Biology and Fertility of Soils (2025) 61:293–309
https://doi.org/10.1007/s00374-024-01885-y

ORIGINAL PAPER

Plant Growth-Promoting Yeasts (PGPYs) as a sustainable solution to mitigate salt-induced stress on zucchini plant growth

Chiara Ruspi[1] · Debora Casagrande Pierantoni[1] · Angela Conti[1] · Roberto Scarponi[1] · Laura Corte[1] · Gianluigi Cardinali[1,2]

Received: 17 September 2024 / Revised: 2 December 2024 / Accepted: 3 December 2024 / Published online: 11 December 2024

Abstract
Among the long-term sustainable solutions to mitigate saline stress on plants, the use of plant growth promoting microorganisms (PGP) is considered very promising. While most of the efforts have been devoted to the selection and use of bacterial PGPs, little has been proposed with yeast PGP (PGPYs). In this study, three PGPY strains belonging to *Naganishia uzbekistanensis*, *Papiliotrema terrestris* and *Solicoccozyma phenolica* were employed singularly and in a consortium to mitigate salt stress of zucchini (*Cucurbita pepo*). The results demonstrated that these yeasts, when applied to salt-amended soil, mitigated the growth inhibition caused by NaCl. Among the three species, *N. uzbekistanensis* and *P. terrestris* showed the most significant improvements in plant performance, with *N. uzbekistanensis* exhibiting hormetic effects under salt stress by improving root length and dry plant biomass. In general, the root system was the most affected part of the plants due to the presence of the yeasts. The entire rhizosphere bacterial microbiota was significantly influenced by the addition of PGPYs, while the mycobiota was dominated by the introduced yeasts. Metabolomic fingerprinting using FTIR revealed modifications in hemicellulose and silica content, indicating that PGPY inoculation impacts not only the plant but also the soil and rhizosphere microorganisms.

Keywords Plant Growth Promoter Yeasts (PGPYs) · Salt stress · Zucchini · FT-IR · Rhizosphere microbiome · Rhizosphere mycobiome

Introduction

Salinization and draught induce stress to crops and consequently reduce the productivity due to the reduced amount of free water in the soil. Both phenomena pose serious threats to food security. In particular, soil salinity affects some 4.4% of the world's arable land at depths ranging from 0 to 300 mm depth (FAO et al. 2021) and is increasing due to both climate change and human activities (Okur and Örçen 2020). Furthermore, high salt concentrations have a negative effect on microbial metabolic activity, reducing the rates of nutrient and organic matter recycling in soil (Rima et al. 2018). Consequently, this process makes saline areas less productive and difficult to use. Additionally, the rising demand for food requires the extension of agricultural activities toe previously unused soil, some of which is naturally saline (FAO 2021).

Among all possible eco-friendly strategies, the application of microbial inoculants has been demonstrated to be a sustainable solution, enhancing plant growth and biomass, crop productivity and tolerance to abiotic stresses (Novello et al. 2021; Rouphael and Colla 2020). These microorganisms, defined as Plant Growth Promoters (PGPs), include bacteria, archaea and fungi (El-Saadony et al. 2022). PGPs act directly on the root system by increasing the uptake of mineral nutrients and by synthetizing phytohormones, or indirectly, by increasing plant tolerance to environmental stresses and pathogens (Novello et al. 2021; Zhang et al.

Chiara Ruspi and Debora Casagrande Pierantoni contributed equally to this work and the order of authorship was determined randomly.

✉ Laura Corte
laura.corte@unipg.it

[1] Department of Pharmaceutical Sciences, University of Perugia, Via Borgo 20 Giugno 74, 06121 Perugia, Italy

[2] CEMIN, Centre of Excellence on Nanostructured Innovative Materials, Department of Chemistry, Biology and Biotechnology, University of Perugia, Via Elce di Sotto 8, 06123 Perugia, Italy

2023). PGPs can play important roles in both seed germination and seedling growth, which are critical phases in plant development and crucial for subsequent growth stages (Shultana et al. 2020). While bacteria have been extensively studied for their PGP potential and are being applied in numerous agricultural preparations, recent attention has focused on PGP properties of yeasts (Jiang et al. 2023).

Yeasts are commonly found in soil and interact with the root system to improve plant growth, through several mechanisms (Yurkov 2018), such as the expression of phyto-beneficial compounds such as phytohormones, and improved phosphate solubilization, siderophore production and plant protection (Nimsi et al. 2023; Ruspi et al. 2024). While some bacterial PGPs can be health-threatening (Pramanik et al. 2017) the majority of yeasts are innocuous or Generally Recognized as Safe (GRAS) and easy to grow under industrial conditions, representing an important source of variability for field application in sustainable agricultural practices (Singh and Gaur 2021).

Plant Growth Promoting Yeasts (PGPYs) are mainly isolated from the rhizosphere, but several studies have also reported beneficial yeasts in other environments (Abdel-Kareem et al. 2021; Bright et al. 2022; Fu et al. 2016; Lonhienne et al. 2014). The primary yeast genera that exhibit PGP traits include *Rhodotorula* spp., *Candida* spp., and *Cryptococcus* spp. The last two genera are undergoing extensive taxonomic reclassification, by splitting into additional genera. In particular, *Cryptococcus* species are now reclassified into genera such as *Cutaneotrichosporon*, *Cystofilobasidium*, *Filobasidium*, *Hanalea*, *Holtermanniella*, *Naganishia*, *Papiliotrema*, *Solicoccozyma*, *Vishniacozyma*, and *Vanrija* (Bertout et al. 2022; Oliveira et al. 2021).

Yeasts may play a crucial role in alleviating salinity stress, by activating plant growth-promoting mechanisms (Kumar et al. 2023). PGPYs have the ability to regulate plant cell water content by transmitting intracellular signals to increase water uptake, reduce oxidative stress, stimulate stomatal closure, and increase plant enzyme production (Hammad and Ali 2014). Yeasts produce phytohormones such as abscisic acid (ABA), auxins (IAA), cytokinins, and gibberellins (GA), promoting plant cell division, root elongation, and epigeal plant development, as shown in maize growth studies (Nimsi et al. 2023). Moreover, PGPYs alleviate nutrient scarcity in saline soils by solubilizing phosphate, and producing siderophores for iron uptake (Ramos-Garza et al. 2016), making these nutrients available for root absorption (Kuo et al. 2018; Nimsi et al. 2023). Yeast mitigate salinity stress indirectly by secreting volatile organic compounds (VOCs) or antifungal agents to inhibit plant pathogen attack which further stresses plants in saline soils (Kowalska et al. 2022; Li et al. 2024; Nimsi et al. 2023). Inoculation with yeasts exhibiting PGP traits can increase yield quality and quantity without significant environmental impacts (Nimsi et al. 2023).

Yeast species isolated from the rhizosphere in saline environments are putatively more resistant to this stress and may have developed particular mechanisms to mitigate the salt stress of the plants hosting them. Based on this rationale, the ideal inocula for mitigating salt stress should be capable of interacting positively with plants as plant growth-promoting agents and thriving in saline conditions. Three strains from our collection meet both criteria. As already demonstrated by our group (Ruspi et al. 2024), these strains exhibit PGP traits and were isolated from bushes growing in highly saline environments. Specifically, strains of the species *Solicoccozyma phenolica*, *Papiliotrema terrestris* and *Naganishia uzbekistanensis* were inoculated separately and as a consortium on zucchini seeds, a horticultural test plant chosen for its economic and nutritional importance (Novello et al. 2021), to assess their impact on early stages of plant growth and their ability to mitigate salt-induced stress.

Materials and methods

Experimental set-up

The experimental design was designed to assess the effects of four yeast inocula on zucchini plant growth (*Cucurbita pepo* L., var. Nano verde di Milano), in two different soil conditions: control- and saline-soil. The experiment was carried out in mesocosm in a greenhouse with automatic temperature control (25°C), which was made available by Bavicchi S.p.a. (Perugia, Italy). Zucchini seeds were planted in 1 L pots with "*Vulcamix"* commercial soil as the substrate (mineral substrate consisting of a mixture of natural volcanic materials composed of 25% volcanic lapilli sand and 75% pumice sand, granulometry 0–3 mm; C and N present in traces; https://www.europomice.it/prodotti/vulcamix/, EUROPOMICE, Milan, Italy). The soil used for saline condition (treated soil) was supplemented with 2 g L^{-1} NaCl (EC_e=12.63 ± 0.11; pH = 6.81 ± 0.09), while the plain soil was used as a control (untreated soil) (ECe = 5.25 ± 0.03; pH = 6.70). For each soil condition, seeds were planted with or without yeast inoculation for a total of 4 inocula tested. Three Basidiomycetes species, previously isolated from soil, were used: *Naganishia uzbekistanensis* (Nu, CMC 1643), *Papiliotrema terrestris* (Pt, CMC 1688) and *Solicoccozyma phenolica* (Sp, CMC 1669). The fourth inoculum corresponds to the microbial consortium (Cons) of the aforementioned species.

The zucchini seeds were soaked for three hours in each cellular suspension (Nu, Sp, Pt and Cons), while the control seeds were soaked in YEPD medium, the same as that used

to obtain the cellular suspensions. Three zucchini seeds, previously soaked in each suspension, were placed into each of the 9 pots used as biological replicates. This resulted in a total of 27 seeds being tested per thesis and a total of 90 pots(45 for NaCl added soil and 45 for control soil). The pots were placed on a cart and watered every 3 days, to maintain 20% soil humidity.

Cellular suspension preparation for seed soaking

Three yeast species, isolated from saline soil, were selected for the study from the Microbial Collection (CMC) of the Microbial Genetics and Phylogenesis Laboratory of CEMIN. Yeasts were prepared individually and as a microbial consortium.

Yeasts were grown in YEPD liquid medium (10 g L^{-1}yeast extract, 10 g L^{-1} peptone and 20 g L^{-1}dextrose) at 25 °C for 18 h, under shaking conditions (120 rpm). The cellular suspensions used for seed soaking (*Nu*, *Pt* and *Sp*) were prepared by calibrating the OD_{600nm} to a final cell density of 10^8 cells mL^{-1} in the same medium. The microbial consortium was prepared by adding 10^8 cells mL^{-1} of each strain in the same volume.

