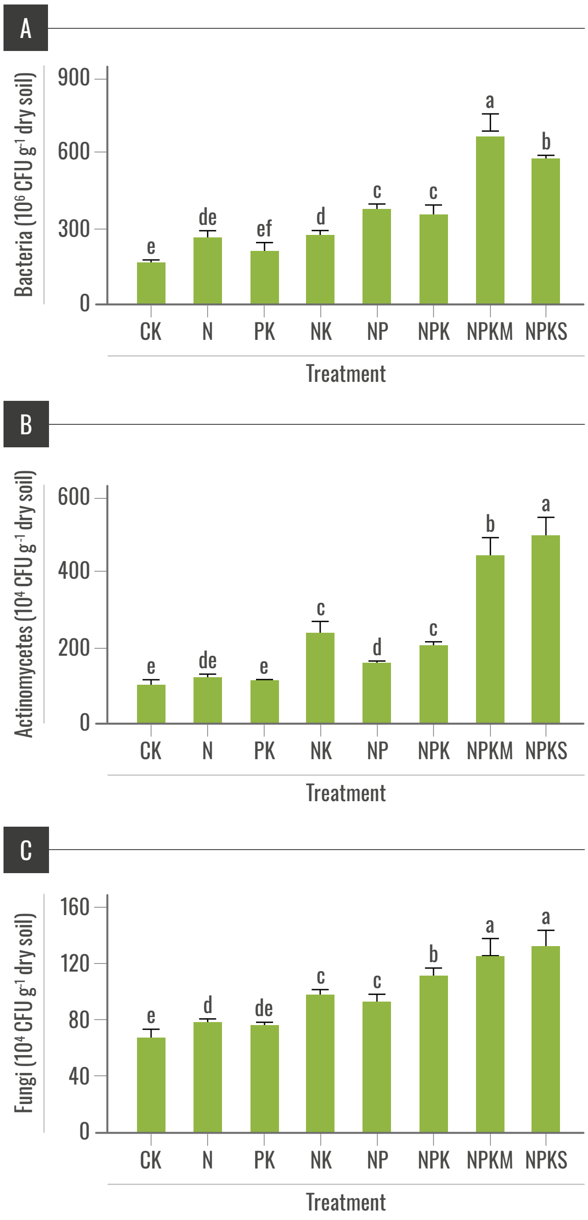
CK = Control (no fertilization) – N = Nitrogen –
PK = Phosphate and potassium – NK = Nitrogen and potassium
NP = Nitrogen and phosphate – NPK = Nitrogen-phosphate-potassium
NPKM = Nitrogen-phosphate-potassium + pig manure
NPKS = Nitrogen-phosphate-potassium fertilizer + rice straw return

Adapted from Zhao et al., 2015.

FIGURE 27.

POPULATIONS OF MAJOR SOIL MICROBES IN PURPLE PADDY SOIL AS AFFECTED BY LONG-TERM FERTILIZATIONS.

Microbes measured in colony-forming units (CFUs), used to estimate the number of microbe cells in a sample per gram of dry soil. Vertical bars with end points are error bars. Different lower case letters in the same set indicate significant differences at P<0.05 level.



CK = Control (no fertilization) – N = Nitrogen
PK = Phosphate and potassium – NK = Nitrogen and potassium
NP = Nitrogen and phosphate – NPK = Nitrogen-phosphate-potassium
NPKM = Nitrogen-phosphate-potassium + pig manure
NPKS = Nitrogen-phosphate-potassium fertilizer + rice straw return

Adapted from Zhao et al., 2015.

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[34] cbbL is a gene responsible for coding Rubisco enzymes, which are crucial in the process of carbon fixation.

Effects of organic fertilization can result in reduced nitrous oxide emissions

Organic fertilization can enhance soil fertility and ecological factors compared to inorganic fertilizers. One aspect is that N mineralization from natural degradation processes fosters low reactive N and thus the potential to reduce N_2O emissions through coupling of C and N. For example, Bhowmik *et al.* (2016) showed that long-term, organic mixed-compost had the best potential (compared to broiler litter and a perennial pasture system) to minimize N_2O emissions by immobilizing excess NH_3^- with the build-up of soil C pools. Krauss *et al.* (2017) recorded the highest N_2O emissions with application of inorganic fertilizer (calcium ammoniate), followed by dairy slurry and then manure compost. Soil N_2O emission in the first two treatments of their study correlated positively to AOB abundances. Lower N_2O production was associated with *nosZ* abundance and higher C levels, as evidenced in the organic treatments. A second, related, aspect is the enhancement of abundance and diversity of soil microorganisms, whose activities influence soil quality and biogeochemical cycling (Calleja-Cervantes *et al.*, 2015). A field experiment in Spain indicated that soil microbial communities involved in biological N-cycling (fixation and denitrification, in particular) were more abundant after 10 years of organic fertilization (combination of grape prunings, combined with sheep manure or legume cover) than conventional management (Pereg *et al.*, 2018). The experiment also identified a significant increase in abundances of bacterial *nifH*, *nosZ*, *nirS* and *nirK* genes in the organic treatments, which were likewise linked to increased SOC. The abundance of *nifH* was lower with use of fertilizers rich in NH_3 and NH_3^-. The authors suggest that its increase under organic fertilization was more related to the availability of organic C than to the nature of the organic amendment. AOA abundance did not correlate with the type of fertilization, and AOB abundance was more dependent on the availability of soluble N than on the type of management. According to the authors, the increase in *nosZ* under organic fertilization may suggest a greater abundance of denitrifiers with the ability to reduce N_2O to N_2, thus potentially reducing soil emission of this GHG.

Effects of organic fertilization can result in increased nitrous oxide production

Organic amendments have nevertheless been observed to exert a stronger influence on denitrification potential and N_2O to N_2 ratio compared to inorganic fertilization (Calleja-Cervantes *et al.*, 2015). The range of such fertilizers is wide, and includes compost, manure, and crop residues. Litter quality, in particular, has been described as a major driver of strongly correlated N_2O and CO_2 emissions from crop residues, especially when soil mineral N is limited, attributed to plant-litter-associated anaerobic microsites (Rummel *et al.*, 2020). For example, given an observation of high N_2O production from faba beans during the growing season in a Mediterranean environment, using their residues for green manure may constitute a weak point from an ecological point of view (Badagliacca *et al.*, 2018). Soil amendment with combined mulch and inorganic N fertilizer has been reported to promote N_2O emission because it created favourable nutrient and soil moisture soil conditions, promoting growth of microbial biomass that included gene copies involved in N-cycling (Fracetto *et al.*, 2017). Dong *et al.* (2018) found that pig manure-treated soil stimulated N_2O emissions even more than in straw-returned and mineral fertilizer- treated soils. Most N_2O emissions occurred within three weeks following fertilization, mainly attributed to denitrification. The authors describe that this response demonstrated positive correlation with *narG* and a negative correlation with soil NO^-_3, suggesting their combined potential use as a predictor variable for N_2O emissions.

A meta-analysis of 47 field studies by Ouyang *et al.* (2018), **demonstrated that organic fertilizer, had a much stronger effect than inorganic fertilizer due to the addition of C in conjunction with N**. Organic inputs provide SOC and other nutrients, thereby further stimulating microbial populations, including soil N-cycling microbes and, in particular, heterotrophs such as denitrifiers. The authors suggest that this was revealed by the correlation between SOC and genes involved in N-fixation and denitrification. In addition, the two fertilizer forms had similar effects in increasing AOB abundance, but organic fertilizer significantly increased both AOA and AOB abundances. Ouyang *et al.* (2018) hypothesized that this suggests that organic fertilization may not be an effective practice to control NH_3^- production in cropping systems, although another meta-anlaysis found contrasting results (Carey *et al.*, 2016).

Ammonium oxidation releases fixed N to the atmosphere as N_2 (dinitrogen gas) by directly oxidizing NH_4^+ and nitrite (NO_2^-) to N_2 under anoxic conditions. **Organic C sources have been seen to reduce active ammonium oxidation (anammox) bacterial abundance** (Gu *et al.*, 2017; Zhang *et al.*, 2018b), **suggesting that their abundance and activity might be affected by fertilizers with different C sources**. For example, Zhang *et al.* (2018b) compared treatments with no C, urea, straw, pig manure, starch, and glucose in a rice wheat cropping system. The different organic C sources reduced active anammox bacteria abundance from 12.8 to 96.6 percent. The anammox bacteria in the soil were in the *Candidatus* genus (*Brocadia* and *Kneunenia*) and three uncultured anammox clusters, but the active bacteria were those closely related to *Candidatus Brocadia*. In a similar study, Gu *et al.* (2017) found that all of the anammox hydrazine oxidase (*hzo*) genes were identified with *Candidatus Brocadia*, while the hydrazine synthase β subunit (*hzs*-β) gene was found in in *Candidatus* Scalindua, and *Candidatus Jettenia* and *Candidatus Brocadia.* They suggest that *hzs*-β is therefore a better indicator of anammox bacterial diversity. They also report that AOB and AOA seemed to share the role of providing NO^-_2 for ammonium oxidation in the cropping system studied.

Soil amendment with organic fertilizer granules can cause N_2O emissions, appearing to be caused primarily by fungal contribution. Wei *et al.* (2014) tested organic granular fertilizer (a mixture of food manufacturing residues such as feather meal, fish meal, rapeseed meal, rice bran, oil palm ash and poultry litter ash) covered with fungal mycelia. By applying the fungal inhibitor cycloheximide and the bacterial inhibitor streptomycin, and the subsequent, respective suppression of N_2O emissions of 84 and 20 percent, **they were able to determine that fungi had provided the main contribution to their measured N_2O emissions**. They furthermore isolated and analyzed 34 fungal strains from the fertilized soils, confirming their N_2O-producing activities. The dominating strains in the fertilized soil were *Actinomucor elegans*, *Bionectria ochroleuca*, *Fusarium avenaceum*, *Fusarium equiseti*, *Fusarium oxysporum*, *Fusarium solani* and *Nectria* sp..

Overall, it appears that organic fertilizers can be associated with increased N_2O production. The nutrients provided by organic inputs can stimulate soil microbial populations and their activities, including heterotrophic denitrifiers. It also seems that organic C sources can reduce anammox bacterial abundance.

Effects of organic fertilizer on soil microorganisms and consequences for soil methane emission

Praeg *et al.* (2014) studied the effects of fertilization with and without cattle manure on CH_4 production and oxidation in subalpine soils in laboratory conditions. **They found that fertilization with cattle manure slightly increased soil microbial biomass. It also increased CH_4 emission but with no statistical significance**. The authors suggested that these increases were likely due to the C and methanogens introduced into the microbiome with the dung, rather than due to changes in the initial soil microbial community. They also found that when combined with water saturation, cattle manure application resulted in the highest CH_4 emission rates. In summary, fertilization with manure did not affect potential CH_4 oxidation significantly. The authors suggest that was probably due to the low application of 1 percent; with an increased moisture and increased manure application the subalpine soils do have the potential to emit CH_4. A 103-year experiment involving regular application of cattle manure found increased archaeal biomass but reduced archaeal diversity, selecting for methanogenic *Methanoculleus* and *Methanosarcina* strains that may have been inoculated by use of cattle manure (Gattinger *et al.*, 2007). The authors concluded that **the long-term, high rates of manure fertilization with such effects resulted in soils that no longer functioned as CH_4 sinks at the investigated site.**

Effects of anaerobic digestates and thermophilic digested sewage sludge on soil microorganisms and consequences for greenhouse gas fluxes

Anaerobic digestates, by-products of renewable energy biogas production, may be a viable organic fertilizer that may not only help mitigate GHG emissions, but also contribute to crop nutrition. Johansen *et al.* (2013) compared applications of raw cattle slurry, anaerobically digested cattle slurry/maize, anaerobically digested cattle slurry/grass-clover, or fresh grass-clover to soils. They found that grass-clover comparatively caused an increase of approximately 10-times in N_2O and CO_2 emissions and a large change in microbial diversity. In comparison, the anaerobically digested materials induced only minor changes in microbial diversity and showed no significant increase in GHG emissions.

Thermophilic digested sewage sludge can likewise supply OM and nutrients. Calleja-Cervantes *et al.* (2017) studied 20 years of sludge application and annual mineral N fertilization in a Mediterranean location, measuring CH_4, N_2O and CO_2 emission. The highest dose (80 t ha^{-1}) delivered the most C, OM and N, but also increased GHG emissions and reduced yield. The effect on yield was attributed to lodging of the crop. It was also the only treatment that altered the soil microbial community structure. The next highest (40 t ha^{-1}) sludge application was suggested as the best to promote soil microbial activity, based on the enhanced C sequestration potential and unchanged GHG emissions, compared to the highest dose and urea-only treatments.

Effects of biofertilizers on (or as) soil microorganisms and consequences for greenhouse gas emissions

Vinasse, a by-product of bio-ethanol production, is rich in C, N and potassium and therefore used as a biofertilizer in sugar cane fields. It is known to increase soil N_2O emissions. Lourenço *et al* (2018) tested straw-covered, sugarcane soils amended with concentrated or nonconcentrated types of vinasse, either before or at the same time as mineral fertilizers. Unsurprisingly, the concentrated and nonconcentrated vinasse with mineral N produced higher N_2O emission than N alone owing to stimulation of soil N-cycling. **Applying vinasse 30 days before mineral N, however, reduced N_2O emissions by 65 percent for concentrated vinasse but not for nonconcentrated.** This interval likely permitted two developments, both contributing to the observed decreases in N_2O emission. First, it gave time to ameliorate the anaerobic conditions thus decreasing heterotrophic denitrification. Second, nutrients introduced by vinasse would have had some time to be decomposed (C) and taken up by plants or mineralized (N). The microbial processes of nitrification, driven by AOA and AOB, and denitrification, driven by both bacteria and fungi, differed depending on soil moisture, soil pH and N sources. In addition, *nirK* was significantly correlated with N_2O emissions, suggesting that fungi contributed to denitrification in the soils that received straw and vinasse. This corroborates with Yamamoto *et al.* (2017), who found that crop residues stimulated fungal denitrification as part of N_2O production process, compared to mineral N alone. Another study also concluded that sugarcane straw amendment increased functions related to decomposition of C compounds, and that application of vinasse increased functions related to spore-producing microorganism phylum of Firmicutes, including the orders of Bacillales and Selenomonadales (Suleiman *et al.*, 2018). While all treatments with straw addition increased abundance of microorganisms related to N metabolism, **vinasse with straw had a synergetic effect, resulting in the highest N_2O emissions.** It was suggested that thermophilic bacteria in vinasse may have contributed to N_2O production, as well as the nitrification pathway in straw decomposition.

Chitin is the second-most abundant polymer after cellulose in nature. It is major component of fungi cell walls as well as insect exoskeletons, and is a source of N and C. It can be used as a slow-release N (bio)fertilizer. A study that combined chitin (or its monomer, N-acetylglucosamine) with mineral fertilizers, manure or rice straw determined that chitin with mineral fertilizer produced the highest CO_2 emissions, as well as mineralization and fungal abundance (Hui *et al.*, 2019). Several chitinolytic bacteria were identified, such as Streptomycetaceae, Oxalobacteraceae, Gemmatimonadaceae, Acidobacteria and Herpetosiphonaceae. Their populations varied amongst the different treatments.

Lastly, in a pot experiment with tea plantation soil, Xu *et al.* (2018) report that *Trichoderma viride* biofertilizer reduced soil N_2O emission by 67.6 percent. Analysis of functional genes involved in N_2O generation and reduction (*amoA*, *nirK*, *nirS* and *nosZ*) showed increased *nirS* and *nosZ* in denitrifying bacteria, which were linked to reduced N_2O emission.

Potential of biochar to stimulate soil microorganisms and soil carbon storage

Biochar is the result of biomass pyrolyzed at temperatures up to 500°C. It is considered a universal sorbent due to its ability to retain organic and inorganic contaminants. Amending agricultural soils with **biochar can potentially offer benefits to soil functioning and ecosystem services such as stimulating soil C storage, increasing the pH of acidic soils, and reducing soil GHG emissions via changes in soil microbial communities. These effects, however, may vary according to the physical (e.g. surface area, microporosity) and chemical properties of biochar, as well as the soil environment to which they are applied.** Many different organic materials can be pyrolized within a range of temperatures, thereby creating different biochar properties that can provoke different soils microbial responses. Brassard *et al.* (2018), for example, tested six biochar combinations from different sources (wood, switchgrass and pig manure) applied to loamy sand and silt loam at two percent. For each type of biochar studied, those produced at the highest temperature with low O/Corg and H/Corg ratios resulted in the lowest increase in CO_2 emissions. The authors report that this supports the idea that high-temperature pyrolized biochar has a higher C stability, thereby increasing the potential soil C storage. They also found that, compared to control soils without biochar, N_2O emissions were decreased by 42 to 90 percent, though this was only in the silt loam amended with biochars made from wood and switchgrass. These biochars had a high C/N ratio (>30), and there were lower N-NH4+ and N-NO3- concentrations in these treatments compared to the control soil, therefore demonstrating the influence of biochar and soil environmental conditions. Senbayram *et al.* (2019) also tested two types of biochars in two different soil types, finding that compared to corn cob, pistachio shell, and cotton stalk biochars, only the olive biochar stimulated both N_2O and CO_2 emissions in the acidic sandy soil. It also altered the bacterial community structure in this treatment, but not in the alkaline clay soil, possibly related to the liming effect (Senbayram *et al.*, 2019).

Converting fresh organic material into biochar results in a product still composed of organic material, but that mineralizes (i.e. decomposes) more slowly. Therefore, in theory at least, it can increase the stability of OM, or in other words, remove CO_2 from the atmosphere for long periods of time. Some studies have found that addition of C in the form of biochar application provoked significantly higher soil microbial metabolic C activity (Xu *et al.*, 2015) and increased the abundance of CO_2-fixing microbes in paddy soil (Huang *et al.*, 2018). Another demonstrated that while biochar amendments significantly increased soil CO_2 emission, there was also an increase in soil C storage and C uptake by rice plants (Qi *et al.*, 2020).

Overuse of N fertilizers can result in acidic agricultural soils, which inhibit the synthesis of N_2O reductase, a key enzyme that breaks down N_2O in the soil. While several studies have demonstrated biochar's potential to increase soil pH (Lin *et al.*, 2017; Senbayram *et al.*, 2019), **studies have also demonstrated that biochar amendments can provoke changes in the soil microbial denitrifying community structure and activities** (Lin *et al.*, 2017), **which reduce overall soil N_2O emission** (Brassard *et al.*, 2018; Ji *et al.*, 2020) **or yield-scaled N_2O emission** (Li *et al.*, 2020a). **The enhancement of microbial N_2O reduction is generally concluded from the association between reductions in soil N_2O emission and increases in *nosZ* abundance** (Harter *et al.*, 2014; Xu *et al.*, 2014). In their pot experiment that illustrates an effect of biochar on both pH and N_2O emission, Xu *et al.* (2014) found that biochar applied to soil cultivated with rape increased the soil pH as well as the soil C, N and the C/N ratio, while simultaneously reducing

N_2O emissions. The biochar amendments increased the microbial species diversity, as seen in other studies (e.g. Xu *et al.*, 2015), as well as abundance of some microorganisms involved in C- and N-cycling. Particularly, the biochar stimulated both nitrification and denitrification processes, including a significant increase in *nosZ* transcription. According to the authors, this effect may suggest that reduced emissions were due to further reduction of N_2O to N_2. Ji *et al.* (2020) report that the biochar amendment enriched *nirK* and *nosZ* gene abundance by enhancing the soil DOC and pH in the acidic soils of their study, stimulating denitrification processes, thereby explaining the measured decrease in soil N_2O emission. Another study, found that two bacterial classes, Deltaproteobacteria and Thermoleophilia, were correlated with a decrease in N_2O emissions (Brassard *et al.*, 2018).

Other studies, however, have found that biochar addition did not affect emissions of total N_2O or CH_4 (Qi *et al.*, 2020). Castaldi *et al.* (2011), for example, observed an initial increase in N mineralization, soil microbial respiration and denitrification activity in the first three months following biochar amendment. However, biochar showed a minimal impact on microbial parameters and GHG fluxes (N_2O, CH_4, CO_2) over the first 14 months in total, and at month 14 the treated and control plots had no significant differences.

In contrast, yet another study found that biochar amendment actually provoked increased soil N_2O emission. In their experiment with wheat straw biochar in paddy soils, Lin *et al.* (2017) observed an increase in soil pH, stimulating the abundance and diversity of AOB, which was correlated with the increased N_2O emissions. The biochar amendment decreased, albeit not significantly, the abundance of the N_2O reductase-encoding gene *nosZ*, but did not alter the abundance of nitrite reductase encoding genes *nirK* and *nirS*. The AOB diversity increased, and the AOB community structure shifted from *Nitrosospira*-dominated toward *Nitrosomonas*. The latter cluster has been associated with fertilization and increased oxygenation, two possible effects of biochar addition to the soil.

It is of interest to know whether applying biochar along with N fertilizers might offer the benefits of nutrient inputs while mitigating N losses through soil N_2O emission. Senbayram *et al.* (2019), mentioned above, found that different biochars applied in combination with mineral N fertilizer in acidic and alkaline soils did not mitigate N_2O emission. In comparison to dairy or poultry manure, however, biochar in combination with urea in a different study did result in a slight decrease in N_2O emissions, primarily due to bacterial denitrification, while also significantly improving soil C contents (Malghani *et al.*, 2020).

In sum, depending on the physical and chemical properties and soil environmental conditions, biochar can minimize soil quality loss (increased soil C storage) without an additional environmental cost (reduced CO_2 and N_2O emission).

Slow release fertilizers can help reduce soil nitrous oxide emissions

Using slow release fertilizers can help reduce N_2O emissions while maintaining and promoting soil health and crop yields. Neem-coated urea with its nitrification-inhibitory effect was observed to reduce soil N_2O emission and maintain significantly higher soil microbial biomass C, populations of NH_3 oxidizers *Azotobacter* and *Azospirillum*, and microbial enzyme activities (Mohanty *et al.*, 2017). Suleiman *et al.* (2016) compared swine slurry with and without dicyandiamide (a nitrification inhibitor sometimes applied with animal manure to reduce N_2O emissions) and mineral fertilizers. They found that dicyandiamide did not change the overall microbial structure, but did influence specific groups related to N-cycling, resulting in reduced N_2O emissions. There was a reduced abundance of the phylum Nitrospirae and the order Nitrosomonadales, which includes the genus *Nitrosomonas*, responsible for first step of the nitrification pathway. Their findings are consistent with others that demonstrated the N_2O-reducing effect of dicyandiamide applied in combination with urea (Jumadi *et al.*, 2008; Soares *et al.*, 2016). However, it has also been reported that both a polymer-coated urea and polymer-coated urea with dicyandiamide lowered the NH_3 oxidation potential compared to urea, but did not reduce soil N_2O emission (Akiyama *et al.*, 2013).

Effects of fertilization in combination with intercropping or tillage practices on biogeochemical cycling

This section focuses on fertilizer use. However, agricultural practices are typically applied in combination. A few examples of paired practices help illustrate the interactions between agricultural practices and the trade-offs to be considered in fertilization management decisions.

N fertilization in the right doses in intercropping systems can offer environmental benefits though impacts on N and C cycling. Chen *et al.* (2019e) found that compared to monoculture treatments, N application in a maize and soy intercropped system reduced N_2O emission and NH_3 volatilization. Intercropping increased N-use efficiency by improving utilization efficiency, and not uptake efficiency, by improving ammonifying and nitrifying capacities to increase the N retained in the soil. The authors explain that this effect shaped the soil environment, for example, by optimizing soil water distribution and increasing soil macroporosity, which may have been responsible for the increased *amoA* and *nirS* abundances compared to monocultures. They report that this gene functionality implies that relay intercropping promoted the conversion of ammonium nitrogen (NH_4^+-N) into nitrate-nitrogen (NO_3-N), and in addition, intercropping increased the conversion ratio of nitrite (NO_2-N) to nitric oxide (NO), rather than N_2O. Another study suggested that reduced N application in an intercropping treatment was more beneficial to improving C sequestration in view of maintaining soil stability and mitigating CO_2 emission than conventional N fertilizer levels (Yu *et al.*, 2020).

