Plant Soil
https://doi.org/10.1007/s11104-024-06806-4

RESEARCH ARTICLE

Interactions between an arbuscular mycorrhizal inoculum and the root-associated microbiome in shaping the response of *Capsicum annuum* "Locale di Senise" to different irrigation levels

Alice Calvo · Thomas Reitz · Fabiano Sillo · Vincenzo Montesano · Eva Cañizares · Elisa Zampieri · Roghayyeh Mahmoudi · Gholamreza Gohari · Walter Chitarra · Luca Giovannini · Adriano Conte · Carmelo Mennone · Gianniantonio Petruzzelli · Mauro Centritto · Miguel González-Guzmán · Vicent Arbona · Vasileios Fotopoulos · Raffaella Balestrini

Received: 6 April 2024 / Accepted: 10 June 2024
© The Author(s) 2024

Abstract

Background and aims The use of root-associated microorganisms emerge as a sustainable tool to enhance crop tolerance and productivity under climate change, particularly in drought-affected areas. Here, the impact of an inoculum based on arbuscular mycorrhizal fungi (AMF) was evaluated on pepper (*Capsicum annuum* L.) cultivation at varying water irrigation treatments (well-watered, reduced irrigation and rain-fed) under open-field conditions.

Methods Agronomic and ecophysiological parameters, as well as biochemical analyses on stress markers and phytohormones in leaves and on fruit quality traits, were evaluated, along with the shifts in soil- and root-associated microbial communities.

Results Rain-fed water treatment caused reduced fruit sizes, while no differences were detected among well-watered and reduced irrigation. Reduced irrigation did not cause a reduction in stomatal conductance. The highest AM fungal colonization rates were observed under reduced irrigation, and the enhanced flavonoid content and reduced oxidative stress markers in AMF-inoculated plants suggested a synergistic effect of AM fungal inoculation in boosting plant tolerance against stress. A shift in microbial community

Responsible Editor: Gaowen Yang.

Alice Calvo and Thomas Reitz contributed equally to this work.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11104-024-06806-4.

A. Calvo · F. Sillo · E. Zampieri · L. Giovannini · M. Centritto
Institute for Sustainable Plant Protection (IPSP), National Research Council of Italy, Turin, Italy

A. Calvo
Department of Agriculture and Desertification Research Centre, University of Sassari, Sassari, Italy

T. Reitz
Department of Soil Ecology, Helmholtz Centre for Environmental Research GmbH – UFZ, Halle, Germany

T. Reitz
German Centre for Integrative Biodiversity Research (iDiv), Halle-Jena-Leipzig, Germany

V. Montesano · A. Conte
Institute for Sustainable Plant Protection, National Research Council of Italy, Metaponto di Bernalda, MT, Italy

E. Cañizares · M. González-Guzmán · V. Arbona
Departament de Biologia, Bioquímica i Ciències Naturals, Universitat Jaume I, Castelló de La Plana, Spain

R. Mahmoudi · G. Gohari · V. Fotopoulos
Department of Agricultural Sciences, Biotechnology and Food Science, Cyprus University of Technology, Limassol, Cyprus

Published online: 02 July 2024

composition in the different irrigation treatments, associated with different enzymatic activity, highlighted the potential role of microbial dynamics in plant stress response under water-limited conditions.
Conclusion The study suggests that a reduced irrigation comes along with beneficial impacts on pepper root associated microbes, while not impairing crop performance and yields, indicating a potential of saving water. All together, our results imply that optimization of irrigation and beneficial plant–microbe interactions, such as AM fungal symbiosis, can improve pepper physiological and productivity features under climate change.

Keywords Pepper · AM symbiosis · AM fungal inoculation · Drought · Irrigation · Tolerance

Introduction

Drought is one of the most impactful abiotic stresses, significantly affecting crop yield and production, thereby posing risks to global food security (Food Insecurity 2022). In addition, as a consequence of global climate change, drought is expected to intensify in the near future, representing a further significant threat to agricultural areas increasing the risks of crop production decline. This underscores the urgent need to explore novel sustainable practices aimed at improving water use efficiency in agroecosystems (Bhattacharyya et al. 2021). Over the last decades, plant responses occurring during drought and recovery phases have been largely studied at many levels, from cell signaling to the whole ecophysiological traits for both herbaceous and woody crops (Zia et al. 2021). However, it is worth noting that plants share their environment with several soil microbes that live associated to the roots and in the rhizosphere, establishing with their hosts a complex network of exchanges crucial for the maintenance of plant health (Berendsen et al. 2012). These intimate associations between plants and their inhabiting microbes allow mutual responses with the surrounding environment *e.g.,* tolerance to abiotic stresses and improved nutrient uptake (Sandrini et al. 2022; Zhang et al. 2021). Beyond the well-known detrimental effects of drought on plants, including reductions in photosynthetic and stomatal conductance rates as a consequence of hydraulic impairment (Haworth et al. 2021), as well as metabolic (*e.g.*, abscisic acid – ABA production and oxidative burst by reactive oxygen species – ROS) (Marino et al. 2017) and nutritional imbalances, stressed plants, in turn, activate responses to cope with stress (*e.g.,* osmoprotectants and antioxidant accumulations) (Ahluwalia et al. 2021; Mahmood et al. 2021; Sandrini et al. 2022; Tang et al. 2023). These responses span from molecular signaling to phenotypic adaptation characteristics (Naseem et al. 2018; Nerva et al. 2023). Among these traits, root traits play key roles in protecting plants from desiccation. This involves root apparatus growth to explore a larger soil volume and the release of exudates, whose composition has been reported to change during stress, influencing the shifting of rhizospheric microbiome structure and dynamics (de Vries et al. 2020). Drought alters both the quantity and quality of root exudates, exerting selective pressure on favorable rhizospheric microbiomes capable of mitigating drought-induced damages in stressed plants (Williams and de Vries 2020). Beneficial soil microorganisms have the capacity to mitigate the effects of water deficit in plants by influencing several processes (Fadiji et al. 2022) *e.g.,* via osmolyte, antioxidant and phytohormone production (Brunetti et al. 2021). Beneficial soil bacteria can enhance soil micropores through the secretion of various compounds, such as exopolysaccharides. This phenomenon results in increased water retention in the soil, thereby fostering plant resilience against water deficit conditions (Philippot et al. 2024). Soil microbiome can have a positive effect also on nutrient uptake, facilitating the availability of elements that are not accessible in other forms or producing molecules able

W. Chitarra
Council for Agricultural Research and Economics, Research Centre for Viticulture and Enology (CREA-VE), 31015 Conegliano, TV, Italy

C. Mennone
Azienda Pantanello, ALSIA Research Center Metapontum Agrobios, Metaponto di Bernalda, MT, Italy

G. Petruzzelli
Institute of Research on Terrestrial Ecosystems (IRET), National Research Council, Pisa, Italy

R. Balestrini (✉)
Institute of Biosciences and Bioresources (IBBR), National Research Council, Bari, Italy
e-mail: raffaellamaria.balestrini@cnr.it

to mitigate water stress (Ali et al. 2022; Caddell et al. 2023; Philippot et al. 2024). In addition to soil bacteria, numerous studies have demonstrated that Arbuscular Mycorrhizal Fungi (AMF) can improve plant growth as they facilitate the absorption of nutrients and water, particularly in challenging environmental conditions such as drought (Augé et al. 2015). The fungal hyphae of AMF have the capability to explore soil pores that are beyond the reach of root hairs, thus enhancing the plant water uptake and ultimately boosting crop productivity under water deficit conditions (Chitarra et al. 2016; Kakouridis et al. 2022). Currently, both AMF and beneficial soil bacteria are representing promising bio-based tools to improve plant tolerance to stresses (Abdelkhalik et al. 2023; Sandrini et al. 2022; Zhang et al. 2022a).

Pepper (*Capsicum annuum* L.), a member of the Solanaceae family, is a globally grown crop (typically annual cycle) with an estimated production exceeding 40 million tons *per* year and cultivated over 3.6 million hectares, making it the second most cultivated vegetable after tomato (FAOSTAT 2021; Olmstead et al. 2008). Additionally, pepper berries are known for their high nutritional value as a source of antioxidant and nutraceutical chemicals (Soare et al. 2017). Due to a wide transpiring leaf surface and high stomatal conductance, as well as to the high-water requirements needed during its growing period, pepper is considered as a drought susceptible crop (Alvino et al. 1994; Delfine et al. 2001). The application of different AM fungal inocula has proved to alleviate water and salt stresses by triggering various molecular (*e.g.*, aquaporin and dehydrin genes) and biochemical (*e.g.*, antioxidant enzymes) pathways in several crops (Chitarra et al. 2016), including different pepper cultivars, *i.e.,* the commercial Sera Demre 8 and the Polish sweet pepper hybrid Roberta F1 (Güneş et al. 2023; Nurzyńska-Wierdak et al. 2021). Under water deficit conditions, an increase in root width, length, and weight due to the application of AM fungal inocula has been observed in pepper plants, enabling them to deeply explore larger volumes of soil (Tang et al. 2023; Türkmen et al. 2008).

Interestingly, it has been already reported that natural soil microbiota seems to be affected by AM fungal inoculation (Jing et al. 2022; Nerva et al. 2022). A study on *Cucurbita pepo* L. rhizosphere has recently revealed a higher presence of plant growth-promoting microorganisms in presence of a *Funneliformis mosseae* inoculation, compared to not-inoculated plants (Wang et al. 2022). A similar scenario was observed in *C. annuum* rhizosphere after *F. mosseae* inoculation (Tang et al. 2023). Hence, it is evident that a deeper understanding of plant–microbe and microbe-microbe interactions during water deficit, with a specific emphasis on the rhizosphere compartment, is essential. This knowledge is pivotal for enhancing crop tolerance to drought (Santos-Medellín et al. 2021).

Here, by using *C. annum* "Locale di Senise" plants as a typical crop of Mediterranean area, a study in small plots under open-field condition was conducted, providing a realistic representation of production environments. The study aimed to investigate the impact of AM fungal inoculation on pepper plants, subjected to different irrigation treatments considering plant physio-chemical responses and AM fungal interaction with the natural soil microorganisms.

Materials and methods

Experimental design

The open-field experiment took place at the Lucan Agency of Innovation and Development in Agriculture (ALSIA) research center "Azienda Pantanello", in Metaponto di Bernalda, Southern Italy (40°23′031.4″ N, 16°47′010.9″ E). Early growth stage pepper plants (cv "Locale di Senise", 3–4 expanded leaves) were transplanted on June 18, 2022. For approximately 40 days after transplantation, till the plant architecture was completely formed, water was supplied with amounts equivalent to 100% of the effective crop evapotranspiration (ETc). After that stage (from July 22, 2022) a plot was kept in well-watered conditions (100% of ETc—R1), a second plot was kept under reduced water conditions (75% of ETc—R2), while the third plot was kept in rain-fed condition (R3). The daily ETc was calculated according to the formula $ETc = ET_0 \times Kc$, where ET_0 is the reference evapotranspiration according to Penman–Monteith equation (Allen et al. 1998) and Kc is the crop coefficient, based on recommendations for bell pepper production and adjusted for the environmental conditions (Ávila-Dávila et al. 2021; Rodríguez Padrón et al. 2015). The water supply was monitored by three MultiJet water meters RMM DN40 (Raphael Valves Industries Ltd, Isr.).

From transplantation to end of the experiment, well-watered parcels (R1) received 50.2 m^3 of water, parcels subjected to reduced irrigation (R2) received 35.3 m^3 of water, while the R3 treatment received 9.5 m^3 of rainfall water. Well-watered parcels and parcels subjected to reduced irrigation received both irrigation and rainfall water. Meteorological data from a weather station positioned at the Azienda Agricola Pantanello were recorded to follow the climatic conditions during the crop growth (Table S1 and Fig. S1). Irrigation was performed using an in-line drip irrigation system (1.3 L/h; 16 mm inside diameter; 20 cm spacing) designed to ensure full water coverage and uniform distribution.

Each single main plot was subsequently divided into two sub-plots, assigned to two different inoculation treatments (I and NI) of AM fungal mixed inoculum (INOQ GmbH, Germany), thus resulting in the following six treatments: IR1, IR2, IR3, NIR1, NIR2 and NIR3. Each single sub-plot was replicated three times (I, II and III) for a total of 18 parcels (six for each irrigation level) spaced 1 m apart. Each parcel consisted of 54 plants, arranged in 5 lines, 4.8 m long for a total of 19.5 square meters (Fig. S2).