Yeast growth under saline stress conditions

To assess microbial halotolerance or halophilic characteristics, we used scalar NaCl concentrations. First, yeasts were grown in YEPD liquid medium (10 g L^{-1}yeast extract, 10 g L^{-1} peptone and 20 g L^{-1}dextrose) at 25 °C for 18 h, under shaking conditions (120 rpm). The yeast cultures were then collected and centrifuged at 4500x g for 5 min. After the supernatant was removed, the pellet was resuspended in RPMI medium (RPMI-1640, Sigma Aldrich, Saint Louis, MO, USA, specifically designed for yeasts) to achieve a final $OD_{600\ nm}$ of 0.1. Next, 100 µL of this standardized cell suspension was plated in triplicate in a 96-well microtiter plate, along with 100 µL of NaCl (M) scalar solution. The NaCl concentrations used were as follows: M 1, 0.5, 0.25, 0.125, 0.0625, 0.031, 0.015, and 0. One line was left uninoculated and served as the negative control. The plates were incubated at 25 °C for 24 h and the Abs at 600 nm were collected every 5 min via a TECAN Infinite F200 plate reader (Tecan Trading AG, Mannedorf, Switzerland). The data were processed via Excel, and the mean values were calculated. To assess microbial growth under different saline concentrations, we specifically focused on the exponential growth phase, which was determined via visual inspection of the growth curve obtained. To quantify growth, the Growth Rate (GR, h^{-1}) was determined via formula 1:

$$GR\left(h^{-1}\right) = \frac{\log_2 ODf - \log ODi}{\Delta t_{\min}} \quad (1)$$

where *ODf* and *ODi* refer to the final and initial cell optical density measured at 600 nm, respectively, and Dt refers to the time interval between the two readings.

Soil and plant parameter collection

After 46 days of growth (third stage of leaf growth) the plants were eradicated and the soil from the rhizosphere was collected and stored at -80 °C for metagenomic and meta-metabolomic analyses.

Plant height and dry weight were measured for hypogeal and epigeal portions along with leaf pigments (carotenoids, chlorophyll a and b).

Dry weight was determined after drying the hypogeal and epigeal portions of the plant, at 70 °C for 48 h (Turhan and Eris 2005). The pigment leaf content was determined following the protocol proposed by Siebeneichler et al. (2019).

Rhizosphere metagenomic soil analysis

DNA extraction

For each treatment and the controls, three bulk samples were obtained from the initial nine replicates (pots). From each bulk sample, 400 mg of soil was homogenized in PBS (800 µL) for 5 min at 0.15 x g in an orbital shaker and then centrifuged for 5 min at 4030 x g. The supernatant was discarded, and the pellet was resuspended in 800 µL of CD1 reagent provided with the kit used for DNA extraction (DNeasy PowerSoil Pro Kit, QIAGEN, Hilden, Germany). DNA extraction was performed according to the manufacturer's instructions given with the abovementioned kit.

Barcode amplification

Metagenomic DNA was used as a template for PCR amplification of the standard barcode regions currently employed in microbial taxonomy: 16S for prokaryotes and ITS together with D1/D2 for eukaryotes. The whole 16S gene was amplified with the primer pair 8F (5'-AGAGTTTGATCCTGGC TCAG) (Edwards et al. 1989) and 1492R (5'- GGTTACC TTGTTACGACTT) (Stackebrandt 1993). The marker loci ITS1, 5.8 S, ITS2 and D1/D2 domains of the 26 S subunit were amplified in contiguity by using the primers ITS1 (5'-TCCGTAGGTGAACCTGCGG)/NL4 (5'-GGTCCGTGTT TCAAGACGG) (Schoch et al. 2012).

Platinum™ SuperFi II PCR Master Mix (Invitrogen™, Waltham, Massachusetts, USA) was chosen for amplification because its Platinum SuperFi II Buffer enables universal

primer annealing. For both barcodes the PCR protocol was carried out as follows: initial denaturation at 98 °C for 30 s, 30 amplification cycles (98 °C for 30 s., 60 °C for 1 min and 72 °C for 45 s) and a final extension at 72 °C for 5 min. Amplicons were checked on a 1% agarose gel.

Library preparation

Library preparation for MinION (Oxford Nanopore, Oxford, UK) sequencing was performed following the procedure of Conti et al. (2023).

Sequence analysis

FASTA5 produced with MinION were basecalled with Guppy (version 6.4.6) on a supported NVIDIA GPU. The sequences analysis pipeline works in a *conda* environment built in Ubuntu. The filtering of the raw reads was carried out via the function *seqtk*, which removes sequences less than 400 bp and greater than 1800 bp in length. Filtered reads were merged into one file that was used as input for the alignment program minimap2 (version 2.24). This tool allows the alignment of sequences against a large reference database. The algorithm was tuned to support the alignment of long-noisy reads by using the option map-ont, which uses ordinary minimizers as seeds.

UNITE version sh_general_release_dynamic_25.07.2023 (https://unite.ut.ee/) and SILVA v138.1 (https://www.arb-silva.de/) were used as reference databases. The former comprises 58,440 ITS sequences among the RepS/RefS sequences of all SHs, thus it supported the identification of eukaryotes sequences, whereas SILVA provided comprehensive datasets of aligned small (16 S/18S, SSU) and large subunit (23 S/28S, LSU) ribosomal RNA (rRNA) sequences. ITS and 16 S MiSeq sequences are stored in the SRA archive with the BioProject ID PRJNA1161850.

Rhizosphere meta-metabolomic soil analysis with FT-IR spectroscopy

For each treatment and the controls, one bulk sample of rhizosphere soil was obtained from the initial nine replicates (pots). These samples were subjected to FT-IR in quintuplicate technical replicates, to evaluate the influence the of yeast inoculum on the soil metabolic profile. Each rhizosphere soil bulk sample was air-dried, and 2 g of dry soil was suspended in HPLC grade water at a ratio of 1:5 w v^{-1}, vortexed and allowed to settle for 10 min. Each sample (35 µL) was used for five independent readings and plated on an FT-IR silica plate (Essendoubi et al. 2005). A TENSOR 27 FT-IR spectrophotometer, equipped with an HTS-XT accessory (Bruker, Billerica, Massachusetts, USA), was used for the measurements performed in transmission mode, with the following settings: wavelengths ranging between 3400 and 700 cm^{-1}, spectral resolution of 4 cm^{-1}, and 256 scans per sample. The final spectra were elaborated with OPUS v. 7.0, by water subtraction, baseline correction, vector normalization, second derivative calculation and spectral averaging (Tinti et al. 2015). The final values were expressed as the second derivative of the absorbance intensity.

Data analysis

Plant parameters and Mitigation Index (MI)

The data from the plant parameter measurements ($n = 9$) were evaluated for their normal distribution and homoscedasticity in the R environment (R Core Team (2021)), via the Shapiro-Wilk (*stats* package) and Breusch-Pagan (*lmtest* package) tests, respectively. The statistical significance was assessed via Wilcoxon (Fig. 1) and Kruskal-Wallis (Fig. 2) tests in R environment, via the *dplyr* package. The Mitigation Index (MI) quantifies the impact of microbes on plant parameters, represents the ratio of two effects (the microbial effect on the control, NaCl-added soil, and salt effect on uninoculated soil) and it is calculated as follows:

$$MI = \frac{(\text{Inoculum}_{\text{NaCl}} - \text{Control}_{\text{NaCl}})}{(\text{Control}_{\text{Untreated}} - \text{Control}_{\text{NaCl}})} \quad (2)$$

The numerator indicates the microbial effect under salt conditions, whereas the denominator represents the salt effect without inoculum on zucchini growth. Statistical analyses for all the MI indices were conducted in R. Random sampling and replica picking were used for selection, evaluation of normality, homoscedasticity, and subsequent Kruskal-Wallis test. The results obtained performing the statistical analyses on plant parameters are presented in the Supplementary Material SI-1, of which only significant results are discussed in the Results section.

Metagenomic statistical and bioinformatic analyses

After alignment with reference databases, the resulting unmapped reads were not considered in downstream analysis for both the ITS and 16SrRNA genes. Sequencing of the ITS marker sequence resulted in an average number of reads of 16,800 with an average of 53% unmapped reads. On the other hand, sequencing of the 16 S marker sequence resulted in an average number of reads of 800 with a negligible number of unmapped reads.

Statistical analyses were carried out in the R environment (https://www.R-project.org/) (Team 2022) via the *microeco*, *phyloseq* and *vegan* packages, as well as functions from

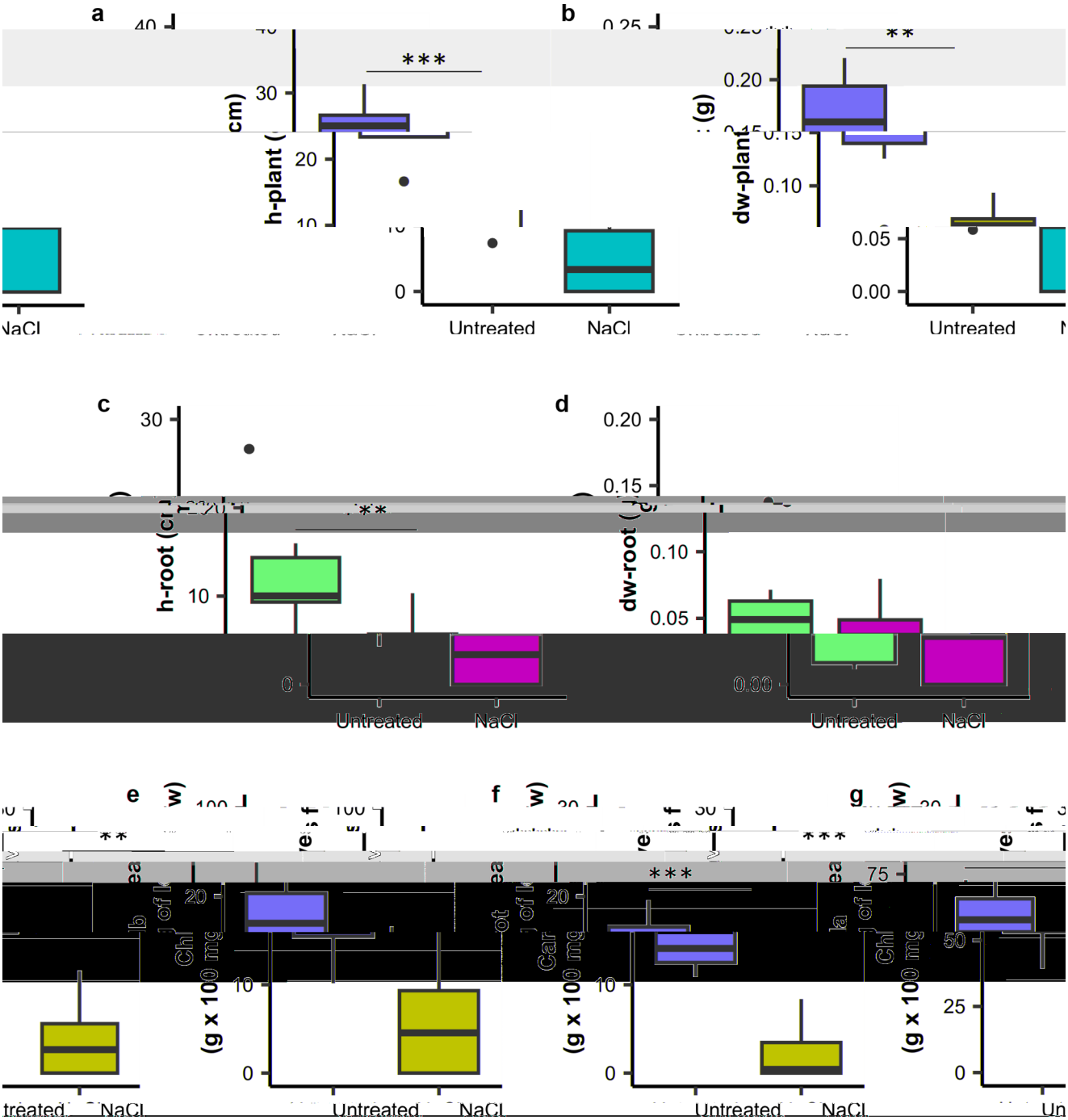

Fig. 1 Plant growth parameters after 46 days of growth. Plant parameters of uninoculated controls (Ctrl) cultivated in soil with and without NaCl addition. The aerial plant portion (panels (**a**) and (**b**)) was evaluated for epigeal height (h) and dry weight (dw). Moreover, the hypogeal plant sections (panels (**c**) and (**d**)) were analysed for root length (**h**) and dry weight (dw). Additionally, the content of pigments (panels (**e**), (**f**), and (**g**)), including chlorophyll a (Chla), chlorophyll b (Chlb), and carotenoids (Carot), were measured. Statistical significance was denoted as *** for $p < 0.001$, ** for $p < 0.01$, and * for $p < 0.05$ (Wilcoxon test)

base R. Shapiro-Wilk (*Shapiro.test()*) and Breusch-Pagan (*ncvTest()*) tests were performed to assess data normal distribution and homoscedasticity, respectively. The Shannon index was calculated to investigate microbial alpha diversity, whereas beta diversity was evaluated via the Bray-Curti's index.