While no-till (NT) systems may contribute to enhanced soil C, the effects on soil N dynamics are less clear. A meta-analysis of 57 studies found that N fertilization had a significant impact on the NT effect on soil denitrification: there was a greater increase in denitrification with N fertilizer than without, resulting in increased N_2O emission (Wang and Zou, 2020). The authors attributed this effect to the increased size and activity of denitrifying soil microbial community. Piazza *et al.* (2020) suggest that N fertilization combined with reduced tillage may be an optimal combination of practices to maintain and increase SOC conservation in the Mediterranean area, as it can drive shifts in the microbial community to taxa that combat soil degradation. In their long-term study, they found that N fertilization under minimal tillage increased the SOC in occluded microaggregates (+16 percent at 0–15 cm soil depth and +84 percent at 15–30 cm) while conventional tillage (CT) decreased SOC in these microaggregates (-46 percent at 0–15 cm and -15 percent at 15–30 cm). Moreover, the enzymatic activities involved in C cycling were the least reduced under N fertilization combined with minimal tillage.

Effects of mineral nitrogen fertilization in rice paddies on greenhouse gas emissions mediated by methanogens and methanotrophs

Increasing the N supply to rice plants in paddies is generally thought to inhibit the activities of methanotrophs and stimulate soil CH_4 emission. Studies have nevertheless reported that N fertilization may actually create favourable conditions for CH_4-oxidizing bacteria (Bodelier *et al.*, 2000), even while increasing CH_4 production in the bulk soil (Kruger and Frenzel, 2003). Bodelier *et al.* (2000) reported that type II CH_4 oxidizers (methanotrophs) dominated CH_4 oxidation processes in unplanted and unfertilized soils. In the long-term fertilization of the planted soils, both type I and II methanotroph abundance was enhanced in the rhizosphere, but growth occurred mainly for type I. The authors suggest that because there was enough available CH_4 to sustain them, it seems that neither the methanotroph activity nor abundance were inhibited in the rhizosphere. In addition, both methanotroph types require NH_4^+, supplied through the N fertilizer, to be active and grow in the rice rhizosphere. Fertilization with ammonium nitrate (NH_4NO_3), however, does not necessarily result in significant changes in methanotroph community compositions (Zhao, Cai and Jia, 2020). Ji, Conrad and Xu (2020) also reported that in their study, urea showed only a negligible effect on the functional genes related to both CH_4 and N_2O emissions, as well as on archaeal and bacterial community composition.

N fertilization in rice paddies might decrease or increase soil CH_4 emission,

depending on the response of soil methanotrophs and methanogens and their interaction with rice plant response to N inputs. Chen *et al.* (2019e)found that increasing the N fertilizer application rate at an early stage of plant development increased the root activity and contents of malic, succinic and citric acids in root exudates. The acids were associated with a higher abundance of methanotrophs in the rhizosphere, and the authors suggested that they promoted CH_4 oxidation and thus reduced CH_4 emission from the rice paddy fields. Overall, they concluded that the interaction between stronger root systems thanks to a timely N input, the higher oxygen delivery capacity by the improved root system, and suitable root exudates established a favourable habitat for soil methanotroph populations, leading to reduced CH_4 emission from rice paddies during the mid-growing period of their experiment. In contrast, a field experiment with and without urea fertilizer found that urea application significantly stimulated CH_4 emissions from paddies (Liu *et al.*, 2019). The authors attributed this to a changed oxygen state, but this time in the opposite direction. They observed a decrease of redox potential in the rhizosphere as well as a reduction of dissolved oxygen concentration at the soil-water interface. Furthermore, urea fertilization was associated with a sharp increase in both active methanogens and methanotroph bacteria, but their diversity was decreased. Urea fertilization appears to have stimulated the genera *Methanoregula* (methanogens) and *Methylococcus* (type I methanotroph), and inhibited the genus *Methlocystis* (type II methanotroph). The authors suggest it probable that because methanotrophs require N, and as N can be limiting in paddies, fertilization overcame this limiting effect. They propose that methanogen communities were likely influenced by the urea-stimulated rice plant growth, which released more root exudates, plant litter and other organic C substates, as pointed out in other studies (e.g. Bodelier *et al.*, 2000). The collective responses in this experiment resulted in a net increase in soil CH_4 emission. Yet another study found that N, P and K amendments to rice paddy soil in incubation experiment reduced the activity of CH_4 oxidation of methanotrophs even while enhancing their abundance (Zheng, Zhang and He, 2013). The authors suggest that the chemical-induced stress may have changed the ratio of type I to type II methanotrophs, creating shifts in activities and abundances. This negative correlation between methanotroph abundance and CH_4 oxidation by methanotrophs is an example that cautions against inferring microbial activity from simple gene abundance (Zheng, Zhang and He, 2013). The authors caution against overuse of potassium chloride (KCl) fertilizers in paddy soils, as Cl toxicity was seen to completely inhibit CH_4 oxidation. Zheng (2008) similarly report that 16 years of N fertilization showed an inhibiting effect on methanotroph abundance, while NK and NPK + crop residues demonstrated stimulating effects. Finally, differently from all of these studies, Zhao *et al.* (2020) observed no significant changes in methanotroph community composition with NH_4NO_3 fertilization.

The population dynamics of methanogens and methanotrophs can also vary with nutrient deficiency, **which can affects plant growth and soil properties.** Sheng *et al.* (2016) suggest that P-deficiency-induced changes in soil physiochemical properties, in tandem with rice plant growth, might constrain the activity of methanogens. Methanotrophs, in comparison, might be better adapted to this soil environment. The authors demonstrated that a P-deficient soil significantly reduced CH_4 fluxes, whereas a K-deficient soil did not. Moreover, there was a lower transcript abundance of both methanogens and methanotrophs in K-deficient soils.

Soil pH is one of the main determinants of soil microorganisms that control C and N fluxes. Zhao, Cai and Jia (2020) for instance, observed significant inhibition of CH_4 oxidation activity in low-pH soils. Providing a more comprehensive view on the subject, Zhao *et al.* (2020) worked with six physiochemically contrasting paddy soils across China and described that in soils with relatively low pH (5.44 to 6.10), CH_4 was primarily consumed by *Methylocystis*-affiliated type II methanotrophs, while in soils with a high pH (7.02–8.02), *Methylobacter*- or *Methylosarcina*-affiliated type I methanotrophs dominated CH_4 consumption. The high or low pH conditions did not cause any significant difference regarding contribution to SOC from CH_4 consumption.

Finally, a last study to mention explored a different approach to mitigating GHGs from rice paddies. Guo *et al.* (2019a) concluded that **applying polymer-coated urea or nitrapyrin-treated urea to rice paddies could be a feasible strategy to mitigate NH_3 volatilization, CH_4 and N_2O emission while maintaining or even increasing grain yields.** The polymer coating and nitrapyrin contents inhibit the first step of nitrification, thereby slowing the ammonium (NH_4^+) oxidation process and reducing N_2O emissions from the soil (Guo *et al.*, 2019a).

In summary, on the one hand, an increased N supply in rice paddies may either stimulate or inhibit CH_4-oxidation by methanotrophs as influenced by the soil oxygen state, N limitation, and indirect positive effects of enhanced plant growth. On the other, the increased rice plant biomass and root exudates in response to an increased N supply are likely to stimulate methanogen activities. Taken together, these interactions may result in net increases or decreases of soil CH_4 emission, depending on other factors such as soil conditions. Soil pH, for example, is a critical influence on methanotroph populations, and this niche specialization is important in regulating CH_4 emissions after intensified input of anthropogenic N fertilizers. **The potential toxicity of other chemicals may also shape methanotroph abundance or activities, such as Cl from KCl fertilizers, which has been seen to completely inhibit CH_4 oxidation** (Zheng, Zhang and He, 2013). **Finally, bacterial methanotrophs receive the majority of attention, but the less-explored role of archaeal methanotrophs such as *Methanoperedens nitroreducens* may nonetheless play an important role in NO_3^--dependent anaerobic oxidation of CH_4** (Vaksmaa *et al.*, 2016).

Effects of organic fertilizers in rice paddies on greenhouse gas emissions mediated by methanogens and methanotrophs

Long-term manure fertilization in rice paddies, even in combination with mineral fertilizer, can substantially enhance the soil microbial abundance and richness and activity, producing positive effects on SOM and soil C (Chen *et al.*, 2017; Guo *et al.*, 2019b). One 32-year long experiment demonstrated that a treatment of 70 percent manure and 30 percent mineral fertilizer sustained high yields while increasing SOM, microbial biomass and bacterial diversity while also alleviating soil acidification (Chen *et al.*, 2017). Bacterial community compositions may offer indications about the particular roles of certain bacterial populations in soil processes and functions in response to fertilizer management, with implications on biogeochemical cycling. For example, in the same study there was a higher abundance of *Nitrospira* in mineral-fertilized soil than manure + mineral-fertilized soils, which could lead to heavy N loss along with a major N_2O loss in the former treatment. In a 22-year long experiment comparing combinations of manure and mineral fertilizers, it was determined that the SOC and soil microbial biomass N were the most important factors influencing the variance in microbial community composition (Guo *et al.*, 2019b). These variances did not have a significant influence on C mineralization, but the abundance of several non-dominant bacteria did (*Gemmatimonadetes* and *Latescibacteria*). There were strong increases in CO_2 emission, potential mineralized C and turnover rate in both of the organic fertilizer treatments, relative to the control, while mineral fertilizer alone had no significant effect on these parameters. Overall in this experiment, the authors report that organic fertilization provided more C input than mineral fertilizers, thereby enhancing microbial activity and the C mineralization process. Daquiado *et al.* (2016) studied the bacterial succession in response to several combinations of mineral and compost inputs for 45 years in paddy soils. In contrast, they surprisingly found only minor differences and no significant overall effect on the soil microbial structures. Nevertheless, they report that organic fertilizer, compared to several combinations of mineral fertilizers, activated diverse groups of gram-positive microorganisms. The compost-amended soils hosted high abundances of *Rhizobiales*, involved in nutrient cycling, and *Methylococcales*, which may contribute to reduced CH_4 emission.

While manure can thus offer benefits to soil health and plant productivity, it can also introduce microorganisms that can impact the local soil GHG emissions. Nguyen *et al.* (2015) demonstrated that swine manure introduced active CH_4-producers in the early stages of a paddy field, resulting in increased CH_4 emission. Rapidly composted manure, however, could be a viable alternative organic fertilizer for rice paddies that provides nutrients while reducing CH_4 emission (Zhou *et al.*, 2016). This type of manure is treated with a sanitizer, a catalyser rich in sulphate (SO_4^{2-}) and N, and an NH_3-fixing synergist at 120°C. The output is a nutrient-rich compost, produced in only three hours. The authors reported that the significantly lower CH_4 emissions of this product, compared to non-composted manure and commonly composted manure, were due to a decreased abundance of total soil methanogenic archaea (specifically, the two guilds *Methanosarcina* and *Methanosaeta*).

Effects of phosphorus or silicate fertilizer in rice paddies on greenhouse gas emissions and soil carbon storage

Zhang *et al.* (2015) report that **P fertilization in a rice paddy stimulated C sequestration**. P fertilization in rice paddy induced a decline in the C:P stoichiometric ratio of soil microbial biomass because microbes invested in C instead of P acquisition. They suggest that this, in turn, drove a shift in soil resource availability by increasing the bacterial community richness and diversity, and furthermore stimulated C sequestration by promoting bacteria involved in C degradation, which broke down C from plant matter.

Si fertilizer can impact the soil microbiome, with potential consequences on local soil nutrient cycling and GHG emissions. Si amendment has been seen to increase soil pH, nutrient availability, and crop production, while decreasing N availability and CH_4 and N_2O emissions (Das *et al.*, 2019a). In their study, the Si input affected the soil microbiome composition and abundance by increasing functional genes responsible for C degradation, C and N fixation, P utilization, CH_4 oxidation and metal detoxification, while CH_4 production and denitrification genes were diminished. Another experiment demonstrated that short-term slag silicate fertilizer significantly enhanced saprotrophic fungal communities and stimulated SOM mineralization (Das *et al.*, 2019b). The authors suggest this could have negative feedback impacts on soil C storage in submerged rice paddies. The increase in saprotrophic fungi was mostly attributed to greater labile C availability and Si availability.

The effect of nitrogen fertilization in ground cover rice systems on soil nitrogen cycling and losses

Ground cover rice systems involve laying a plastic film to reduce use of irrigation water. Chen *et al.* (2018b) described the microbial mechanisms underlying risks associated with this technique for increased N mineralization (illustrated by the great increase in levels of the gene *chiA*), increased nitrification and N_2O emissions (increased *qnorB* and AOA transcripts) and decreased biological N fixation (reduced *nifH* transcripts). Nevertheless, they argue that their analysis of topsoil N stocks provided evidence that, at least under N fertilizer application, this system might overall maintain soil N stocks. They speculate that this effect might result from an increase in fertilizer N-use efficiency, root development and C and N return via residues. These effects appear to outbalance the observed effects on nitrification, gaseous N losses and biological N fixation, thereby preventing a net loss of total soil N.

4.7.10

Systematic review: Effects of fertilization on the soil microbiome, and their combined impact on human health

Three separate searches were performed for studies linking fertilization, the soil microbiome and human health. They returned a total of 53 articles, though none were appropriate for this section (see **Annex I** for search terms).

Nevertheless, as discussed in detail in **Section 4.7.6** about organic fertilizer and AMR, there is substantial research that tries to address critical knowledge gaps in this subject. The line of enquiry follows the suspected pathway of ARGs known to be present in manure from livestock treated with antibiotics, to their introduction into the soil through manure fertilization practices, the response of the soil resistome to the introduced ARGs (including potential acquisition of ARGs, or their increase in abundance and diversity in the soil), and their potential migration into the food chain thereby posing a possible threat to public health.

4.8
PEST MANAGEMENT

HIGHLIGHT BOX 13 Impacts of pest management on the soil microbiome, climate change, and human health.

NARRATIVE REVIEW What are the impacts of pest management on the soil microbiome?

- Pesticides can impact the soil microbiome, significantly increasing or decreasing soil microbial communities, activities and biomass; less commonly, they can also have no detectable effect.
- There are currently no robust data on how much antimicrobials (pesticides like fungicides and bactericides, including antibiotics) are used globally by the plant sector. That some antimicrobials are used to treat both human and plant diseases is cause for concern regarding potential human-pathogen resistance.
- There is a lack of data on the contents and concentrations of pesticide residues in agricultural soils worldwide. Pesticide residues frequently occur in mixtures, which should be taken into account when assessing risks to soil organisms.
- The One Health perspective emphasises that human, animal and plant health are related, thereby expanding the potential field of risk concerning antimicrobial resistance.
- There remain many critical knowledge gaps regarding antimicrobial resistance gene transfer and inheritance in the soil resistome.
- How glyphosate affects soil microorganisms remains controversial, even if it is a widely used and studied herbicide.
- Better understanding of the soil immune response, which is mediated by soil microorganisms, may be an opportunity to reduce pesticide use while promoting crop health.

SYSTEMATIC REVIEW What are the impacts of pest management on the soil microbiome, and their causal impacts on climate change?

- Pesticide applications can impact the soil microbiome, and can also increase or decrease nitrous oxide, methane and carbon dioxide emissions, depending on the biogeochemical pathways and doses involved.
- Pesticides can directly suppress the abundance of certain microorganisms (e.g. methanogens, methanotrophs), resulting in changes to biogeochemical pathways.
- Soil environmental factors, microbial-produced enzymes, and microbial electron transport activity also play key roles in mechanisms explaining shifts in greenhouse gas emission rates with pesticide application.
- Pesticides addressed in this section are: chloropicrin, dimethyl disulfide, allyl isothiocyanate, chlorothalonil, methyl viologen dichloride, bensulfuron methyl, pretilachlor, bensulfuron methyl, pretilachlor Pentachlorophenol, and copper.

SYSTEMATIC REVIEW What are the impacts of pest management on the soil microbiome, and their causal impacts on human health?

- Overall, few studies were found drawing direct links between use of pesticides, their effects on the soil microbiome, and subsequent consequences to human health. No relevant studies were found specifically for herbicides.
- Organochlorine pesticide residues that have accumulated in soils from past use were demonstrated not to have a strong impact on soil microorganisms and soil ecosystems; they were evaluated as posing very low risks to human health through consumption of vegetables grown in such soils.
- Similar soil microbiome profiles under long-term organic and conventional management suggest that the soil microbiome can develop stability, even when subjected to continuous pesticide applications. This may be related to their adaptability to the introduction of chemical substances that originate in organisms like themselves. In this particular study, there was no significant transfer of antimicrobial resistance genes to soils under either system, though it is not clear what level of farming intensity under conventional management might significantly affect the soil microbiome in this regard.

4.8.1
Key issues regarding pesticides in agricultural soils

According to the Code of Conduct of Pesticide Management, a pesticide is “any substance, or mixture of substances of chemical or biological ingredients intended for repelling, destroying or controlling any pest, or regulating plant growth” (WHO and FAO, 2014, p. 6). A pest may be a plant, animal or other pathogenic agent that causes harm to plants or their products or environments, including animal or human pathogen and parasite vectors (WHO and FAO, 2014). They enable increased crop production (by protecting crops and commodities until they reach consumers) but pose potential toxicity risks to organisms in surrounding ecosystems as well as animal and human consumers, pesticide applicators and bystanders. They may be classified according to their target organism (e.g. fungicide, insecticide, herbicide), or their chemical structure. Commonly used chemical families include organophosphates, pyrethroids and neonicotinoids (these three families typically being insecticides and nematicides), and carbamates (some of which are fungicides or herbicides).

It has been estimated that the amount of pesticides reaching targeted pests is actually extremely small, even when properly applied (Pimentel and Burgess, 2012).[35] Factors that influence the percentage of sprayed pesticides reaching target pests, for instance, include drift, volatization, contact with abiotic and biotic non-targets degradation by abiotic and biotic processes (Duke, 2017). It is therefore suspected that a large percentage of applied pesticides have the potential to contaminate air, water, soil thereby impacting soil biota and ecosystems. **Pesticides work by targeting systems or enzyme synthesis in pests. Since these same systems or enzymes may be present in other organisms – including microorganisms – the concern is how they will affect non-target organisms and ecosystems. This has also,**

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mixtures, rather than as a single compound is important to highlight. The interaction of multiple compounds could different effects on soil microorganisms and should be taken into account in risk evaluation for soil biota (e.g. during approval processes of new products).

It is clear that pesticides can shape the soil microbiome composition, activities and abundance. In some cases this includes selecting for species that can actually degrade pesticide compounds. Though that may seem positive in one sense**, this response has led to concerns about the presence of increased antibiotic resistance genes (ARGs) in the soil, and how they may find their way into animals or humans through the food chain.** These questions are discussed in further detail below.

Given the concern about pesticide use and antimicrobial resistance (AMR), there is surprisingly no current, robust data on how much antimicrobials – meaning pesticides like fungicides and bactericides, including antibiotics – are used globally by the plant sector. A recent survey conducted by Taylor and Reeder (2020) using the Plantwise Online Management System (POMS) revealed that all six WHO regions of the world use antibiotics in plant production, with the exception of Africa (no data for Europe were available). The main crop on which they were used was rice. Furthermore, the survey found that eleven different antibiotics were recommended for crops, and that they were frequently blended together. Lastly, the authors speculate that antibiotics are often used as prophylactics in some regions, as a significant number of recommendations used antibiotics to target insects or mites (against which they have no effect). More specifically, at least 20 countries outside the EU authorize antibiotic use for plants to control fire blight and citrus greening disease. In some countries, the antibiotic *Streptomycin* is authorized to control certain bacterial disease in pip fruit, stone fruit, seedling tomatoes and kiwifruit. Kasugamycin, oxytetracycline and oxolinic acid are other antibiotics used to control plant pests (Ponce de León-Rosales, Arredondo-Hernández and López-Vidal, 2015; Stockwell and Duffy, 2012). The EU does not approve of any antibiotic as an active ingredient in pesticides, except for emergency use, which must be reported to the European Commission and to the other EU Member States on an annual basis. Some antibiotics used on a regular basis for the control of plant infections are also used in human and veterinary medicine (aminoglycosides, quinolones and tetracycline). The same applies to triazole antifungals, which are broadly used for the control of infectious diseases in both humans and plants. Potential consequences of such use is cause for concern regarding risks to human health. For example, the emergence of azole resistance is already challenging the management of human aspergillosis, a potentially life-threatening infection (Rivero-Menendez *et al.*, 2016). Nevertheless, according to results of a recent questionnaire jointly published by the WHO, FAO and OIE (2018), the number of countries that monitor the use of antibiotics in plant production is very low compared to monitoring of those used in veterinary and medical sectors. Only three percent of the 158 countries questioned indicated any kind of regular assessment of types and amounts of antibiotics used on crops (WHO, FAO and OIE, 2018).

4.8.2
Impact of pesticides on non-target soil microorganisms

There are multiple aspects to consider in the evaluation of the effects of pesticides on the soil microbiome. These include (i) the particular pesticide mechanism (i.e. how does it work), (ii) application management (e.g. fumigation, use of nanocarriers), (iii) effects on nontarget organisms, and (iv) what happens over time if it remains in the soil. Whether pesticide compounds break down quickly or remain in the soil for long periods depends on factors such as the type of pesticide, the soil type and the soil microorganisms in the surrounding environment.[36] The main degradation processes are biological processes

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[36] How long a pesticide remains in the soil is referred to as persistence and is expressed in terms of half-life values (DT50) (FAO, 2000). Half-life DT50 is a measure of the amount of time it takes for 50 percent of the parent compound to disappear from soil or water by transformation into mineral compounds and intermediate substances.

(biodegradation) and physicochemical processes (e.g. hydrolysis, when a compound is split by contact with water; and photolysis, breakdown of a compound by irradiation).

On the one hand, pesticides have been demonstrated to drive shifts in the soil microbiome by inducing changes in microbial activities (measured by enzymes), abundance (measured by biomass), and community structure (or diversity); on the other, research has yet to clearly demonstrate the link between these effects and significant, long-lasting decreases in soil functions (FAO and ITPS, 2017).

Two extensive reviews have reported mixed results regarding how pesticides impact microbial enzyme activities, according to their mechanism of action (Puglisi, 2012; Riah *et al.*, 2014). In their review of microcosm studies, Riah *et al.* (2014) reported that fungicides indeed had their intended deadly effect on fungi, thereby having a generally negative response on enzyme activity. This was frequently accompanied by an increase in bacteria populations, which likely feed upon the nutrients provided by dead fungal hyphae. Insecticides and herbicides, however, showed both positive and negative effects on microbial enzyme activity. Organochlorine insecticides generally caused a positive response in microbial enzymatic activity, while organophosphate insecticides generally induced a negative response. While photosynthesis-inhibiting herbicides and those that inhibit the acetolactate synthase enzyme induced either minor or no effects, another group that inhibits 5-enolpyruvylshikimate-3-phosphate synthase led to negative responses in 77 percent of the experiments. This latter group includes glyphosate, which is discussed further in **Box 7**. Effects of pesticides on microbial abundance also appear to have mixed results. In his review of 3 405 case studies, Puglisi (2012) reported that the majority of studies did not cause any significant difference in microbial biomass, while one third caused an decrease.