For the treatments involving AM fungal inoculation (IR1, IR2 and IR3), a commercial AM fungal inoculum was applied during the transplantation. In detail, each plant was dipped in a water suspension of the AM fungal inoculum provided as wettable powder at recommended doses (INOQ ADVANTAGE; INOQ GmbH, Schnega, Germany). The inoculum contained *Rhizoglomus irregulare*, *Funneliformis mosseae*, *Funneliformis geosporum* with a minimum of 150,000 mycorrhiza units, *i.e.,* propagules composed by spores, hyphae, and root residues (*per* gram). Not-inoculated treatments were named NIR1, NIR2 and NIR3. Weeding was carried out by hand and integrated production agronomic management for sweet pepper was applied. In detail, the total amount of applied NPK fertilization, corresponding to 70, 45 and 160 kg/ha, respectively, was split into five rates during the crop cycle: 30% at transplanting (as ammonium sulfate and urea phosphate), 10% at 30 and 50 days after transplanting (as urea phosphate, ammonium nitrate, and calcium nitrate for fertilization), 30% at full fruit set and 20% during fruit development (as 20/20/20 fertilizer and potassium nitrate). Plant protection was carried out according to the standard cultivation protocols of the Basilicata Region (Italy) for integrated production, using different commercial products, applied at different times, at the recommended doses on the product labels: oil-based suspension of *Beauveria bassiana*, suspension concentrate of *Bacillus thuringiensis* subsp. *kurstaki*, suspension concentrate of Azadirachtin, and water-dispersible granules of *Bacillus amyloliquefaciens*.

Sampling procedure

At the beginning of the experiment, after transplanting, a representative soil sample for each parcel was collected. Samples collected from the same inoculation treatment were pooled (one for I and one for NI treatment) and were analyzed for chemical-physical properties. During and at the end of the experiment, two soil sampling time points were established, in August and in October 2022, respectively. For chemical-physical analysis, three replicates for each treatment were pooled to obtain 12 soil samples (six collected in August and six collected in October). Thirty-six bulk soils samples (approximately 800 g *per* sample), collected twice (August and October 2022), were analyzed for soil enzymatic activity, soil moisture content and assessment of fungal and bacterial community through metabarcoding. In October, at the end of the experiment, roots were sampled for both assessment of mycorrhizal colonization and metabarcoding analysis (three replicate for each treatment, for a total of 18 samples). Leaves (three replicates for each treatment) were collected in August to perform biochemical markers and phytohormones analysis. Fruits (three replicates for each treatment) were collected during both sampling times to perform antioxidant and nutritional analysis.

Soil moisture and extracellular enzyme activities

Moisture content was evaluated using a halogen moisture analyzer on the 36 bulk soil samples (Mettler Toledo, Gießen, Germany). A modified fluorometric assay of Sinsabaugh et al. (2003) was used to determine extracellular enzyme activities of the 36 soil samples. The activity potentials of six hydrolytic soil enzymes, involved in the degradation and acquisition of carbon, nitrogen, phosphorous, and sulfur (β-glucosidase, xylosidase and cellobiohydrolase, N-acetylglucosaminidase, acid phosphatase and sulfatase), were measured as turnover rate of 4-methylumbelliferon (MUF)-coupled substrates. The amount of released MUF was directly related to enzymatic activity potentials. A comparable high concentration

of 300 µM was used for all substrates, to prevent an underestimation of activity due to substrate limitation. For each sample, a black 96-well microplate was used. The plates contained the six enzyme substrates, MUF dilutions to calculate quench and extinction coefficients (1.25 and 2.5 µM), as well as substrate and soil suspension controls. Approximately 250 mg of fresh soil was suspended in 50 ml of 50 µM acetate buffer (pH 5) and sonicated for 5 min to break up soil aggregates. Afterwards, the soil suspensions were transferred to the prepared microplates and incubated at 25 °C for 60 min. After the addition of 30 µl 1 M NaOH to stop the enzymatic reactions, fluorescence was measured for eight replicates using a Tecan Infinite 200 PRO plate reader (Tecan Group, Männedorf, Switzerland) at 360 nm excitation and 465 nm emission wavelengths. Enzyme activities were calculated as turnover rate of substrate in nmol *per* gram dry soil and hour (nmol/g_{soil} h) (German et al. 2011).

Ecophysiological measurements and agronomic parameters

During the phenological stage corresponding to 'visible pepper berry', non-destructive measurement was performed using a steady-state Licor LI-600 Porometer/Fluorometer apparatus (LI-COR Biosciences, Lincoln, NE, United States) to assess stomatal conductance (g_s). The measurements were taken on five randomly selected plants for each parcel, measured on the newly fully expanded leaves, in sunny days at noon (between 10:00 and 12:00). At the end of the experiment, ten randomly selected plants from each parcel were selected to determine: plant height (cm), fruit dry matter (g/100 g), fruit length (cm), width (cm) and thickness (mm), fruit number (n) and weight (g) *per* plant. Yield (t/ha) was evaluated by summing up the two fruit harvests, in August and October.

Assessment of mycorrhizal colonization in roots

Collected roots were decolored with KOH 10% for 30 min at 80 °C, then stained with 0.1% cotton blue in lactic acid overnight and 1-cm-long root portions were used. Fifty-four slides (three slides for replicate of each treatment, for a total of 54 slides) were prepared and analyzed according to the Trouvelot system (Trouvelot 1986) to evaluate the frequency of mycorrhization (F%), the intensity of mycorrhizal colonization in the root system (M%) and the arbuscule abundance in the whole root system (A%).

Biochemical markers and phytohormones quantification in leaves

A total of 17 samples of leaves, corresponding to three replicates for each of the six treatments, except for the replicate III of NIR3, were used to perform the biochemical analyses. The evaluated biochemical markers included malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and proline. Lipid peroxidation was determined in leaves from the measurement of MDA content resulting from the thiobarbituric acid (TBA) reaction (Christou et al. 2013; Heath and Packer 1968) using an extinction coefficient of 155 mM^{-1} cm^{-1}. Hydrogen peroxide content was quantified using the KI method (Velikova et al. 2000), while free proline levels were determined using the ninhydrin reaction (Bates et al. 1973). Concerning phytohormones, the concentration of abscisic acid (ABA), jasmonic acid (JA), salicylic acid (SA), indole-3-acetic acid (IAA) and 12-oxophytodienoic acid (OPDA), was determined by targeted LC/MS approach as previously reported (De Ollas et al. 2021). Briefly, prior the extraction, samples were spiked with 50 ng of the following surrogate analytes to correct for analyte loses and potential analytical drift in the mass spectrometer (ABA-$^2H_6$, IAA-$^2H_5$, dihydrojasmonic acid, SA-$^{13}C_2$). Extraction was carried out in 1 mL ultrapure water for 10 min in a ball mill at room temperature using 2 mm glass beads. Homogenates were centrifuged at 10,000 rpm for 10 min at 4 °C and supernatants recovered and partitioned twice against an equal volume of diethyl ether after adjusting pH to 3.0 with 30% acetic acid. The combined organic layers were evaporated under vacuum in a centrifuge concentrator (Jouan, Sant Germaine Cedex, France) and the dry residues reconstituted in 0.5 mL of a 10% aqueous methanol solution. Prior to injection, extracts were filtered through 0.20 µm PTFE syringe membrane filters and filtrates recovered in chromatography amber glass vials. Samples were analyzed by tandem LC/MS in an Acquity SDS UPLC system (Waters Corp., USA) coupled to a TQS triple quadrupole mass spectrometer (Micromass Ltd., UK) through an electrospray ionization source. Separations were carried out on a C18 column (Luna Omega Polar C18,

50×2.1 mm, 1.6 µm particle size, Phenomenex, USA) using a linear gradient of ultrapure acetonitrile and water, both supplemented with formic acid to a 0.1% (V/V) concentration, at a constant flow rate of 0.3 mL min^{-1}. During analyses, column temperature was maintained at 40 °C and samples at 10 °C to slow down degradation. Plant hormones were detected in negative electrospray mode following their specific precursor-to-product ion transitions and quantified using an external calibration curve with standard samples of known amount.

Antioxidant and nutritional analysis on pepper fruits

A total of 18 fruit samples from the six experimental treatments, with three replicates *per* treatment, were chopped and analyzed for antioxidant compounds and nutritional content. For antioxidant compound analysis, about 500 mg (fresh weight) *per* sample were used. Antioxidant compounds included anthocyanin (mg/100 g FW), ascorbic acid (g/kg FW), flavonoid (mg/100 g FW), phenol (mg/100 g FW) and 2,2-difenil-1-picrylidrazyl (DPPH, radical scavenging activity – RSA%). The anthocyanin content was measured spectrophotometrically according to Giusti and Wrolstad (2001). Ascorbic acid content was evaluated with the oxidation of ascorbic acid by 2,6-dichlorophenol-indophenol sodium salt dihydrate (103,028 Merck, Germany) that, in acidic conditions, combined returned a yellow-orange color according to Suntornsuk et al. (2002). Total flavonoid content was determined according to the method of Zhishen et al. (1999) with some modifications, and phenol quantification was assessed with Folin-Ciocalteu reagent (Singleton et al. 1999). The DPPH radical scavenging activity was determined according to the method described by Dehghan and Khoshkam (2012). Concerning micronutrients analysis, the remaining fruit samples were collected and oven-dried at 65 °C for 4 days to perform micronutrient analysis. Fruits were analyzed for N%, P%, K%, Ca%, Mg%, Na%, Fe (ppm), Mn (ppm), Cu (ppm), Zn (ppm) and B (ppm) content.

Metabarcoding analysis

Thirty-six soil samples and 18 root samples, corresponding to the six experimental treatments, three replicates each, were used for the assessment of microbial communities via metabarcoding analysis. Each soil sample was homogenized by using bead-beating tubes provided by the DNeasy PowerSoil Pro Kit (Qiagen) and a Vortex Genie ® 2 (Scientific Industries) for 20 min. Total DNA was isolated from approximately 250 mg of soil for each sample using the same kit and quantified with a Nanodrop 2000 (Thermo Scientific). For metabarcoding analysis, 16S rDNA fragment (for Prokaryotes) and ITS2 region (for fungi) were amplified using the KAPA HiFi DNA Polymerase and primers (515f: GTGYCA GCMGCCGCGGTAA, 806r: GGACTACHVGGG TWTCTAAT for16S rDNA and ITS4: TCCTCCGCT TATTGATATGC and fITS7: GTGARTCATCGA ATCTTTG for ITS2 region). The PCR-amplification was conducted in triplicates for each sample and target region (40 ng DNA template used *per* PCR reaction). The temperature profile for prokaryotic amplicon PCR was the following: initial denaturation at 95 °C for 3 min, 25 cycles of denaturation at 98 °C for 20 s, annealing at 55 °C for 15 s, and extension at 72 °C for 15 s, followed by the final extension at 72 °C for 5 min. The temperature profile for fungal amplicon PCR was: initial denaturation at 95 °C for 3 min, 30 cycles of denaturation at 98 °C for 20 s, annealing at 56 °C for 20 s, and extension at 72 °C for 20 s, followed by the final extension at 72 °C for 5 min. Success of PCR was checked with agarose gel electrophoresis. Amplicons from the PCR-triplicates were pooled and purified with AmpPure XP Beads, indexed in an additional PCR (Illumina Nextera XT v2 index primers) and purified again with AmpPure XP Beads. Concentration of indexed and purified PCR-products was determined with NanoDrop ND-8000 spectrophotometer. DNA of fungal and prokaryotic amplicons were equimolarly pooled. Exact concentrations of the final pools were determined with Qubit dsDNA-HS Assay and fragment length and quality were additionally checked with Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). The prokaryotic and fungal amplicon pools were combined for Illumina MiSeq paired-end sequencing. Sample libraries and the control library were diluted and denatured following the MiSeq Illumina kit instructions and injected into an Illumina MiSeq flow-cell for paired-end sequencing. Metabarcoding data were analyzed with QIIME 2 (Bolyen et al. 2019). Sequences were trimmed with cutadapt v3.4, denoised through dada2 v2021.8.0, and assembled into Amplicon Sequence Variants (ASVs).

The Greengenes2 2022.10 (McDonald et al. 2023) and UNITE v8-99 (Kõljalg et al. 2013) databases for bacteria and fungi, respectively, were utilized to train Naive Bayes classifier on ASVs (99% identity) sequences for taxonomic assignment. Chloroplast and mitochondrial sequences were removed by filtering from the resulting ASV table with the "*filter-features*" parameter of QIIME 2. The output was elaborated to obtain a relative abundance (%) of each ASV in the total amounts of the entire sample. Raw reads from metabarcoding of soil and root samples were submitted to NCBI SRA under BioProject PRJNA1024920 for bacteria and fungi, respectively. The ASV tables were generated for fungal and bacterial communities detected in soil collected in August, as well as root and soil collected in October. The tables were used as input for Microbiome Analyst (Chong et al. 2020; Dhariwal et al. 2017) for data visualization and statistical assessment. Diversity within samples at feature level (alpha diversity) was calculated using the Chao1 index, while diversity among samples at feature level (beta diversity) was calculated with the Bray–Curtis index and reported in a two-dimensional principal coordinates analysis. Taxon abundance was visualized through stacked barplots at the phylum level. Core microbiome heatmap was generated at phylum level for each ASV table. Taxon abundance was investigated specifically for root bacterial communities, to assess roots-microbiome interactions under water-deficit conditions. A single-factor comparison was performed for each analysis at family level, using DESeq2 method embedded in Microbiome Analyst (Love et al. 2014), considering the different conditions separately (inoculation, irrigation treatment, source and sampling period) and their interaction, to evaluate statistical differences of fungal and bacterial communities associated to each sample.