Microbial relative abundances were calculated and the statistical significance of their differences was assessed via the non-parametric test Kruskal Wallis, *kruskal.test()*. The

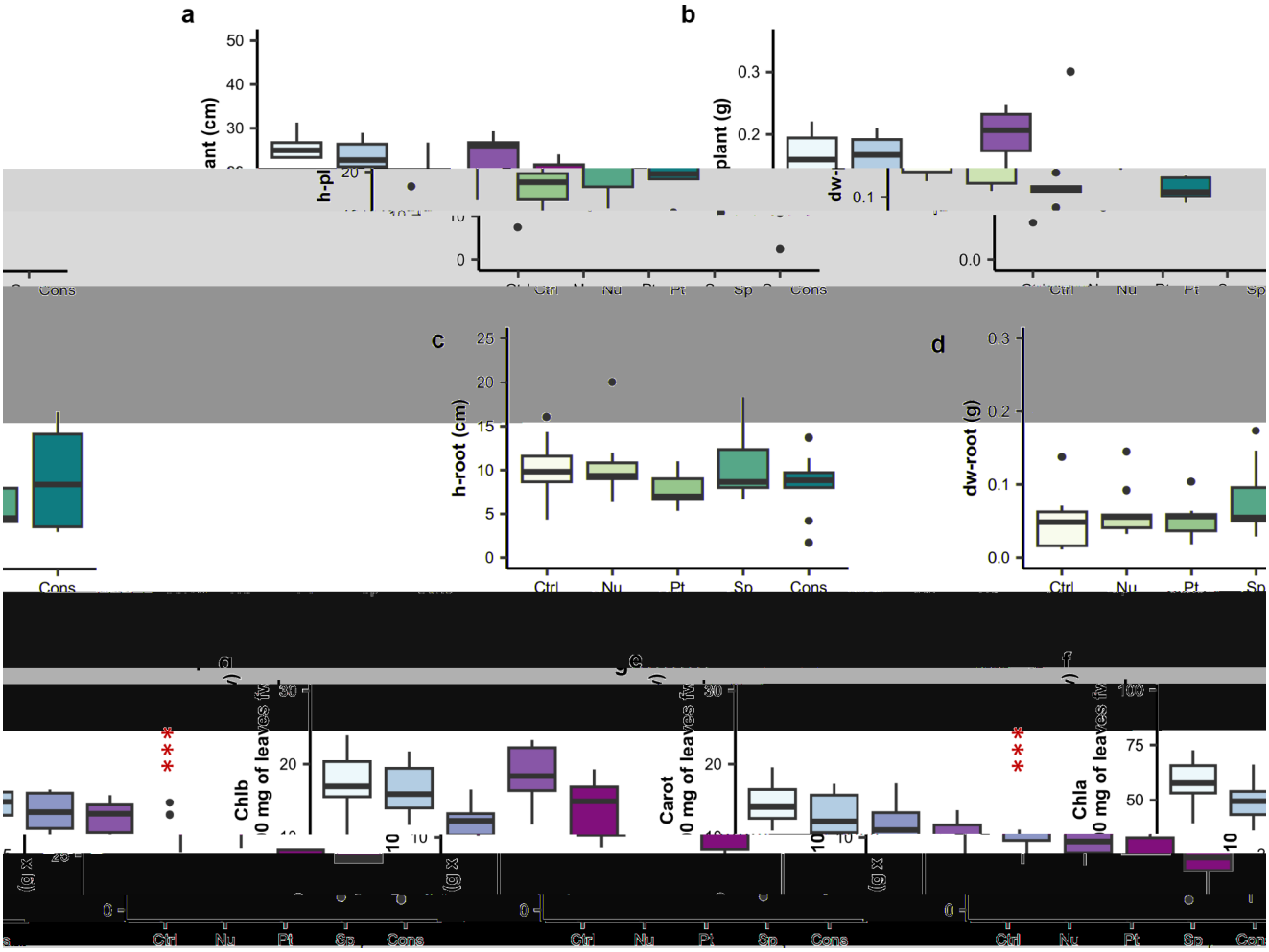

Fig. 2 Impact of microbial inoculation on plant growth and pigment content in untreated soil. Effects of yeast inoculation on zucchini growth after 46 days in untreated soil. The aerial portion was assessed for **a**) height (h) and **b**) dry weight (dw). The same parameters were collected for the root system in panels **c**) height (h) and **d**) dry weight (dw). The contents of the pigments in the leaves were evaluated **e**) chlorophyll a (Chla), **f**) chlorophyll b (Chlb), and **g**) carotenoids (Carot). Boxplots depict values based on a sample size of $n = 9$. The statistical significance between the uninoculated (Ctrl) pot and the microbial inoculum is denoted as ***, for $p < 0.001$, and **, for $p < 0.01$ (Kruskal Wallis – Dunn test)

core microbiome was evaluated as a proxy of the persistence of the fungal inoculum (*phyloseq* package) and Redundancy Analysis (RDA) was performed to assess the relationship between the microbial communities and the plant growth parameters. After the initial analyses, one replicate from the uninoculated control and one from the Nu treatment were removed from the dataset because they were identified as outliers.

FT-IR spectra analyses

Primary peaks were selected from the normalized spectra (Roscini et al. 2010) via the "peak-picking" function of Brucker Opus and the peak base extension was determined from the second derivative, obtaining eleven spectral regions (p1-11), assigned to the bond vibrations listed in Supplementary Material SI-2 (Artz et al. 2006; Bartos et al. 2020; Linker et al. 2005; Meng et al. 2019; Pandey et al. 2024; Parikh et al. 2014; Pärnpuu et al. 2022; Peltre et al. 2017; Sánchez-Sánchez et al. 2019; Teng et al. 2018; Xu et al. 2020). Spectra from NaCl-added rhizosphere soil were subjected to Principal Component Analysis (PCA), to assess the spatial distribution of the treatments, on the basis of the defined spectral regions. Only the regions where the PC1 axis was greater than 50% are presented in the results section and were used for further considerations, as these regions accounted for the most significant variability between the inoculated samples and the control. Student's t-Test was performed between the uninoculated control (Ctrl) and each fungal inoculum, to assess the number of significantly different wavenumbers.

Results and discussion

Effect of salt addition on zucchini plant development

In this study, the effects of soil salinity on zucchini plant (*Cucurbita pepo*) development were investigated using untreated soil and NaCl-added soil in a mesocosm setup, by comparing plant growth parameters after 46 days, specifically during the third stage of leaf development (Fig. 1). Following NaCl addition, the soil attained a final salinity level of 12.63 dS m^{-1}, which is classified as highly saline (FAO. 2021).

Compared with those in untreated soil, only 48.1% of the seeds in NaCl-supplemented soil presented an average seven-day delay (96.3% germination rate), (Supplementary Material SI-3), whereas the remaining 51.9% of the seeds did not germinate. After 46 days of growth, exposure to salt significantly reduced plant height by 79.63% (Fig. 1a), and dry weight by 73.63% (Fig. 1b). Compared with that in the untreated substrate, the length of the roots in the hypogeal portion of the plant decreased significantly, by 69.20%, in NaCl-supplemented soil (Fig. 1c). The root weight also decreased, albeit not significantly, with a mean reduction of 37.41% (Fig. 1d). Additionally, a notable decrease in the leaf pigment content was observed: the content of chlorophyll a, chlorophyll b, and carotenoids decreased by 79.13%, 66.89%, and 85.07%, respectively (Fig. 1e, f and g).

Cucurbita pepo is a horticultural crop of relevant economic interest and is sensitive to soil salinity; in fact, the presence of salt during germination and during the early stages of growth are crucial aspects affecting plant development and final yield (Çulha and Çakırlar 2011; Irik and Bikmaz 2024). Our results confirmed that, compared with untreated soil conditions, zucchini seed germination and plant development are affected by salt addition. It can be hypothesized that the significant reduction in root length led to a decrease in nutrient and water uptake, limiting the aerial development of the plant.

Effects of salt on the fungal and bacterial communities of the rhizosphere

The fungal and bacterial microbiomes of the rhizosphere are crucial for nutrient absorption and resistance to abiotic stresses, such as salinity or drought. Specifically, fungal communities are involved in the formation of mycorrhizae, which are essential for proper root function and plant development (Kottke 2002).

In this study, the fungal communities of the rhizosphere from the control samples were dominated by the genera *Preussia* (30%) and *Candida* (25.6%) in the NaCl-added samples and by the genera *Candida* (36.3%) and *Thelephora* (18.9%) in the untreated soil (Supplementary Material SI-4). The relative abundance of the genus *Preussia* significantly increased in NaCl-added soil (30% vs. 8.5%, $p = 0.005$), whereas the genus *Thelephora* was negatively affected by NaCl addition, decreasing from 18.9% (untreated soil) to 1.3% ($p = 0.05$). These two genera are common inhabitants of the endophytic root mycobiota and are both considered able to tolerate saline stress conditions (Mapperson et al. 2014; Thiem et al. 2018, 2023). The members of the genus *Preussia* are predominantly isolated from soil, plant material and, occasionally, as endophytes and commonly adapted to arid soils (Wei et al. 2021). The genus *Thelephora* is populated by moderately halotolerant endophytes that can form ectomycorrhizae (Thiem et al. 2023). While the results to *Preussia* are in line with expectations, the reduction in the relative abundance of *Thelephora* could be explained by the fact that the salt concentration in this study was higher than that in the papers reporting the halotolerance of this genus.