Several studies have reported decreases in bacterial diversity, following chloropicrin soil fumigation (Li *et al.*, 2017) or in organochlorine-contaminated soils (Regar *et al.*, 2019), for instance. However, investigating such effects on community structure is extremely challenging because most of the microorganism diversity and their functions remain to be studied (FAO and ITPS, 2017). A review by Jacobsen and Hjelmsø (2014) suggests that decreases in microorganism species diversity actually had a minor effect on soil functions because the immense number of species ensured soil functions, or functional redundancy. Puglisi (2012) similarly noted that a change in community structure does not always equate a reduction in biodiversity, but rather may reflect shifts to adapt to the pesticide. He stressed that **the complexity of interacting factors in a farming system or in the soil can make it difficult to predict precisely how pesticides may affect the soil microbiome**. For example, although many studies point out marked effects of pesticides on the soil microbiome, a long-term study that compared organic and conventional systems reported that pest management and pesticide application were of minor importance when compared to the amount and quality of fertilizers (Hartmann *et al.*, 2015). In another example, Satapute *et al.* (2019) report that propiconazole can paradoxically be used to increased soil microbial abundance at low doses, but at high doses it is severely damaging for the soil microbiome. Furthermore, Puglisi (2012) found that effects on the soil microbiome were dose dependent: there were less impacts when applied at recommended field doses and more significant effects with increasing doses. Also, like other studies in this section, the review identified temporary effects where the soil microbial community structure was initially impacted but later returned to its original conditions. This occurred in 11 percent of cases for herbicides, seven percent for fungicides, but curiously, there was no such trend for insecticides. **Nevertheless, pesticide effects on the soil microbiome are indeed real, thus soil microbial biomass, activity and structure should be used as pattern-identifying parameters to assess different pesticide effects on soil microorganisms, the ultimate goal being to protect these non-target organisms.**

4.8.3

Pesticide-degrading soil microbes and concerns about antimicrobial resistance

Pesticide degradation in the soil may occur by chemical or biological means. Biodegredation refers to the transformation of pesticides when microorganisms use them as a source of energy, or nutrition, thereby converting them into nontoxic metabolites. The soil environment can have low nutrients levels because of many factors, for example, the soil type, management practices, or the phase of cropping cycle. To survive, soil microorganisms may simultaneously consume the multiple sources of energy that are available in the nutrient-limited environment. These include unbound pesticide compounds, available for direct interactions with soil microbiota. Many factors influence pesticide bioavailability (i.e. whether the compound is available to cross an organism's cellular membrane at any given moment) throughout the biodegradation process. These factors include water solubility, volatility, concentration, method, and the timing and frequency of application. What is not degraded is bound into the soil as residues.

Multiple studies have demonstrated the pesticide-degrading capabilities of certain soil microorganisms. The study by Li *et al.* (2017), for example, reports that there were clear shifts in soil microbiota populations in response to chloropicrin fumigation. One was an increase in the abundance of certain species, understood to be due to their role in biodegradation of that particular pesticide. This phenomenon has been observed in several studies (Bragança *et al.*, 2019; Regar *et al.*, 2019), causing some to ask whether can introducing genes known to degrade specific pesticide compounds to the soil microbiome play a role to help remediate polluted soils (Regar *et al.*, 2019). Similarly, Petric *et al.* (2016) identified bacteria with nicosulfuron (NS) tolerance, demonstrating that exposure to the herbicide drove selection for NS-tolerant bacteria. Increasing levels of NS created increased bacterial abundance and diversity. Also, different NS-tolerant bacteria showed different levels of sensitivity to the herbicide. Their study did not, however, specifically identify NS degradation by NS tolerant bacterial strains. Though the mechanisms to explain the shifts in bacterial community were unclear, they concluded that this particular bacterial community could be useful as a bioindicator of NS exposure in the assessment of ecotoxicity towards soil microorganisms. Furthermore, it was suggested that sulfonylurea herbicides are not persistent in soils due to degradation by bacteria.

Microorganism communities growing in pesticide-polluted soil are often resistant to multiple antibiotics, making the soil microorganisms themselves an important source of multiple antibiotic resistance (AR) (Ramakrishnan *et al.*, 2019). This was observed, for example, in several studies in citrus groves (where glyphosate is frequently used) where results suggest that not only was there cross-resistance between glyphosate and penicillin, but that it was more frequent than between glyphosate and streptomycin or tetracycline (two commonly used clinical antibiotics) (Van Bruggen *et al.* 2018). It should be pointed out that soil microorganisms are a natural original source of antibiotics, having been developed as part of a survival strategy against other microorganisms, so they are expected to be found in soil microbiome. However in this case, the concern is about elevated levels of antimicrobial resistance owing to pesticide contamination in soils. How antimicrobial resistance can be transferred between microorganisms is a wide subject of ongoing debate. For example, glyphosate resistance has been identified in many bacterial genera since intensification of its use, but by what exact mechanisms this occurs is not yet conclusive. One hypothesis is through horizontal gene transfer by natural means. Another is gene mutation (related to target site EPSP synthase). Others yet include selection of bacteria that produce enzymes that degrade glyphosate, and bacteria that manage to escape some harmful effects by producing "molecules that scavenge free radicals" (Van Bruggen *et al.* 2018). Whether resistance in the microorganisms leads to heritability likewise remains an open question.

Looking towards the future, several trends predict increased herbicide-resistance in the soil microbiome, likely associated with increases in multi-AMR. One is that combined use of herbicides may intensify soil contamination (e.g. combined dicamba and glyphosate applications used on 'Roundup Ready 2Xtend' soybeans and 'Bollgard II XtendFlex' cotton). Dicamba and 2,4-D are herbicides that will likely replace glyphosate, due to notable weed-resistance to the latter. 2,4-D and Dicamba are actually compounds that were introduced to the market in 1945 and 1976, respectively. As they are already registered, they are therefore already approved for use. There are, for example, Dicamba-resistant soy beans. It has already been observed that soil microbial insensitivity to dicamba and 2,4-D is often accompanied by AR. Therefore it is expected that resistance these two herbicides will also increase in microbiomes with intensification of use. Added to that are the expanding crop surface areas under other genetically engineered, herbicide-tolerant varieties. Also, soil persistence of herbicide chemicals will increase the probability of microorganisms developing degrading abilities, as well as adaptive resistance to multiple antimicrobial agents.

Ramakrishnan *et al.* (2019) are amongst researchers who highlight the consequences to humans and other organisms of pesticide-degrading microbes that harbour antimicrobial resistance. **They advocate the 'One Health' strategy, which acknowledges that the same microbes can infect both humans and animals.** In response, One Health promotes the design and implementation of programmes through cooperation between different sectors. **From this perspective, it follows that pesticides and pesticide-degrading microorganisms may contribute indirectly to negative health effects in humans and animals.** The evolution of antimicrobial resistance may depend profoundly on the types and bioavailable concentrations of pesticides in natural and agricultural ecosystems. Soil microbiome responses are also difficult to predict given the many influential factors, including different soil environments, high or low pesticide concentration that may switch on or off certain genes or have harmful or beneficial effects on microbial functions.

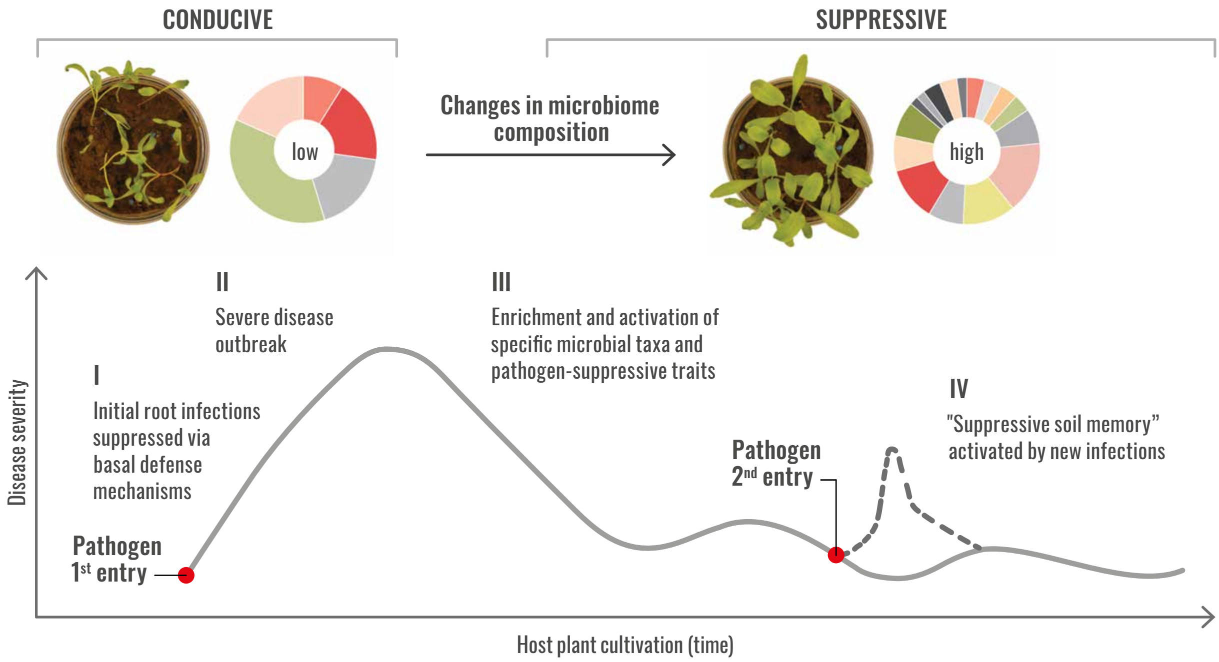
4.8.4
Soil microorganisms mediate the soil immune response: an opportunity to reduce pesticide use?

Intensive pesticide use is linked to potential risks to human health and – from a One Health perspective – other ecosystems, prompting interest in other pest management strategies. Furthermore, the production and transportation of agricultural pesticides is very energy-demanding. Could using biological control agents help reduce this energy use, as well as help avoid development of pesticide resistance and preserve natural enemies that would otherwise be killed as non-target organisms (Beed *et al.*, 2011)? Raaijmakers and Mazzola (2016) present an interesting approach that responds to these concerns by **drawing a parallel between soil and human immune systems**. They describe the capacity of the soil microbiome to provide a soil immune response and the need to harness this capacity for plant health. There are two kinds of pest suppression: general suppression based on competitive activities of the biome; and specific suppression, which is mediated by a specific subset of microbes. As in humans, general suppression provides a fast and non-specific defensive response. Specific suppression involves complex interactions between the pathogen, host plant and the soil microbiome, and also requires enrichment and stimulation of microbes that have antagonist traits towards the given pest. Specific suppression therefore requires time to react, is pathogen-specific, and has a memory of previously encountered pathogens. **The overall idea is thus to manage soils and associated microbiomes to build up suppressive, rather than conducive, soils** (**Figure 28**)**, thereby contributing to agricultural sustainability and food security.**

FIGURE 28.

LINES OF DEFENCE: CONDUCIVE VS CONDUCTIVE SOILS.

If a pathogen can circumvent the basal defences (innate immunity) in both soil and plant, a severe disease outbreak may occur. This disease outbreak can last for years but will ultimately enrich for specific microbial consortia and pathogen-suppressive traits in the soil and plant microbiome. This specific suppression can dissipate but is rapidly regained in the presence of the original host plant and inducing pathogen. The images show plants exposed to a fungal pathogen in disease-conducive and -suppressive soils. In the conducive soil with a low abundance of antagonistic microbial consortia, the fungal pathogen causes disease (left), whereas in the suppressive soil with a high abundance of antagonistic microbial consortia, most seedlings remain healthy (right).



Source: Raaijmakers and Mazzola, 2016.

One approach to disease management using biological management has been to isolate a single microbial species and apply it to soil or plant seed as pathogen control. However these introduced microbial species often fail to establish and implement the desired effect due to competition with the indigenous soil microbiome. In response to this limited, single-species approach, that neglects complex interactions in the soil microbiome, Raaijmakers and Mazzola (2016) describe two more holistic strategies that could offer a more stable soil memory and have the desired effect of limiting pathogen infestations. One is the introduction of synthetic microbial communities (SynComs), which are a consortia of specifically selected microbial species designed to mimic the soil microbiome under natural conditions (de Souza, Armanhi and Arruda, 2020). Not only can the spectrum of functions provided by the consortia be tailored, but it has also been suggested that their synergistic interactions may improve soil microbial community stability (McCarty and Ledesma-Amaro, 2019). A second strategy is to identify and enhance the indigenous disease-suppressive soil communities. The latter might be done by plant breeding programmes that select for specific root traits that are able to attract pathogen suppressive microorganisms. Also, specific inputs such as compost and seed meal could be used much like prebiotics are in humans; they help select and encourage beneficial bacteria. Finally, the authors draw a last comparison between use of vaccines in animals, which introduce specific pathogen molecules to trigger an immune response, to a mechanistic understanding of specific metabolites that could likewise activate the adaptive immune response of the soil. Altogether, such an approach evidently implies much more knowledge than we currently have about individual soil microbes and their communities. However, the strategy of creating communities with customized activities holds great potential to improve sustainable agricultural production through promoting crop health (Sergaki *et al.*, 2018).

Concluding remarks

Pesticides can clearly, even if unintentionally, impact soil microbial abundance, activities and community structure with mixed results, depending on their mode of action. For the moment, however, there is still not enough evidence that unequivocally shows that these effects are directly linked to long-term negative impacts on soil functions. Indeed the consequences for soil health appear difficult to predict with certainty, the multitude of interacting factors in the soil environment making for complex circumstances. Nevertheless, promoting a soil immune response by carefully managing soils and associated microbiomes is an intriguing, even if challenging, proposition to reduce pesticide use while contributing to food security and sustainable agroecosystems. Another concern is that some pesticides select for soil microorganisms that can degrade the potential toxicants, thus creating communities that could be important sources of multiple AMR. This has incited yet-answered questions about eventual negative effects on animal and human health.

4.8.5

Systematic review: Effects of pest management on the soil microbiome, and their combined impact on climate change

A search for studies linking pest management, soil microbiome and climate change returned 184 articles, nine of which were relevant for this section (see **Annex I** for search terms).

Application of certain pesticides has been shown to alter the soil microbiome. These pesticides can also increase or decrease nitrous oxide (N_2O), methane (CH_4) and carbon dioxide (CO_2) emissions, depending on the biogeochemical pathways and doses involved. Although soil microorganisms can undoubtably be a connecting factor between pesticides and greenhouse gas (GHG) emission rates, there is more to this relationship than a simple, direct correlation between functional gene abundance and emission rates. Soil environmental factors, microbial-produced enzymes, and microbial electron transport activity also play key roles in the explanatory mechanisms. Nevertheless, some compounds found in pesticides can directly suppress the abundance of certain microorganisms (e.g. methanogens, methanotrophs), resulting in changes to biogeochemical pathways.

Effects of pesticides on soil microorganism-mediated nitrous oxide-related pathways

Two similar studies showed that fumigation with broad-spectrum, multi-use pesticides chloropicrin (CP), dimethyl disulfide (DMDS), and allyl isothiocyanate (AITC) increased soil N_2O emissions (Fang *et al.*, 2018, 2019). Both found that the **N_2O emissions in pesticide fumigated soils correlated with soil environmental factors rather than the abundance of functional genes.** This conclusion came partly from their observations that the increase of N_2O production did not correspond with the decreased abundances of 16S rRNA and N-cycling functional genes with fumigation. This suggests that **the abundance of nitrification and denitrification genes may not always be related to N_2O production**. Fumigation with CP, DMDS and AITC led to shifts from a nitrification to a denitrification pathway, thereby contributing to increased N_2O emissions. AITC, for example, suppressed most genes involved in the N cycle, but increased the expression of genes that transform nitrogen dioxide (NO_2^-) to nitric oxide (NO) and organic decomposition, resulting in an overall shift to a denitrifying pathway (Fang *et al.*, 2019). Finally, in both studies, **when the effects of fumigation disappeared, so did the inhibitory effects, and the soil microbial community returned to previous levels**. The rate of return, however, depended on soil conditions (Fang *et al.*, 2018).

High doses of the fungicide chlorothalonil, widely used in tea plantations, resulted in its accumulation in the soil and were seen to inhibit the denitrification process while strikingly promote N_2O emissions by 380–830 percent (Su *et al.*, 2020). More specifically, the fungicide significantly inhibited N_2O reductase activity (part of the denitrification process that produces N_2O) without affecting *nosZ* abundance (the functional genes responsible for producing N_2O reductase). Therefore, use of chlorothalonil in this study promoted soil N_2O accumulation by perturbation of NOS activity (i.e. one of the four denitrifying enzymes), rather than *nosZ* gene abundance. This may be because NOS is located in periplasm and therefore easier to be disrupted by chlorothalonil. Nevertheless, the fungicide did impact the soil microbiome by downregulating denitrifying functional genes (*nar*G, *nirS*, and *nor*B) and also decreasing the relative abundances of potential denitrifiers (*Pseudomonas* and *Streptomyces*). That *nosZ* did not decline suggests that *nosZ*-harbored denitrifiers that could adapt to chlorothalonil toxicity under this experiment's conditions. Lastly, high doses of the fungicide also influenced soil denitrification via soil microorganisms by directly suppressing microbial electron transport system activity: there were decreased levels of electron donor nicotinamide adenine dinucleotide and energy source adenosine triphosphate.

In contrast to these studies, Li *et al.* (2014) found that the herbicide **methyl viologen dichloride applications to soils with corn roots significantly suppressed N_2O production by active pseudomonad denitrifying bacteria**. They suggest that methyl viologen dichloride likely non-selectively inhibited the redox enzymes associated with the denitrification process, thereby inhibiting N_2O production. In the same study, though, they also found that other **herbicides such as amitrole and other triazole-type chemicals may have potential to activate soil N_2O emissions by accelerating activity by particular pseudomonad denitrifying bacteria**.

Das, Ghosh and Adhya (2011) applied the herbicides bensulfuron methyl and pretilachlor separately and in combination to a flooded rice paddy. They found that **single applications of either herbicide resulted in a significant reduction of N_2O emissions**. **This was linked to lower mineral N, lower denitrifying and nitrifying activity, and low denitrifier and nitrifier populations.** In stark contrast, **applications of combined herbicides clearly increased N_2O emissions**. **The explanation lies in the reversal of the factors that inhibited emissions in single applications.** For instance, while the pretilachlor application decreased nitrifying and denitrifying bacterial abundance, the combined application of pretilachlor and bensulfuron methyl increased their abundance. It is possible that the larger C input through increased dead weed biomass and rhizodepositions resulting from a combined herbicide treatment favoured microbial proliferation.

Effects of pesticides on soil microorganism-mediated methane-related pathways

Indigenous microbial communities can be affected differently during the degradation process of pesticides, leading to different biogeochemical processes. **Pentachlorophenol, applied in anoxic mangrove soils, was observed to significantly inhibit suphate (SO_4^{2-}) reduction, resulting in high CH_4 emissions** (Xu *et al.*, 2019). The pesticide applications inhibited classical SO_4^{2-} and S-reducing bacteria in the families Desulfarculaceae, Desulfobulbaceae and Desulfobacteraceae, thereby decreasing the SO_4^{2-} reduction process. In contrast, pentachlorophenol had no significant influence on Fe reduction. In this case, therefore, two microbial communities associated with terminal electron accepting pathways were differently affected during pentachlorophenol dichlorination.

Cu can also affect members of the soil microbiome differently, with consequences for soil CH_4 emissions. Mao, Yin and Deng (2015) found that **the diversity and abundance of both methanotrophs and methanogens were decreased following copper ion (Cu^{2+}) applications, resulting in decreased CH_4 emission.** This decrease was attributed specifically to the negative effects on methanogens, as well as on rice growth – the reduced shoot and root growth inhibited transportation of CH_4. However, the lowest dose (200 mg kg^{-1}) did not significantly decrease the diversity of methanotrophs and methanogens in the rhizosphere, likely owing to adsorption of Cu^{2+} by rice roots and exudates. Lastly, that the diversity of methanotrophs was more affected than that of methanogens led the authors to suggest that the former were more sensitive to Cu^{2+} additions.

In their study already described above, Das, Ghosh and Adhya (2011)reported that **single applications of bensulfuron methyl or pretilachlor herbicide resulted in a significant reduction of CH_4 emissions**. This was explained by several factors, including a prevention of the drop of redox potential. In other words, weeds help increase the redox potential of soil by transferring atmospheric oxygen to the rhizosphere, which leads to less CH^4 production. Other factors were lower readily mineralizable C, and MBC and lower methanogenic and higher methanotrophic bacterial population. Like for N_2O emissions, **applications of the combined herbicides clearly increased CH_4 emissions, the explanation also lying in the reversal of the factors that inhibited emissions in single applications.**

Effects of pesticides on soil microorganism-mediated carbon dioxide emissions

Two studies, using different pesticides, investigated CO_2 emissions, concluding that only at higher-than-normal-use concentrations did they have an impact on the soil microbial community. Gigliotti *et al.* (1998) report that the herbicide bensulfuron-methyl, at concentrations greater than used in normal agricultural practices, can affect the structure and activity of the soil microbial community. They did not, however, detect any significant changes in soil respiration (CO_2 emissions). Allievi *et al.* (1996) did find that bentazon inhibited soil nitrification and CO_2 emission, but only at the highest dose tested.

Concluding remarks

Altogether, these studies describe how certain pesticides can impact the soil microbiome and GHG fluxes, while pointing to several questions. For instance, what are long-term effects of continuous applications or high-doses – both of which can potentially lead to high concentrations of a given pesticide in the agricultural soil – on the soil microbiome structure and activities, and in turn, GHG emissions? It is possible that N_2O emissions in tea plantations, for instance, may be vastly underestimated. Given that it is not an uncommon practice, what are the different effects of combined pesticide applications, in different farming systems which feature different soil environments? Regarding pesticides that are no longer in use, but still present in soils, it seems that the impact of bioremediation processes such as chlorinated pollutants can have an impact on soil biogeochemical processes.

4.8.6

Systematic review: Effects of pest management on the soil microbiome, and their combined impact on human health

Only three of the twenty studies found were selected for this section. The other studies addressed slightly different topics that are out of the scope of this section. They ranged from the impact of pesticides on the insect gut microbiome, to the effect of pesticides application on human health, to the capacity of bio-fertilisers to degrade pesticides in soils.

The studies included here address soil pesticide pollution, remediation of pesticide polluted soils, and presence of anti-microbial resistant genes in the resistome, with implications on human health. Overall, few studies were found that drew direct links between use of pesticides, effects on the soil microbiome, and subsequent consequences to human health.

Do soils contaminated with previously banned pesticides demonstrate human health risks via an impact on the soil microbiome?

Organochlorine pesticides (OCPs) were produced and extensively used in China between the 1950s and 80s. Although their use has been abandoned for the last three decades, it resulted in widespread soil and vegetation pollution. Phthalate esters (PAEs) are used in the production of a range of plastic products, in everything from building materials, polyvinyl chloride (PVC) resins, food packaging and the growing sector of controlled-environment agriculture (e.g greenhouses, use of plastic films).

Sun *et al.* (2018a) investigated the presence of OCPs, specifically, DDTs and HCHs, and six priority control PAEs in soils and vegetables in Jiansu province and Shangai municipality (Eastern China).[37] They found that the OCP residues accumulated in the surface plough layer, whereas PAEs infiltrated easily into the soil, thereby impacting deep soil layers and groundwater. They also observed that PAEs significantly reduced the soil fungal abundance. Conversely, no significant correlation between OCPs and soil microbiota was found. **Overall, the soil ecosystem functions did not appear significantly influenced by OCP or PAE pollution, and they concluded that there was marginal non-carcinogenic and low carcinogenic risk to human health.**

Can continuous pesticide use and organic fertilizer applications change the soil resistome?