Statistical analysis

R software (version 4.3.1) was used to perform statistical analysis. Two-way ANOVA was employed to examine the impact of the irrigation treatment, inoculation, and their interaction on agronomic and ecophysiological parameters, biochemical marker content and hormones in pepper leaves, levels of antioxidant compounds and micronutrients in fruits, and data of soil enzyme activity. Post-hoc statistical analysis was conducted using the Tukey Honestly Significant Difference (HSD) test, with a significance level of $p \leq 0.05$.

Results

Soil chemical-physical characteristics

Soil chemical-physical characteristics did not show any relevant differences at the beginning of the experiment between I and NI treatments (Table S2). Both soil samples showed high sand content (78–82%) and low clay content (9–10%). The organic matter content was rather low (around 1.37–1.44%) and the pH of both soils was neutral (7.5–7.6). Both soil samples showed a slight level of salinity, as well as low levels of nitrogen and organic matter (Table S2). Similarly, soil chemical-physical characteristics during and at the end of the experimental period did not significantly differ among treatments (Table S3). In IR1 samples, both in August and October, the exchangeable P levels (21.4–20.5 ppm, respectively) were slightly higher compared to all the other treatments (17.88–17.78 ppm, on average, in August and October, respectively), although not significantly. In addition, both nitrogen and organic matter percentages showed a slight decrease in August and October compared to the beginning of the experiment (Table S2-S3). Soil moisture was affected by the irrigation treatment at both sampling times (August and October). Additionally, R3 was always associated to a significantly lower moisture content (Table S4).

Soil enzyme activities

Results on collected soils showed no significant effect of inoculation and inoculation x irrigation interaction on enzyme activity (Table S4). However, a significant effect of irrigation conditions was detected on enzyme activity in samples collected during both sampling times *i.e.,* bulk soils collected in August and in October (Table S4). Particularly, in August, a differential activity of cellobiohydrolase, β-glucosidase and N-acetyl glucosaminidase was detected (Table S4). For all these enzymes, a significant difference was found comparing R1 and R2 to R3, with the R3 samples showing a lower enzymatic activity compared to the others. The same

analysis performed on bulk soils collected in October highlighted that most of the enzymes (*i.e.,* xylosidase, cellobiohydrolase, β-glucosidase, N-acetyl glucosaminidase and acid phosphatase) showed a variation in their activity and, as observed in the first sampling time, the enzymatic activity was significantly lower in R3 (Table S4). Sulfatase did not show any variation in its activity in both times. A higher phosphatase activity was obtained from the samples collected in October compared to those collected in August, whereby the increase was particularly evident for the R1 and R2 irrigation treatments. As stated before, the inoculation showed no impact on soil enzyme activities in the two sampling months. However, although not significant, an increased β-glucosidase activity was observed in I samples when compared to NI ones during both sampling points (Table S4).

Ecophysiological measurements, plant development and production

Stomatal conductance was significantly affected by irrigation, with plants under R1 and R2 showing g_s values that were significantly higher compared to R3 ones. However, no significant difference was observed between R1 and R2 themselves (Table S5 and Fig. 1). Conversely, interaction between inoculation and irrigation did not significantly affect stomatal conductance (Table S5). Concerning the agronomic parameters, inoculation, irrigation and the interaction between these two factors did not result in significant differences in plant height, which ranged between 63.2 ± 8.8 and 73.2 ± 6.2 cm. By contrast, fruit length, fruit width, fruit dry matter, and fruit fresh weight *per* plant were significantly affected by the irrigation treatment (Fig. 2a-d and Tables S6-7). Under R3, these parameters were significantly reduced. Additionally, also the total estimated yield (Fig. 2e) significantly decreased in R3 condition. On the other hand, fruit thickness and fruit number *per* plant were not significantly different among treatments (Table S6-7).

AM fungal colonization in pepper roots

In pepper roots, AM fungal colonization was observed independently from the inoculation (Fig. S3). On the other hand, the irrigation treatment significantly affected M% (intensity of the mycorrhizal colonization

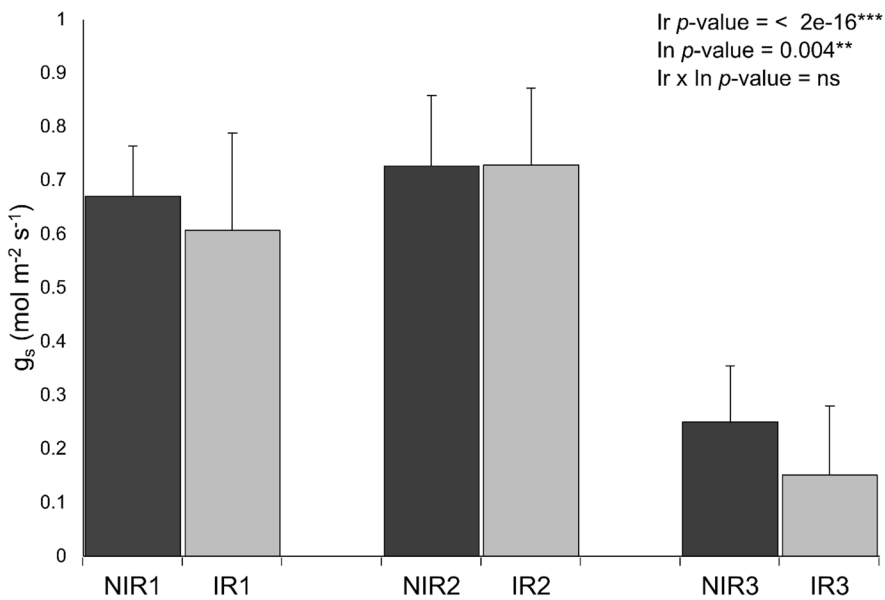

Fig. 1 Stomatal conductance (g_s, mol m^{-2} s^{-1}) of pepper leaves. All results are reported as mean ± standard deviation. Analysis of variance on the single variables is reported in Table S5. Dark grey bars represent not-inoculated samples (NI) and grey bars inoculated ones (I), each subjected to three irrigation treatments (R1 well-watered condition, R2 reduced irrigation, and R3 rain-fed condition, respectively). ns, *, **, and ***: non-significant or significant at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively. Ir: irrigation treatment, In: inoculation, Ir x In: interaction between irrigation treatment and inoculation

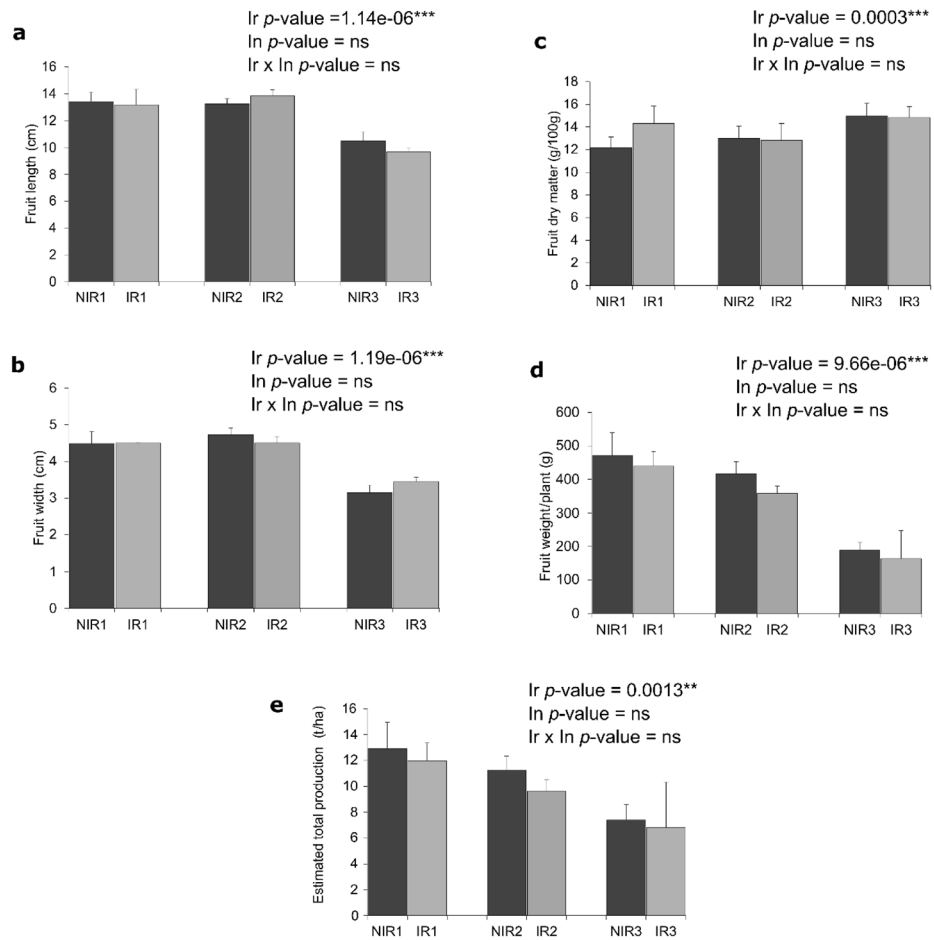

Fig. 2 Agronomic parameters and pepper production. Pepper fruit length (**a**), fruit width (**b**), fruit dry matter (g/100 g) (**c**), fruit weight/plant (**g**) (**d**), estimated total yield (t/ha) (**e**). All results are reported as mean ± standard deviation. Analysis of variance on the single variables is reported in Tables S6-S7. Dark grey bars represent not-inoculated samples (NI) and grey bars inoculated ones (I), each subjected to three irrigation treatments (R1 well-watered condition, R2 reduced irrigation, and R3 rain-fed condition, respectively). ns, *, **, and ***: non-significant or significant at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively. Ir: irrigation treatment, In: inoculation, Ir x In: interaction between irrigation treatment and inoculation

in the root system) and A% (arbuscule abundance in the root system), with A% also affected by the irrigation x inoculation interaction (Table S8). The mycorrhizal assessment revealed that both treatments IR3 and NIR3 showed a lower M% and a decreased A% compared to the other treatments (IR1, IR2, NIR1, and NIR2), suggesting a negative impact of the water deficit on the root colonization by the AMF. Specifically, M% was significantly higher in the IR2 treatment than in the NIR3 and IR3 treatments (*p*-value 0.007), whereas A% was significantly higher in the IR2 treatment compared to all other treatments (*p*-value 0.001). No significant differences were observed among treatments regarding F% (frequency of mycorrhiza in the root system), although F% was higher in the IR2 treatment compared to the other treatments (Table S8).

Analysis on biochemical markers and phytohormones in leaves

The results highlighted a significant difference among samples in H_2O_2 considering irrigation x inoculation interaction (Fig. 3, Table S9). Additionally, MDA content and proline were significantly affected by the irrigation and inoculation factors, as well as by the irrigation x inoculation interaction (Table S9). Specifically,

the MDA content was significantly lower in IR2 and NIR1 compared to NIR2 and NIR3 treatments (Fig. 3a), while H_2O_2 content was significantly lower in IR2 and NIR1 treatments compared to the others (Fig. 3b). The highest level of proline content was detected in IR1 and NIR1 (Fig. 3c). However, proline content was also significantly higher in IR2 compared to IR3, NIR2 and NIR3 samples (Fig. 3c). It was possible to observe an inverse trend in proline content with increasing water stress, *i.e.,* samples subjected to R1 showed a higher proline level compared to samples under R2 and R3, both in inoculated and not-inoculated plants. Analysis of plant phytohormones, with a putative role in tolerance and defense against stressful factors, showed that IAA (indole-3-acetic acid), OPDA (12-oxo-phytodienoic acid) and jasmonic acid (JA) concentrations were significantly affected by inoculation x irrigation interaction (Fig. 4), while abscisic acid (ABA) and salicylic acid (SA) did not show any significant differences considering all the variance factors (Table S10). Considering IAA, a significant difference was found between NIR1 and NIR2 treatments (Fig. 4a). The OPDA concentration was significantly higher in IR2 compared to IR1 and NIR3 (Fig. 4b), while JA content was significantly higher in IR1 compared to all the other treatments (Fig. 4c).