The bacterial communities of the control samples were dominated by the genera *Bacillus* and *Rhodobacter* (Supplementary Material SI-4) which did not significantly differ between the two conditions. Four bacterial genera, *Mesorhizobium*, *Luteimonas*, *Lysobacter* and *Devosia*, were negatively affected by soil salinity, and decreased in the NaCl-treated samples from 2.3 to 3.8% to 1.5–2.5% (Supplementary Material SI-4). The plant growth-promoting traits of *Mesorhizobium*, *Luteimonas*, *Lysobacter*, and *Devosia* have been extensively investigated, but the detailed mechanisms of action are not fully understood (Peng et al. 2021). Previous studies have indicated that *Luteimonas* is a member of the rhizosphere microbiome and serves as a microorganism that promotes plant growth (Gu et al. 2020). Furthermore, it is negatively influenced by increases in soil electrical conductivity (EC), which also affect the overall bacterial community (Zhang et al. 2024). Conversely, *Mesorhizobium*, *Lysobacter*, and *Devosia* respond favourably to increased soil salinity in the rhizosphere (Monjezi et al. 2023; Peng et al. 2021; Sehrawat et al. 2020; Wang et al. 2022). However, in this specific experimental context, a slight decrease in relative abundance was observed. Once again, this could be explained by the harsh conditions employed in this study, as well as by the different species and strains dwelling in the soils of the various analyses. This observation suggests the presence of some variability and calls for a more detailed analysis of the salt resistance phenotypes of isolates from different soils. The Alpha and Beta diversity indices (Shannon diversity and Bray-Curtis distance) used to investigate the community composition did not differ among the untreated and NaCl-added soil rhizosphere samples (Supplementary Material SI-4), both for the fungal and bacterial communities.

Impact of yeast inoculum on plants and the rhizosphere in untreated soil

Salt impacted negatively on the growth of zucchini, affecting plant development, root extension and the pigment content of leaves.

Three yeast strains belonging to our collection have already demonstrated their potential as PGPYs acting as phosphate solubilizers and indol-acetic acid (IAA) producers (Ruspi et al. 2024). These strains: *Naganishia uzbekistanensis* CMC 1643 (referred to as Nu), *Papiliotrema terrestris* CMC 1688 (referred to as Pt) and *Solicoccozyma phenolica* CMC 1669 (referred to as Sp), were tested for their ability to grow in the presence of NaCl, ranging from 0 to 1 M for a possible use in the mitigation of salt effects on plants. Their growth velocity was subsequently calculated, as shown in Supplementary Material SI-5.

Nu exhibited halotolerance but not halophily, displaying its maximum growth velocity at 0 M NaCl, with only a 45% decrease in velocity at 1 M NaCl. Pt and Sp showed a halophilic profile, i.e. a greater growth rate with salt than without salt. In fact, the optimum salt concentration was 15 mM for Pt and 62 mM for Sp. Both species were practically unable to grow at 1 M, showing their halophily at moderate salt concentrations but the absence of halotolerance at high salt concentrations, confirming the presence of a complex relationship between halotolerance and halophily in yeasts (Corte et al. 2006). While these salt concentrations are far from those of extremophiles, they fall well within the general conditions of soils associated with the presence of salt. In fact, in this experiment, we used a salt concentration equivalent to 126 mM NaCl, corresponding to 12.63 dS m^{-1} according to the FAO guidelines (FAO and ICBA 2023). Under this conditions, all three strains presented growth rates ranging from 56 to 67% of their respective maximum growth rates, suggesting that their growth in salt containing soils is not severely impaired by sodium chloride.

Assessment of the presence of yeast inoculum in the rhizosphere of zucchini plants

Zucchini seeds were planted in 1 L pots and inoculated with the selected yeasts and their consortium. After 46 days of growth the plants were eradicated, and the rhizosphere soil was used for metabarcoding analysis to assess the persistence of the inoculum. The fungal community of each thesis was populated predominantly by the corresponding yeast inoculum (Supplementary material SI-6). *N. uzbekistanensis* and *P. terrestris* were detected in the corresponding inoculated samples as 69.5% and 62.6%, respectively, of the total fungal community. In the corresponding samples, Sp constituted 66.6% of the total fungal community. Among the Consortium-inoculated samples, 39.5% of the total reads were identified as Pt, whereas Sp and Nu accounted for 12.7% and 7.9%, respectively. *S. phenolic*a could not be detected as such because the database used (Unite) does not include a reference sequence for this species but rather that of *Solicoccozyma fuscescens*, which is the closest taxonomic match.

We confirmed that the reads marked as *S. fuscescens* must instead be attributed to *S. phenolica* by aligning the sequences to a custom database that includes references for all identified species including *S. phenolica*. Owing to this analysis, all the reads initially identified as *S. fuscescens* could be subsequently reassigned to *S. phenolica*. Conti et al. (2023) demonstrated that the absence of taxa in databases leads to an ill attribution of reads to the closest species present in the database, indicating a serious problem in environmental mycology in which not all species are represented in databases.

Inoculum impact on plant growth without salt addition

The impact of microbial inoculation on seed germination (Supplementary Material SI-3) and on most plant growth parameters was not evident, as the differences between treated and control seeds/plants were not statistically significant (Fig. 2). Across all inoculant treatments, the average plant height and dry weight remained relatively consistent (Fig. 2a and b).

Yeast inoculation did not significantly affect root length or dry weight (Fig. 2c and d). The chlorophyll a and carotenoid contents were significantly reduced by *S. phenolica* which caused a reduction of 31.8% in chlorophyll a and 36.6% in carotenoids, whereas the microbial consortium led to a more substantial decrease of 56.2% in chlorophyll a and 53.0% in carotenoids (Fig. 2e and g). Taken together, these data indicate that inoculation is innocuous for plants, with some problems only for the consortium.

Inoculum impact on the rhizosphere fungal and bacterial microbiomes

Reduced Dimensionality Analysis (RDA) of the bacteriome revealed separation of the control and the consortium samples along the first dimension, which explained 53.9% of the total variance. All other samples of single species used to inoculate the seeds were not clearly separated in either component (Supplementary material SI-7). The relative lack of differences observed mirrors the low level of variation detected in the plant parameters. Considering the response vs. explanatory variables, *Paludisphaera borealis* plays a pivotal role in driving spatial separation between

the consortium and control bacterial communities, whereas *Bacillus* species are negatively correlated with the presence of yeasts. In fact, the relative abundance of the top-three species of *Bacillus* decreased from 10.2 to 2% with Nu, to 1.1% with Pt, to 1.8% with Sp and finally to 0.9% in the consortium samples.

The RDA revealed more pronounced spatial separation within the fungal communities of the inoculated and control theses (Supplementary material SI-7), with the exception of Nu, which closely resembled the control. This phenomenon is in line with the lack of differentiation in plant parameters between Nu and the control, as highlighted in the preceding paragraph (Fig. 2).

Yeast mitigation effects on NaCl-induced stress

Persistence of yeast inoculum on the zucchini rhizosphere under saline soil conditions

As already observed in the untreated rhizosphere samples, the fungal community of each thesis was populated predominantly by the corresponding yeast inoculum (Fig. 3). *N. uzbekistanensis*, *P. terrestris* and *S. phenolica*, were detected in the corresponding inoculated samples as 73.4%, 77.6%, and 80.5% respectively, of the total fungal community. When these results were compared with those in untreated samples, the relative abundances of all inoculated yeasts increased by 15% (Pt), 13.9% (Sp) and 3.9% (Nu). These figures can be explained by the halophilic nature of Pt and Sp, as already evidenced in the Supplementary Material SI6. Moreover, these two species dominated the rhizosphere fungal community of the salt-added soil more than they did in the untreated samples. This can be explained by two different but not alternative mechanisms: salt increases the growth of Pt and Sp or decreases that of the resident mycobiota. Conversely, Nu, a halotolerant but not halophilic strain, did not increase its dominance over the rest of the fungal community, in comparison to the untreated samples. Among the consortium-inoculated samples, 52.6% of the total reads were identified as Pt, whereas Sp and Nu accounted for 11.8% and 6.9%, respectively. Taken together, these data suggest that in the presence of salt at the studied concentrations, halophily plays a more important role than halotolerance in the competitive success of the inoculated strain.

Mitigation effect of yeast inoculation on NaCl-stressed plants

This study aimed to investigate the potential of microbial inoculation to mitigate the adverse effects of NaCl on plant growth as previously reported. To quantify and visualize

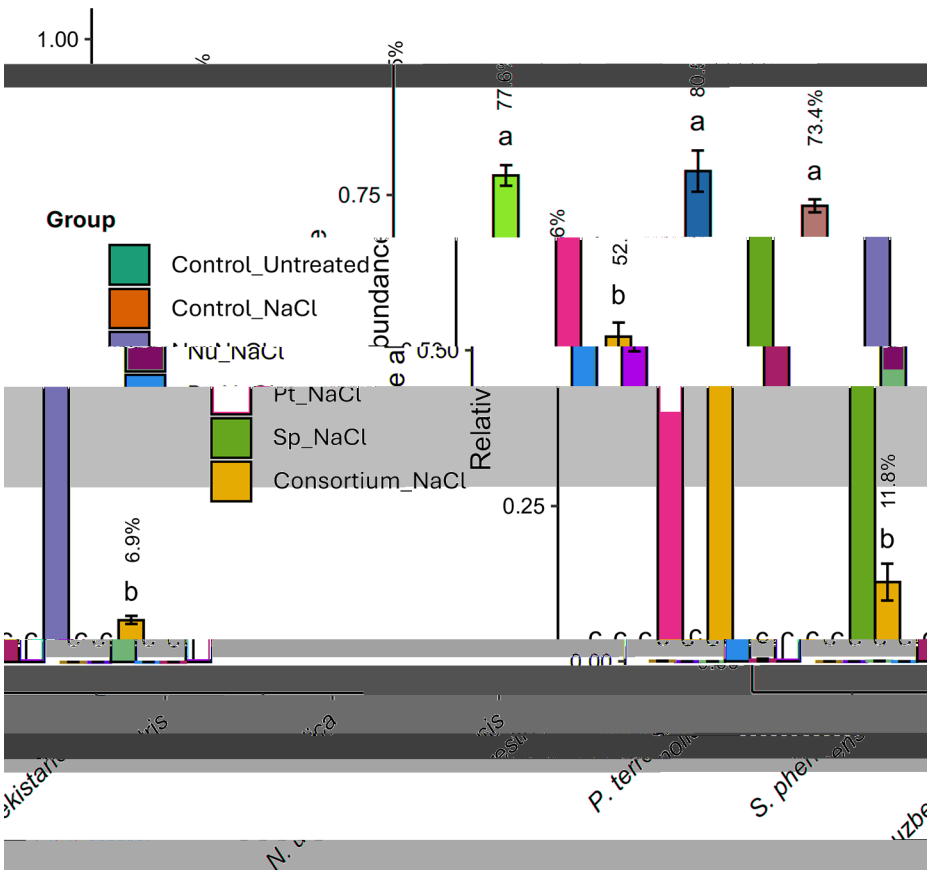

Fig. 3 Persistence of yeast inoculum on zucchini rhizosphere, in saline soil conditions. Relative abundance of yeast inocula, for each treatment under saline soil conditions and in the untreated control. The numbers indicate the yeast inoculum abundance in the total mycobiome

the mitigating effects of inoculated yeasts on plant growth parameters, we introduced the Mitigation Index (MI) ranging from negative to positive values. Specifically, (i) MI values less than 0 indicate an exacerbation of the salt effect; (ii) 0 indicates a lack of effect, i.e., the plant suffers the stress as the treated control; (iii) values between 0 and 1 indicate a mitigation effect with growth parameters ranging from the treated and the untreated control; (iv) finally, values greater than 1 indicate a synergistic effect of the microorganisms with the salt, resulting in better growth than that observed in the untreated control. The data in Fig. 4 are presented as boxplots between a dotted red line (average of the NaCl-added control) and a dotted green line (average of the untreated control).