The following study did not include a direct analysis of impacts on human health, but questioned the role of pesticides and agricultural soils in the current trends of clinical multi-drug AMR. Armalytė *et al.* (2019) compared microbiota diversity in soils from organic and conventional management in Central Europe, with specific regard to antimicrobial resistance gene transfer (AMRG). They found that the soil bacterial profiles between management styles were similar, with only very minor taxonomic differences (**Figure 29**). Microorganism diversity indexes were comparable between management styles, too. **These results implied that continuous pesticide use under conventional management did not significantly change the soil microbial community in this study.**[38] The authors concluded that the similar profiles under organic and conventional management suggest that the soil microbiome was quite stable. This may be due to their ability to adapt to environmental changes such as introduction of chemical substances that originate from organisms like themselves. Lastly, they found only a small number, and in low variety, of clinically important genes that encode resistance to antimicrobials. **They therefore concluded that there was no transfer of AMRGs to soils under either management system. However, they emphasize that what level of farming intensity under conventional management could change the soil microbiome remains unclear.**

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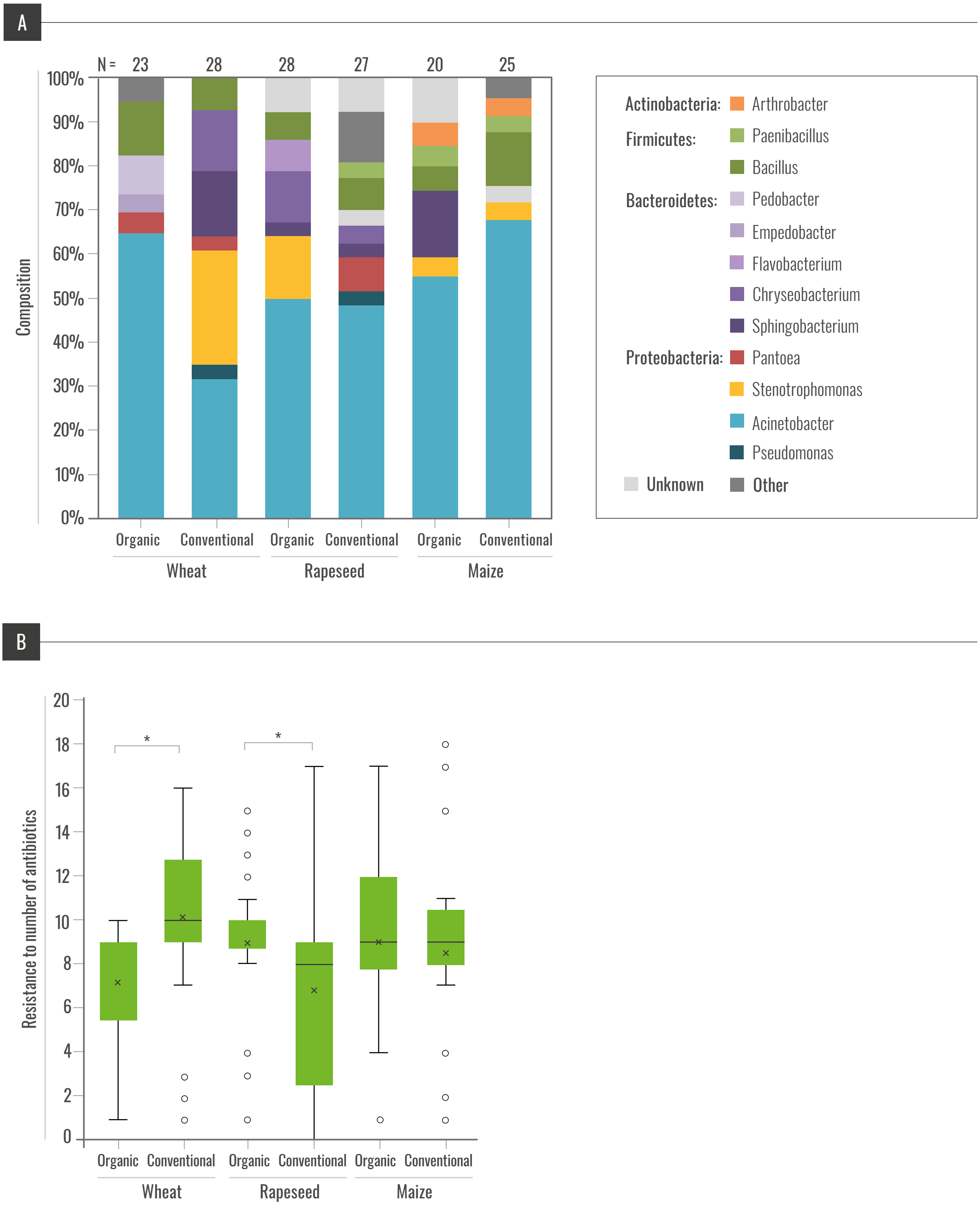
[37] Dichlorodiphenyltrichloroethanes (DDTs) and hexachlorocyclohexanes (HCHs).

[38] Pesticide compounds or names were not specified.

FIGURE 29.

CULTIVABLE ANTIBIOTIC RESISTANT SOIL BACTERIA ISOLATED FROM SIX SOILS UNDER DIFFERENT FARMING STYLES.

(A) The abundance of resistant bacterial genera (grouped by phylum) isolated from soils under different farming styles. (B) The abundance of antibiotic resistant bacteria isolated from various soils. Boxes indicate upper and lower quantities, and the vertical bars with end points indicate minimum and maximum values excluding outliers, circles depict outliers, and crosses indicate mean values. * Indicates statistical significance calculated as non-parametric Mann-Whitney test for two independent samples (p<0.05; one-tailed).



Source: Armalytė et al., 2019.

BOX 7. THE IMPACT OF GLYPHOSATE ON THE SOIL MICROBIOME REMAINS CONTROVERSIAL

Glyphosate is the most widely used herbicide in the world (Benbrook, 2016), the total surface area treated having increased rapidly between 1995 and 2014 due to introduction of glyphosate-resistant crops, use as a harvest aid, and increased application rates to combat weed-resistance. **Nevertheless, despite being a widely used and substantially studied herbicide, precisely how it affects soil microorganisms remains controversial**. This may be due to several factors, including the limited resolution of techniques to characterize more subtle changes in soil microbial communities, how the many different location and soil characteristics influence microbial communities, as well as the small effect of glyphosate treatments at the field scale (Schlatter *et al*., 2017a).

The review by Van Bruggen *et al*. (2018) reports that **different studies have demonstrated that glyphosate negatively affected plant-growth promoting microorganisms such as *Rhizobia* species, AMF, *Burkholderia* and *Pseudomonas* species. In contrast, other studies found no or only minor effects on the soil microbiome**. These included some observations of microbial communities that seemed to recover from short-term glyphosate treatment. The authors of the review suggested this was likely due to the diversity and compensatory ability of soil microorganisms. **Indeed, a meta-analysis found no significant effect on soil microbial biomass or soil microbial respiration overall** (Nguyen *et al*., 2016). There was actually an increase in these parameters at higher rates of glyphosate application (>100 mg kg-1), because the herbicide provides sources of nutrients. Nevertheless, they concluded that the glyphosate dose was influential, as mid-range concentrations could suppress microbial biomass. **Somewhat in contrast to these observations,** Riah *et al*. (2014) **found that the group of herbicides that includes glyphosate induced negative responses in soil microbial enzyme activities in 77 percent of the microcosm experiments reviewed.**

Shifts in soil microbial community structure are notable amongst studies that have shown significant but minor effects of glyphosate (Schafer, Hallett and Johnson, 2014; Schlatter *et al*., 2017, 2018). Schlatter *et al*. (2017) , for instance, studied the effects of repeated glyphosate treatments on soil and rhizosphere bacterial communities in wheat plots with and without long-term history of glyphosate applications. They found that glyphosate overall had small impacts on fungal community composition or diversity. Chou *et al*. (2018) likewise identified soil fungal and bacterial community changes with glyphosate application in vineyards. Schlatter *et al*. (2018) tested glyphosate effects on fungal communities using soils from four different locations in a dryland wheat production area of eastern Washington and Idaho. Results showed that only a small percentage of taxa showed differences following glyphosate application, and that glyphosate was not the main driver of a fungal community shift. Nevertheless, their findings suggested that glyphosate can affect interactions of fungi competing for dying roots, without impacting fungal community composition in wheat cropping.

When assessing glyphosate's effects, it is important to consider that minor changes triggered by glyphosate on the soil community composition can have much broader, indirect impacts on soil functioning, plant health and animal pathogens. For instance, species in the fungal genus Fusarium that cause certain, serious plant diseases have been observed to be non-responsive to glyphosate, while their antagonistic microorganisms are very sensitive to it. This implies that exposure to glyphosate might negatively affect microorganisms that help control *Fusarium* species populations and activities, without directly affecting *Fusarium species* itself (Van Bruggen et al., 2018). Indeed, pathogenic *Fusarium* species that cause root rot in glyphosate-treated soils have been observed repeatedly. Glyphosate can also have indirect effects through changes in plant endophytic (i.e. within the plant) and rhizosphere microbiomes. For example, herbicide use can increase plant production, resulting in root exudation of carbohydrates and amino acids, which can enhance attraction of plant pathogens (Van Bruggen *et al*., 2018).

4.9
MICROPLASTICS IN AGRICULTURAL SOILS

HIGHLIGHT BOX 14 **Impacts of microplastics in agricultural soils on the soil microbiome, climate change and human health.**

NARRATIVE REVIEW **What are the impacts of microplastics in agricultural soils on the soil microbiome?**

- Research investigating effects of microplastics on soil microorganisms remains relatively underdevelOped.
- There are multiple possibilities for direct and indirect effects of microplastics on soil microorganisms. Microplastics can alter the soil environment, potentially selecting for microorganisms with certain traits that imply evolutionary consequences as they are passed on to the following generations (direct effect). Microplastics and nanoplastics, in particular, can accumulate in and have adverse effects on soil organisms. This may in turn impact soil microorganism communities and functioning, through complex, trophic relationships.
- Microplastics can enter agricultural soils by a wide variety of possibilities, including direct release from agricultural products, fragmentation of macroplastic litter, treated wastewater and municipal waste, fertilizers, seed coatings, inappropriate disposal and transportation of atmospheric particles.
- Plastic fragments can clearly form a distinctly different habitat, compared to the bulk soil, for microorganisms.
- Looking towards the future: Microplastics may have evolutionary implications on soil microbiota with a range of selection pressures; recent studies have clearly identified gaps and opportunities for research.

SYSTEMATIC REVIEW **What are the impacts of microplastics in agricultural soils on the soil microbiome, and their causal impacts on climate change?**

- Microplastics can have selective effects on soil microbes, such as different responses by fungal and bacterial abundance and diversity to different MP particle sizes. Such selective effects show potential to exert a substantial impact on soil microbial ecology and terrestrial biogeochemical cycles.

SYSTEMATIC REVIEW **What are the impacts of microplastics in agricultural soils on the soil microbiome, and their causal impacts on human health?**

- No relevant literature was found

4.9.1
What are microplastics and where do they come from?

The manufacture, use and disposal of plastics is increasing globally. Insufficient recycling and improper disposal contribute to plastic waste that ends up in oceans and in soil. Global plastic waste was estimated to total 6 300 tonnes as of 2015, with 79 percent of that accumulated in terrestrial systems (Geyer, Jambeck and Law, 2017). Extensively used types of plastic are polystyrene, polypropylene, polyethylene, polyvinylchloride, polyurethane, and polyethylenterephthalat (Awet *et al.*, 2018). Microplastic (MP) pollution was first observed in marine environments, and has generated a substantial body of literature indicating their potential for physical and toxicological harm (Law and Thompson, 2014; Thompson, 2015). Only relatively recently, however, have effects of MPs on terrestrial ecosystems been studied (Huang *et al.*, 2019; Rillig, 2012). In agricultural systems, plastics are commonly used in mulch films, greenhouses and plastic tunnels, nets, solarization and silage films, packaging bags and bottles for fertilizers and chemicals (De Lucia and Pazienza, 2019).

Plastics can be classified by size. At present, formal definitions are still being debated, but the following descriptions are commonly used (GESAMP, 2015; Lusher, Hollman and Mendoza-Hill, 2017).

- Macroplastics are plastic items larger than 5 mm in size (e.g. plastic litter or debris).
- **Microplastics are particles between 0.1 and 5 000 µm (micrometers) in their longest dimension. They present in a variety of shapes, including beads, fragments, fibres and films.**
- **Nanoplastics (NPs) are even smaller, ranging from 1 to 100 nm (nanometers).**

Furthermore, there are two categories of MPs and NPs. Primary MPs and NPs enter the environment in small sizes (e.g. microbeads used in cosmetic products or industrial processes). Secondary MPs and NPs are by-products of fragmentation and weathering of larger plastics (GESAMP, 2015; Lusher, Hollman and Mendoza-Hill, 2017). Biological degradation by microorganisms, mandibulate insects, and geophagous soil fauna - especially earthworms - can all contribute to fragmentation when ingested and broken down in the digestive tract. Larger organisms such as moles and rodents can also break macroplastics into smaller pieces. Exposure to ultra-violet (UV) radiation and elevated temperatures may render plastics on soil surfaces more brittle, and hence more likely to fragment (Horton *et al*., 2017).

Plastics are often made to be inert and take a long time to degrade, thus even in their degraded forms as MPs or NPs, they are environmentally persistent (Rillig *et al*., 2019). **Their presence is a world-wide problem that is causing global change through MP presence in ocean, freshwater, and terrestrial ecosystems. It has even been strongly suggested that soil could be a major sink of NPs, compared to air and water ecosystems** (Awet *et al*., 2018).

The nature of soil media presents a challenge to studying MP pollution and its adverse effects. Although some methods to extract and sort plastic waste from the soil have been developed, there are still several limitations such as size detection, absence of standard protocol and high costs (Bläsing and Amelung, 2018; Chae and An, 2018). This presents a limitation to all kinds of studies on relationships between MPs and soil (micro)organisms and their interrelatedness. Of the studies investigating MP effects on soil organisms, many have focused on larger soil organisms such as earthworms and collembola (Chae and An, 2018) rather than microorganisms. **This section nevertheless offers an overview of how MPs and NPs may impact the soil microbiome, directly or indirectly.**

Microplastics can find their way into the (agricultural) soil environment by a variety of means. One is by direct release from agricultural plastic mulch (Steinmetz *et al*., 2016; Zhang *et al*., 2019b). It is used extensively as it blocks weeds, increases water use efficiency by regulating the soil microclimate, therefore generally improves crop production. Its use is very popular and has increased every year for the last 30 years (Geyer, Jambeck and Law, 2017), but the complete retrieval of films from the soil is very difficult, especially in the case of thin films that tend to rip easily. Those film fragments can be further damaged by ploughing, and their continued breakdown can eventually result in MPs. Processed municipal waste resulting in mixed-waste organic output (MWOO), mixed municipal waste-derived compost, or compost-like output (CLO) are other sources (Judy *et al*., 2019). It has been demonstrated, for instance, that laundry washing can discharge large amounts of MPs into wastewaters (De Falco *et al*., 2019; Napper and Thompson, 2016), and synthetic fibres have been reported in sewage sludge and effluents during wastewater treatment (Habib, Locke and Cannone, 1998). Considering the quantity of plastics present in municipal waste streams, there is substantial concern about the NPs created during the mechanical-biological treatment process. Use of coated fertilizers for controlled release, use of seeds coated in plastic-like material for ease of handling and pesticide encapsulation, inappropriate waste disposal, and atmospheric particles transported over long distances are all additional sources.[39]

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[39] Applying a thin, plastic-like coat can improve seed handling while simultaneously encapsulating pesticides. Mechanical abrasion during handling and planting of coated seeds can create microplastic coating fragments and dust-off. Accinelli **et al.** (2019) found that though the fragments did not last very long, their degradation rate was highly variable. Of note, there was a higher degradation rate for biodegradable seed coatings incorporated with plant growth promoting bacteria.

4.9.2
In what ways can microplastics potentially impact soil microorganisms?

There are multiple possibilities for direct or indirect effects of MPs on soil microorganisms. A few are introduced here:

- Microplastics can affect soil organisms, and therefore indirectly affect soil microorganisms (see next point below). Chae and An (2018) investigated current research trends on plastic pollution and their impacts on the soil ecosystem. A few highlights from their study are: i) Microplastics can accumulate in and have adverse effects on soil organisms ii) Microplastics can cause changes in chemical contents of soil organisms; iii) Responses of soil organisms can cause changes in soil characteristics; iv) Chemicals (including pesticides) adsorbed on MPs can enter the soil ecosystem; and v) Microplastics can move horizontally and vertically in the soil.
- Chemical functionalities of MPs are different from macro fragments (Judy *et al.*, 2019). For instance, the type of polymer used in the plastic is a known factor in the rate of degradation in the environment. During degradation, plastics can release toxic additives such as plasticizers, retardants, stabilisers, antioxidants and photostabilizers and other substances introduced during manufacture of the plastic (Zhang *et al.*, 2020).
- Owing to their small size (<0.1 µm), some NPs can pass biological barriers such as membranes (de Souza Machado *et al.*, 2018). It has been suggested that in such cases, NPs could interfere with cellular or organism activities in soil animals, developing toxic effects that could select for genotypes with better resistance (Rillig *et al.*, 2019). **As soil microorganisms interact strongly with their animal hosts, this type of change in soil animal community structure could in turn impact soil microbial processes** (Rillig *et al.*, 2019).
- Alterations to the soil environment as a result of MP pollution could influence soil microbial community structures by selecting for microorganisms according to certain traits. Rillig *et al.* (2019) have suggested that this may shape the following generations, and ask what traits will be selected and perpetuated.

4.9.3
Studies show mixed results on the impact of microplastics on soil microorganisms

A review by Chae and An (2018) **found that research demonstrated mixed results regarding the impact of MPs on soil microorganisms, some studies showing no significant effects**. We discuss three different studies as examples of this variance.

Polystyrene nanoparticles (PS-NPs) can negatively affect microorganism biomass and extracellular enzyme activities. A short-term study reported that 28 days after addition of PS-NPs to soil, microbial enzyme activities related to nutrient cycling were reduced and that microbial biomass decreased, suggesting a broad and detrimental impact of PS-NPs on those enzymes and the soil microbiota (Awet *et al.*, 2018). A possible explanation could be that the small size and high surface area to volume ratio of NPs enabled them to interact closely with microbial cells, thereby exercising potentially negative effects. Also, NPs can have a strong sorption affinity for toxic compounds in soil, and could have negatively affected bacteria through their toxicity. The authors furthermore found that basal cell respiration increased with an increased PS-NP application rate, perhaps due to the death of some microorganism groups and subsequent feeding upon them by other surviving organisms. This is one of the few studies on soil microorganisms involving nanoplastics.

Huang *et al.* (2019) found that **amendment of polyethylene (PE) MPs stimulated microbial catalase and urease activities in soil**. Catalase has been suggested as an indicator of aerobic microorganisms (Liu *et al.*, 2017b), thus the increase in catalase activities could be attributed to increased porosity by PE micro-fragments. Urease has a close relationship with the soil N cycle, though the authors did not measure the composition of dissolved organic matter and so could not report on any direct effects. However, they speculate that PE MPs may have a similar effect as observed by Liu *et al.* (2017b): that the addition of polypropylene MPs significantly increased the dissolved organic carbon (C), total dissolved nitrogen (N), and dissolved organic N in soil (Liu *et al.*, 2017b).

The following is an example of research which did not find a significant effect of MPs on soil microorganisms. In their study in New South Wales Australia, Judy *et al.* (2019) used treatments of unamended soil, MWOO-amended soil, and MWOO-amended soil with high density PE (HDPE, from shopping bags), PE terephthalate (PET, from drink bottles), or polyvinyl chloride (PVC, from tablecloths). Tests were conducted at zero, three and nine months. When introduced into the experimental media, the MPs measured <2 mm in size and were irregularly shaped, consistent with MP properties previously observed in MWOO. They found that **measurements of microbial community structure were highly variable, demonstrating no clear trends, therefore concluding that there was little evidence in their study that MPs affected the microbial community structure or functions.** The main differences found were actually attributed to soil type and incubation length. They suggested that further research is necessary, experimenting with different concentrations, particle sizes, different soil types and different MWOO types.

4.9.4
Plastic fragments can form a distinct habitat for soil microorganisms

Several studies have clearly demonstrated that plastic fragments can form a distinct habitat from the bulk soil.[40] We return to the study by Huang *et al.* (2019), which found that the microbial communities on the PE plastic fragments showed significantly lower diversity indexes than in the control and amended soil. The bacterial community structure on the fragments showed distinct assemblage; several taxa such as plastic-degrading bacteria and pathogens were more abundant. Other studies have reported that *Streptomyces* (the largest genus of Actinobacteria) can degrade PE (Abraham *et al.*, 2017; Huerta Lwanga *et al.*, 2017); they were indeed enriched on the MP fragments after 90 days, suggesting they may have plastic degrading capacities. The relative abundance of the genus *Norcardia* increased on PE fragments after 90 days; they are known to be causal agents of suppurative and granulomatous human and animal diseases. Findings in this study resemble similar dynamics in studies on MPs in water habitats. Hence, MPs offer a new habitat in the soil to colonize. The authors suggest that **this may result in alterations of biogeochemical processes and ecological functions, and may serve as vectors supporting disease-causing bacteria. Furthermore, changes in communities may affect N and C cycles by changing metabolic functional diversity.**

In a study with similar conclusions, Zhang *et al.* (2019b) used soils from cotton fields in China where plastic mulch had been applied for more than 30 years. They compared microorganisms on plant litter, macroplastic and MPs particles using SEM and DNA sequencing. Results also demonstrated that MPs were a distinct habitat for the microbial community. For one, pits and flakes were especially colonized. The microbial community on MPs was also distinctly different from surrounding soil, plant litter and macroplastics, results indicating a larger and more complex bacterial network. Given that the smaller particle size of MPs (compared to those of plastic residues or plant litter) increased the surface area in contact with the soil, the MPs likely supported more complex interactions between microorganisms. In addition, MPs remain in the soil in their form longer than plant litter or macroplastics, and therefore colonizing microorganisms have more time to form their core community, metabolizing their own substrates. Finally, the authors propose that MPs may be a "special microbial accumulator", offering particular spaces and substrates for colonizing bacteria (as seen in other studies in aquatic environments). This study looked only at three replicate plots in a small area of a cotton field. More studies taking into account soil types, plant varieties, and management decisions such as mulching duration or fertilization systems are needed to further explore these findings.

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[40] Bulk soil is soil outside of the plant root zone, or rhizosphere.

4.9.5

Microplastics can affect soil microorganisms through their host soil organisms

Microplastics can also affect other soil organisms, potentially affecting food webs through complex trophic relationships. Microplastics are known to be consumed by soil animals (e.g. earthworms, collembolans) with ensuing negative effects. They can also affect microorganisms inside the host's gut. For example, microbes specialised in degrading MP compounds have been isolated from earthworm guts. Rillig *et al.* (2019) project a cascading effect of MPs on microbiota through effects on host animals.

Ju *et al.* (2019) studied effects of PE MPs (<500 µm) at different concentrations for 28 days on soil springtail *Folsomia candida.* Results demonstrated that the MPs had significant toxic effect on springtails: they exhibited avoidance behaviours, there was decreased bacterial diversity in their gut microbial community, and their reproduction was inhibited at 0.1 percent MP amendment. Their reproduction was further reduced by about 70 percent at the highest concentration of MPs (1 percent).

Xiang *et al.* (2019), investigating effects of polystyrene MPs (2–2.9 µm) likewise on the collembolan *Folsomia candida*, also found that the MPs altered the gut microbiome. PVC is one of the most common polymers used world-wide. Zhu *et al.* (2018b) studied effects of PVC MP exposure (80 and 250 µm) for 56 days on the same collembolan species, *Folsomia candida*. They observed that exposure to MPs altered microbial community composition and structure in the gut. These changes were hypothesized to result from alterations in feeding behavior incurred by exposure to MPs. The inhibition of collembolan growth and reproduction by MP exposure may also have contributed to gut microbiome changes. The gut microbial diversity was actually enhanced by exposure to MPs, likely owing to more ingested bacterial taxa.

In another study by Zhu *et al.* (2018a), the authors fed soil worms (*Enchytraeus crypticus)* oatmeal with polystyrene NPs (0.05–0.1 µm). Results showed a significant shift in the gut microbiome when worms were fed at a rate of 10 percent nanoplastics. Specifically, there were significant decreases in the relative abundance of families that contain key microbes contributing to N-cycling and organic matter (OM) decomposition (Rhizobiaceae, Xanthobacteraceae and Isosphaeraceae). Individual worms also demonstrate significant reductions in weight at 10 percent. Results of this study imply that NPs are pollutants with potential for detrimental influences on terrestrial ecosystems by inhibiting key bacteria in the microbiome of invertebrates that provide fundamental ecosystem services.

4.9.6

A potential connection between microplastics and antimicrobial resistant genes

The following two examples describe interactions between MPs, soil microorganisms and antibiotic resistance genes (ARGs) from two different perspectives.