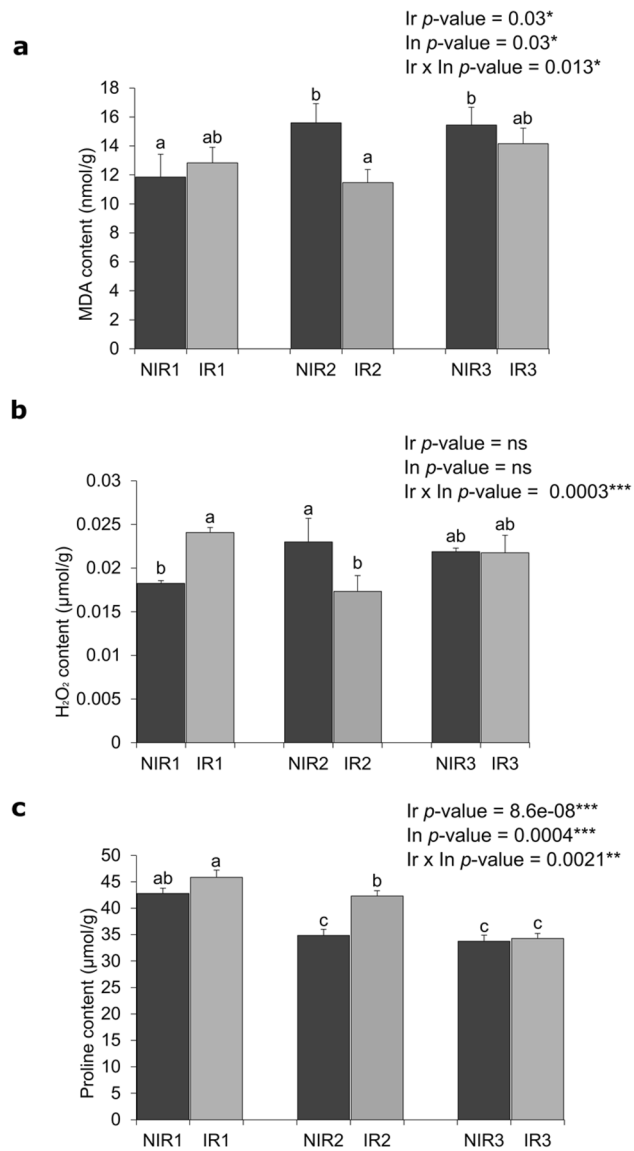

Fig. 3 Biochemical markers (hydrogen peroxide – H_2O_2, malondialdehyde – MDA, proline) content in leaves of the different treatments. MDA content (nmol/g) (**a**), H_2O_2 content (µmol/g H_2O_2) (**b**) and proline content (µmol/g) (**c**) in pepper leaves. All results are reported as mean ± standard deviation. Different letters represent significant differences according to Tukey HSD test ($p < 0.05$), considering the inoculation x irrigation interaction. Analysis of variance on the single variables is reported in Table S9. Dark grey bars represent not-inoculated samples (NI) and grey bars inoculated ones (I), each subjected to three irrigation treatments (R1 well-watered condition, R2 reduced irrigation, and R3 rain-fed condition, respectively). ns, *, **, and ***: non-significant or significant at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively. Ir: irrigation treatment, In: inoculation, Ir x In: interaction between irrigation treatment and inoculation

Analysis of antioxidant compounds and micronutrients in fruits

In fruits, all the considered antioxidant compounds were significantly affected by the irrigation x inoculation interaction (Fig. 5, Table S11). In the inoculated condition, anthocyanin content increased in IR2 and IR3 treatments compared to IR1 one (Fig. 5a). On the other hand, under the not-inoculated condition, fruits from NIR3 showed the highest content, followed by NIR1, with NIR2 having the least. Concerning ascorbic acid content (Fig. 5b), NIR3 showed a significantly higher content compared to all the other treatments. Flavonoid content (Fig. 5c) showed a trend considering the irrigation and the inoculation. Indeed, in the inoculated condition, samples subjected to R1 treatment showed a lower flavonoid content, compared to both R2 and R3 treatments. In fruits of the not-inoculated plants, the lowest flavonoid content was observed in NIR2, while the highest content was recorded in NIR3 treatment. Phenol content (Fig. 5d) increased progressively from IR1 to IR3, and the same trend was also observed in the not-inoculated samples. Overall, the inoculated samples showed a lower phenol content compared to the not-inoculated ones. The DPPH assay results (Fig. 5e) highlighted that, under identical irrigation treatments, both inoculated and not-inoculated samples showed a similar pattern. Indeed, samples

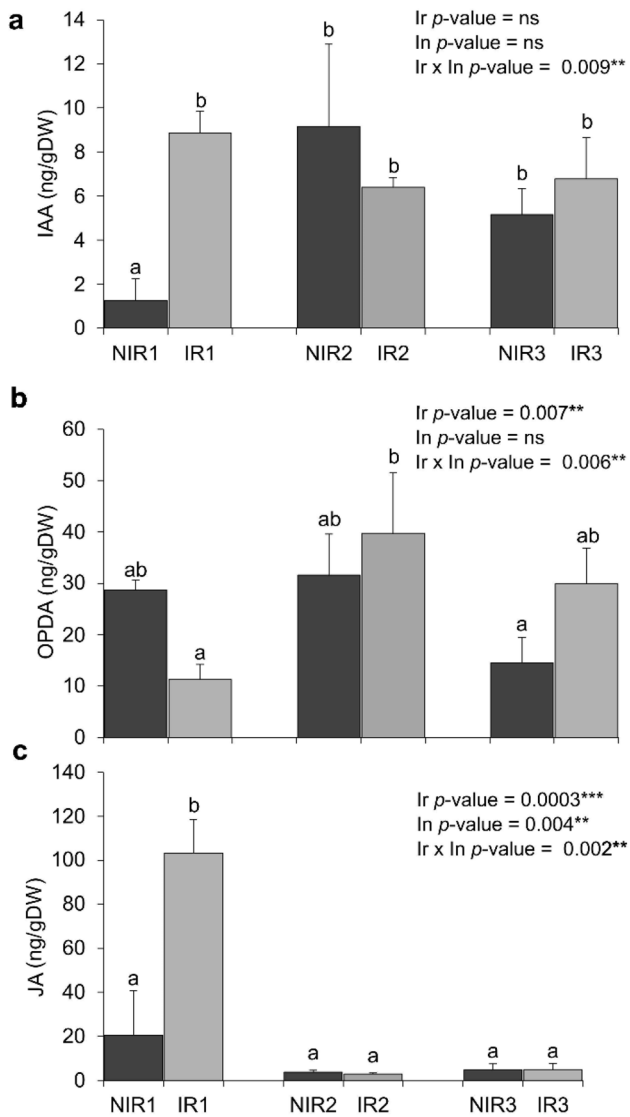

Fig. 4 Hormonal content in pepper leaves (Indole-3-acetic acid – IAA, 12-oxo-phytodienoic acid – OPDA, jasmonic acid – JA). IAA content (ng/g DW) (**a**), OPDA content (ng/g DW) (**b**) and JA content (**c**). All results are reported as mean ± standard deviation. Different letters represent significant differences according to Tukey HSD test ($p < 0.05$), considering the inoculation x irrigation interaction. Analysis of variance on the single variables is reported in Table S10. Dark grey bars represent not-inoculated samples (NI) and grey bars inoculated ones (I), each subjected to three irrigation treatments (R1 well-watered condition, R2 reduced irrigation, and R3 rain-fed condition, respectively). ns, *, **, and ***: non-significant or significant at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively. Ir: irrigation treatment, In: inoculation, Ir x In: interaction between irrigation treatment and inoculation

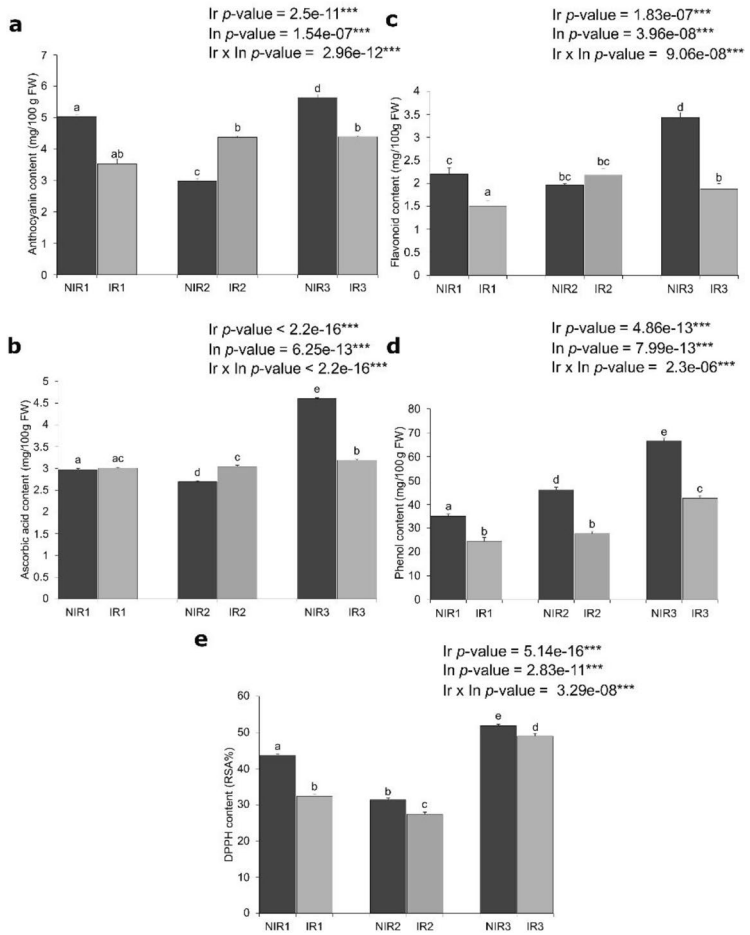

Fig. 5 Antioxidant compounds (anthocyanin, ascorbic acid, flavonoid, phenol, 2,2-difenil-1-picrylidrazyl – DPPH) in pepper fruits. Anthocyanin content (mg/100 g FW) (**a**), ascorbic acid content (g/kg FW) (**b**), flavonoid (mg/100 g FW) (**c**), phenol content (mg/100 g FW) (**d**) and DPPH content (RSA%) (**e**) in fruits. All results are reported as mean ± standard deviation. Different letters represent significant differences according to Tukey HSD test ($p < 0.05$), considering the inoculation x irrigation interaction. Analysis of variance on the single variables is reported in Table S11. Dark grey bars represent not-inoculated samples (NI) and grey bars inoculated ones (I), each subjected to three irrigation treatments (R1 well-watered condition, R2 reduced irrigation, and R3 rain-fed condition, respectively). RSA: radical scavenging activity. ns, *, **, and ***: non-significant or significant at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively. Ir: irrigation treatment, In: inoculation, Ir x In: interaction between irrigation treatment and inoculation

subjected to R2 treatment showed a lower DPPH radical scavenging activity compared to samples under R1 and R3 treatments. Moreover, the highest DPPH radical scavenging activity content was found in R3. Overall, a difference between inoculated and not-inoculated condition was identified. A significant lower DPPH radical scavenging activity was found in inoculated samples in all water treatments compared to the not-inoculated ones (Fig. 5e).

Regarding the analysis of fruit micronutrients, the potassium content was influenced by the irrigation condition, with significantly higher levels observed in samples under R1 compared to those under R3. All the other fruit micronutrients did not significantly differ among treatments (Table S12).

Fungal and bacterial communities in bulk soil and roots over time

The total number of reads obtained from metabarcoding analysis of bulk soil samples were 1,326,631 for fungi and 6,121,663 for bacteria. On the other hand,

in roots, 564,933 and 3,598,961 reads were obtained for fungi and bacteria, respectively. In total, 710 fungal ASVs and 1330 bacterial ASVs were identified and reported in Tables S13 and S14, respectively.

For fungal community, the predominant phyla of bulk soils were represented by phyla Ascomycota, Mortierellomycota, Chytridiomycota and Basidiomycota. In bulk soils collected in August, alpha diversity was affected by the irrigation (p-value 0.043) and by the interaction between the irrigation treatment and the inoculation (p-value 0.029, Fig. 6a). The inoculated samples showed a higher alpha diversity under R1 and R2, while no differences were found comparing the inoculation treatments under R3. Moreover, an increased diversity in terms of taxon abundance from R1 to R3 condition, as well as in I condition compared to NI, was observed (Fig. 6a). Comparative analysis of single factors highlighted that the Ascomycota families Sarocladiaceae and Onygenales were more prevalent in inoculated samples (Table S15). In bulk soils collected in August, beta diversity revealed significant difference in the fungal communities among different irrigation treatments (p-value 0.026, Fig. S4a) with changes in the relative abundance of the phyla Basidiomycota, Mortierellomycota, Chytridiomycota, and Mucoromycota in relation to the irrigation condition (Fig. 6a). Particularly, the relative abundance of the phylum Chytridiomycota increased from R1 to R3, showing a trend towards dryer conditions (Fig. 6a). Similarly, the relative abundance of Ascomycota was related to the irrigation treatment (Sporormiaceae associated to R1), while relative abundance of Basidiomycota *i.e.,* Piskurozymaceae was associated to R3 (Table S15). Moreover, a difference in relative taxon abundance for Chaetomiaceae and Glomerallaceae (Ascomycota), Calcarisporiellaceae (Mucoromycota), Filobasidiaceae (Basidiomycota) and Chytridiaceae (Chytridiomycota) was observed among specific treatments (Table S15).