The positive impact of inoculants in saline soil conditions was evident through increased seed germination rates. The inoculated pots consistently outperformed the uninoculated pots, with germination rates increasing from 48.1% in the control to 96.3% under *N. uzbekistanensis* inoculation, which was the most effective treatment (Supplementary Material SI-3). Under NaCl soil conditions, the plant growth parameters in the inoculated pots were greater than those in the NaCl-uninoculated pots and are therefore between the dotted red and green lines; only the consortium exacerbated the salt effect on root length (MI -0.09). The consortium and *Papiliotrema terrestris* (Pt) had small mitigating effects, whereas *Solicoccozyma phenolica* (Sp) was generally able to mitigate the salt effect. *N. uzbekistanensis* (Nu) significantly improved all the growth parameters over those of the NaCl control, and in four cases, it even had a synergistic effect: the root length improved by 17% (MI 1.25), the root dry weight increased by 180% (MI = 5.81), and the chlorophyll b content increased, with an MI of 1.31. The fact that two out of three parameters are related to roots suggests a direct effect of the inoculation on this part of the plant. Interestingly, the dry weight increased much more than the length did, indicating a possible thickening of the roots, or their ramification, induced by this yeast. However, a more detailed analysis of the changes in root morphology could not be carried out under these experimental conditions, and further studies focusing on this topic are needed. In general, an improvement in the radical apparatus has been positively correlated with the resistance to NaCl (Liu et al. 2020).

N. uzbekistanensis was the species with the greatest ability to mitigate salt effects under the experimental conditions studied, which is also in line with recent literature were another *Naganishia* species isolated from extremophilic environments showed PGP traits in saline stress conditions (Raklami et al. 2024). Its halotolerant and non-halophilic behaviour was likely a key factor in this success. In fact, the two halophilic species showed fewer mitigation effects and practically no synergy, probably because their more abundant growth required more organic matter from the soil, inducing competition with plants that somewhat reduced the mitigation effect in comparison to Nu. Once confirmed by other studies, halotolerant and non-halophilic behaviour could be considered positive criteria to identify further microorganisms able to mitigate salt stress in plants. On the other hand, investigating how the joint presence of salt and Nu induced a strong and significant effect is interesting.

Impact of bacterial and fungal communities on plant parameters

In the context of long-term environmental sustainability, it is crucial that microbial inoculations designed to promote plant growth do not disrupt the existing microbiota and mycobiota in soils. To address this concern, we conducted metabarcoding analysis on the bacterial and fungal communities present in the rhizosphere soil under various tested conditions The presence of the inoculum significantly influenced the fungal community in each inoculated sample. This effect was evident through a distinct separation observed in the PCoA spatial ordination based on the Bray-Curtis index (as shown in Fig. 5a). Since inocula were predominant over the fungi present in the rhizosphere (Fig. 3), the fungal community was necessarily shaped primarily by the added yeasts. This explains why different inoculations resulted in different mycobiota under the different treatments. On the other hand, the yeast inoculum had some effect on the bacterial community composition, since uninoculated and inoculated samples were clearly separated by PCoA but no specific effect of the different inocula could be observed (Fig. 5b).

To better understand the alterations in the prokaryotic taxonomic composition induced by the inocula, the bacterial genera whose relative abundances significantly differed from those of the control were analysed (Fig. 5c-f). A positive effect was observed on *Mesorhizobium*, leading to an increase of 2.1–3.7% (Fig. 5c), whereas other bacterial genera presented a small increase in the rhizosphere microbiome; however, these effects were not consistent across all inocula and were not inoculum specific. All inoculations had a negative impact on the relative abundance of *Bacillus* members resulting in a decrease ranging from 17.8 to 26.4% of the total abundance. When the species of this genus were studied specifically, the inoculations in the absence of salt decreased the relative abundance from 10.2% of the control to approximately 1.3% (average of the four inoculations), whereas in the presence of salt, values decreased from 29.6% of the control to 6.9% of the mean value of all four inocula. The apparent increase in *Bacillus* abundance in the salt-added group compared with that in the non-treated control group (29.6% vs. 10.2%) does

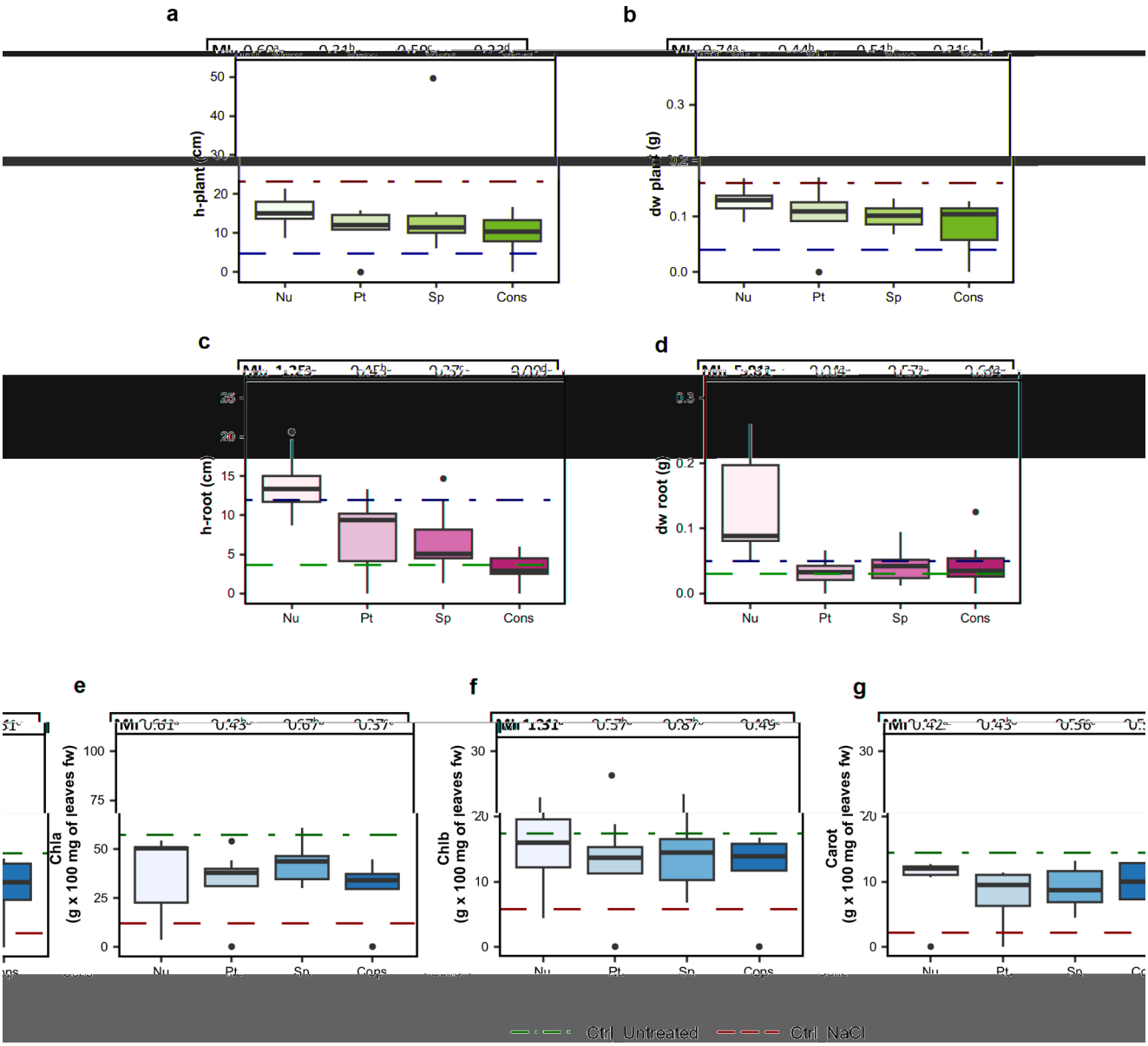

Fig. 4 Impact of microbial inocula on plant growth parameters under salt conditions. The plant growth parameters ($n = 9$) were evaluated after 46 days of growth under salt stress. The study examined both aerial (height (**a**) and dry weight (**b**)) and root (height (**c**) and dry weight(**d**)) portions as well as pigment contents (chlorophyll a (**e**), chlorophyll b (**f**) and carotenoids (**g**)). The green line represents the mean value of uninoculated and untreated samples (Ctrl_Untreated), whereas the red line corresponds to uninoculated pots supplemented with NaCl (Ctrl_NaCl). Statistical significance was determined by comparing the salt effect in uninoculated pots to the salt effect in inoculated pots. The Mitigation Index (MI), which quantifies the microbial effect in minimizing salt-induced plant damage, is reported over each boxplot. Statistical significance was assessed via the Kruskal-Wallis and post-hoc Dunn test ($p < 0.05$). Samples with the same letters were not significantly different

not necessarily indicate that there is a greater absolute cell density of this genus in the presence of salt but rather that salinity negatively affected the other bacterial species rather than *Bacillus*. On the other hand, the addition of inoculants negatively affected these bacteria in both cases, suggesting some sort of competition between them and yeasts. The fact that *Bacillus* is considered an r-strategist (or zymogenous organism), similar to most yeasts, further reinforces the possibility of competition between taxa with similar ecological and physiological habits (Kunito et al. 2001).