It is known that MPs habitats can be hotspots of gene transfer by providing favourable conditions for community interactions, including the potential horizontal transfer of ARGs. In their study using polystyrene MPs (2–2.9 µm), Xiang *et al.* (2019) observed **alterations in the collembolan gut microbiome, which were associated with changes in the microbial ARG profile. Specifically, ARGs were increased, and even more so when the polystyrene MPs beads were associated with sulfamethoxazole, an antibiotic typically used to treat bronchitis and urinary tract infections.** Amongst potential explanations, the authors refer to the observation that bacteria can grow as a biofilm on MPs, thereby potentially changing their ecological functions such as protecting themselves from antibiotics.

Questions have been raised about the potential negative effects of mixed contamination MPs and ARGs in greenhouses. The MPs can result from degraded residual films, and the ARGs from organic fertilizers (see **Section 4.7.6 about organic fertilizer and AMR**). It is known that bacteria transmit ARGs to other bacteria**. More recently, though, it has been proposed that bacteriophages may be an even more crucial source of ARG transmission,**

serving as vectors to transmit antimicrobial resistance (AMR) within microbial populations.[41] Bacteriophages are likely even more abundant than bacteria (Ashelford, Day and Fry, 2003; Cesar Ignacio-Espinoza, Solonenko and Sullivan, 2013). Microplastics have been observed to reduce dissipation of ARGs. To counter this effect, environmentally friendly facilitators (biofactants) have been used to improve the bioaccessibility of organic pollutants, thereby increasing the dissipation of ARGs. In their study, Sun *et al.* (2018b) found that MPs presence inhibited dissipation of the antibiotic tetracycline and ARGs in greenhouse soils. However, application of an antimicrobial biofactant (a sophorolipid) outweighed the inhibiting effect of MPs, and led to the highest dissipation of tetracycline and ARGs. Soil bacteria and phages were identified as crucial reservoirs of ARGs.

4.9.7
Evolutionary implications of microplastics for soil microbiota

An interesting study explored evolutionary implications of MPs on soil microbiota (Rillig *et al.*, 2019), **describing a range of selection pressures that are likely to act upon soil microorganisms**. To start with, MPs are a source of C and other nutrients. The abundance of genotypes with the ability to access these resources, or other chemically or physically bound additives, can be expected to increase in certain communities. In addition, though sorption capacities vary with different MPs, they have been seen to have high adsorption capacities for specific antibiotics, because of their elevated ability to adsorb or absorb trace elements and other chemical substances. Those two points put together explain how MPs can serve as hot spots of microbial evolution and horizontal gene transfer. Microplastics can change the soil physical environment by altering soil aggregates, which provide very specific, distinct temporary habitats and are known to be microbial evolutionary incubators. It is possible that changes in rates of formation, stabilisation or disintegration of soil aggregates could therefore affect microbial evolution. The authors point to the impact of linear fibres, in particular, as they have already been demonstrated to have effects on soil aggregation. As already mentioned, NPs can pass through biological membranes of macro-organisms. It would therefore not be surprising to observe increased abundance of genotypes with better resistance to NP toxic properties. The cascading effect of MPs on soil microorganisms through their larger hosts has also been mentioned above.

4.9.8
Future directions for research on microplastics and soil microorganisms

Rillig *et al.* (2019) highlighted **favoured approaches to further studies on these evolutionary responses to MP presence in soils**. One is experimental evolution using serial transfers (i.e. testing whether traits that are expected to be favoured do indeed increase over time). A second is resurrection ecology, which involves comparing current populations with reanimated populations from archived soil samples that date to before widespread plastic use.

Looking towards the future, Chae and An (2018) **and** Rillig *et al.* (2019) **make recommendations regarding obstacles to overcome and issues to focus on if research is to reflect real-world conditions**. To begin with, Chae and An (2018) call for development of advanced techniques for sampling, extraction, and detection of plastic wastes in soil. This goes hand in hand with the need for a better understanding of plastic pollution in soils, including ecotoxicity. One obstacle that needs to be addressed is the diversity of MPs, in terms of shapes, sizes, surface properties and chemical forms (Rillig *et al.*, 2019). For example, the effect of beads, films, and fibres may be quite different. Indeed, round-shaped beads are often used in studies due to their convenience (Chae and An 2018). Another issue is the effect of plastic product additives (e.g. plasticizers, retardants, antioxidants, photostabilizers) and adsorbed chemicals in the soil environment that will need

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[41] A bacteriophage is a virus that infects bacteria, destroying its host in the process.

to be accounted for (Chae and An 2018). Also, MPs enter the soil gradually through degradation processes, and not through sudden introductions as simulated in many studies (Rillig *et al.*, 2019). Finally, soil trophic transfers are very complex and diverse, raising questions about effects of long-term exposure to soil food webs and reproductive systems of (micro)organisms (Chae and An 2018; Rillig *et al.*, 2019).

Concluding remarks

To conclude, though studies regarding the effects of MPs on soil microorganisms show mixed results, it is clear that they have potential to impact soil microbial communities and functioning as well as complex trophic relationships. It has been undeniably demonstrated, in addition, that plastic fragments form distinctly different microhabitats. Their specific chemical functionalities, capacity to pass biological barriers and accumulate in macro-organisms, combined with their wide use and presence in soils around the world give reason to continue critical research to understand their impacts, including evolutionary implications for soil microbiota.

4.9.9

Systematic review: Effects of microplastics on the soil microbiome, and their combined impact on climate change

A search for studies linking MPs in agricultural soils, the soil microbiome and climate change returned one article (see **Annex I** for search terms).

The following study suggests that MPs have selective effects on soil microbes and appear to show a substantial, detrimental impact on soil microbial ecology and terrestrial biogeochemical cycles. Ren *et al.* (2020) investigated the influence of two different MP particle sizes (<13 µm and <150 µm) on dissolved organic C and relative functional groups, greenhouse gas (GHG) fluxes, and bacterial and fungal communities in fertilized soils in a thirty day trial. They used MPs from polyethylene, typically used for mulching film, at a concentration of five percent.

Microplastic presence in fertilized soils decreased the Global Warming Potential, mainly owing to the reduction of nitrous oxide (N_2O) emissions in the first three days of the trial. According to the authors, a mechanism that may explain this initial decrease in N_2O emissions is the MP inhibition of the phylum *Chloroflexi* (which possesses denitrification genes such as *nirK* and plays a key role in N removal) and genera *Rhodoplanes*, combined with MP enhancement of the abundance of *Thermoleophilia* (which have been observed to correlate with a decrease in N_2O emissions). At thirty days, however, there was an increase in N_2O emissions, related to the reduction of ammonia (NH_3^-), an increase in fungal denitrifiers, and a decrease in *Gemmatimonadacea* (which are related to *nosZ* clade II – one of the two N_2O reductase genes – a large fraction of which do not produce N_2O).

Finally, this study suggests that MPs may also influence microbial networks through their different capacities to metabolize nutrients, and therefore possibly influence biogeochemical cycling in this way, too. In soils with MPs, for instance, Actinobacteria replaced Proteobacteria as the dominant phylum, perhaps because the former are potentially able to degrade PE.

4.9.10

Systematic review: Effects of microplastics on the soil microbiome, and their combined impact on human health

The literature search returned one article, but it was not relevant to this section.

CHAPTER

5

CONCLUSIONS

5.1
WHY DOES THE SOIL MICROBIOME MATTER FOR A HEALTHY PLANET?

The soil microbiome is fundamental to a healthy planet because its immense biodiversity is responsible for carrying out a multitude of fundamental ecosystem functions. These activities are the foundation of essential biogeochemical processes on earth. The soil microbiome is, for example, a direct controller of greenhouse gas (GHG) fluxes and soil carbon (C) storage and sequestration. In this way, the soil microbiome also plays an essential role in ecosystem restoration. For instance, soil microorganisms are critical in the processes that enable healthy ecosystems to store huge volumes of carbon. Ecosystem functions that benefit human society directly are called ecosystem services (ESS). These include provision of food and water, soil formation, and production of atmospheric oxygen.

Soils host more than one-quarter of global biodiversity, including microorganisms, but we still have much to explore regarding soil microorganisms and their simultaneously performed functions. Nevertheless, there is firm evidence of a link between loss of microbial diversity and a reduction in multifunctionality. Furthermore, a positive link between soil biodiversity and multifunctionality has also been firmly established. These concepts are pivotal to understand the potential consequences for ecosystem functions related to biodiversity loss. They also offer opportunities to better design sustainable agriculture systems. The evidence-based knowledge that we already have is enough to promote the message that soil microorganism biodiversity and functions, and their complex interactions in terrestrial agroecosystems, can contribute to achieving food security targets as described in SDG 2 of the 2030 Agenda for Sustainable Development.[42]

In addition to harbouring sustainable food production systems, a healthy planet includes a healthy climate and healthy humans. Continued exploration of the soil microbiome and its functions will contribute to improving agroecosystems' potential to mitigate and adapt to climate change. The relationship between the soil microbiome and human health has generally been less studied, with many intriguing questions as yet unanswered.

Leaving no one behind is a central narrative in FAO's Strategic Framework 2022–2031 (FAO, 2021). The new strategy aims to achieve this vision through sustainable, inclusive and resilient agri-food systems for better production, better nutrition, a better environment and a better life. Emerging knowledge about the soil microbiome and cropping systems could play a major role in better production and better environment.

5.2

WHAT ARE THE IMPACTS OF CROP PRODUCTION PRACTICES ON THE SOIL MICROBIOME?

There is solid evidence that crop production practices can impact the soil microbiome, with varying predictability and significance depending on the practice and farming context. These effects are often described without qualification as negative or positive, because the interactions within and between the soil microbiome and farming systems are extremely complex and characterized by trade-offs. In our literature review, we explored how the following practices can impact the soil microbiome: land use, tillage, agro-system diversification (including plant diversity, crop rotations, cover crops), crop residue management, plant variety selection, irrigation, fertilization, pest management, and microplastics in agricultural soils. The following are selective highlights that illustrate such effects:

- **Land-use** changes such as deforestation can affect the soil microbial biomass, diversity and functional roles. In some cases, the impact of land-use change can be stronger than extreme meteorological events such as drought.
- **Excessive tillage** usually negatively affects soil microbial communities, causing changes in their composition by affecting both bacterial and fungal populations, thereby influencing soil functioning, plant productivity and provisioning of certain ecosystem services.
- A central point regarding **agroecosystem diversification** is that plants can influence the soil microbiome through their different biochemical compositions (e.g. nutrients and metabolites), introduced into the soil via plant litter, root exudates and rhizodeposits. This creates a plant legacy, or soil memory effect, whereby the plants continue to influence the soil environment even after they have disappeared. Though studies have reported positive or no effects of **plant diversity** on soil microorganisms, it appears that sown plant diversity increases microbial reproduction and necromass, which may play a key role in soil organic carbon storage. **Crop rotations** seem to result in improved soil health and related ecosystem services via effects on the soil microbiome. **Cover crops** can impact soil microorganism communities, including fungi, with possible improved ecosystem resilience.
- The impact of **crop residues** on the soil microbiome remains inconclusive, studies reporting positive, minor or no effects.

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[42] SDG 2 aims to end hunger, achieve food security and improved nutrition, and promote sustainable agriculture.

- **Plant variety selection** impacts the soil microbiome because plant genotypes influence root exudate composition and plant architecture, shaping the selection of rhizosphere microorganisms. Plant domestication has led to potential "missing microbes" in the root microbiome.
- **Irrigation** directly changes soil abiotic properties and can indirectly modify the rhizosphere through plant rhizodeposition (i.e. root exudates and root litter). The latter effect can shape communities of microorganisms that promote crop growth and disease control. In addition, treated wastewater can carry antimicrobial resistant elements, causing concerns about how irrigation with such water sources impacts the soil resistome.
- Both inorganic and organic **fertilizers** can affect the soil microbiome directly (e.g. as a source of nutrients) and indirectly (e.g. through positive effects on plant growth and development). Organic fertilizers can contribute microorganisms (e.g. through manure or compost applications), substantial carbon and other nutrients and are therefore considered important for long-term soil fertility, soil functions and multifunctionality. However, there are concerns and unanswered questions regarding how trace element accumulation, antimicrobial resistant genes and antibiotic residues, introduced through manure fertilizer, impact the soil resistome.
- **Pesticides** can impact the soil microbiome, significantly increasing or decreasing soil microbial communities, activities and biomass; less commonly, they can also have no detectable effect. These effects can drive selection of soil microbial species that can degrade pesticide compounds, leading to concerns about the presence of increased antibiotic resistance genes in the soil.
- Studies on the effects of **microplastics** in agricultural soils on soil microorganisms are relatively few. However, plastic fragments and microplastics form a distinct microhabitat. There is some evidence and some strong theoretical reasoning indicating that microplastics and nanoplastics, in particular, can potentially impact soil microorganism communities and functioning.

5.3

DO WE HAVE SOLID EVIDENCE OF THE IMPACT OF CROP PRODUCTION PRACTICES ON THE SOIL MICROBIOME, WITH CLEAR CAUSAL LINKS TO CLIMATE CHANGE AND HUMAN HEALTH?

Related to climate change

Results from our systematic literature review of over 2 000 publications demonstrate solid evidence for strong connections between certain agricultural practices, the soil microbiome, and their combined, causal effects on GHG fluxes and soil C storage. These include, for example, tillage, fertilization, crop residue management, agroecosystem crop diversification and plant variety selection. **These findings drive home the message that the soil microbiome thus plays pivotal roles in ecosystem health, agroecosystems and the climate system.** This must be taken into account for agroecosystem design and agronomic management in order to combine goals related to both food security and the environment.

In addition, **trade-offs** incurred by the choice of practices need to be considered. For example, on the one hand, increased soil microbial biomass and activities result in increased carbon dioxide (CO_2) production through respiration. On the other, those same factors can lead to benefits such as improved soil functioning (e.g. nutrient cycling, improved soil health, better soil structure), which lead to long-term gains in soil organic C (SOC) storage, biodiversity and multifunctionality, and better resilience to climate-induced stress. It is thus critical to take into account short-term versus long-term benefits. Furthermore, this review explored agricultural practices in isolation and does not capture the complexity of farm management and the interaction of crop production practices. For instance, effects on the soil microbiome of farming systems that combine specific practices, such as conservation agriculture, were beyond the scope of this study. In real-life contexts, analyses should consider the sum effect of interactions across practices on the soil microbiome, while also calculating hidden GHG emission costs (e.g. global warming potential (GWP) of mineral fertilizers). Life cycle analysis offers a suitable method because it provides a framework for estimating the environmental impacts of all components of a farming system. The approach considers the entire life cycle of farm inputs and activities – from obtaining raw materials, their transformation and use in the production system, and including flows of matter and energy – thereby offering more than a simple sum of practices.

The following are selected highlights to illustrate connections between agricultural practices, the soil microbiome and the climate system:

- Different types of **land use** directly impact soil conditions and soil microorganisms with consequences for GHG fluxes and C storage. As land use shifts from less-disturbed to more-disturbed soils (e.g. natural forest to cropland), soil C storage potential and methane (CH_4) oxidation reduce while nitrous oxide (N_2O) emission increases. Related to the soil C storage potential, there is generally higher soil microbial biomass and microbial enzyme activities in forests than croplands.
- **Tillage** directly impacts soil structure, which partially but significantly regulates soil microbial activities of GHG production and release from soils. Well-aerated soils with good drainage are recommended to reduce surface N_2O fluxes, though under certain conditions wet, anaerobic soils do not necessarily enhance N_2O emission. Well-aerated, moist soil conditions favour CH_4 oxidation by methanotrophs and CO_2 exchange, while water-blocked soil pores hinder CO_2 escape to the surface. Many studies show that no-till (NT) increases denitrification in the short term, seemingly linked to increased N_2O emissions. However, the duration of this practice should be considered because its positive impact on denitrification may decline in the long run. In addition, there is a lack of data from places where NT is practised widely. Finally, the effects of tillage-influenced soil structure on soil microorganisms in addition to bacteria, archaea and fungi – such as protists – should also be considered.
- **Intercropping** has been observed to reduce as well as increase N_2O (potential) emissions. To get a clearer picture will require additional field experiments with different soil types, crop combinations, and a much more precise understanding of the microbe–microbe and plant–microbe responses to those management choices. Intercropping can have a short-term legacy effect on soil microbial responses regarding soil C and N dynamics, while potentially more persistent effects remain yet undefined.
- **Crop rotations** can impact nutrient cycling of C and N via soil microbial activities, owing primarily to different crop chemical compositions.
- It has been suggested that **cover crop** mixtures can improve microbial ability to degrade substrates, potentially resulting in reduced GHG emissions. Cover cropping may also be a strategy to increase subsoil arbuscular mycorrhizal fungi (AMF) inoculum and abundance, in turn helping improve fertilization efficiency, C sequestration, and reduction of GHG emissions.
- **Crop residue management** can affect soil environmental conditions, influencing soil microbial abundance, community structures and activities that are directly related to soil biogeochemical processes. Some subjects remain inconclusive, such as whether retaining or removing residues from the soil surface results in net increases of GHGs or not. Other subjects seem quite clear, such as the enhanced microbial functional diversity or activity induced by mixed crop residues, and the promotion of soil C stabilization through

stoichiometric balancing of C residue inputs with other nutrients.

- Most studies discussed regarding the **selection of crop variety** were related to rice production, suggesting that CH_4 soil emissions from paddy fields could potentially be altered by manipulating rice plant genotypes. High-yielding and hybrid cultivars, for instance, may offer a means to reduce CH_4 rice field emissions while increasing rice productivity. However, potential hidden costs such as mineral fertilizer requirements should be included in Global Warming Potential (GWP) calculations. There are also other opportunities such as plant breeding programmes that make use of varietal relationships with specific microbes (missing microbes).
- Factors that influence **irrigation** effects on the soil microbiome and consequences for GHG emissions include timing (night vs day), delivery mechanisms (drip or subsurface vs furrow, or aerated vs non-aerated), reduced quantities of water and water quality, and the context of the farming system. For instance, drip irrigation and subsurface irrigation could both save water and reduce N_2O emission in a greenhouse setting. Also, there are potential ecological benefits (e.g. reuse of water resources, increased soil microbiome resilience) of using reclaimed water.
- **Fertilizer**: *Inorganic nitrogen (N) fertilizer* appears to generally reduce soil microbial biomass, particularly with heavier loads and longer durations. N input can also improve soil fertility and enhance the overall soil bacterial community composition and functioning, resulting in increased microbial production of N_2O and CO_2. Microbial communities and activities in different soil layers can have different responses regarding CH_4 dynamics. *Organic fertilizers* can enhance soil fertility and ecological factors compared to inorganic fertilizers (e.g. enhanced SOC; natural degradation processes foster low reactive N and thus potential to reduce N_2O emissions; enhancement of abundance and diversity of soil microorganisms), and can also be associated with increased N_2O production owing to stimulated soil microbes and activities. Depending on environmental conditions, biochar can increase soil C storage with reduced CO_2 and N_2O emission. *In rice paddies*, an increased inorganic N supply may either stimulate or inhibit CH_4-oxidation by methanotrophs, while the increased rice plant biomass and root exudates in response to an increased N supply are likely to stimulate methanogen activities. Taken together, these interactions may result in net increases or decreases of soil CH_4 emission, depending on other factors such as soil conditions.
- **Pesticides** can impact the soil microbiome structure, abundance and activities with consequences for GHG fluxes. Studies raised questions about the temporality of these effects, and the different impacts of combined pesticide applications in farming systems which feature their own soil environments.
- **Microplastics in agricultural soils** can have selective effects on soil microbes, and may also influence microbial networks through their different capacities to metabolize nutrients, thereby potentially incurring a substantial impact on terrestrial biogeochemical cycles.

Related to human health

There was little to no literature returned that identified clear links between crop production practices, the soil microbiome, and human health. We therefore conclude that the relationship between the soil microbiome and human health remains to be clarified, although questions about possible connections abound. One publication suggested that manipulating crop rotations may offer a strategy to reduce aflatoxin severity. The few other studies that did address this connection focused on concerns about risks to human health posed by:

- Irrigation-introduced pathogens transferred to humans by consumption of raw vegetables; and
- Changes to the soil resistome caused by **pesticide** application and **organic fertilizer** of animal origin, and the subsequent possible transfer of antibiotic resistance genes (ARGs) to humans through crop consumption. Though the studies did not find evidence to substantiate these concerns, many questions remain unanswered.

CHAPTER

6

NEXT STEPS: HOW TO BRIDGE THE GAP BETWEEN SCIENCE, INNOVATION AND POLICY?

6.1

PRIORITY R&D CHALLENGES RELATED TO THE SOIL MICROBIOME: WHICH ISSUES NEED URGENT, FURTHER INVESTIGATION?

Even as more knowledge about the soil microbiome is generated, knowledge gaps and research opportunities continue to grow. In keeping with FAO's role to both monitor the latest scientific research and inform policy on issues that need further investigation, the two literature reviews conducted for this report enabled identification of a number of research issues listed below concerning the soil microbiome and agricultural practices and their relationship with climate change and human health. Research issues identified during the focus group workshops held in July 2020 with international soil microbiome experts (from research, industry and policymaking backgrounds) are also included here.

Related to soil microbial diversity and functions

- The diversity of organisms drives the maximum exploitation of resources available in a given habitat. **There is a massive, yet relatively unidentified, diversity of genes and functions in the soil microbiome.** It is estimated that of 160 million unique microorganism genes identified in worldwide topsoil samples, only 0.51 percent are recognized in published genomes and gene catalogues. **The volume and diversity of unstudied soil microorganisms and their functions hold huge potential concerning design and management of sustainable agroecosystems,** putting global soil **biodiversity and biosphere integrity** at the centre of attention. Furthermore, understanding the link between soil biodiversity and multifunctionality is absolutely fundamental to discern the potential consequences of biodiversity loss. There is a need to better describe the determinants of the current biodiversity loss (e.g. what are the interactions between agricultural practices and agroecosystem products and the soil microbiome?).

- **Identification** of microbial species that could be used as **biological indicators** for ecosystem functions could help with long-term monitoring activities.

Related to the role of soil microbiota in biogeochemical cycling and climate change

- **Improved knowledge of soil microbial functions would help improve the accuracy of models that seek to predict outcomes of biogeochemical cycling processes.** For example, from modelling of farming systems that aim to harness microbial functions for sustainable practices to predictive climate change modelling at regional or national scales.
- **More precise understanding of mechanisms involved in soil biogeochemical cycling would help guide nutrient management strategies, benefitting crop production and long-term healthy soil functioning, as well as improve understanding of related consequences for greenhouse gas emissions from agroecosystems.** For instance, soil nutrient management could be enhanced by more knowledge about long-term biogeochemical processes related to crop rotations, about soil microbiome decomposition dynamics related to cover crops and crop residues, and about effects on the soil microbiome and plants of legume intercropping. In addition, plant nutrient content also concerns impacts on animal and human health. **The ability to quantify relationships between soil microbial communities and ecosystem functions, with predictability over time and space, would contribute to better understanding of greenhouse gas fluxes and, thereby, climate change.**
- **How changes in the soil microbiome can affect climate change remains a challenging question overall, but there is solid evidence of direct links. How to exploit positive feedbacks appears to be even more challenging to answer.** This is due, in part, to the complex and multilateral biogeochemical interactions in terrestrial ecosystems, and to the knowledge gaps about soil microorganism biodiversity and functions. How to engineer the soil microbiome to control carbon mobilization and storage, for instance, remains an outstanding question.
- **Soil microbial necromass may be a potentially underestimated contributor to soil carbon storage and carbon sequestration.** The products of soil microbial activities, such as their necromass, can accumulate in the slow-cycling soil organic matter pools and contribute to recalcitrant soil carbon. There is debate about the influence of soil microbial activity and crop residue degradation on soil carbon storage.