Fungal alpha diversity of bulk soil samples collected in October did not reveal any differences between treatments (Fig. 6b), while beta diversity highlighted a significant difference among R1, R2 and R3 (p-value 0.001, Fig. S4b). In bulk soils collected in October, among Ascomycota, Pezizaceae family was relatively more abundant in samples subjected to R1 condition, while Microdochiaceae was relatively more abundant in samples subjected to R2 and R3 condition (Table S16). On the other hand, among Basidiomycota, Symmetrosporaceae, Holtermanniales (family *incertae sedis*) and Chrysozymaceae were uniquely associated to samples subjected to R3 condition (Table S16). A different taxon composition among treatments was also identified (Table S16).

The core microbiome of fungal community associated to pepper root samples was represented by phyla Ascomycota, Basidiomycota, and Mortierellomycota (Fig. 6c). Additionally, phylum Mortierellomycota was associated only with NI samples. Alpha diversity showed a significant difference between I and NI condition (p-value 0.033; Fig. 6c). A decreasing trend in the taxon abundance considering the different irrigation treatments was observed (Fig. 6c). Beta diversity revealed a significant difference inside the fungal communities at different irrigation treatments (p-value 0.008; Fig. S4c). In addition, the inoculated samples showed an overall higher taxon abundance compared to the NI ones, and the irrigation treatment showed a selective effect, *i.e.,* taxa progressively decreased from R1 to R3. In particular, NIR3 treatment was characterized by the lowest associated fungal diversity mostly represented by Basidiomycota (Fig. 6c). The families Microdochiaceae of Ascomycota and Filobasidiaceae of Basidiomycota showed a higher taxon abundance in NI samples (Table S17). A trend was observed in relation to the irrigation treatments. Ascomycota as Glomeraceae and Didymosphaeriaceae were less abundant in R3, while Chaetomiaceae and Didymellaceae were more abundant. Similarly, Glomerellaceae were associated to R1. The lowest abundance of Mucormomycota *i.e.,* Mortierellaceae was detected in NIR3 treatment. The different treatments significantly affected taxon distribution, as reported in Table S17.

Concerning bacterial communities, both bulk soils collected in August and October showed diverse abundances of different phyla *i.e.,* Proteobacteria, Bacteroidota, and Actinobacteriota, except for Firmicutes uniquely associated to samples collected in October (Fig. 7a-b). In August, beta diversity highlighted a significant difference between I and NI, while no significant differences were detected in samples collected in October (p-value 0.004, Fig. S5a-b). The NIR1 treatment showed the higher number of taxa compared to the other treatments, mainly due to the abundance of members of the phyla Thermoproteota, Acidobacteriota, Chloroflexota and

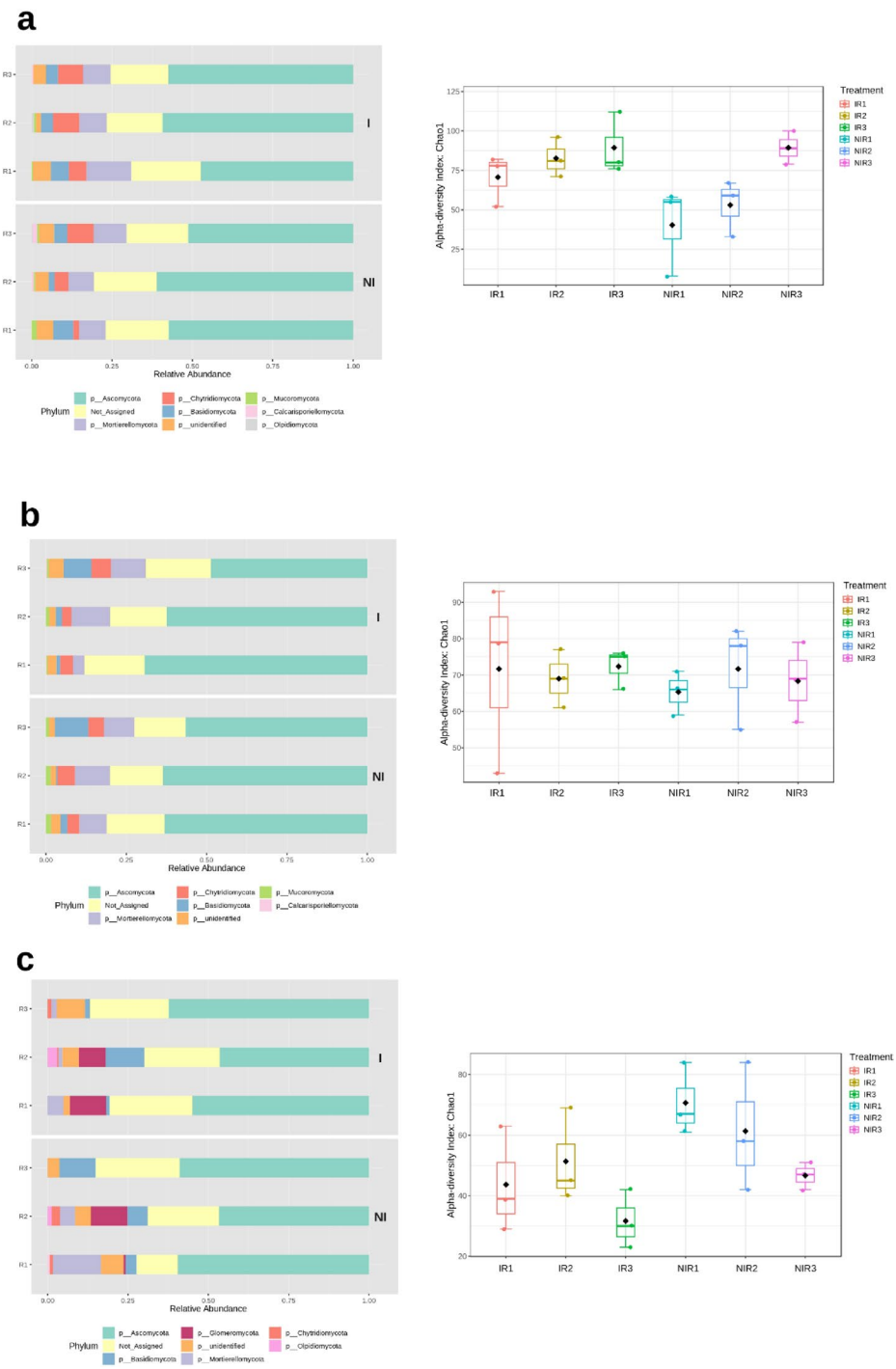

Fig. 6 Assessment of fungal diversity within samples. Taxon bar plot and alpha diversity of bulk soils collected in August (**a**), bulk soils collected in October (**b**) and roots collected in October (**c**). Taxon bar plots were produced at phylum level considering the inoculation treatment (inoculated samples – I and not-inoculated samples – NI, each subjected to three irrigation treatments (R1 well-watered condition, R2 reduced irrigation, and R3 rain-fed condition, respectively). Alpha diversity was performed considering all treatments *i.e.,* IR1-inoculated samples in well-watered condition, IR2—inoculated samples in reduced irrigation, IR3—inoculated samples in rain-fed condition, NIR1—not-inoculated samples in well-watered condition, NIR2—not-inoculated samples in reduced irrigation, NIR3—not-inoculated samples in rain-fed condition

Gemmatimonadota, while in I samples, members of the phylum Bacteroidota were more abundant (Fig. 7a). The Rhizobiaceae family within Proteobacteria was the most abundant one in all I samples (Table S18). Bulk soils collected in October showed a higher abundance of Proteobacteria and Bacteroidota in NI samples and a higher abundance of Firmicutes and Acidobacteriota in I ones. Notably, Bacteroidota was more abundant in IR3 compared to IR1 and IR2 (Fig. 7b). In October, the inoculation significantly changed taxon distribution among treatments (Table S19). Geodermatophilaceae family (Actinobacteria) was more abundant in inoculated samples. On the contrary, Marinilabiliaceae (Bacteroidota) and Lacipirellulaceae (Planctomycetota) were more abundant in NI. Notably, Marinilabiliaceae were also abundant in IR3 treatment. The irrigation treatment had a significant effect on the bacterial communities for both sampling times (Table S18-19). In August, the highest abundance of Burkholderiaceae, Azospirillaceae and Xanthobacteraceae was observed in R3 (Table S18). In October, a family within Actinobacteria, *i.e.,* Gaiellaceae, was more abundant in R1 and R2, while a family within Proteobacteria *i.e.,* Beijerinckiaceae was associated to R3 condition (Table S19). Moreover, a shift in taxon distribution among treatments was observed in samples collected in October (Table S19).

The core microbiome at phylum level of bacterial communities in roots was represented by Proteobacteria, Actinobacteriota, Firmicutes, and Acidobacteriota. Bacteroidota were more abundant in NI, while Acidobacteriota, Actinobacteriota, Gemmatimonadota, Chloroflexota and Firmicutes were more abundant in I samples (Fig. 7c). Beta diversity showed a significant difference in taxon abundance among treatments (p-value 0.009; Fig. S5c). Both the inoculation and the irrigation resulted in a significant effect

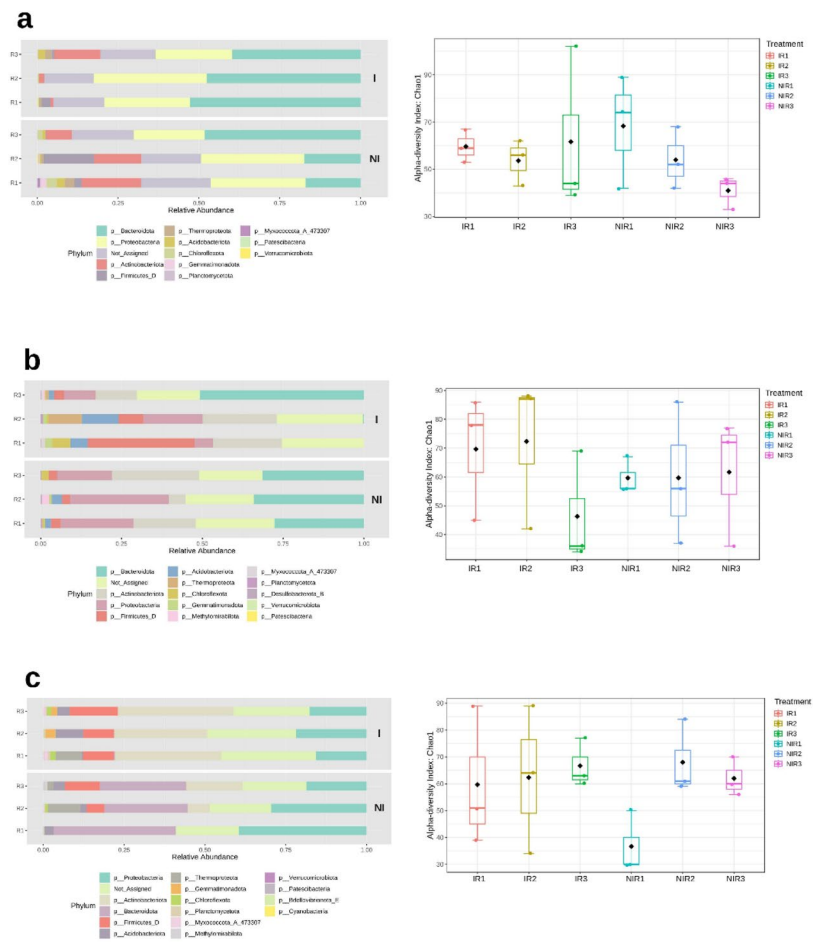

Fig. 7 Assessment of bacterial diversity within samples. Taxon bar plot and alpha diversity of bulk soils collected in August (**a**), bulk soils collected in October (**b**) roots collected in October (**c**). Taxon bar plots were produced at phylum level considering the inoculation treatment (inoculated samples – I and not-inoculated samples – NI, each subjected to three irrigation treatments (R1 well-watered condition, R2 reduced irrigation, and R3 rain-fed condition, respectively). Alpha diversity was performed considering all treatments *i.e.,* IR1- inoculated samples in well-watered condition, IR2—inoculated samples in reduced irrigation, IR3—inoculated samples in rain-fed condition, NIR1—not-inoculated samples in well-watered condition, NIR2—not-inoculated samples in reduced irrigation, NIR3—not-inoculated samples in rain-fed condition

on taxon distribution (Table S20). Solirubrobacteraceae family (Actinobacteria) was more abundant in R3. Marinilabiliaceae (Bacteroidota), Rhizobiaceae and Cellvibrionaceae (Proteobacteria) were more abundant in NI, while Gaiellaceae family (Actinobacteria) was associated to I samples (Table S20).