Meta-metabolomic analysis of rhizosphere soil under saline conditions

The state of the rhizosphere was studied at the end of the 46-day experiment without considering intermediate stages,

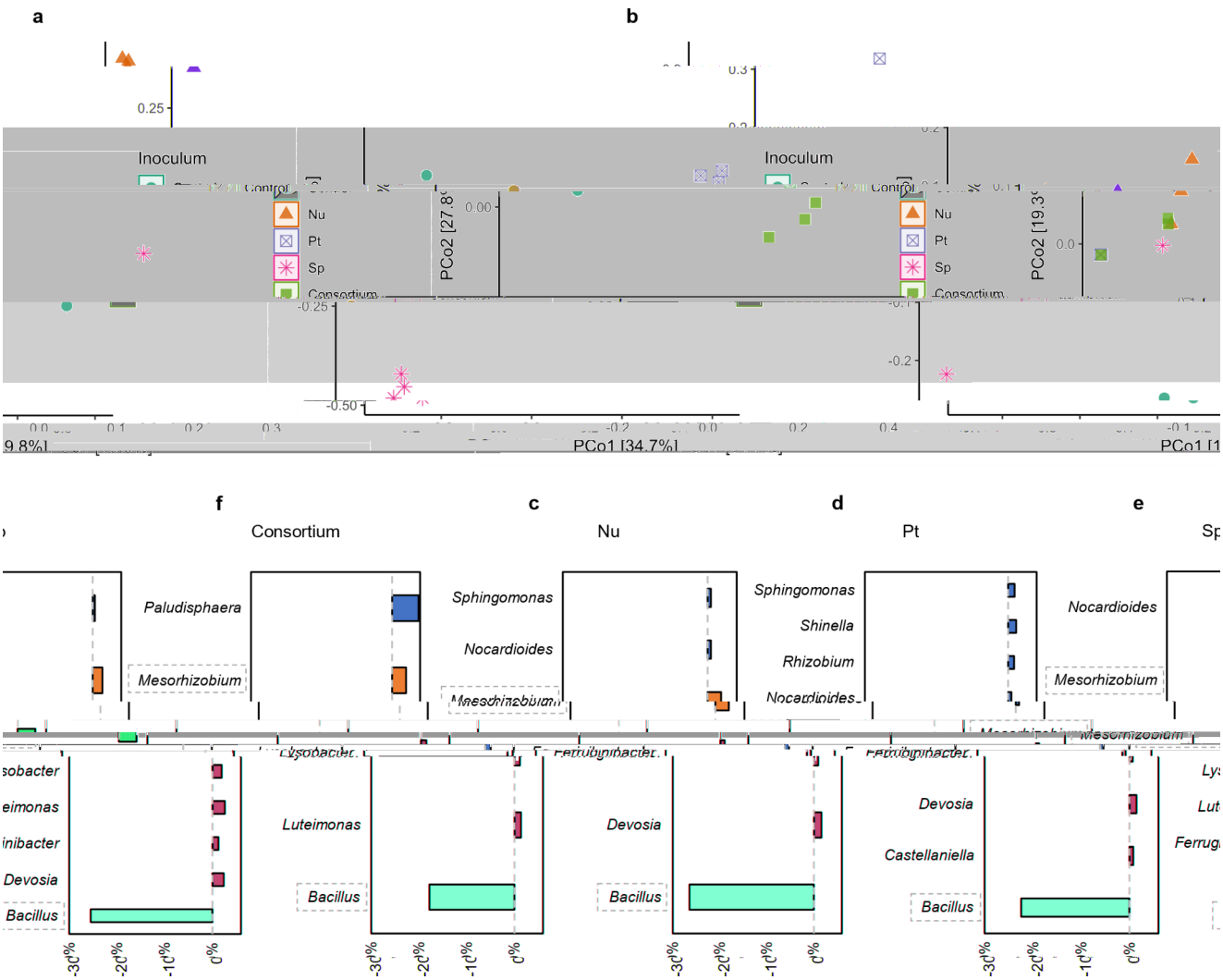

Fig. 5 Impact of microbial inoculation on bacterial community variation and composition, in the rhizosphere of salt-added soil. Principal Coordinates Analysis (PCoA) reveals the spatial distribution of inoculated samples on yhe basis of both fungal (**a**) and bacterial (**b**) community compositions. Each point represents a sample, and the axes capture the major sources of variation. PCo1 explains 34.7% and 19.8% of the variance (**a**, **b**), whereas PCo2 accounts for 27.8% and 19.3% of the variance (**a**, **b**). Panels **c**-**f** highlight the relative abundance of bacterial genera significantly different from that of the control (uninoculated pot), in each inoculated rhizosphere soil sample

as our aim was not to study the entire plant growth cycle but to picture the rhizosphere microbiota at the early stage of growth. Sandy soils typically contain a limited number of microorganisms, as their presence is not buffered by abundant organic matter, resulting in inherently unstable microbiota. The plant rhizosphere acts as an oasis, fostering edaphic communities that continuously evolve, along with their metabolome, throughout plant growth.

FT-IR spectroscopy revealed that the carbohydrate peak of the rhizosphere soil of the NaCl-added samples (approximately 1200–900 cm^{-1}) was the prominent peak of the spectrum rather than the amide I peak, as in the case of microbial cells (Corte et al. 2010, 2014) (Supplementary Material SI-8). Given the complexity of these spectra, all analyses described below were restricted to the wavenumbers corresponding to the bases of the peaks selected as described in the Material and Methods section. The complex of the spectrum spanning through these selected wavenumbers is referred to as the "Selected Spectrum" (SSpec).

Since the purpose of this study was to compare the effects of each inoculant with the control, a series of pairwise comparisons were performed to define which spectral peaks were significantly different to assess the differential impacts of the treatments on the various peaks observed (Supplementary Material SI-8). Specifically, the peaks from p4 to p9 presented greater numbers of significantly different wavenumbers in the comparisons of all inoculants vs. the control. Peak p10 presented few significantly different wavenumbers. Peaks p1-3 and p11 were not significantly different in terms of wavenumbers between the consortium and Sp

vs. the control, but there were strong differences in both Nu and Pt vs. the control. (Supplementary Material SI-8). In general, the differences between the control and both Nu and Pt treatments were greater than those between the other two treatments (Supplementary Material SI-8). This finding was corroborated by the PCA carried out on the SSpec regions (Fig. 6a). Pt and Nu replicates were distributed quite distantly from the control and were separated mainly along the first component (PC1), which accounted for 30.5% of the total variability (Fig. 6a). The consortium and Sp were poorly separated along PC1 from the control but showed some discrimination along the second dimension (PC2) which accounted for 14.6% of the total variability.

This evidence further confirmed that, in comparison with the control, Pt and Nu significantly changed the metabolome in the rhizosphere, which is in agreement with the plant performances described above. The differences observed for Nu and Pt were mostly influenced by two sets of wavenumbers, observable as loadings in the PCA plot (Fig. 6a). The first set, located to the rightmost part of the PCA refers to the spectral section around peak p6 (nitrate region), whereas the second set of wavenumbers, to the left, refers to the carbohydrate region around peak p7 (hemicellulose and polysaccharide region).

A more detailed analysis of the peaks covering SSpec showed two different patterns, as reported in Fig. 6b-e. Peaks p6 (Fig. 6b) and p5 (carbonates, Fig. 6c) presented lower intensities of Nu and Pt than did the control. Conversely, peaks p8 (silicates, Fig. 6d) and p7 (Fig. 6e) displayed control intensities significantly lower than those of Nu and Pt. In all four cases the intensities of the consortium and Sp were not different from those of the control, which further reinforces of the scarce effects exerted by these two inocula on the plant and rhizosphere metabolome.

Low intensity of p6 and p5 spectral regions in Nu and Pt, corresponding to the signals of nitrate and carbonates, respectively (Fig. 6b, c), were observed at the end of the experiment, i.e., during the latest plant growth stage (Linker et al. 2005; Pärnpuu et al. 2022). This evidence could be attributed to increased plant uptake stimulated by the inoculants, which would explain the overall improvement in growth parameters under saline soil conditions (Fig. 4) due to the presence of Nu and Pt in the rhizosphere soil (Senbayram et al. 2020). However, further information is needed to quantify the plant N content and assess its ability to assimilate this nutrient.

The signal at 1403–1426 cm^{-1} (p5), attributed to the vibration of carbonate bonds, decreases in Nu and Pt compared with the control (Pärnpuu et al. 2022; Peltre et al. 2017). Carbonate is the most abundant form of inorganic carbon in soil and can be derived from Microbially-Induced Carbonate Precipitation (MICP) by using soil CO_2 as a precursor for carbonate synthesis (Robles-Fernández et al. 2022). This reduction in carbonate due to the presence of Nu and Pt more than Sp and the consortium is likely due to an imbalance of the microbiota responsible for MICP, but the mechanism is not clear in light of the current knowledge, and further experiments are necessary to elucidate this phenomenon (Miltner et al. 2004).

Both Nu and Pt exhibited notable increases in relative the absorbances of peaks p8 (Si-O silicates) and p7 (C-O hemicellulose) (Fig. 6d, e) (Meng et al. 2019; Xu et al. 2020). Hemicellulose is an important polysaccharide component in vegetable tissues, and its presence in soil is influenced by the lytic activity of the microbiota (Kögel-Knabner 2002). A previous study demonstrated that carbohydrates serve as key inputs for microbial degradation. Additionally, the increased rate of carbohydrate decomposition is associated with a reduction in CO_2 production (Artz et al. 2006). The increase in hemicellulose signals in Nu and Pt could be attributed to the ability of these strains to hydrolyse both L-arabinose and D-xylose, which are the main components of hemicellulose and polysaccharides (Kurtzman et al. 2011). These findings suggest that these two species may play a significant role in soil carbon turnover by acting as potential hydrolytic agents (Artz et al. 2006). Additionally, further research is necessary to determine whether microbial lytic activity can be directly linked to promoting plant growth.

Finally, peak p8 was more intense in the Pt and Nu treatment than in the control and the other two treatments. The increased intensity in this region is attributed primarily to the depolymerization of silicate species, which creates Si-O free broken bonds, resulting in absorption at 915 cm^{-1} (Lenza and Vasconcelos 2001). Silicate compounds in the rhizosphere are essential for plant growth and resistance to abiotic stresses, including drought and salinity (Lenza and Vasconcelos 2001). Additionally, silicates promote microbial activity by inducing the activity of Silicate-Solubilizing Microorganisms (SSM), which convert insoluble silicates into forms that are usable by plants (Rangaraj et al. 2014).