Related to underexplored fields where the soil microbiome plays a central role

- **The importance of certain microorganisms in microbial interactions in soil may have been underestimated, such as the role of protists in nutrient dynamics and bacteriophages in antibiotic resistance gene transmission.** Protists play key roles in food webs as consumers of bacteria, fungi and other small eukaryotes, but have received relatively little attention in scientific literature. Protistology should be incorporated along with studies of bacteria and fungi in terrestrial ecosystems. For example, soil protists have been observed to be more sensitive to nitrogen fertilization than bacteria and fungi. For another example, amoebae, the most abundant soil protozoa, release nitrogen and carbon from the bacteria they feed upon. This implies that their interactions may well have an impact on soil nutrient dynamics. Lastly, bacteriophages may be a crucial source of **antibiotic resistant gene** transmission in the soil, partly due to the potential acquisition of genes from bacterial hosts.[43]

- **The association between plant variety and rhizosphere microorganisms has not received enough attention in breeding programmes.** Genetic make-up of a plant species (or variety or cultivar) influences the chemical composition of its root exudates and its root architecture, which in turn influence selection of rhizosphere microorganisms. So-called missing plant microbes, resulting from genotypic changes that have occurred during plant domestication, could play a critical role in developing solutions to global, environmental challenges. Indigenous crop microbiomes could also play a similar role, with potential to increase crop resistance to biotic and abiotic stress and reduce agrochemical inputs. In addition, how, and to what degree, might transgenic plants influence the soil microbiome in comparison to conventional varieties?
- **Deeper soil layers have generally received less attention than topsoils, leaving unanswered questions about vertical distribution of biodiversity and functioning and deep-soil carbon dynamics.** Deeper soil layers (<20 cm) have been estimated to store about half of the global soil organic carbon stocks owing to their increased mass. Changes in subsoil horizons have potentially severe consequences, and because subsoil organic carbon pools react more slowly than those of topsoils, they may require more difficult restoration strategies. It is known that soil microorganism communities, activities and nutrient pools change with soil depth. However, there is a lack of information on ecosystem contributions of even those microorganisms well known to play vital roles in ecosystem functioning, such as arbuscular mycorrhizal fungi. Other research gaps include: what impact might agricultural practices such as organic fertilization have on nutrient dynamics mediated by soil microorganisms? And what is the potential for long-term carbon stabilization and the sensitivity of stored deep-soil carbon, likewise mediated by soil microorganisms? How might rhizospheric microbial activities influence deep-soil carbon dynamics with deep rooting plant?
- **There is a need to better understand the effects of micro- and nanoplastics on soil microorganisms.** Knowledge gaps include how micro- and nanoplastics affect biogeochemical processes, ecological functions, complex trophic relationships, and selection pressures that exercise evolutionary implications. There is also a need to understand impacts of different micro- and nanoplastic properties, such as particle shape, chemical composition and ecotoxicity, as well as the influence of different soil types, plant varieties and management decisions (e.g. mulching duration, fertilization systems). It has been clearly demonstrated, however, that microplastic particles form distinct habitats, thereby driving selection of soil microorganisms that can use the plastic particles as a source of energy.

Related to using soil microbiota to directly engineer improved soil and plant health

- More knowledge about bioinoculants, biostimulants and synthetic microbial community (SynCom) applications and their interactions with soil and plant microbiomes could help further technological opportunities in the field of **microbiome biofortification**.
- **More knowledge about the soil immune response, mediated by the soil microbiome, may be an opportunity to reduce pesticide use and improve other forms of biological control.** Engineering specific suppression in soils (comparable to the adaptive immune response in animals) requires understanding the specific interactions between pathogen, host plant, and soil organisms. This implies

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[43] A bacteriophage is a virus that infects bacteria, destroying its host in the process.

approaching sustainable disease management from a perspective that accounts for co-evolutionary histories between plants and soil microorganisms. In addition, it promotes a holistic approach that encourages use of synthetic or indigenous soil microbial communities. This is in contrast to a single-species approach, which neglects complex interactions in the soil microbiome, and consequently the introduced microbes frequently fail to establish.

- **Regarding soil restoration and remediation**: there is a need to understand more about how the soil microbiome could contribute to improving degraded soils by increasing soil carbon and, specifically, more bioavailable carbon. Similarly, how could it be harnessed to contribute to soil clean-up (remediation) following damage caused by industrial activities and certain types of fertilizers? And how can microbiome industrial applications contribute to soil remediation?
- **More knowledge about resistance and resilience of soil microorganisms to climate stress (e.g. drought, heavy rain, extended or increased heat), and the interactions between soil microorganisms and crops under these conditions, could help inform adaptive management strategies in the context of climate change.** For example, how might inoculation with beneficial microorganisms such as arbuscular mycorrhizal fungi help plants cope with stress? Or how might plant legacy impact carbon and nitrogen-related soil microbial activities, resistance and resilience after heat disturbance? And what are the links between water stress, nitrogen inputs, and soil microbial activities? How might plant-diverse agroecosystems such as agroforestry systems and legume intercropping enhance soil microbial resistance and/or resilience under climate stress conditions?

Related to the soil microbiome and antimicrobial resistance

- **There is a need for better monitoring of antimicrobial quantities** (e.g. fungicides and bactericides, including antibiotics) used globally by the agricultural sector. That some antimicrobials are used to treat both human and plant diseases may be problematic regarding potential human-pathogen resistance.
- **Comprehension of precisely how the introduction of antibiotic residues, antibiotic resistant bacteria and antibiotic resistance genes to agricultural soils affects the soil microbiome is still inconclusive.** Questions centre around entry pathways to agricultural soils (e.g. irrigation, manure fertilizer), degree and temporality of changes to the native soil resistome upon introduction of additional antibiotic resistance genes, potential cross-talk between soil and phyllosphere resistomes, and possible health risks posed by consuming treated crops. In conjunction, further studies are needed to address concerns about selection for pesticide- and plastic-degrading soil microbial species that could potentially lead to increased antibiotic resistance genes in soils. These issues are difficult to assess because: i) there are technical difficulties in detection and quantification of antibiotic resistant bacteria and antibiotic resistance genes; ii) the number of antibiotic resistant bacteria required to start successful colonization in the human body is unknown; and iii) there is very limited information regarding dissemination paths and transmission from the environment to humans. The One Health approach offers a framework for cross-cutting, interdisciplinary collaboration to pursue this issue.

Related to interconnections between ecosystems and between elements of human society

- **Connections between ecosystems must be explored through interdisciplinary collaboration** in order to respond to questions such as: what are the links between the air microbiome and the soil microbiome? What is the direct connection between the soil microbiome and human gut microbiome? How can soil biodiversity be leveraged to support crop production and human health?
- **There is a need for better understanding of the direct and indirect links between the soil microbiome and human health.** Possible direct links include effects on food quality (both safety and nutrition status), the role of antibiotic resistant gene abundance, and risks concerning human pathogens. Indirect links connect factors related to soil health (e.g. soil capability to remove pesticide residues or other pollutants) and provisioning of ecosystem functions and services.
- **Both field and laboratory-based research are required to improve knowledge about microorganisms, their microbe–microbe interactions as well as the influence of biotic and abiotic factors in their environment.**

6.2

KEY OPPORTUNITIES AND CHALLENGES: INNOVATIVE SOLUTIONS FOR GLOBAL PROBLEMS

Knowledge about what microbiomes do in the soil, and how they interact among each other and with the environment is a new scientific frontier that has led to many research findings and many more research questions. What we know and understand so far is that microorganisms have critical impacts on soil health, on human health, on plants and animals, and on ecosystems as a whole. Unravelling their complexities promises immense potential for innovation. Microbiome Science, Technology, and Innovation will be a major game changer in the way we manage our planet's resources to obtain our food and improve our health and the health of our ecosystems. It is these kinds of innovations that enable us to transform our economy into a more sustainable bioeconomy.

The bioeconomy is the production, utilization, conservation, and regeneration of biological resources, including related knowledge, science, technology, and innovation, to provide sustainable solutions (information, products, processes and services) within and across all economic sectors and enable a transformation to a sustainable economy (International Advisory Council on Global Bioeconomy, 2020). The sustainable bioeconomy has in fact been recognized as a leading framework for agri-food systems transformation by the Scientific Group of the recently convened UN Food Systems Summit. It is against this background that the FAO takes a holistic and inclusive approach to the application of bio-based innovations – including agri-food innovations – across our entire agri-food systems.

Microbiome Science, Technology, and Innovation is already showing potential to provide sustainable solutions that leverage the knowledge and concrete applications emerging from the fast-growing microbiome research and development field. Agri-food systems around the world stand to gain from the enormous potential of microbiome science, technology and innovation, supported by a circular and sustainable bioeconomy framework.

Indeed, our growing knowledge of microbiomes is not receiving attention from scientists alone, but also from industry, funders, regulators, and end users such as the public. Scientists build knowledge of soil and plant microbiomes, ultimately informing farming practices and helping develop biotechnologies such as biopesticides and biofertilizers to enable more sustainable farming.

Today, our ability to manipulate soil microbial diversity for improved production is limited to altering agricultural management practices, the addition of microbial-based products, or a combination of both strategies. The use of microbial inoculants as biostimulants and biofertilizers to colonize the rhizosphere promises great benefits for plant health and production while respecting sustainable agroecosystem practices (Beed *et al.*, 2011; Okoth, Okoth and Jefwd, 2013; Uzoh and Babalola, 2018), but has so far limited success in field conditions – mainly due to competition with the indigenous microflora of soils (Compant *et al.*, 2019). Another, approach is *in situ* microbiome engineering by using microbial-based inputs, which creates the possibility to manipulate the microbiome without culturing and thus move beyond many current technologies (Sheth *et al.*, 2016). Examples include use of selective antibiotics, probiotics, and bacteriophages to modify an existing soil microbiome. Similarly, plant host-mediated microbiome selection could be leveraged to encourage microbiome functions that impact plant fitness (Mueller and Sachs, 2015).

6.2.1

From nature-based solutions for healthier microbiomes to microbiome innovations

Engineering microbiomes does not necessarily require new, high-tech innovations. As this literature review shows, sustainable agricultural practices alone can positively modify the soil microbiome.[44] A recent review on emerging trends in microbial applications to improve plant health likewise concluded that appropriate agro-management practices can improve the soil microbiome, as can plant breeding for enhanced interaction with microbiota (Compant *et al.*, 2019). So-called nature-based solutions thus aim to restore and maintain ecological balances in the soil by virtue of soil microorganism activities. Among such practices that stand out from our review are use of organic fertilizers, reduced tillage, increasing on-farm plant diversity, and plant variety selection:

- Organic fertilizers further enhance soil organic carbon (C), generally contributing to sustaining biological soil fertility and mitigation of greenhouse gas (GHG) emissions.
- Reduced or no-tillage is associated with positive effects on arbuscular mycorrhizal fungi, which are known to be beneficial to plants through scavenging and nutrient transfer activities. Reduced root soil disturbance can promote the selection of species that are adapted to intact roots or that grow extensive mycelia. For example, a metanalysis found that low-intensity tillage saw an 11 percent increase in arbuscular mycorrhizal fungi species richness, and a 25 percent increase in root colonization (Bowles *et al.*, 2017).
- Plant diversity and plant variety selection can be thought of in terms of 'plant legacy' (de Oliveira *et al.*, 2020) or a 'soil memory effect' (Babin *et al.*, 2019), similar to the 'plant–soil feedback' concept (van der Putten *et al.*, 2013). These concepts explain that effects from a crop can leave an imprint on the soil microbiome due to species or cultivar-specific root exudates and rhizodeposits that select for certain soil microorganisms, with potential impacts on subsequent crop(s) and long-term soil quality. This is relevant to key sustainable crop production practices such as cover cropping, intercropping, and encouraging on-farm plant diversity. The same premise upholds the theory of 'missing plant microbes' resulting from genotypic changes that have occurred during plant domestication, and which could play a critical role in developing solutions to global, environmental challenges (Pérez-Jaramillo *et al.*, 2018).

Another approach to microbiome engineering is the use of so-called agricultural biologicals. These include a wide range of products and can even be used in conjunction with agricultural practices as described above. Agricultural biologicals refer not only to microbiological applications such as biofertilizers, biopesticides and biostimulants, but also macrobials, semiochemicals, and natural products, including plant extracts.[45] These products are used in

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[44] Sustainable agricultural practices include actions to protect, sustainably manage, and restore natural or modified ecosystems. They also address societal challenges effectively and adaptively, simultaneously providing human well-being and biodiversity benefits.

[45] Macrobials are beneficial macro-organisms used in crop production, e.g. insects used for pest control. Semiochemicals are chemicals produced by living organisms that have an effect on other individuals of the same or other species, e.g. pheromones that attract beneficial insects.

crop protection, biocontrol, crop enhancement, biofertilization and biostimulation. The global agricultural biologicals market size was estimated to account for a value of USD 8.8 billion in 2019 (MarketsandMarkets, 2020). This figure is small compared to the overall agrochemical market, valued at roughly USD 208 billion in 2020 (Research and Markets, 2021), but its contribution is growing. In fact, it has been projected to expand at a Compound Annual Growth Rate (CAGR) of 13.6 percent to reach a value of USD 18.9 billion by 2025 (MarketsandMarkets, 2020). Key factors propelling the growth of this market are the upward, global trend of sustainable agriculture, a drive towards a sustainable and circular bioeconomy, low residue levels of microbial products, and supportive regulations. North America and Europe are the two largest markets for agricultural biologicals, largely owing to the development of pest resistance to current products and the demand for organic products (Fortune Business Insights, 2019). The Farm to Fork strategy, at the heart of the European Green Deal, promises to take action to reduce the use and risk of chemical pesticides by 50 percent, and increase the area under organic farming by 25 percent by 2030 (European Commission, 2020).

Furthermore, a report by the McKinsey Global Institute (2020) that assessed the future of bio-innovations identified "agriculture, aquaculture and food" as one of the four domains that will generate the most value related to bio-innovations in the next one to two decades.[46] They estimated that the annual direct impact from biomolecules and biosystems over the next ten to twenty years in agriculture, aquaculture and food could be USD 800 billion to USD 1.2 trillion, globally.[47]

According to the market research platform MarketsandMarkets™ (2020), the microbials segment is projected to dominate the agricultural biologicals market in the near future. Similarly, the McKinsey Global Institute (2020) highlighted three types of bio-innovations that could change farming, one being the microbiome. Valued at approximately USD 4.6 billion in 2020, the global market size for agricultural microbials is projected to reach USD 11.8 billion by 2027, with a CAGR of 14.27 percent (Fortune Business Insights, 2020). Microbial agricultural biologicals include biopesticides, biofertilizers, and biotimulants. Drivers include farmers' desire for credible, beneficial, natural, and cost-effective options (Fortune Business Insights, 2021) as well as the availability of strains, ease of production and handling, and a higher rate of adoption among farmers (MarketsandMarkets, 2020). Furthermore, their use does not require the user to have specific technical knowledge and high-end distribution logistics. This factor has already led to a higher acceptance of microbial-based solutions than any other type of agricultural biologicals among growers. Other important interrelated drivers are the focus on resistance to currently used pesticides, stricter pesticide regulatory requirements, the growing demand for organic food products, government initiatives, and the general increasing awareness about the need for sustainability in modern agriculture.

It is telling that key players in the microbials market include large agrochemical companies such as BASF SE (Germany), Syngenta (Switzerland), Marrione Bio Innovation (US), Isagro (Italy), UPL (India), Evogene (Israel), Bayer (Germany), and Vegalab (US). Ag-tech companies are working on another area of development: soil-microbial based services. Biome Makers (California, USA), for example, aims to help farmers and agronomists assess and improve soil biological health by using the soil microbiome as a monitoring tool. The company offer a service that uses DNA sequencing to perform a functional soil analysis tailored to specific crops, which can then be used to make farm management decisions.

All types of farmers and farming systems across the spectrum stand to benefit from the use of microbial agricultural products: from smallholders to large farmers in wealthy countries, and from conventional to organic farming and other approaches that operate with agroecological principles (e.g. permaculture, biodynamic, agroforestry systems). Bender,

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[46] The four categories identified were 1) agriculture/aquaculture and food, 2) human health, 3) consumer products and services, and 4) materials, chemicals and energy.

[47] In this report, biomolecules refers to the mapping, measuring, and engineering of molecules; and biosystems refers to the engineering of cells, tissues, and organs (Chui *et al.*, 2020).

Wagg and van der Heijden (2016) describe a 'soil ecological engineering' approach as one that promotes combining management practices to enhance biodiversity in agroecosystems, target soil biota functioning, and enhance overall ecosystem service delivery. They argue that while the green revolution focused on external manipulation of ecosystems, the focus today should be on manipulating internal ecosystem processes: not only does this offer enormous potential to enhance ecosystem performance, but it also implies lower environmental consequences. Thus, in conventional farming, microbial agricultural products offer the possibility to integrate more sustainable practices by partially replacing some types of pesticides and synthetic fertilizers, both of which can have negative effects on human, non-target organisms, and environmental health. Organic farming, with its nature-oriented approach, also clearly stands to gain from such products, constituting a considerable market for microbial agricultural biologicals. Contributing factors include global trends of increasing area under organic farming in conjunction with increasing retail markets, as well as a farming philosophy that promotes use of environmentally friendly farming practices.

Key players in the field are the millions of small and large organic farmers who work without conventional agrochemicals such as synthetic fertilizers and chemical pesticides. In 2018, there were 2.8 million organic farmers globally (Research Institute of Organic Agriculture (FiBL), Frick and IFOAM - Organics International, 2020). India continues to be the country with the highest number of producers (1 149 000), followed by Uganda (210 000), and Ethiopia (204 000). Globally, only 1.5 percent of farmland is organic. However, many countries have far higher shares. Those with the largest organic proportion of their total farmland are Liechtenstein (38.5 percent), Samoa (34.5 percent), and Austria (24.7 percent). In sixteen countries, ten percent or more of all agricultural land is organic. In terms of organic agricultural area by continent, Oceania ranks highest (owing to Australia) (36 million ha), followed by Europe (15.6 million ha), then by Latin America (8 million ha).

The research institute FiBL, in its latest survey in 2018 on organic agriculture from 186 countries (2020), reported that organic farmland had increased by 2.0 million hectares, attributed to increases in all continents. In parallel, organic retail sales have also continued to grow. The market research company Ecovia Intelligence estimates that the global market for organic food surpassed USD 100 billion (almost EUR 97 billion) for the first time in 2018. In the same year, many major markets continued to show double-digit growth rates, and the French organic market, in particular, grew by more than 15 percent. Danish and Swiss consumers spent the most on organic food (EUR 312 per capita in 2018). Denmark also had the highest organic market share at 11.5 percent of its total food market.

6.2.2
Bio-innovations can provide ecosystem health and climate benefits beyond crop productivity

As described above, many examples of how to capitalize on recent research findings concerning the soil microbiome are already on the market. For obvious reasons, most commercial applications of products aim to increase crop productivity, improve resistance to disease, increase resistance to environmental stresses, and promote plant growth. Nonetheless, research could also lead to solutions that target multiple challenges using a systems approach that promotes ecosystem health. For instance, bio-sequestration and bioremediation innovations, many of which rely on soil microbiome functions, have been estimated to have potential impact of USD 15-30 billion over the next ten to twenty years (MGI, 2020).

Within the category of microbiological applications, most products contain one or several microbial species and rely upon microbes that can be cultured. However, culturable microbes constitute only about one percent of the total microbial population, implying prodigious and unrealised potential in the remaining microorganism species (Singh, 2017). Moreover, that type of species-specific, dose-application approach contrasts with potential solutions in the near future where the entire microbiome is manipulated *in situ* (Sheth *et al.*, 2016; Singh, 2017). This latter field of research, which implies a dramatic shift in soil management, could lead to microbiome applications with consortia of microorganisms that restore degraded soils, promote plant health and growth, increase C storage, reduce GHG emissions, contribute to adaptive management strategies in the context of climate change and, finally, enhance human health.

Restoring degraded soils and bioremediation of polluted soils. Wubs *et al.* (2016) demonstrated that manipulation of the soil microbiome is key to successful restoration of terrestrial ecosystems. They demonstrated that soil inoculation is a powerful tool to both restore disturbed terrestrial ecosystems and steer plant community development. Applying these findings in the context of soil restoration holds great promise for the millions of hectares of degraded soils worldwide. The soil microbiome can also play a crucial role in the bioremediation of polluted soils (FAO *et al.*, 2020). This can be done by biostimulation (creating the environmental conditions able to sustain the growth and biodegradation activity of indigenous specialized microbiomes) and by bioaugmentation (addition of microorganisms capable of degrading certain pollutants) (Goswami *et al.*, 2018). Soil bacteria and fungi, for example, have been observed to reduce petroleum hydrocarbons following a spill by up to 85 percent (FAO *et al.*, 2020). These approaches offer techniques that are cheaper and more environmentally and socially friendly than current physical–chemical methods, although there is still need to improve the performance, reproducibility and robustness of these technologies.

Promoting plant health with biofertilizers and by building disease suppressive soils. Biofertilizers are substances containing microorganisms that are expected to colonize plant roots and promote plant growth. Perhaps one of the best known examples of biofertilizers is *Rhizobia*, which are responsible for biological N fixation in the root nodules of legume plants. The seed microbiome is a growing area of study; it has been observed that biofertilized seeds can pass on beneficial microorganisms to subsequent generations (Malinich and Bauer, 2018).

More knowledge about the soil immune response, mediated by the soil microbiome, may be an opportunity to reduce pesticide use (Raaijmakers and Mazzola, 2016). Engineering specific suppression in soils requires understanding the specific interactions between pathogen, host plant, and soil organisms. It is comparable to the adaptive immune response in animals. One approach to disease management using biological management has been to isolate a single microbial species and apply it to soil or plant seed as pathogen control. However, these introduced microbial species often fail to establish and implement the desired effect due to competition with the indigenous soil microbiome. In addition, identifying single inoculants capable of performing across the widely varying soil environments is highly unlikely; this may promote the development of microbial precision agriculture, similar to the concept of human personalized medicine (Sergaki *et al.*, 2018). Another approach to disease management with biological control is to use a holistic perspective. One option is the introduction of synthetic microbial communities (SynComs), which are a mixture of specifically selected microbial species. Another is to identify and enhance the indigenous disease-suppressive soil communities. This might be accomplished, for instance, by plant breeding programmes that select for specific root traits able to attract pathogen-suppressive microorganisms. Research has also demonstrated that soil transplantation could result in the transfer of microbiome-mediated plant protection from one generation to the next (Wei *et al.*, 2019b).

Reducing greenhouse gas emissions and increasing soil C storage. Jansson and Hofmockel (2020) outline soil microbial innovations that could help mitigate nitrous oxide (N_2O) emissions and promote soil C sequestration. They suggest that N_2O emissions might be attenuated by promoting certain arbuscular mycorrhizal fungi species that can acquire ammonium, or by inoculating soil with N_2O-consuming microbial communities.

There are a number of innovations that rely upon soil microbial responses to increase the deposition of C in the soil. One is to modify the soil microbiome *in situ* with soil amendments or crop production practices that encourage storage of C in deeper soil layers through production of microbial polymers and necromass (Chenu *et al.*, 2019; Lange *et al.*, 2015; Zhu *et al.*, 2020). Another area of interest is manipulating plant rhizodepositions. This might be done so that roots exude more C, which could then be used by soil microorganisms and incorporated into biomass or stable metabolites. Another option is to breed plants that produce rhizodepositions attractive to beneficial microbes that trap specific C exudates. This latter direction would require close collaboration between plant breeders and soil microbiologists in order to find appropriate plant–microbe pairings.

Lastly, from a broader perspective, it has been argued that a tremendous amount of energy is needed to produce food which contributes substantially to GHG emissions. This includes the production and transportation of chemical inputs, as well food production itself and the subsequent transportation and preservation of food produce (e.g. cold-chain and freezing). It is thus essential to produce energy and food at a lower cost to the environment. In the numerous ways described throughout this report, soil microorganisms could help contribute to this goal. Factoring in the microorganism contributions to greenhouse gas budgets is therefore critical for several reasons (Beed *et al.*, 2011). One is because they likely contribute significantly to climate change mitigation by improving plant productivity and by virtue of the lower greenhouse gas emissions associated with their use compared to fertilizer production and transportation. Another is because this would help make policies and management decisions to help conserve and encourage presence of important soil microorganisms.

Adaptive management strategies in the context of climate change. More knowledge about how soil microorganisms can help crops under stressful conditions (e.g. drought, heavy rain, extended or increased heat) could help inform such strategies. For example, inoculation with beneficial microorganisms such as arbuscular mycorrhizal fungi (AMF) may help plants cope with water stress (Bernardo *et al.*, 2019) through improved access to water and regulation of plant-produced, water stress-reducing molecules. Beneficial, rhizospheric plant growth-promoting bacteria can optimize plant growth even under stressful conditions by producing hydrophobic biofilms that protect plants from desiccation, phytohormones that stimulate plant growth or other protective compounds (Jansson and Hofmockel, 2020).