Discussion

The impact of the irrigation treatments on pepper ecophysiological and agronomic parameters

Our field trial study provided a comprehensive understanding on the responses of pepper to water deficit and AM fungal inoculation. The study revealed that g_s was not affected by reduced irrigation (R2), while it declined significantly in rain-fed pepper (R3). Results of the agronomic parameters showed that fruit length and width were significantly impacted by the irrigation treatment, as previously observed in pepper (Feng et al. 2019; Khazaei et al. 2020), highlighting that the water treatment is one of the key drivers affecting pepper growth and yield features. Indeed, the reduction in fruit size and weight under rain-fed (R3) compared to well-watered conditions (R1) or reduced irrigation (R2) underscores the reported responses of pepper plants to limited water availability (Ali et al. 2022; Khazaei et al. 2020). It has been reported that bell pepper cv Aristotle irrigated at 67% ETc had similar fruit yield and quality compared to plants at 100% ETc (Kabir et al. 2021). Interestingly, our results showed no significant differences in pepper production between R1 and R2 condition, thus suggesting a potential application of a reduced water supply to cultivate pepper plants.

The role of the AM fungal inoculum on plant responses to irrigation treatments

In addition to exploring the effects of water treatments in pepper physiology, the main aim of this study was to investigate the interactions between AMF and the colonized pepper plants in response to diverse levels of water availability, including the structure and dynamics of root- and bulk soil-associated microbiomes. In our experimental field, not-inoculated plants also showed root colonization by native AMF. This is in line with a recent comparative analysis on barley and sorghum AMF-inoculated plants showed no significant differences in fungal colonization by native soil AM fungal communities and commercial inoculum (Frew 2021). However, a combined effect of the native and externally applied AM fungal inoculum was detected for some measured plant parameters, probably due to a boost by the commercial inoculum. It is worth noting that although AM fungal inoculation has been reported to have a positive impact on plant growth and productivity in diverse species, including several crops, the effect of field inoculations with AMF is highly unpredictable and variable (Lutz et al. 2023). The results of the assessment of AM fungal colonization in root samples revealed that the IR2 treatment exhibited the highest colonization levels. This finding is in keeping with the agronomic and ecophysiological results, suggesting that the reduced irrigation applied in this study (*i.e.*, 75% of the well-watered treatments—R2) may be considered optimal also for AM fungal colonization. Conversely, plants subjected to the R3 treatment showed lower level of AM fungal colonization, with a reduced presence of arbuscules.

Despite the negative effect that water deficit may have on AM fungal colonization (Wu and Zou 2017), results demonstrated that AM fungal inoculation improved several plant parameters. Notably, flavonoid content in pepper leaves was higher in inoculated (I) condition, indicating a potential enhancement in the plant stress response and production of secondary metabolites, such as flavonoids, due to the symbiotic relationship with AMF, as previously reported (Castellanos-Morales et al. 2010; Pal et al. 2024). The analysis of stress markers MDA, H_2O_2, and proline showed variations based on the interaction between irrigation and inoculation. Based on our proline data, we can hypothesize that the well-watered treatment (100% of ETc—R1) may result in excess water retention in the considered soil. Consequently, plants might perceive a mild excess of water in the rhizosphere and activate responses to water stress, leading to proline accumulation in R1 leaves (particularly in IR1 and NIR1 treated plants). This is coupled with slightly lower g_s rates compared to R2 treated plants, similarly to previous observations in green sweet pepper plants subjected to both drought and flooding stresses (Masoumi et al. 2021). These findings, in agreement with the susceptibility of pepper to both water deficit and water excess (Masoumi et al. 2021), suggest that precise estimation of ETc for a specific crop is a critical issue for

effective irrigation management in soils with diverse water storage capacities. This highlights the necessity of defining tailored crop coefficients (Kc) for particular climatic areas (Miranda et al. 2006). Notably, the higher proline content in IR2 treatment compared to NIR2 may suggest the ability of AMF in improving plant stress response, as previously observed (Zheng et al. 2020; Zou et al. 2021). In this line, the significant high content in IR2 of the phytohormone OPDA, a precursor of JA, known to have a key role in triggering signaling pathways that regulate JA-responsive genes (Liu and Park 2021), may also suggest a role of AMF in the observed OPDA increase. Additionally, IAA content in IR1 treatments can also be linked to the presence of AMF. It has been previously reported that inoculation with *F. mosseae* can induce the production of OPDA and IAA in tomato plants and in trifoliate orange, respectively (Liu et al. 2018; López-Ráez et al. 2010).

Fruit antioxidant compounds were affected both by irrigation and inoculation. Plants grown in the R3 treatment showed a higher antioxidant compounds abundance compared to R1 and R2. Moreover, considering the inoculation, a common trend was observed: NI samples showed a higher content of antioxidant compounds, compared to I samples. Despite several studies have tried to correlate AM fungal inoculation and antioxidant content in fruits (Abdelhalim et al. 2022; Grozić et al. 2021), there is not a definitive answer, because the presence of antioxidants may depend on multiple parameters (*e.g.*, exposure to extreme temperatures, drought, salinity) (Baslam and Goicoechea 2012; Mishra et al. 2023; Torres et al. 2018). In our case, it is worth noting that anthocyanin and ascorbic acid increased in IR2 treatment compared to NIR2, suggesting a pivotal role of the irrigation treatment combined with AM fungal inoculation in pepper plants. This relation is inverted for phenol and DPPH content: IR2 treatment showed a lower content compared to NIR2. The DPPH radical scavenging activity indicates the antioxidant plant capacity and is activated under abiotic stresses, as water deficit conditions (Abdalla et al. 2019), therefore its reduction under AM fungal inoculation may suggest a reduced stress level in inoculated samples. A similar role has been proposed for phenol (Rosa-Martínez et al. 2023), suggesting that, in our conditions, pepper plants in IR2 treatment produced more anthocyanin and ascorbic acid and less DPPH and phenol, compared to NIR2, shaping their response in relation to the different conditions. Flavonoid content highlighted a similar trend among the irrigation treatments and between the two inoculation treatments. In this case, there were no significant differences between IR2 and NIR2, but it is noteworthy that: i) IR2 is associated to the highest flavonoid abundance among the inoculated samples, and ii) NIR2 is associated to the lowest flavonoid abundance among the not-inoculated ones. These data suggest that IR2 may be the optimal condition to boost flavonoid content in pepper fruits and the associated antioxidant potential (Hernández-Pérez et al. 2020). Concerning pepper fruit nutrients, the only nutrient showing significant variation was potassium, with a decreasing content from well-watered (R1) to rain-fed conditions (R3). This could indicate that water availability plays a crucial role in nutrient uptake, especially for potassium. It has been reported that the potassium content can be related to water availability in pepper plants, showing a significant decrease under water deficit conditions (Kabir et al. 2021). Overall, our results suggested that antioxidant compound production may vary depending on multiple factors, and that AM fungal inoculation might trigger specific compound production, as recently documented (Shalaby and El-Sayed Ramadan 2024). Further studies are needed to understand how application of AM fungal inoculation may shape pepper fruit quality.

The impact of the irrigation and the AM fungal inoculum on the soil and root-associated microbial communities

Metabarcoding analysis revealed that Chytridiomycota phylum was more abundant in bulk soils collected in August mainly in R3 treatment and, in agreement with this result, this phylum is known to include members showing the ability to cope with water deficit condition (Dacal et al. 2022). Moreover, R3 bulk soils collected in August revealed an enrichment in Burkholderiaceae, Azospirillaceae and Xanthobacteraceae. Burkholderiaceae family was also detected in bulk soil collected in October in NIR2 and NIR3, as well as Azospirillaceae in R3 samples. These data are in line with previous studies (Jang et al. 2020; Maestro-Gaitán et al. 2023; Munoz-Ucros et al. 2022; Yasuda et al. 2022). Burkholderiaceae and Xanthobacteraceae are reported to increase under water deficit condition, resulting in

improving plant in carbon cycling and nitrogen fixation and plant response to phytopathogen (Jang et al. 2020; Maestro-Gaitán et al. 2023; Munoz-Ucros et al. 2022). Conversely, Azospirillaceae family has been reported as a plant growth promoting bacteria (PGPB) able to improve nitrogen mineralization under water deficit conditions (Yasuda et al. 2022). A high abundance of Myxococcaceae was detected in IR1 and IR3, both in bulk soils and in root samples collected in October. Myxococcaceae are known to be able to survive in adverse environments, producing antibiotics and important secondary metabolites, such as carotenoids, able to improve plant tolerance against several phytopathogens (Zhou et al. 2021). The order Myxococcales seems to be associated to AM fungal hyphae (Emmett et al. 2021), and our results showed that this order was more abundant in inoculated samples. Notably, pathogenic fungal taxon Didymellaceae (Chen et al. 2017) was detected in roots of R3 and IR3 plants. The concurrent detection of Myxococcales and fungal pathogen taxa, specifically Didymellaceae, in the roots of inoculated plants, may suggest that AMF can recruit biological control agents. Interestingly, according to Lutz et al. (2023), the presence of plant pathogens may be used to assess AMF potential in restoring plant health, as it has been documented that AM fungal inoculation has a relevant impact on pathogen-infected soil, enhancing the plant response to pathogens and overall performance compared to not-inoculated soils. In our experiment, in addition, it is worth noting that bulk soil from inoculated plants showed a high abundance of Geodermatophilaceae, a bacterial family that has been identified as a component of the mycorrhizosphere and hyphosphere microbiome of AMF (Zhang et al. 2022b).

Results on soil enzyme activity suggested that irrigation treatments are the primary determinant of the enzyme activity, as previously reported in other field experiments with maize (Muhammad et al. 2022) and alfalfa (Deng et al. 2022). The consistently lower enzymatic activity observed in R3 across both sampling times for most of the enzymes studied is of particular note. This trend suggested that the R3 irrigation treatment may not be favorable in increasing microbial activity and enzyme production, as instead observed in R1 and R2. The high fungal alpha diversity in R1 and R2 could be associated to an enhanced enzyme activity, putatively linked to organic matter decomposition and nutrient cycling. The differential activity of cellobiohydrolase, β-glucosidase, and N-acetyl glucosaminidase in August, with the addition of xylosidase, cellulase, and acid phosphatase in October, suggested that soil enzyme activities are also subjected to fluctuations influenced by a range of factors, including soil moisture levels and climatic changes. Overall, soil enzymatic activity was significantly affected by irrigation treatments, while AM fungal inoculation has no relevant effect, with the exception of β-glucosidase activity. Hence, we cannot exclude that the strong effect of water regime may have potentially masked the effects mediated by AM fungal inoculation. It is worth noting that not-inoculated treatments also showed the presence of native AM fungal communities, resulting in similar soil enzymatic activity in comparison with inoculated ones. Nevertheless, inoculation predominantly affected β-glucosidase activity. This outcome could be explained by the presence of bacteria of the Rhizobiaceae family being more abundant in inoculated treatments than in not-inoculated and known for a high production of β-glucosidase (Duan et al. 2022). Lastly, an increased phosphatase activity was observed in October compared to August, especially under the R1 and R2 treatments. Members of Firmicutes, often associated with high phosphatase activity (Cui et al. 2019; Li et al. 2020), and exclusively found in October samples, may be indicative of the enhanced enzymatic activity recorded in this period.

Looking at the roots, our results showed that rain-fed condition (R3) was associated with an enrichment in plant-beneficial taxa such as Firmicutes and Gemmatimonadota, as already reported in other crops (Faghihinia et al. 2023; Santos-Medellín et al. 2021). In addition, Actinobacteria phylum was abundant in inoculated roots under R3 treatment, underlining a hypothetical role of AMF in recruiting beneficial taxa as PGPB under stressed conditions, as previously reported (Garbaye 1991; Sbrana et al. 2022). Concerning fungal communities, mycorrhizal assessment in roots showed that IR2 was the most AMF-colonized treatment. Focusing on AM fungal communities, metabarcoding analysis showed that the Glomeraceae family (Spatafora et al. 2016) was found to be abundant in inoculated roots, although its presence was significantly affected by the irrigation treatment, with the lowest abundance detected in R3, in agreement with previous results reporting that drought negatively affects AM fungal colonization (Wu and Zou 2017). On the other hand, samples subjected to R1 and R2 irrigation treatments showed no statistical differences in Glomeraceae abundance, highlighting that

R2 may be similar to R1 (well-watered), especially from a sustainability point of view where saving water is considered as a key approach.