Conclusions

The addition of yeasts derived from plants growing in saline environments significantly mitigated salt-induced stress in zucchini plants. Interestingly, the roots parameters were more positively affected than the other parameters were, suggesting that the mechanism of mitigation is mediated by an increase in biomass and probably by the functionality of the roots. This phenomenon may explain why inocula in salt-added soil were able to improve epigeal parameters close to those of the plants without salt stress and without inoculum. The fact that root growth was enhanced by the

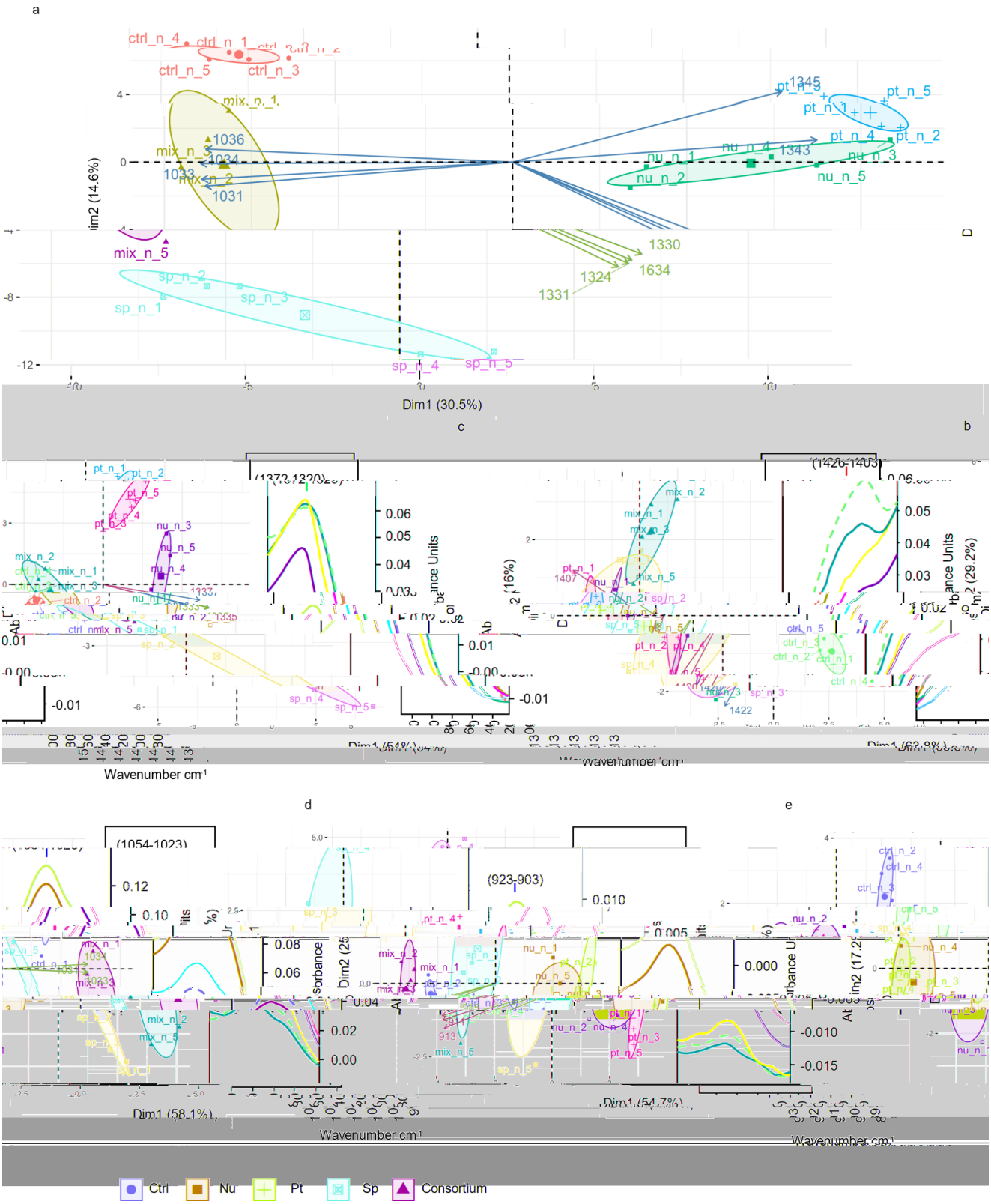

Fig. 6 Impact of the yeast inoculum on the rhizosphere metabolome. Principal Component Analysis (PCA) is performed by combining the second derivative wavenumbers of the spectral regions (p1-11) of each thesis (**a**). PCA revealed the spatial distributions of inoculated samples (Nu, Pt, Sp and Cons) and the control. Panels **b-e** show the PCA of the most significant regions where the PC1 axis is greater than 50% and explains the main sample variation and spatial distribution. Panel b) corresponds to the region of nitrates (p6); panel **c**) corresponds to carbonates (p5); panel **d**) corresponds to silicates (p8) and panel **e**) corresponds to hemicellulose and polysaccharides (p7) (Supplementary Material SI-2)

combination of salt and inoculum well over the values of the control plants, suggests a hormetic effect that requires further investigation. In fact, the possibility that the presence of salt could induce positive effects could lead to improved technologies to transform, as much as possible, a negative factor into a positive factor for the agricultural management of saline soils, improving not only productivity but also long-term sustainability owing to the positive effects noted in the rhizosphere soil and microbiota.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00374-024-01885-y.

Acknowledgements The authors thank Bavicchi S.p.a., for providing the facilities for mesocosm setup.

Author contributions Conceptualization: Gianluigi Cardinali, Laura Corte, Chiara Ruspi, Debora Casagrande Pierantoni; Data curation: Chiara Ruspi, Debora Casagrande Pierantoni, Roberto Scarponi; Formal analysis: Chiara Ruspi, Debora Casagrande Pierantoni, Gianluigi Cardinali; Funding acquisition: Gianluigi Cardinali; Investigation: Gianluigi Cardinali, Laura Corte, Chiara Ruspi, Debora Casagrande Pierantoni; Methodology: Chiara Ruspi, Debora Casagrande Pierantoni, Angela Conti, Roberto Scarponi; Project administration: Gianluigi Cardinali, Laura Corte; Resources: Gianluigi Cardinali; Software: Angela Conti, Roberto Scarponi; Supervision: Gianluigi Cardinali, Laura Corte; Validation: Chiara Ruspi, Debora Casagrande Pierantoni, Gianluigi Cardinali; Visualization: Gianluigi Cardinali, Chiara Ruspi, Debora Casagrande Pierantoni; Writing – original draft: Debora Casagrande Pierantoni, Gianluigi Cardinali, Chiara Ruspi; Writing – review & editing: Gianluigi Cardinali, Laura Corte, Chiara Ruspi, Debora Casagrande Pierantoni.

Funding This research was supported by the Italian Ministry of University and Research (MIUR) National Operational Program on Research and Innovation 2014–2020 (PON RI 2014–2020) in the framework of European Social Fund (ESF), Action IV.4 and IV.6 (CUP J91B21003350006) and action IV.5 “Dottorati su tematiche Green”. The project was partially funded by a funding from EMITA s.r.l. Italy.

Declarations

Competing interests The authors have no competing interests to declare that are relevant to the content of this article.

References

Abdel-Kareem MM, Zohri A-NA, Nasr SAEE (2021) Novel marine yeast strains as plant growth-promoting agents improve defense in wheat (Triticum aestivum) against Fusarium oxysporum. J Plant Dis Prot 128:973–988

Artz RR, Chapman SJ, Campbell CD (2006) Substrate utilisation profiles of microbial communities in peat are depth dependent and correlate with whole soil FTIR profiles. Soil Biol Biochem 38:2958–2962

Bartos A, Szymański W, Klimek M (2020) Impact of conventional agriculture on the concentration and quality of water-extractable organic matter (WEOM) in the surface horizons of Retisols—A case study from the Carpathian Foothills in Poland. Soil Tillage Res 204:104750

Bertout S, Gouveia T, Krasteva D, Pierru J, Pottier C, Bellet V, Arianiello E, Salipante F, Roger F, Drakulovski P (2022) Search for cryptococcus neoformans/gattii complexes and related genera (Filobasidium, Holtermanniella, Naganishia, Papiliotrema, Solicoccozyma, Vishniacozyma) spp. Biotope: two years surveillance of wild avian fauna in southern France. J Fungi 8:227

Bright JP, Karunanadham K, Maheshwari HS, Karuppiah EAA, Thankappan S, Nataraj R, Pandian D, Ameen F, Poczai P, Sayyed RZ (2022) Seed-borne probiotic yeasts foster plant growth and elicit health protection in black gram (Vigna mungo L). Sustainability 14:4618

Conti A, Casagrande Pierantoni D, Robert V, Corte L, Cardinali G (2023) MinION sequencing of yeast mock communities to assess the effect of databases and ITS-LSU markers on the reliability of metabarcoding analysis. Microbiol Spectr 11:e01052–e01022

Corte L, Rellini P, Lattanzi M, Picchetta C, Fatichenti F, Cardinali G (2006) Diversity of salt response among yeasts. Ann Microbiol 56:363–368

Corte L, Rellini P, Roscini L, Fatichenti F, Cardinali G (2010) Development of a novel, FTIR (Fourier transform infrared spectroscopy) based, yeast bioassay for toxicity testing and stress response study. Anal Chim Acta 659:258–265. https://doi.org/10.1016/j.aca.2009.11.035

Corte L, Tiecco M, Roscini L, Germani R, Cardinali G (2014) FTIR analysis of the metabolomic stress response induced by N-alkyltropinium bromide surfactants in the yeasts Saccharomyces cerevisiae and Candida albicans. Colloids Surf B Biointerfaces 116:761–771

Çulha Ş, Çakırlar H (2011) The effect of salinity on plants and salt tolerance mechanisms. AKU-J Sci Eng 11:11–34

Edwards U, Rogall T, Blöcker H, Emde M, Böttger EC (1989) Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. Nucleic Acids Res 17:7843–7853

El-Saadony MT, Saad AM, Soliman SM, Ahmed AI, El-Tahan AM, El-Mageed A, Taia A, Elrys AS, El-Tarabily KA, AbuQamar SF (2022) Plant growth-promoting microorganisms as biocontrol agents of plant diseases: mechanisms, challenges and future perspectives. Front Plant Sci 13:923880

Essendoubi M, Toubas D, Bouzaggou M, Pinon J-M, Manfait M, Sockalingum GD (2005) Rapid identification of Candida species by FT-IR microspectroscopy. Biochim Biophys Acta Gen Subj 1724:239–247

FAO (2021) World Soil Day: FAO highlights the threat of soil salinization to global food security. https://www.fao.org/global-soil-partnership/resources/highlights/detail/en/c/1458974/. 13 September 2024

FAO, ICBA (2023) Thematic 1: Farmers’ guidelines on soil and water management in salt-affected areas. FAO, Rome