Not only soils and plants can benefit from soil microbiome applications, though. In a review of scientific literature, Rook (2013) showed that living close to green spaces helped the regulation of the **human immune system** through microbial inputs. Likewise, Blum, Zechmeister-Boltenstern and Keiblinger (2019) argue that access to more biodiverse areas in urban environments, such as parks, is related to health benefits, which can be associated with the exposure to rich environmental microbiota. While research still has a long way to go, one can imagine that medical doctors might eventually prescribe walks in the park to stimulate the human microbiome through breathing air naturally diffused with soil microorganisms. Brodie *et al.* (2007) showed that aerosols contain at least 1 800 different bacterial types, representing diversity comparable to that seen in some soils.

Moreover, not just the animal immune system seems to benefit. A recent study by Liddicoat *et al.* (2020) found that biodiverse soil dust was linked to reduced anxiety-associated behaviour in mice. Although only studied in a mouse model, the potential applications of such findings for mental health treatments could be immense. The study demonstrated that realistic exposures to trace-level dust, derived from soil rich in microbial diversity, can change mouse gut microbiota, in comparison to dust from low-biodiversity soil. The authors suggested that the findings have potential to inform cost-effective population health interventions through microbiome-conscious green space design and, ultimately, the mainstreaming of biodiversity into healthcare.

While the applications of (soil) microbiome science seems endless, be it for the commercial plant production sector, or the wider public good such as climate change mitigation or human health, there are still quite a few challenges to tackle.

6.2.3

Technological and scientific challenges to upscale microbiome innovations

A recent review by Sergaki *et al.* (2018) lists a series of technological and scientific challenges to the assessment and development of microbiome science and its applications. These include the need to:

- Expand microbiome research in the "omics" area, capitalizing on the reduction in sequencing costs, advances in sequencing technologies, and increased computational power. Areas of focus could be the characterization of complex microbial communities, the assessment of functions to individual microbiome members or groups, and the reconstruction of microbiome communities ex situ.
- Improve experimental set-ups to study microbiomes, including the need to understand interactions between soil microbes, and between soil microbes and other living soil biomass (e.g. plants, macrofauna, mesofauna) and abiotic factors.
- Overcome limitations on the experiments performed in controlled conditions, as lab studies generally do not capture the complexity of microbe–microbe interactions that occur in a natural setting such in agricultural soils.

Challenges, however, go far beyond research and development alone. According to Singh (2017), current non-scientific hurdles include:

- Competitiveness with agrochemical alternatives, meaning that biologicals need to be economically and logistically competitive in terms of price, transportation and storage.
- Compliance with regulations and standards, including through self-monitoring.
- Public acceptance, requiring that not only industry and farmers benefit from the innovations, but also civil society and any others concerned by environmental and social impacts. Microbiome engineering will strongly benefit from advances in the field of synthetic biology, which aims to create new biological organisms for specific tasks, but it is equally important to develop safety mechanisms that ensure these organisms can be controlled (Sonnenburg, 2015). Without these controlling mechanisms, public acceptance is likely to be low.

As these challenges can be (partially) addressed by policy, they will be discussed in more detail in the next section.

6.3

ACTION AREAS AND POLICY RECOMMENDATIONS TO ADDRESS RESEARCH GAPS AND SUPPORT BIO-INNOVATIONS

Growing knowledge on the role of microbiomes could have significant impacts and implications for society. Scientists therefore need to work with policymakers to ensure that the potential of this emerging science and the innovations emerging from it are communicated accurately. Policymakers should be equipped to make informed decisions regarding opportunities and risks. Other areas of public support concern capacity building, education and market creation. The latter includes supporting the commercialization of microbiome applications and stimulating the demand of these products and related services.

To this end, policymakers are increasingly committed to developing national and/or regional bioeconomy strategies and programmes that valorise and efficiently use biological resources. They are also more attuned to planning and preventing detrimental trade-offs. Policymakers are also increasingly engaged in creating the conditions for new bio-based technologies, business models, and good environmental and socio-economic practices. Yet, the development of a bioeconomy does not necessarily happen sustainably, nor in a circular manner.

More than 50 countries have included bioeconomy in their political agendas, although only 20 have developed an explicit and holistic bioeconomy strategy. They include Austria, Costa Rica, East Africa, European Union, Finland, France, Germany, Ireland, Italy, Japan, Latvia, Malaysia, Nordic Countries, Norway, South

Africa, Spain, Thailand, United Kingdom, United States of America and Uruguay. A few of these strategies specifically refer to the importance of policy support for microbiome research, development and innovation (R&D&I). Uruguay's strategy, for example, explicitly refers to the microbiome, with a focus on the soil microbiome.

In the Updated EU Bioeconomy Strategy (European Commission, 2018), policymakers highlight that key discoveries concerning microbiomes offer the potential to improve primary production and food systems, to protect our crops, to restore and better manage soils, to improve human and planetary health, and to spawn new sustainable solutions and economic opportunities for growing bioeconomies – while preserving the intrinsic value and biodiversity of our ecosystems.

6.3.1
Public support for research, development and innovation

Among the different policy support areas, public support for R&D&I has been the principle policy area to bridge the gap between microbiome science, the private sector and policymakers to date. Policy instruments used are primarily market-based mechanisms such as research grants and the promotion of public–private partnerships, on the one hand, and communication and outreach activities such as the establishment of networks and organization of conferences and workshops with researchers and policymakers at national, regional and international level, on the other.

The EU, in particular, strongly supports research and innovation actions. It does so under the premise that microbiomes are known to regulate the productivity and health of major food sources such as plants and animals of both terrestrial and aquatic origins, therefore playing a major role in food and nutrition security. The EU also recognizes that the microbiome plays a crucial role in food and feed processing and metabolisms in different organisms throughout the evolutionary trophic scale, ultimately influencing human health.

At the global level, the European Commission launched the International Bioeconomy Forum (IBF) in 2017. Harnessing microbiomes for food and nutritional security is the first and key component, along with regional economic growth and job creation. The working group on the food systems microbiome includes members from the European Commission, Canada, USA, New Zealand, Australia, Argentina, Brazil, China, India, South Africa and FAO.

Another global network is the *Phytobiome Alliance*, an industry–academia collaborative initiative focused on building a phytobiome-based foundation for accelerating the sustainable production of food, feed, and fibre. The US-based alliance facilitates and coordinates international efforts toward expanding phytobiome research. It is jointly sponsored by the private sector, including companies such as Bayer, Novozymes and Indigo, and research institutes and universities such as Colorado State University, Penn State, the University of Maryland, the French National Research Institute for Agriculture, Food and Environment (INRAE), and the Austrian Institute of Technology.

The *Global Microbiome Initiative of Crop Microbiome and Sustainable Agriculture* promotes development and implementation of innovative farming and farm management practices based on system approaches that harness natural resources (through utilization of crop and soil microbiomes) to sustainably increase farm productivity, food quality and environmental health. Led by Western Sydney University, the initiative is a non-profit, public–private partnership body, comprised of academia, industries, learned societies and governmental stakeholders.

At the European level, the European Commission has allocated over EUR 1.5 billion over the last ten years through the framework programme FP7 and Horizon 2020 to more than 500 international projects for research related to microorganisms. These include projects within various technical areas related to agriculture and food systems, such as soils, plants, animals, marine environments, food and nutrition. They also include projects that address human health, such as immunology, diabetes/metabolic disorders, infection control, oncology, neurodegeneration; and cross-cutting areas, such as evolution and biodiversity. Most are conducted within large consortia, involving not only researchers from university and public research organisations, but also actors from the private sector, civil society and public administration.

One such ongoing research call specifically focuses on plant–soil interactions, called *Small organisms, big effects for plants – Belowground biodiversity interaction with plants.* Another, *Microbiome applications for sustainable food systems*, targets food systems more holistically, building on research results from soil and plant microbiome studies.

Demonstrating the increasing interest in the soil microbiome, the European Commission's Joint Research Centre is currently undertaking the largest systematic assessment to date on the soil microbiome. LUCAS (Land Use/Cover Area frame statistical Survey), an extensive and regular topsoil survey, has been carried out across the European Union in different phases since starting in 2009. From 2018, the survey includes analysis of soil biodiversity, including microorganisms, that will be integrated with land use information and soil properties (Orgiazzi *et al.*, 2018). It will be used to develop policy indicators that describe the impact of land management on soil health and GHG emissions.

Moreover, at the national level, too, there are many ongoing microbiome initiatives of significant volume. In the **United States of America**, the government launched the National Microbiome Initiative (NMI) in 2016 with an investment of USD 450 million to enhance innovation and commercialization as well as to develop new, related industries (Bouchie, 2016). The initiative is a public–private partnership between federal agencies, the private sector and the Bill and Melinda Gates Foundation. The NMI aims to generate knowledge from microbial systems in an attempt to impact healthcare, agriculture, environmental science and industrial processes. This includes research on how the gut microbiome affects malnutrition and stunting and which components of the soil microbiome can be used to mitigate crop pests that affect sub-Saharan Africa. The Interagency Strategic Plan for Microbiome Research offers opportunities for microbiome research collaboration across the US government, and for the application of microbiome discoveries to address societal problems. Over 20 different agencies were connected to areas where microbiomes are known to play a critical role. In **Brazil**, the Brazilian Microbiome Project, founded in 2013, aims to assemble a Brazilian Metagenomic Consortium/Database, while also coordinating microbiome projects within the country. Research domains include different ecosystems (soil, plant, water/ocean, human, animal) and bioprospecting, and there is a strategic focus on bioinformatics. **Canada** launched the Canadian Microbiome Initiative in 2007. In 2020, the National Committee for Biosafety, Biotechnology and Life Science in **Italy** presented the industrial implementation action plan 'Italian Microbiome Initiative for improved human health and agri-food production'.

The following are policy recommendations regarding research and development, related either to the subject or the approach:

- **Research policy should encourage studies and infrastructure that call for a unification of microbiome knowledge and actions across ecosystems.** For instance, studying parallels between agroecosystems (where increased plant diversity is associated with increased microbial mass and diversity) and the human gut (where higher quantities of fermentable fibre and more diversified diets are associated with improved human health). These two ecosystems are both part of a larger system, and it appears that there are clear complementarities. The One Health approach is useful in this regard: although the interconnections of these interacting ecosystems may not always be evident, they clearly exist and it must be acknowledged. For another example, one critical issue that links different food system domains is the use of antibiotics, the potential transfer of antimicrobial resistance (AMR) genes, and risks to animal and human health. This is particularly important in ecosystems that are connected to livestock production or that feature an abundance of trace elements, as well as those impacted by the use of human hygiene products.
- **Research policy should help unify or standardize research protocols on how to study the microbiome.** This process should, however, take into account that different ecosystems or soil types may require particular protocols. Agreeing on the methodological standards to measure

microbiome-dependent end points could help interpret soil health. These may include, for instance, microbial biodiversity, structure, functions, and ecosystem services. The African continent, for example, is unmapped for soil microbiome structure.[48]

- **Research policy should support action in response to calls to establish a biobank for the microbiome bank, similar to the concept of a seed bank**. The required infrastructure does not currently exist for biobanking of microbiomes, but the value of such a resource could support research by preserving microbiome samples and their functional potential.[49]
- **Research policy should encourage more soil microbiome research under field conditions, in addition to laboratory studies.** There is a large gap between laboratory and field conditions. In the field, the effects of complex interactions with other living biomass in the soil, climate and edaphic conditions, can influence results and conclusions with consequences for the development and application of new technologies.
- **Research policy should encourage participatory approaches, whether involving citizens or farmers.** This may be with end goals of co-creating policy options or collecting data through voluntary participation.
- **Research policy should promote investigation into the impacts of micro- and nanoplastics in soils, and any potential links to other ecosystems such as the human gut microbiome.** This would be especially appropriate, given that the EU has recently launched their Circular Economy strategy. Research so far has demonstrated that micro- and nanoplastics have effects on soil microbial functionality but has yet to identify effects on the soil microbiome as a whole.

POLICY RECOMMENDATIONS 1.
PUBLIC SUPPORT FOR RESEARCH, DEVELOPMENT AND INNOVATION

- Policy should channel funding into research on what constitutes a healthy microbiome and into exploring the causal relations between microbiomes and human, plant, animal, environmental health throughout life spans.
- Policy should encourage expanding soil microbiome research from laboratory studies to conditions that reflect the complex interactions with other living biomass in the soil.
- Policy should further encourage national and international interdisciplinary research collaboration linking microbiome research communities such as plant, environmental, animal, marine and human research. At the same time, policy should encourage participatory approaches to include collaboration with farmers and citizens.

6.3.2
Education and communication

In line with support for microbiome research, development and innovation, policy supports education and capacity building related to the microbiome. Many university curricula of bachelor, master and postgraduate programmes do include courses on the microbiome, spanning medical, biological, agronomic and engineering schools. More focus on multi- and transdisciplinary approaches, though, would be beneficial to highlight the microbiome connections between ecosystems (e.g. linking agriculture, biology and environment). Science educators need to develop targeted early-career training and education in areas such as bioinformatics and basic microbiology skills (Microbiological Society, 2017).

From a broader societal perspective, too, there is a need for microbiology literacy in the general population (Timmis *et al.*, 2019). What is missing in civil society is a tangible understanding of how the microbiome impacts lives on a daily basis, and in the most basic ways. For instance, although microorganisms are frequently negatively associated with disease,

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[48] The African soil microbiology (AfSM) project, launched in October 2016 by a consortium of soil microbiology researchers, is a key step in creating a broad survey of soil microbiology across different regions and climates in Africa (Wild, 2016).

[49] See the publication by Ryan *et al.* (2020) for a more detailed analysis.

they are, in fact, all around us, providing many benefits and essential functions (Shamarina *et al.*, 2017). Grasping this will inspire people to give it value. Concepts such as Planetary Health or One Health can help give context to these ideas. Citizen science initiatives are also a way to raise awareness through participation, while also collecting data that can be translated back into meaningful messages.[50] Awareness could even start at an early age by inciting curiosity in school-aged children (McGenity *et al.*, 2020).

The private sector facilitates capacity building and awareness-raising regarding applications that stimulate plant growth and support plant health, offering alternatives to the current agrochemical product market. Policy, too, could promote a systemic approach that prioritizes agricultural practices that can stimulate soil microbiome to complement these microbiome products supplied by the private sector. In tandem, the government could provide incentives to promote management for ecosystem service provisioning. To make the case with policymakers, it is crucial to emphasize that microbiome research can provide solutions to many global challenges, while addressing true cost accounting and emphasizing the value of a long-term approach. For example, rotations with temporary grass-cereal-clover result in ecosystem functions that are good for long-term soil fertility, crop production, and climate change. However, the immediate short-term benefits are less obvious.

Less common, despite the huge potential, is capacity building of microbiome-related soil management practices that can contribute to the larger common good. Examples are climate change mitigation through the sequestration of C and the reduction of soil GHG emissions. This is where public policy support is most needed, particularly in working with farmers to raise awareness and to co-discover knowledge to harness advantages of beneficial microbes. Farmer field schools, for instance, could be an opportunity to do so using a participatory approach. It has also been suggested that developing countries have more potential for bigger gain regarding the impact of soil microbiome knowledge, as the majority of degraded soils are located in these countries where access to fertilizers is also limited. For example, marginal lands could be made economically viable through soil microbiome services. Similarly, there are currently no chemical solutions to fight against certain plant diseases in some (sub)tropical regions, and moreover, their abundance is increasing and predicted to do so with climate warming. Using microbial solutions could be an opportunity to demonstrate a tangible effect, as even a ten percent difference would already show huge economic impact from broad-scale and long-term perspectives.

POLICY RECOMMENDATIONS 2.
EDUCATION AND COMMUNICATION

- Policy should encourage training of the current and future workforce to build capacity for the field. This concerns school-aged children, early-career training, as well as university curricula of bachelor, master and postgraduate programmes.
- Policy should provide information about microbiomes to consumers and citizens in a clear and understandable way, engaging concerted action from all responsible actors – the research community, food and healthcare professionals, industry, regulatory opinion leaders, the media and policymakers. This information should include the angle that microbiome research can provide solutions to many global challenges. Promoting microbiology literacy will help individuals grasp the direct relevance of the microbiome to their daily lives.
- Policy should also make sure that local communities have the capacity to benefit from new microbiome innovations, including those in developing countries. Research and industrial infrastructure to develop and provide biological solutions for the agricultural sector should also be developed in rural and coastal areas, to provide employment opportunities, including to the rural youth. This could contribute to stopping rural migration and support public acceptance of these new microbiome-based technologies.

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[50] The American Gut Project is an example of such an initiative.

6.3.3
Commercialization of microbiome innovations and increasing the demand for microbiome practices, products and services

Existing policy initiatives should further support the commercialization of microbiome innovations and stimulate the demand for microbiome services and products. Most of this work is done by the private sector without government support, albeit limited to those applications that promise high economic returns. This is particularly evident in the pharmaceutical sector, but also increasingly in agriculture. Although still in its infancy, the use of and market for microbiome applications in agriculture is growing rapidly (see **Section 6.2** on innovations).

One of the central issues regarding microbiome products for use in agriculture is quality regulation, as there is currently no robust testing or certification process. There are ample commercial microbiome products available that are advertised with misleading and unfounded claims, such as that they contain live microorganisms when it is not the case. Even if these products have no apparent beneficial results, there is not apparent adverse effect in the market. In addition, the regulation that does exist pertains to single strains, rather than microbial communities, reflecting the lag between regulations and more recent product developments.

In response to such needs, the European Commission has adopted the Fertilising Products Regulation (FPR), a product of the Circular Economy Action Plan (European Commission, 2019). The FPR stipulates the definition of a plant biostimulant, permitted microbial plant biostimulants, as well as conditions of inclusion of microorganisms or strains.[51] However, the procedures to ensure compliance with the conditions are yet to be defined. The FPR follows a conformity assessment procedure, where the manufacturer needs to demonstrate whether specific requirements have been fulfilled. Additional work in this area is also ongoing standardization work on plant biostimulants at the European market level (CEN/TC 455), and requirements concerning the visual appearance of the label on EU fertilizing products (European Commission, 2019, 2021).

Another regulatory need is to address the impact of pesticides on the soil microbiome, as well as other physical soil treatments, even if more research is urgently needed to better describe such effects before they can be translated into policy. In Europe, for example, regulations for biopesticides are based on chemical regulations, despite major differences in the products. Concerning microbial pesticides, however, the International Code of Conduct on Pesticide Management has published recommended data requirements concerning the registration of microbial pesticides (FAO and WHO, 2017). Regarding the registration of active substances and formulated products, they recommend data on: the identity, composition, physical and chemical properties of the active substance or formulated product; analytical methods; efficacy data (formulated products only); human health and toxicology; metabolism and residues; environmental fate; and ecotoxicology.

Finally, across the regulatory needs mentioned, there is currently no synchronization between the regulatory frameworks of countries.

Public programmes that target private sector initiatives usually consist of funds that can be accessed by the private sector through different mechanisms. These are usually in the form of grants or loans and require a process of consideration. These programmes prioritize eligible activities or types of projects, according to the objective of the public policy, and demand the achievement of certain indicators by the financed entity. Their scale of application can be very diverse (from business ideas to full projects) and they aim to promote investment in infrastructure and soft, intangible skill development. Examples

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[51] Conditions include: potential of significant trade on the market; safe use to humans, animals, plants, and environment; safe production and conservation; identity and residue levels of residual intermediates, toxins or microbial metabolites; natural occurrence, survival and mobility in environment (European Commission, 2019).

include entrepreneurship and business incubation, and the promotion of innovations. There are several programmes of this kind that specifically target bioeconomy, particularly in the European Union. In fact, the biggest public–private partnership for R&D is the Bio-based Industries Joint Undertaking (BBI-JU), a EUR 3.7 billion partnership between the EU and the Bio-based Industries Consortium, which comprises EUR 975 million in EU funds (through the Horizon 2020 programme) and EUR 2.7 billion in private investments. It furthermore helps to leverage capital markets and additional private and public funds (e.g. synergies with EU Structural Funds).

POLICY RECOMMENDATIONS 3. COMMERCIALIZATION OF MICROBIOME INNOVATIONS AND INCREASING THE DEMAND FOR MICROBIOME PRACTICES, PRODUCTS AND SERVICES

- Policy should particularly support the development and commercialization of those microbiome applications that are destined for the common good, such as C sequestration, rehabilitation of degraded or contaminated soil, and adaptive management to climate change.
- Policy should furthermore support the competitiveness of biological applications with agrochemical alternatives, making sure that the biologicals are economically and logistically competitive in terms of price, transportation and storage. Quality regulation is also necessary to ensure efficacy of microbiome products in the field.

6.3.4
Framework conditions

Last, but not least, framework conditions such as regulations and laws in the context of soils are scarce. Only a few countries have specific legislation on soil protection.[52] Soil is often addressed in legislation related to other areas, such as chemical law, waste law and in legislation on facilities and industrial installations. To our knowledge, none of these address the soil microbiome.

Furthermore, agricultural policies do not venture into the importance of soil microorganisms, let alone the microbiome. For example, one of the world’s largest agricultural policies is the EU’s Common Agricultural Policy (CAP). The policy instruments within the CAP are predominantly economic and fiscal, providing subsidies of various kinds. Although the CAP originally focused mostly on supporting production and farm income, the policy has progressively integrated instruments to address environmental and climate change concerns. Regarding soil management, direct payments are currently available to farmers if they comply with so-called greening measures, which are expected to contribute to soil health. These measures, which account for 30 percent of direct payments, require farmers to diversify crops, maintain permanent grassland and to dedicate five percent of arable land to ‘ecological focus areas’. These actions have been set to make soil more resilient, conserve soil C and protect biodiversity. Criticisms have been raised, however, that these measures have not been very effective in protecting soils.

The next phase of the CAP will therefore put more emphasis on environmental care, climate change action, and landscapes and biodiversity preservation – three of the nine future CAP objectives. The future CAP will support the sustainable management and efficient use of our soils using an evidence- and performance-based approach, underpinned by digitalisation. Legislative proposals for the future of the

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[52] SoiLEX is a global database that aims to facilitate access to information on existing legal instruments on soil protection and prevention of soil degradation. **http://www.fao.org/soils-portal/soilex/en/**

CAP, including better soil management, were presented in 2018. It remains to be seen whether measures eligible for CAP support will be based on microbiome research findings, corresponding to the significant amount of financial resources already invested in EU microbiome research.

The recent launch of the new **EU Farm to Fork Strategy** gives reason for optimism. At the heart of the European Green Deal, it comprehensively addresses the challenges of sustainable food systems and recognises the inextricable links between healthy people, healthy societies and a healthy planet (European Union, 2020). The strategy aims to develop solutions for restoring soil health and functions. New knowledge and innovations will also scale up agroecological approaches in primary production through a dedicated partnership with agroecology living laboratories. This will contribute to reducing the use of pesticides, fertilizers and antimicrobials. An approach to facilitating this transition is more microbiome research, as explicitly mentioned in the strategy.

Another positive development is that at its Seventeenth Regular Session in 2019, the FAO-led **Commission on Genetic Resources for Food and Agriculture (CGRFA)** adopted a Work Plan for the Sustainable Use and Conservation of Microorganism and Invertebrate Genetic Resources for Food and Agriculture. It agreed to address functional groups of invertebrates and/or microorganisms as follows:

- CGRFA-18: (a) Pollinators, including honey bees, and (b) Biological control agents and biostimulants
- CGRFA-19: (a) Soil microorganisms and invertebrates, with emphasis on bioremediation and nutrient cycling organisms and (b) Microorganisms of relevance to ruminant digestion
- CGRFA-20: (a) Edible fungi and invertebrates used as dietary components of food/feed and (b) Microorganisms used in food processing and agroindustrial processes (Commission on Genetic Resources for Food and Agriculture, 2020a)

As the only permanent intergovernmental body that specifically addresses biological diversity for food and agriculture, the CGRFA plays an important role in developing policies and commitment to action plans. Furthermore, in their Eighteenth Session planned for 2021, the Commission was due to discuss microorganisms and – as a different agenda item – climate change (Commission on Genetic Resources for Food and Agriculture, 2020b).