In conclusion, our results contribute to enhance the understanding of pepper plant traits affected by AM fungal inoculation and water deficit conditions. Our findings confirmed a positive impact of the application of an AM fungal inoculum on pepper plants and suggested that, at least for the considered pepper genotype, reduced irrigation (75% of ETc) could not cause stress conditions, ensuring significant water savings. Additionally, our study sheds light on the intricate interactions among bacteria, fungi (AMF and non-AMF), and pepper roots under water limited conditions. Overall, our study underlines the interconnected nature of these factors, emphasizing the need to consider their interdependence rather than evaluating them alone.

Acknowledgements The authors thank Francesca Brescia and Yulia Kiryakova for the help in DNA extraction and ecophysiological measurements, respectively, in addition to Beatrix Schnabel from the Department of Soil Ecology (UFZ) for the support in the sequencing analysis.

Author contribution Conceptualization: RB, CM, VM; Methodology: RB, TR, FS, WC; Investigation: ACa, TR, FS, VM, EC, EZ, RM, GG, LG, ACo, CM, GP, MGG, VA, VF; Formal analysis: FS, TR, LG; Writing—Original Draft: ACa, FS, VM, MGG, EZ; Writing—Review & Editing: ACa, TR, FS, ACo, WC, MC, VF, RB; Supervision: RB.

Funding Open access funding provided by Consiglio Nazionale Delle Ricerche (CNR) within the CRUI-CARE Agreement. The work was partially funded by the PRIMA RESCHEDULE project (Italian MUR DD 1293/2021 and German BMBF 01DH21019) and by CNR project FOE-2021 DBA.AD005.225.

Data availability Reads from metabarcoding of pepper soil and root samples were submitted to NCBI SRA under BioProject PRJNA1024920.

Declarations

Competing interests The authors declare that there are no conflicts of interest.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

Abdalla MUE, Taher M, Sanad MI, Tadros LK (2019) Chemical properties, phenolic profiles and antioxidant activities of pepper fruits. J Agric Chem Biotechnol 10:133–140. https://doi.org/10.21608/jacb.2019.53475

Abdelhalim TS, Tia NAJ, Sir Elkhatim KA, Othman MH, Joergensen RG, Almaiman SA, Hassan AB (2022) Exploring the potential of arbuscular mycorrhizal fungi (AMF) for improving health-promoting phytochemicals in sorghum. Rhizosphere 24:100596. https://doi.org/10.1016/j.rhisph.2022.100596

Abdelkhalik A, Abd El-Mageed TA, Mohamed IAA, Semida WM, Al-Elwany OAAI, Ibrahim IM, Hemida KA, El-Saadony MT, Abu Qamar SF, El-Tarabily KA, Gyushi MAH (2023) Soil application of effective microorganisms and nitrogen alleviates salt stress in hot pepper (*Capsicum annum* L.) plants. Front Plant Sci 13. https://www.frontiersin.org/articles/10.3389/fpls.2022.1079260

Ahluwalia O, Singh PC, Bhatia R (2021) A review on drought stress in plants: implications, mitigation and the role of plant growth promoting rhizobacteria. Resour Environ Sustain 5:100032. https://doi.org/10.1016/j.resenv.2021.100032

Ali S, Tyagi A, Park S, Mir RA, Mushtaq M, Bhat B, Mahmoudi H, Bae H (2022) Deciphering the plant microbiome to improve drought tolerance: mechanisms and perspectives. Environ Exp Bot 201:104933. https://doi.org/10.1016/j.envexpbot.2022.104933

Allen R, Pereira L, Raes D, Smith M (1998) Crop evapotranspiration guidelines for computing crop requirements. FAO Irrig. Drain. Report modeling and application. J Hydrol 285:19–40

Alvino A, Centritto M, De Lorenzi F (1994) Photosynthesis response of sunlit and shade pepper (*Capsicum annuum*) leaves at different positions in the canopy under two water regimes. Funct Plant Biol 21:377–391. https://doi.org/10.1071/PP9940377

Augé RM, Toler HD, Saxton AM (2015) Arbuscular mycorrhizal symbiosis alters stomatal conductance of host plants more under drought than under amply watered conditions: a meta-analysis. Mycorrhiza 25:13–24. https://doi.org/10.1007/s00572-014-0585-4

Ávila-Dávila L, Molina-Martínez JM, Bautista-Capetillo C, Soler-Méndez M, Robles Rovelo CO, Júnez-Ferreira HE, González-Trinidad J (2021) Estimation of the evapotranspiration and crop coefficients of bell pepper using a removable weighing lysimeter: a case study in the southeast of Spain. Sustainability 13:747. https://doi.org/10.3390/su13020747

Baslam M, Goicoechea N (2012) Water deficit improved the capacity of arbuscular mycorrhizal fungi (AMF) for inducing the accumulation of antioxidant compounds in lettuce leaves. Mycorrhiza 22:347–359. https://doi.org/10.1007/s00572-011-0408-9

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant Soil 39:205–207. https://doi.org/10.1007/BF00018060

Berendsen RL, Pieterse CM, Bakker PA (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17:478–486. https://doi.org/10.1016/j.tplants.2012.04.001

Bhattacharyya A, Pablo CH, Mavrodi OV, Weller DM, Thomashow LS, Mavrodi DV (2021) Rhizosphere plant-microbe interactions under water stress. Adv Appl Microbiol 115:65–113. https://doi.org/10.1016/bs.aambs.2021.03.001

Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodríguez AM, Chase J, Cope EK, Da Silva R, Diener C, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibbons SM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley GA, Janssen S, Jarmusch AK, Jiang L, Kaehler BD, Kang KB, Keefe CR, Keim P, Kelley ST, Knights D, Koester I, Kosciolek T, Kreps J, Langille MGI, Lee J, Ley R, Liu YX, Loftfield E, Lozupone C, Maher M, Marotz C, Martin BD, McDonald D, McIver LJ, Melnik AV, Metcalf JL, Morgan SC, Morton JT, Naimey AT, Navas-Molina JA, Nothias LF, Orchanian SB, Pearson T, Peoples SL, Petras D, Preuss ML, Pruesse E, Rasmussen LB, Rivers A, Robeson MS 2nd, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song SJ, Spear JR, Swafford AD, Thompson LR, Torres PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ul-Hasan S, van der Hooft JJJ, Vargas F, Vázquez-Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber KC, Williamson CHD, Willis AD, Xu ZZ, Zaneveld JR, Zhang Y, Zhu Q, Knight R, Caporaso JG (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 37:852–857. https://doi.org/10.1038/s41587-019-0209-9

Brunetti C, Saleem AR, Della Rocca G, Emiliani G, De Carlo A, Balestrini R, Khalid A, Mahmood T, Centritto M (2021) Effects of plant growth-promoting rhizobacteria strains producing ACC deaminase on photosynthesis, isoprene emission, ethylene formation and growth of *Mucuna pruriens* (L.) DC. in response to water deficit. J Biotechnol 331:53–62. https://doi.org/10.1016/j.jbiotec.2021.03.008

Caddell D, Louie K, Bowen B, Sievert J, Hollingsworth J, Dahlberg J, Purdom E, Northen T, Coleman-Derr D (2023) Drought shifts sorghum root metabolite and microbiome profiles and enriches the stress response factor pipecolic acid. Phytobiomes J 4:449–463. https://doi.org/10.1094/PBIOMES-02-23-0011-R

Castellanos-Morales V, Villegas J, Wendelin S, Vierheilig H, Eder R, Cárdenas-Navarro R (2010) Root colonisation by the arbuscular mycorrhizal fungus *Glomus intraradices* alters the quality of strawberry fruits (*Fragaria* × *ananassa* Duch.) at different nitrogen levels. J Sci Food Agric 90:1774–1782. https://doi.org/10.1002/jsfa.3998

Chen Q, Hou LW, Duan WJ, Crous PW, Cai L (2017) Didymellaceae revisited. Stud Mycol 87:105–159. https://doi.org/10.1016/j.simyco.2017.06.002

Chitarra W, Pagliarani C, Maserti B, Lumini E, Siciliano I, Cascone P, Schubert A, Gambino G, Balestrini R, Guerrieri E (2016) Insights on the impact of arbuscular mycorrhizal symbiosis on tomato tolerance to water stress. Plant Physiol 171:1009–1023. https://doi.org/10.1104/pp.16.00307

Chong J, Liu P, Zhou G, Xia J (2020) Using MicrobiomeAnalyst for comprehensive statistical, functional, and meta-analysis of microbiome data. Nat Protoc 15:3. https://doi.org/10.1038/s41596-019-0264-1

Christou A, Manganaris GA, Papadopoulos I, Fotopoulos V (2013) Hydrogen sulfide induces systemic tolerance to salinity and non-ionic osmotic stress in strawberry plants through modification of reactive species biosynthesis and transcriptional regulation of multiple defense pathways. J Exp Bot 64:1953–1966. https://doi.org/10.1093/jxb/ert055

Cui Y, Fang L, Guo X, Wang X, Wang Y, Zhang Y, Zhang X (2019) Responses of soil bacterial communities, enzyme activities, and nutrients to agricultural-to-natural ecosystem conversion in the Loess Plateau. China J Soils Sediments 19:1427–1440. https://doi.org/10.1007/s11368-018-2110-4

Dacal M, García-Palacios P, Asensio S, Wang J, Singh BK, Maestre FT (2022) Climate change legacies contrastingly affect the resistance and resilience of soil microbial communities and multifunctionality to extreme drought. Funct Ecol 36:908–920. https://doi.org/10.1111/1365-2435.14000

De Ollas C, González-Guzmán M, Pitarch Z, Matus JT, Candela H, Rambla JL, Granell A, Gómez-Cadenas A, Arbona V (2021) Identification of ABA-mediated genetic and metabolic responses to soil flooding in tomato (*Solanum lycopersicum* L. Mill). Front Plant Sci 12:613059. https://doi.org/10.3389/fpls.2021.613059

de Vries FT, Griffiths RI, Knight CG, Nicolitch O, Williams A (2020) Harnessing rhizosphere microbiomes for drought-resilient crop production. Science 368:270–274. https://doi.org/10.1126/science.aaz5192

Dehghan G, Khoshkam Z (2012) Tin (II)–quercetin complex: synthesis, spectral characterisation and antioxidant activity. Food Chem 131:422–426. https://doi.org/10.1016/j.foodchem.2011.08.074

Delfine S, Loreto F, Alvino A (2001) Drought-stress effects on physiology, growth and biomass production of rainfed and irrigated bell pepper plants in the Mediterranean region. J Am Soc Hortic Sci 126:297–304. https://doi.org/10.21273/JASHS.126.3.297

Deng Q, Wu Y, Zhao X, Qiu C, Xia S, Feng Y, Liu H (2022) Influence of different irrigation methods on the alfalfa rhizosphere soil fungal communities in an arid region. PLoS ONE 17, https://doi.org/10.1371/journal.pone.0268175

Dhariwal A, Chong J, Habib S, King IL, Agellon LB, Xia J (2017) MicrobiomeAnalyst: a web-based tool for

comprehensive statistical, visual and meta-analysis of microbiome data. Nucleic Acids Res 45:180–188. https://doi.org/10.1093/nar/gkx295

Duan C, Mei Y, Wang Q, Wang Y, Li Q, Hong M, Hu S, Li S, Fang L (2022) *Rhizobium* inoculation enhances the resistance of alfalfa and microbial characteristics in copper-contaminated soil. Front Microbiol 12:781831. https://doi.org/10.3389/fmicb.2021.781831

Emmett BD, Lévesque-Tremblay V, Harrison MJ (2021) Conserved and reproducible bacterial communities associate with extraradical hyphae of arbuscular mycorrhizal fungi. ISME J 15:2276–2288. https://doi.org/10.1038/s41396-021-00920-2

Fadiji AE, Santoyo G, Yadav AN, Babalola OO (2022) Efforts towards overcoming drought stress in crops: revisiting the mechanisms employed by plant growth-promoting bacteria. Front Microbiol 13. https://www.frontiersin.org/articles/10.3389/fmicb.2022.962427

Faghihinia M, Jansa J, Halverson LJ, Staddon PL (2023) Hyphosphere microbiome of arbuscular mycorrhizal fungi: a realm of unknowns. Biol Fertil Soils 59:17–34. https://doi.org/10.1007/s00374-022-01683-4

FAOSTAT (2021) Food and Agriculture Organization of the United Nations. Accessed 01.09.2023. FAOSTAT. https://www.fao.org/statistics/en

Feng X-H, Zhang H-X, Ali M, Gai W-X, Cheng G-X, Yu Q-H, Yang S-B, Li X-X, Gong Z-H (2019) A small heat shock protein *CaHsp25.9* positively regulates heat, salt, and drought stress tolerance in pepper (*Capsicum annuum* L.). Plant Physiol Biochem 142:151–162. https://doi.org/10.1016/j.plaphy.2019.07.001

Food insecurity (2022) Nat Clim Chang 12:963. https://doi.org/10.1038/s41558-022-01530-2