FAO, IFAD, UNICEF, WFP, WHO (2021) Global map of salt-affected soils. FAO, Rome
Fu S-F, Sun P-F, Lu H-Y, Wei J-Y, Xiao H-S, Fang W-T, Cheng B-Y, Chou J-Y (2016) Plant growth-promoting traits of yeasts isolated from the phyllosphere and rhizosphere of Drosera Spatulata Lab. Fungal Biol 120:433–448
Gu Y, Dong K, Geisen S, Yang W, Yan Y, Gu D, Liu N, Borisjuk N, Luo Y, Friman V-P (2020) The effect of microbial inoculant origin on the rhizosphere bacterial community composition and plant growth-promotion. Plant Soil 452:105–117
Hammad SA, Ali OA (2014) Physiological and biochemical studies on drought tolerance of wheat plants by application of amino acids and yeast extract. Ann Agric Sci 59:133–145
Irik HA, Bikmaz G (2024) Effect of different salinity on seed germination, growth parameters and biochemical contents of pumpkin (Cucurbita pepo L.) seeds cultivars. Sci Rep 14:6929
Jiang M, Delgado-Baquerizo M, Yuan MM, Ding J, Yergeau E, Zhou J, Crowther TW, Liang Y (2023) Home-based microbial solution to boost crop growth in low-fertility soil. New Phytol 239:752–765
Kögel-Knabner I (2002) The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biol Biochem 34:139–162
Kottke I (2002) Mycorrhizae—Rhizosphere determinants of plant communities. Plant roots. CRC, pp 1419–1438
Kowalska J, Krzymińska J, Tyburski J (2022) Yeasts as a potential biological agent in plant disease protection and yield improvement—A short review. Agriculture 12:1404
Kumar A, Behera I, Langthasa M, PrakashNaroju S (2023) Effect of plant growth-promoting rhizobacteria on alleviating salinity stress in plants: a review. J Plant Nutr 46:2525–2550
Kunito T, Saeki K, Nagaoka K, Oyaizu H, Matsumoto S (2001) Characterization of copper-resistant bacterial community in rhizosphere of highly copper-contaminated soil. Eur J Soil Biol 37:95–102
Kuo C, Fu S, Chou F, Chen R, Chou J (2018) Phosphate-solubilizing characteristics of yeasts. Mycosphere 9:1117–1131
Kurtzman C, Fell JW, Boekhout T (2011) The yeasts: a taxonomic study. Elsevier, USA
Lenza RF, Vasconcelos WL (2001) Structural evolution of silica sols modified with formamide. Mater Res 4:175–179
Li Z, Liu Q, Wu C, Yuan Y, Ni X, Wu T, Chang R, Wang Y (2024) Volatile organic compounds produced by Metschnikowia Pulcherrima yeast T-2 inhibited the growth of Botrytis Cinerea in Postharvest blueberry fruits. Hortic Plant J
Linker R, Shmulevich I, Kenny A, Shaviv A (2005) Soil identification and chemometrics for direct determination of nitrate in soils using FTIR-ATR mid-infrared spectroscopy. Chemosphere 61:652–658
Liu A, Qu Z, Nachshon U (2020) On the potential impact of root system size and density on salt distribution in the root zone. Agric Water Manage 234:106118
Lonhienne T, Mason MG, Ragan MA, Hugenholtz P, Schmidt S, Paungfoo-Lonhienne C (2014) Yeast as a biofertilizer alters plant growth and morphology. Crop Sci 54:785–790
Mapperson RR, Kotiw M, Davis RA, Dearnaley JD (2014) The diversity and antimicrobial activity of Preussia sp. endophytes isolated from Australian dry rainforests. Curr Microbiol 68:30–37
Meng T, Wang Q, Abbasi P, Ma Y (2019) Deciphering differences in the chemical and microbial characteristics of healthy and Fusarium wilt-infected watermelon rhizosphere soils. Appl Microbiol Biotechnol 103:1497–1509
Miltner A, Richnow H-H, Kopinke F-D, Kästner M (2004) Assimilation of CO2 by soil microorganisms and transformation into soil organic matter. Org Geochem 35:1015–1024
Monjezi N, Yaghoubian I, Smith DL (2023) Cell-free supernatant of Devosia sp.(strain SL43) mitigates the adverse effects of salt stress on soybean (Glycine max L.) seed vigor index. Front Plant Sci 14:1071346
Nimsi K, Manjusha K, Kathiresan K, Arya H (2023) Plant growth-promoting yeasts (PGPY), the latest entrant for use in sustainable agriculture: a review. J Appl Microbiol 134:lxac088
Novello G, Cesaro P, Bona E, Massa N, Gosetti F, Scarafoni A, Todeschini V, Berta G, Lingua G, Gamalero E (2021) The effects of plant growth-promoting bacteria with biostimulant features on the growth of a local onion cultivar and a commercial zucchini variety. Agronomy 11:888
Okur B, Örçen N (2020) Soil salinization and climate change. Elsevier, London
Oliveira LSS, Pinto LM, de Medeiros MAP, Toffaletti DL, Tenor JL, Barros TF, Neves RP, Neto RGL, Milan EP, Padovan ACB (2021) Comparison of Cryptococcus gattii/neoformans species complex to related genera (Papiliotrema and Naganishia) reveal variances in virulence associated factors and antifungal susceptibility. Front Cell Infect Microbiol 11:642658
Pandey V, Yadav R, Khare P (2024) Adding mineral-enriched biochar to the rhizosphere reduces heavy metal toxicity on plants and soil microbes. J Environ Chem Eng 113972
Parikh SJ, Goyne KW, Margenot AJ, Mukome FN, Calderón FJ (2014) Soil chemical insights provided through vibrational spectroscopy. Adv Agron 126:1–148
Pärnpuu S, Astover A, Tõnutare T, Penu P, Kauer K (2022) Soil organic matter qualification with FTIR spectroscopy under different soil types in Estonia. Geoderma Reg 28:e00483. https://doi.org/10.1016/j.geodrs.2022.e00483
Peltre C, Gregorich EG, Bruun S, Jensen LS, Magid J (2017) Repeated application of organic waste affects soil organic matter composition: evidence from thermal analysis, FTIR-PAS, amino sugars and lignin biomarkers. Soil Biol Biochem 104:117–127
Peng J, Ma J, Wei X, Zhang C, Jia N, Wang X, Wang ET, Hu D, Wang Z (2021) Accumulation of beneficial bacteria in the rhizosphere of maize (Zea mays L.) grown in a saline soil in responding to a consortium of plant growth promoting rhizobacteria. Ann Microbiol 71:1–12
Pramanik K, Mitra S, Sarkar A, Soren T, Maiti TK (2017) Characterization of cadmium-resistant Klebsiella pneumoniae MCC 3091 promoted rice seedling growth by alleviating phytotoxicity of cadmium. Environ Sci Pollut Res 24:24419–24437
R Core Team (2021) A language and environment for statistical computing. https://www.R-project.org/
Raklami A, Babalola OO, Jemo M, Nafis A (2024) Unlocking the plant growth-promoting potential of yeast spp.: exploring species from the Moroccan extremophilic environment for enhanced plant growth and sustainable farming. FEMS Microbiol Lett 371:fnae015
Ramos-Garza J, Bustamante-Brito R, Angeles de Paz G, Medina-Canales MG, Vásquez-Murrieta MS, Wang ET, Rodríguez-Tovar AV (2016) Isolation and characterization of yeasts associated with plants growing in heavy-metal-and arsenic-contaminated soils. Can J Microbiol 62:307–319
Rangaraj S, Gopalu K, Rathinam Y, Periasamy P, Venkatachalam R, Narayanasamy K (2014) Effect of silica nanoparticles on microbial biomass and silica availability in maize rhizosphere. Biotechnol Appl Biochem 61:668–675
Rima FS, Biswas S, Sarker PK, Islam MR, Seraj ZI (2018) Bacteria endemic to saline coastal belt and their ability to mitigate the effects of salt stress on rice growth and yields. Ann Microbiol 68:525–535
Robles-Fernández A, Areias C, Daffonchio D, Vahrenkamp VC, Sánchez-Román M (2022) The role of microorganisms in the nucleation of carbonates, environmental implications and applications. Minerals 12:1562
Roscini L, Corte L, Antonielli L, Rellini P, Fatichenti F, Cardinali G (2010) Influence of cell geometry and number of

replicas in the reproducibility of whole cell FTIR analysis. Analyst 135:2099–2105
Rouphael Y, Colla G (2020) Biostimulants in agriculture. Front Plant Sci 11:511937
Ruspi C, Pierantoni DC, Conti A, Favaro L, Antinori ME, Puglisi E, Corte L, Cardinali G (2024) Beneficial effects of plant growth-promoting yeasts (PGPYs) on the early stage of growth of zucchini plants. Curr Plant Biol. 100357
Sánchez-Sánchez A, Cerdán M, Jordá J, Amat B, Cortina J (2019) Characterization of soil mineralogy by FTIR: application to the analysis of mineralogical changes in soils affected by vegetation patches. Plant Soil 439:447–458
Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Consortium FB, List FBCA, Bolchacova E (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. PNAS 109:6241–6246
Sehrawat A, Khandelwal A, Sindhu SS (2020) Characterization of Mesorhizobium strains for salt tolerance and wilt control: their potential for plant growth promotion of chickpea (Cicer arietinum L). Legume Res 43:146–150
Senbayram M, Well R, Shan J, Bol R, Burkart S, Jones DL, Wu D (2020) Rhizosphere processes in nitrate-rich barley soil tripled both N 2 O and N 2 losses due to enhanced bacterial and fungal denitrification. Plant Soil 448:509–522
Shultana R, Tan Kee Zuan A, Yusop MR, Mohd Saud H, Ayanda AF (2020) Effect of salt-tolerant bacterial inoculations on rice seedlings differing in salt-tolerance under saline soil conditions. Agronomy 10:1030
Siebeneichler SC, Barbosa JS, Cruz AMM, Ramos M, Fernandes H, Nascimento V (2019) Comparison between extraction methods of photosynthetic pigments in Acacia mangium. Commun Plant Sci 9:1–5
Singh N, Gaur S (2021) GRAS fungi: a new horizon in safer food product. Fungi Sustain Food Prod. 27–37
Stackebrandt E, Liesack W (1993) Nucleic acids and classification. In: Goodfellow M (ed) The New Bacterial Systematics. Academic, London, pp 151–194
Team RC (2022) R: A Language and Environment for Statistical Computing. https://www.R-project.org/
Teng Q, Feng Y, Li H (2018) Effects of silicate-bacteria pretreatment on desiliconization of magnesite by reverse flotation. Colloids Surf Physicochem Eng Aspects 544:60–67
Thiem D, Gołębiewski M, Hulisz P, Piernik A, Hrynkiewicz K (2018) How does salinity shape bacterial and fungal microbiomes of Alnus glutinosa roots? Front Microbiol 9:318754
Thiem D, Goebel M, Gołębiewski M, Baum C, Koczorski P, Szymańska S, Hrynkiewicz K (2023) Endophytic microbiota and ectomycorrhizal structure of Alnus glutinosa Gaertn. At saline and nonsaline forest sites. Sci Rep 13:22831
Tinti A, Tugnoli V, Bonora S, Francioso O (2015) Recent applications of vibrational mid-infrared (IR) spectroscopy for studying soil components: a review. J Cent Eur Agric 16:0–0
Turhan E, Eris A (2005) Changes of micronutrients, dry weight, and chlorophyll contents in strawberry plants under salt stress conditions. Commun Soil Sci Plant Anal 36:1021–1028
Wang X, Xu X, Lu A, Li X, Zhang F (2022) Cultivation of two barnyard varieties improves physicochemical properties of saline-alkali land through mediating rhizospheric microbiome and metabolome. Agronomy 12:1322
Wei P, An S, Dong Y, Sun Z, Hou Y, Bieerdawulieti X (2021) Diversity and community structure of Soil Fungi in three typical Desert Plant formations in the Junggar Basin, Northwest China. Eurasian Soil Sci 54:1945–1956
Xu X, Du C, Ma F, Shen Y, Zhou J (2020) Forensic soil analysis using laser-induced breakdown spectroscopy (LIBS) and Fourier transform infrared total attenuated reflectance spectroscopy (FTIR-ATR): principles and case studies. Forensic Sci Int 310:110222
Yurkov AM (2018) Yeasts of the soil–obscure but precious. Yeast 35:369–378
Zhang G, Bai J, Zhai Y, Jia J, Zhao Q, Wang W, Hu X (2023) Microbial diversity and functions in saline soils: a review from a biogeochemical perspective. J Adv Res 59:129–140
Zhang H, Chang D, Zhu Z, Meng C, Wang K (2024) Soil priming effects and involved microbial community along salt gradients. Biogeosciences 21:1–11