Further regulatory tools are monitoring systems that take stock of the soil quality over time. The Land Degradation Assessment in Drylands (LADA) offers tools to assess land degradation at different spatial scales, for instance, considering the biophysical factors including biodiversity and biomass (FAO, 2013). The importance of developing robust, reliable and resilient biological indicators for soil quality monitoring has been emphasized in order to establish an early warning system for potential losses of soil multifunctionality. In response to the need to bring this issue into the policy spotlight, Guerra *et al.* (2021) recently proposed a set of holistic soil ecological indicators. They are based on soil biodiversity and key ecosystem functions, and correspond directly to global targets established under the Convention on Biological Diversity, the Sustainable Development Goals and the Paris Agreement. The indicators not only address societal needs such as nutrient cycling and plant protection, but were also designed to inform policy related to nature conservation and climate action.

Neither tool, however, offers any specific focus on the soil microbiome. The current, existing indicators are mostly traditional parameters like microbial biomass, global or potential microbial activity patterns or assays that determine potential enzymatic activities. Indicators that describe the status of the soil microbiome (or of microbial key players) are, however, still rare (Schloter *et al.*, 2018). An exception is the **ISO standard 17601:2016**. Schloter *et al.* (2018) point out that one of the approximate methods proposed to analyse soil quality through biological characterization aims to determine the abundance of selected microbial gene sequences by quantitative polymerase chain reaction (PCR) using soil microbiome DNA.

Lastly, there is a need for **technical harmonization at international level** in order to share genetic and functional information related to biodiversity. The generation of vast amounts of data from microbiome projects can be difficult to compare because of differing approaches and because many catalogues are not published. Worldwide, soil-specific genomic resources would help translate these immense quantities of data into usable information (Choi *et al.*, 2017). Standardization of methods would allow comparisons using data generated by different sources. It is important to ensure that these inventories of microorganism genetic resources are accessible by all, so as not to exclude researchers in poorer nations (Beed *et al.*, 2011). Other scientists have gone further, calling for an International Microbiome Initiative that would integrate data across institutions, nations and disciplines (Dubilier, McFall-Ngai and Zhao, 2015). After all, as we have argued in this review, microbiomes exist in interconnected ecosystems and so studying any particular microbiome requires cross-cutting, interdisciplinary and international collaboration.

Transparency and accountability must also be considered while developing framework conditions. Manipulating soil microbiomes will inevitably have consequences, but information on these implications is scarce. New technologies that contain microorganisms, such as those presented above, might furthermore be scrutinized by the public, especially when the organisms are genetically engineered or a product of synthetic biology. Transparency and accurate communication with the consumer is therefore of vital importance (Microbiological Society, 2017).

New biological capabilities come with profound and unique risks that need serious debate, and proactive, rather than reactive, approaches toward mitigation, as experience from the past has shown. A recent study by McKinsey (MGI, 2020) found that about 70 percent of the total potential impact of such products could hinge on consumer, societal, and regulatory acceptance. This figure was based on an analysis of areas where regulations exist today in major economies.

The use of engineered microorganisms should therefore follow a risk/safety assessment that takes into account the potential adverse environmental effects of the microorganisms under their conditions of use (OECD, 2015).

One such risk is that biological systems are self-sustaining, self-replicating, and interconnected, with potentially cascading and long-lasting effects on entire ecosystems or species. Access to these tools may be relatively cheap and easy, making the potential for misuse considerable. Privacy and consent issues abound, owing to new forms of biological data. Responding to such challenges through cooperation and coordination may be complicated given competitive and commercial incentives and varying jurisdictional or cultural value systems (MGI, 2020).

POLICY RECOMMENDATIONS 4.
FRAMEWORK CONDITIONS

- Policymakers should develop regulatory frameworks that require the evaluation of health and environmental claims of new food or environmental microbiome-based products.
- To that end, it would be important to bring regulators, farmers, scientists, industry and citizens into the dialogue to highlight the key challenges in this area, and identify the steps necessary to enable innovation and to avoid or adequately mitigate risks.
- Policymakers need to allow public scrutiny of new microbiome-based technologies and ensure adequate safety assessment prior to any introduction, providing guidance on the use and potential misuse of new microbiome-based technologies. While it is important to highlight the opportunities of their applications, it is crucial that users and consumers are aware of the risks and are protected by fit for purpose regulations, where necessary.

6.4
CONCLUDING REMARKS AND NEXT STEPS FOR FAO

Providing useful, scientific advice to policymakers is increasingly important because science, technology and innovation are advancing at a fast pace. Yet, science does not have all the answers – results of experiments or studies can be inconclusive, sometimes even pointing in opposite directions. As the example of microbiome science clearly shows, many complexities and knowledge gaps still exist that need to be explored further. Indeed, even if the science is clear, the question remains how does one translate it into policy actions? One way is to convince policymakers of the importance of enabling policy in guiding scientific research, ensuring that policy questions and priorities are integrated in research agendas.

Recent experience has shown that science-advisory structures can act as intermediaries between scientific communities and policymakers. Their task consists of aggregating and synthesising scientific evidence and framing it in a format that is both accessible and useful to policymakers. The Food and Agriculture Organization of the United Nations (FAO) has a comparative advantage in the field of food and agriculture. It could become an intermediary between the scientific community and policymakers worldwide to identify and share innovative ideas and solutions that can bring rapid and tangible change to agri-food systems. The FAO Strategic Framework 2022-2031 is centred on leaving no one behind through sustainable, inclusive and resilient agri-food systems for better production, better nutrition, a better environment and a better life. From a microbiome science perspective, this entails investing in a dedicated, multidirectional science–policy interface, the purpose of which would be to identify and prioritize scientific information needed for policymakers and establish policy priorities for research agendas.

This interface should provide the basis for actors to:

1. **Perform regular and timely assessments** of how diets, agriculture, the climate, agri-food systems and the bioeconomy more broadly impact the microbiome. This literature review offers an assessment of the effects of crop production practices on the soil microbiome, with causal impacts on climate change or human health.
2. **Inform policymakers** regarding:
   a. Essential R&D challenges and issues that require further investigation from a microbiome perspective. This literature review has pointed to significant gaps.
   b. Innovations based on microbiomes.
   c. Key opportunities and challenges that the new findings and innovations can bring about. For instance, adapting agroecosystems to climate change will require additional knowledge of soil biodiversity and functions. This would contribute to the engineering of agroecosystems that promote resistance and resilience to stress, while strengthening climate change-mitigating ecological processes such as increasing soil organic C storage and reducing greenhouse gas emissions.
3. **Support policy formulation and implementation**, translating the research results into recommendations for policy, including legislation, standards and other normative instruments at national, regional and global levels. One important policy instrument is to promote debate within and between the scientific community, policymakers, private sector actors and consumers about the role of the microbiome. A point of discussion should be how certain agricultural practices could be discouraged, and others be promoted effectively. As this review has shown, intensive crop production practices typically underutilise and often degrade beneficial soil microbiome diversity and functions or promote undesirable microbial activity. This could potentially cause a decline in soil health, reduced crop yields, and increase costs and environmental impacts such as higher greenhouse gas emissions.

4. **Support communication between policymakers, the scientific community and industry** actors to ensure that important policy questions and priorities are integrated into research agendas.
5. And, with FAO as lead global science-advisory institution, **promote long-term partnership with centres for scientific research and innovation**, including South-South and other forms of cross-country cooperation, as a means of promoting greater uptake of new science, innovation, development and application by end users.

More specifically, with regard to FAO, the following suggestions from key, global microbiome experts were compiled during the July 2020 virtual event, *Microbiome: The Missing Link(s) Learning Pathway.* They illustrate an external perspective on FAO's interfacing role between science, innovation, and policy related to the microbiome:

- FAO has a coordinating role, and particularly so concerning the subject of the microbiome. For example, FAO could help coordinate responses to key questions, such as (i) what is known about the soil microbiome?; and (ii) based on what is known, how do we give recommendations to protect the soil microbiome?
- FAO could play a key role in managing a soil microbe bank (similar to the concept of a seed bank), particularly in a context of land degradation and loss of wild crop varieties, which implies the loss of many beneficial microbes, the exact functionality of which we still don't fully understand.
- In developing countries, FAO could promote research on linking microbiomes with productivity and human health (connectivity between ecosystems). This effort could also include training and capacity building of local stakeholders, rather than focusing on research alone.
- FAO could promote and coordinate specific programmes based on participatory approaches (e.g. farmer field schools) to experiment and adapt sustainable and microbiome-friendly farming practices to specific contexts.
- FAO could take the lead in helping to harmonize global policy regarding microbial product regulation. These products are currently subject to the same regulations as chemical products, which does not make sense for a host of reasons. For example, a microbial product would be subject to the same regulations as a chemical pesticide, though their contents and mechanisms may be completely different.
- FAO could help build synergies and collaboration between key organizations that have overlapping work areas (e.g. WHO, UNCCD, IPS, UNEP).
- FAO should continue using key events to discuss of the role of the microbiome for sustainable agri-food systems. Major events in 2021 included the UN Food Systems Summit, the Climate Change COP 26 and the Biodiversity COP 15, as well as the launch of the UN Decade on Ecosystem Restoration 2021–2030.

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ANNEX I

SEARCH STRATEGIES FOR SCOPUS SYSTEMATIC LITERATURE RETRIEVAL

ALL SEARCHES WERE SET TO AN UNRESTRICTED PERIOD WITH TERMS SEARCHED IN THE TITLE, ABSTRACT AND KEYWORDS.

SEARCH TERMS	RESULTS[1]		SEARCH DATE
	TOTAL	INCLUDED	
LAND USE			
micro-organism* OR microorganism* OR microbiota OR microbiome AND “land use” AND “climate change”	148	10	9 March 2020
micro-organism* OR microorganism* OR microbiota OR microbiome AND “land use” AND “climate change” OR emission*	254	23	12 May 2020
“soil microbiome” OR “soil micro-organism*” OR “soil microorganism*” OR “soil microbiota” AND “land use” AND “endocrine system” OR “endocrine disruptor” OR “ obesity” OR “gut” OR “antibiotic resistance” OR “immune system”	10	0	9 March 2020
“soil microbiome” OR “soil micro-organism*” OR “soil microorganism*” OR “soil microbiota” AND “land use” AND “inflammatory bowel disease*“ OR asthma OR allergies OR neurodegenerative OR “human health” OR “auto-immune disease*”	10	0	9 March 2020
“soil microbiome” OR “soil micro-organism*” OR “soil microorganism*” OR “soil microbiota” AND “land use” AND cancer OR diabetes OR “celiac disease” OR “noncommunicable disease*” OR “non-communicable disease*”	0	0	9 March 2020
TILLAGE			
micro-organism* OR microorganism* OR microbiota OR microbiome AND tillage AND “climate change”	22	4	20 May 2019
micro-organism* OR microorganism* OR microbiota OR microbiome AND tillage AND “climate change” OR emission*	100	13	5 June 2020
“soil microbiome” OR “soil micro-organism*” OR “soil microorganism*” OR “soil microbiota” AND “tillage” AND “endocrine system” OR “endocrine disruptor” OR “ obesity” OR “gut” OR “antibiotic resistance” OR “immune system”	1	0	9 March 2020
“soil microbiome” OR “soil micro-organism*” OR “soil microorganism*” OR “soil microbiota” AND “tillage” AND “inflammatory bowel disease*“ OR asthma OR allergies OR neurodegenerative OR “human health” OR “auto-immune disease*”	2	0	9 March 2020
“soil microbiome” OR “soil micro-organism*” OR “soil microorganism*” OR “soil microbiota” AND “tillage” AND cancer OR diabetes OR “celiac disease” OR “noncommunicable disease*” OR “non-communicable disease*”	0	0	9 March 2020

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[1] There may be overlapping results for multiple searches for the same subject. For example, the two different searches for links between land use, the soil microbiome and climate change may have returned some of the same publications. This potential overlap is not reflected in the table.

SEARCH TERMS	RESULTS[1]		SEARCH DATE
	TOTAL	INCLUDED	
PLANT DIVERSITY			
micro-organism* OR microorganism* OR microbiota OR microbiome AND “agroforestry” OR “agro-forestry” AND “climate change”	8	0	1/2/3 February 2019
microrganism* OR microorganism* OR microbiota OR microbiome AND “agroforestry” OR “agro-forestry” AND “climate change” OR emission*	13	3	12 May 2020
micro-organism* OR microorganism* OR microbiota OR microbiome AND “intercropping” OR “inter-cropping” OR “relay cropping” OR “relay-cropping” AND “climate change”	8	1	1/2/3 February 2019
micro-organism* OR microorganism* OR microbiota OR microbiome AND “intercropping” OR “inter-cropping” OR “relay cropping” OR “relay-cropping” AND “climate change” OR emission*)	15	4	12 May 2020
“soil microbiome” OR “soil micro-organism*” OR “soil microorganism*” OR “soil microbiota” AND “plant diversity” AND “inflammatory bowel disease*“ OR asthma OR allergies OR neurodegenerative OR “human health” OR “auto-immune disease*”	2	0	9 March 2020
“soil microbiome” OR “soil micro-organism*” OR “soil microorganism*” OR “soil microbiota” AND “plant diversity” AND cancer OR diabetes OR “celiac disease” OR “noncommunicable disease*” OR “non-communicable disease*”	0	0	9 March 2020
CROP ROTATION			
micro-organism* OR microorganism* OR microbiota OR microbiome AND “crop rotation” AND “climate change”	19	1	1/2/3 February 2019
micro-organism* OR microorganism* OR microbiota OR microbiome AND “crop rotation*” AND “climate change” OR emission*	51	6	17 June 2020
“soil microbiome” OR “soil micro-organism*” OR “soil microorganism*” OR “soil microbiota” AND “crop rotation*” AND “endocrine system” OR “endocrine disruptor” OR “ obesity” OR “gut” OR “antibiotic resistance” OR “immune system”	0	0	9 March 2020
“soil microbiome” OR “soil micro-organism*” OR “soil microorganism*” OR “soil microbiota” AND “crop rotation*” AND “inflammatory bowel disease*“ OR asthma OR allergies OR neurodegenerative OR “human health” OR “auto-immune disease*”	0	0	9 March 2020
“soil microbiome” OR “soil micro-organism*” OR “soil microorganism*” OR “soil microbiota” AND “crop rotation*” AND cancer OR diabetes OR “celiac disease” OR “noncommunicable disease*” OR “non-communicable disease*”	0	0	9 March 2020
COVER CROP			
micro-organism* OR microorganism* OR microbiota OR microbiome AND “cover crop*” OR “cover cropping” OR “cover-crop*” OR “cover-cropping” AND “climate change”	11	1	1/2/3 February 2019
micro-organism* OR microorganism* OR microbiota OR microbiome AND “cover crop*” OR “cover cropping” OR “cover-crop*” OR “cover-cropping” AND “climate change” OR emission*	18	4	12 May 2020
“soil microbiome” OR “soil micro-organism*” OR “soil microorganism*” OR “soil microbiota” AND “cover crop*” AND “endocrine system” OR “endocrine disruptor” OR “ obesity” OR “gut” OR “antibiotic resistance” OR “immune system”	0	0	9 March 2020
“soil microbiome” OR “soil micro-organism*” OR “soil microorganism*” OR “soil microbiota” AND “cover crop*” AND “inflammatory bowel disease*“ OR asthma OR allergies OR neurodegenerative OR “human health” OR “auto-immune disease*”	0	0	9 March 2020
“soil microbiome” OR “soil micro-organism*” OR “soil microorganism*” OR “soil microbiota” AND “cover crop*” AND cancer OR diabetes OR “celiac disease” OR “noncommunicable disease*” OR “non-communicable disease*”	0	0	9 March 2020

SEARCH TERMS	RESULTS[1]		SEARCH DATE
	TOTAL	INCLUDED	
CROP RESIDUE MANAGEMENT			
micro-organism* OR microorganism* OR microbiota OR microbiome AND straw OR “crop residue*” AND “climate change”	25	9	27 May 2019
micro-organism* OR microorganism* OR microbiota OR microbiome AND “crop residue*” AND “climate change” OR emission*	59	14	23 June 2020
”soil microbiome” OR “soil micro-organism*” OR “soil microorganism*” OR “soil microbiota” AND “crop residue*” AND “endocrine system” OR “endocrine disruptor” OR “ obesity” OR “gut” OR “antibiotic resistance” OR “immune system”	1	0	9 March 2020
“soil microbiome” OR “soil micro-organism*” OR “soil microorganism*” OR “soil microbiota” AND “crop residue*” AND “inflammatory bowel disease*“ OR asthma OR allergies OR neurodegenerative OR “human health” OR “auto-immune disease*”	2	0	9 March 2020
“soil microbiome” OR “soil micro-organism*” OR “soil microorganism*” OR “soil microbiota” AND “crop residue*” AND cancer OR diabetes OR “celiac disease” OR “noncommunicable disease*” OR “non-communicable disease*”	0	0	9 March 2020
PLANT VARIETY SELECTION			
micro-organism* OR microorganism* OR microbiota OR microbiome AND “crop variety” OR “crop genotype” OR “cultivars” AND “climate change”	28	2	1/2/3 February 2019
micro-organism* OR microorganism* OR microbiota OR microbiome AND “crop variety” OR “crop genotype” OR “cultivars” AND “climate change” OR emission*	65	8	22 June 2020
“soil microbiome” OR “soil micro-organism*” OR “soil microorganism*” OR “soil microbiota” AND “crop variet*” OR “crop genotype*” OR “cultivar*” AND “endocrine system” OR “endocrine disruptor” OR “ obesity” OR “gut” OR “antibiotic resistance” OR “immune system”	1	0	9 March 2020
“soil microbiome” OR “soil micro-organism*” OR “soil microorganism*” OR “soil microbiota” AND “crop variet*” OR “crop genotype*” OR “cultivar*” AND “inflammatory bowel disease*“ OR asthma OR allergies OR neurodegenerative OR “human health” OR “auto-immune disease*”	1	0	9 March 2020
“soil microbiome” OR “soil micro-organism*” OR “soil microorganism*” OR “soil microbiota” AND “crop variet*” OR “crop genotype*” OR “cultivar*” AND cancer OR diabetes OR “celiac disease” OR “noncommunicable disease*” OR “non-communicable disease*”	0	0	9 March 2020
IRRIGATION			
micro-organism* OR microorganism* OR microbiota OR microbiome AND irrigation AND “climate change”	44	1	1/2/3 February 2019
micro-organism* OR microorganism* OR microbiota OR microbiome AND irrigation AND “climate change” OR emission*	123	12	3 June 2020
“soil microbiome” OR “soil micro-organism*” OR “soil microorganism*” OR “soil microbiota” AND irrigation AND “endocrine system” OR “endocrine disruptor” OR “ obesity” OR “gut” OR “antibiotic resistance” OR “immune system”	11	1	9 March 2020
“soil microbiome” OR “soil micro-organism*” OR “soil microorganism*” OR “soil microbiota” AND irrigation AND “inflammatory bowel disease*“ OR asthma OR allergies OR neurodegenerative OR “human health” OR “auto-immune disease*”	8	0	9 March 2020
“soil microbiome” OR “soil micro-organism*” OR “soil microorganism*” OR “soil microbiota” AND irrigation AND cancer OR diabetes OR “celiac disease” OR “noncommunicable disease*” OR “non-communicable disease*”	0	0	9 March 2020
FERTILIZER			
microorganism* OR microbiota OR microbiome AND fertilizer* AND “climate change”	114	8	2 May 2019
microorganism* OR microbiota OR microbiome AND manure AND “climate change”	38	1	13 May 2019
microorganism* OR microbiota OR microbiome AND fertilizer* AND “climate change” OR emission*	544	87	13 May 2020

SEARCH TERMS	RESULTS[1]		SEARCH DATE
	TOTAL	INCLUDED	
“soil microbiome” OR “soil micro-organism*” OR “soil microorganism*” OR “soil microbiota” AND “fertilizer*” AND “endocrine system” OR “endocrine disruptor” OR “ obesity” OR “gut” OR “antibiotic resistance” OR “immune system”	35	0	9 March 2020
“soil microbiome” OR “soil micro-organism*” OR “soil microorganism*” OR “soil microbiota” AND “fertilizer*” AND “inflammatory bowel disease*“ OR asthma OR allergies OR neurodegenerative OR “human health” OR “auto-immune disease*”	17	0	9 March 2020
“soil microbiome” OR “soil micro-organism*” OR “soil microorganism*” OR “soil microbiota” AND “fertilizer*” AND cancer OR diabetes OR “celiac disease” OR “noncommunicable disease*” OR “non-communicable disease*”	1	0	9 March 2020
PEST MANAGEMENT			
micro-organism* OR microorganism* OR microbiota OR microbiome AND pesticide* OR herbicide* AND “climate change”	44	0	9 March 2020
micro-organism* OR microorganism* OR microbiota OR microbiome AND pesticide* OR herbicide* AND “climate change” OR emission*	140	9	1 June 2020
“soil microbiome” OR “soil micro-organism*” OR “soil microorganism*” OR “soil microbiota” AND pesticide* AND “inflammatory bowel disease*“ OR asthma OR allergies OR neurodegenerative OR “human health” OR “auto-immune disease*”	13	2	29 May 2019
“soil microbiome” OR “soil micro-organism*” OR “soil microorganism*” OR “soil microbiota” AND pesticide* AND cancer OR diabetes OR “celiac disease” OR “noncommunicable disease*” OR “non-communicable disease*”	0	0	30 May 2019
“soil microbiome” OR “soil micro-organism*” OR “soil microorganism*” OR “soil microbiota” AND herbicide* AND cancer OR diabetes OR “celiac disease” OR “noncommunicable disease*” OR “non-communicable disease*”	0	0	30 May 2019
“soil microbiome” OR “soil micro-organism*” OR “soil microorganism*” OR “soil microbiota” AND herbicide* AND “inflammatory bowel disease*“ OR asthma OR allergies OR neurodegenerative OR “human health” OR “auto-immune disease*”	0	0	30 May 2019
“soil microbiome” OR “soil micro-organism*” OR “soil microorganism*” OR “soil microbiota” AND pesticide* AND “endocrine system” OR “endocrine disruptor” OR “ obesity” OR “gut” OR “antibiotic resistance” OR “immune system”	7	1	5 November 2019
“soil microbiome” OR “soil micro-organism*” OR “soil microorganism*” OR “soil microbiota” AND herbicide* AND “endocrine system” OR “endocrine disruptor” OR “ obesity” OR “gut” OR “antibiotic resistance” OR “immune system”	7	0	5 November 2019
MICROPLASTICS IN AGRICULTURAL SOILS			
“soil microbiome” OR “soil micro-organism*” OR “soil microorganism*” OR “soil microbiota” AND nanoplastic* OR nano-plastic* OR microplastic* OR micro-plastic* AND “climate change”	0	0	9 March 2020
“soil microbiome” OR “soil micro-organism*” OR “soil microorganism*” OR “soil microbiota” AND nanoplastic* OR nano-plastic* OR microplastic* OR micro-plastic* AND “climate change” OR emission*	1	1	23 June 2020
“soil microbiome” OR “soil micro-organism*” OR “soil microorganism*” OR “soil microbiota” AND nanoplastic* OR nano-plastic* OR microplastic* OR micro-plastic* AND “endocrine system” OR “endocrine disruptor” OR “ obesity” OR “gut” OR “antibiotic resistance” OR “immune system”	3	1	6 November 2019
“soil microbiome” OR “soil micro-organism*” OR “soil microorganism*” OR “soil microbiota” AND nanoplastic* OR nano-plastic* OR microplastic* OR micro-plastic* AND “inflammatory bowel disease*“ OR asthma OR allergies OR neurodegenerative OR “human health” OR “auto-immune disease*”	0	0	6 November 2019
“soil microbiome” OR “soil micro-organism*” OR “soil microorganism*” OR “soil microbiota” AND nanoplastic* OR nano-plastic* OR microplastic* OR micro-plastic* AND cancer OR diabetes OR “celiac disease” OR “noncommunicable disease*” OR “non-communicable disease*”	0	0	6 November 2019
TOTAL	**2024**	**227**	