Frew A (2021) Contrasting effects of commercial and native arbuscular mycorrhizal fungal inoculants on plant biomass allocation, nutrients, and phenolics. Plants People Planet 3:536–540. https://doi.org/10.1002/ppp3.10128

Garbaye J (1991) Biological interactions in the mycorrhizosphere. Experientia 47:370–375. https://doi.org/10.1007/BF01972079

German DP, Weintraub MN, Grandy AS, Lauber CL, Rinkes ZL, Allison SD (2011) Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. Soil Biol Biochem 43:1387–1397. https://doi.org/10.1016/j.soilbio.2011.03.017

Giusti MM, Wrolstad E (2001) Characterization and measurement of anthocyanins by UV-visible spectroscopy. Curr Protoc 00:F1.2.1-F1.2.13. https://doi.org/10.1002/0471142913.faf0102s00

Grozić K, Pasković I, Radić T, Lukić M, Žurga P, Palčić I, Urlić B, Brkljača M, Ban D, Kaliterna J, Klanjac J, Major N, Matotan Z, Goreta Ban S (2021) Processing tomato response to arbuscular mycorrhizal fungi application under conventional production practice. J Cent Eur Agric 22:72–85. https://doi.org/10.5513/JCEA01/22.1.2856

Güneş H, Demir S, Erdinc C, Furan A (2023) Effects of arbuscular mycorrhızal fungı (AMF) and bıochar on the growth of pepper (*Capsicum annum* L.) under salt stress. Gesunde Pflanz 75:1–13. https://doi.org/10.1007/s10343-023-00897-2

Haworth M, Marino G, Loreto F, Centritto M (2021) Integrating stomatal physiology and morphology: evolution of stomatal control and development of future crops. Oecologia 197:867–883. https://doi.org/10.1007/s00442-021-04857-3

Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Int Physiol Biochem Biophys 125:189–198. https://doi.org/10.1016/0003-9861(68)90654-1

Hernández-Pérez T, Gómez-García MDR, Valverde ME, Paredes-López O (2020) *Capsicum annuum* (hot pepper): an ancient Latin-American crop with outstanding bioactive compounds and nutraceutical potential. A review. Compr Rev Food Sci Food Saf 19:2972–2993. https://doi.org/10.1111/1541-4337.12634

Jang SW, Yoou MH, Hong WJ, Kim YJ, Lee EJ, Jung KH (2020) Re-analysis of 16S amplicon sequencing data reveals soil microbial population shifts in rice fields under drought condition. Rice 13:44. https://doi.org/10.1186/s12284-020-00403-6

Jing J, Cong W-F, Bezemer TM (2022) Legacies at work: plant–soil–microbiome interactions underpinning agricultural sustainability. Trends in Plant Sci 27:781–792. https://doi.org/10.1016/j.tplants.2022.05.007

Kabir MY, Nambeesan SU, Bautista J, Díaz-Pérez JC (2021) Effect of irrigation level on plant growth, physiology and fruit yield and quality in bell pepper (*Capsicum annuum* L.). Sci Hortic 281:109902. https://doi.org/10.1016/j.scienta.2021.109902

Kakouridis A, Hagen JA, Kan MP, Mambelli S, Feldman LJ, Herman DJ, Weber PK, Pett-Ridge J, Firestone MK (2022) Routes to roots: direct evidence of water transport by arbuscular mycorrhizal fungi to host plants. New Phytol 236:210–221. https://doi.org/10.1111/nph.18281

Khazaei Z, Esmaielpour B, Estaji A (2020) Ameliorative effects of ascorbic acid on tolerance to drought stress on pepper (*Capsicum annuum* L) plants. Physiol Mol Biol Plants 26:1649–1662. https://doi.org/10.1007/s12298-020-00846-7

Kõljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AFS, Bahram M, Bates ST, Bruns TD, Bengtsson-Palme J, Callaghan TM, Douglas B, Drenkhan T, Eberhardt U, Dueñas M, Grebenc T, Griffith GW, Hartmann M, Kirk PM, Kohout P, Larsson E, Lindahl BD, Lücking R, Martín MP, Matheny PB, Nguyen NH, Niskanen T, Oja J, Peay KG, Peintner U, Peterson M, Põldmaa K, Saag L, Saar I, Schüßler A, Scott JA, Senés C, Smith ME, Suija A, Taylor DL, Telleria MT, Weiss M, Larsson KH (2013) Towards a unified paradigm for sequence-based identification of fungi. Mol Ecol 22:5271–5277. https://doi.org/10.1111/mec.12481

Li C, Li H, Yao T, Su M, Li J, Liu Z, Xin Y, Wang L, Chen J, Gun S (2020) Effects of microbial inoculation on enzyme activity, available nitrogen content, and bacterial succession during pig manure composting. Bioresour Technol 306:123167

Liu CY, Zhang F, Zhang DJ, Srivastava AK, Wu Q-S, Zou YN (2018) Mycorrhiza stimulates root-hair growth and IAA synthesis and transport in trifoliate orange under drought stress. Sci Rep 8:1978. https://doi.org/10.1038/s41598-018-20456-4

Liu W, Park SW (2021) 12-oxo-Phytodienoic acid: a fuse and/or switch of plant growth and defense responses? Front Plant Sci 12:724079. https://doi.org/10.3389/fpls.2021.724079

López-Ráez JA, Verhage A, Fernández I, García JM, Azcón-Aguilar C, Flors V, Pozo MJ (2010) Hormonal and transcriptional profiles highlight common and differential host responses to arbuscular mycorrhizal fungi and the regulation of the oxylipin pathway. J Exp Bot 61(2589–2601):2589–2601. https://doi.org/10.1093/jxb/erq089

Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 15:550. https://doi.org/10.1186/s13059-014-0550-8

Lutz S, Bodenhausen N, Hess J, Valzano-Held A, Waelchli J, Deslandes-Hérold G, Schlaeppi K, van der Heijden MAG (2023) Soil microbiome indicators can predict crop growth response to large-scale inoculation with arbuscular mycorrhizal fungi. Nat Microbiol 8:2277–2289. https://doi.org/10.1038/s41564-023-01520-w

Maestro-Gaitán I, Granado-Rodríguez S, Redondo-Nieto M, Battaglia A, Poza-Viejo L, Matías J, Bolaños L, Reguera M (2023) Unveiling changes in rhizosphere-associated bacteria linked to the genotype and water stress in quinoa. Microb Biotechnol 16:2326–2344. https://doi.org/10.1111/1751-7915.14337

Mahmood T, Rana RM, Ahmar S, Saeed S, Gulzar A, Khan MA, Wattoo FM, Wang X, Branca F, Mora-Poblete F, Mafra GS, Du X (2021) Effect of drought stress on capsaicin and antioxidant contents in pepper genotypes at reproductive stage. Plants 10:1286. https://doi.org/10.3390/plants10071286

Marino G, Brunetti C, Tattini M, Romano A, Biasioli F, Tognetti R, Loreto F, Ferrini F, Centritto M (2017) Dissecting the role of isoprene and stress-related hormones (ABA and ethylene) in *Populus nigra* exposed to unequal root zone water stress. Tree Physiol 37:1637–1647. https://doi.org/10.1093/treephys/tpx083

Masoumi Z, Haghighi M, Jalali SAH (2021) Flooding or drought which one is more offensive on pepper physiology and growth? Mol Biol Rep 48:4233–4245. https://doi.org/10.1007/s11033-021-06437-3

McDonald D, Jiang Y, Balaban M, Cantrell K, Zhu Q, Gonzalez A, Morton JT, Nicolaou G, Parks DH, Karst SM, Albertsen M, Hugenholtz P, DeSantis T, Song SJ, Bartko A, Havulinna AS, Jousilahti P, Cheng S, Inouye M, Niiranen T, Jain M, Salomaa V, Lahti L, Mirarab S, Knight R (2023) Greengenes2 unifies microbial data in a single reference tree. Nat Biotechnol 1–4. https://doi.org/10.1038/s41587-023-01845-1

Miranda FR, Gondim RS, Costa CAG (2006) Evapotranspiration and crop coefficients for tabasco pepper (*Capsicum frutescens* L.). Agric Water Manag 82:237–246. https://doi.org/10.1016/j.agwat.2005.07.024

Mishra N, Jiang C, Chen L, Paul A, Chatterjee A, Shen G (2023) Achieving abiotic stress tolerance in plants through antioxidative defense mechanisms. Front Plant Sci 14:1110622. https://doi.org/10.3389/fpls.2023.1110622

Muhammad I, Yang L, Ahmad S, Zeeshan M, Farooq S, Ali I, Khan A, Zhou XB (2022) Irrigation and nitrogen fertilization alter soil bacterial communities, soil enzyme activities, and nutrient availability in maize crop. Front Microbiol 13:833758. https://doi.org/10.3389/fmicb.2022.833758

Munoz-Ucros J, Wilhelm RC, Buckley DH, Bauerle TL (2022) Drought legacy in rhizosphere bacterial communities alters subsequent plant performance. Plant Soil 471:443–461. https://doi.org/10.1007/s11104-021-05227-x

Naseem H, Ahsan M, Shahid MA, Khan N (2018) Exopolysaccharides producing rhizobacteria and their role in plant growth and drought tolerance. J Basic Microbiol 58:1009–1022. https://doi.org/10.1002/jobm.201800309

Nerva L, Chitarra W, Fila G, Lovat L, Gaiotti F (2023) Variability in stomatal adaptation to drought among grapevine cultivars: genotype-dependent responses. Agriculture 13:2186. https://doi.org/10.3390/agriculture13122186

Nerva L, Giudice G, Quiroga G, Belfiore N, Lovat L, Perria R, Volpe MG, Moffa L, Sandrini M, Gaiotti F, Balestrini R, Chitarra W (2022) Mycorrhizal symbiosis balances rootstock-mediated growth-defence tradeoffs. Biol Fertil Soils 58:17–34. https://doi.org/10.1007/s00374-021-01607-8

Nurzyńska-Wierdak R, Buczkowska H, Sałata A (2021) Do AMF and irrigation regimes affect sweet pepper fruit quality under open field conditions? Agronomy 11:2349. https://doi.org/10.3390/agronomy11112349

Olmstead R, Bohs L, Migid H, Santiago-Valentin E, Garcia V, Collier S (2008) Molecular phylogeny of the Solanaceae. Taxon 57:1159–1181. https://doi.org/10.1002/tax.574010

Pal SC, Hossain MB, Mallick D, Bushra F, Abdullah SR, Dash PK, Das D (2024) Combined use of seaweed extract and arbuscular mycorrhizal fungi for alleviating salt stress in bell pepper (*Capsicum annuum* L.). Sci Hortic 325:112597. https://doi.org/10.1016/j.scienta.2023.112597

Philippot L, Chenu C, Kappler A, Rillig MC, Fierer N (2024) The interplay between microbial communities and soil properties. Nat Rev Microbiol 22:226–239. https://doi.org/10.1038/s41579-023-00980-5

Rodríguez Padrón R, Guedes J, Swarowsky A, Nogueira C, Cerquera R, Díaz-Pérez J (2015) Supplemental irrigation levels in bell pepper under shade mesh and in open-field: crop coefficient, yield, fruit quality and water productivity. Afr J Agric Res 10:4117–4125. https://doi.org/10.5897/AJAR2015.10341

Rosa-Martínez E, Bovy A, Plazas M, Tikunov Y, Prohens J, Pereira-Dias L (2023) Genetics and breeding of phenolic content in tomato, eggplant and pepper fruits. Front Plant Sci 14. https://www.frontiersin.org/articles/10.3389/fpls.2023.1135237

Sandrini M, Nerva L, Sillo F, Balestrini R, Chitarra W, Zampieri E (2022) Abiotic stress and belowground microbiome: the potential of omics approaches. Int J Mol Sci 23:1091. https://doi.org/10.3390/ijms23031091

Santos-Medellín C, Liechty Z, Edwards J, Nguyen B, Huang B, Weimer BC, Sundaresan V (2021) Prolonged drought imparts lasting compositional changes to the rice root microbiome. Nat Plants 7:1065–1077. https://doi.org/10.1038/s41477-021-00967-1

Sbrana C, Agnolucci M, Avio L, Giovannini L, Palla M, Turrini A, Giovannetti M (2022) Mycorrhizal symbionts and

associated bacteria: potent allies to improve plant phosphorus availability and food security. Front Microbiol 12. https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2021.797381

Shalaby OA, El-Sayed Ramadan ME-S (2024) Mycorrhizal colonization and calcium spraying modulate physiological and antioxidant responses to improve pepper growth and yield under salinity stress. Rhizosphere 29:100852. https://doi.org/10.1016/j.rhisph.2024.100852

Singleton VL, Orthofer R, Lamuela-Raventos RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol 299:152–178. https://doi.org/10.1016/S0076-6879(99)99017-1

Sinsabaugh R, Saiya-Cork K, Long T, Osgood M, Neher D, Zak D, Norby R (2003) Soil microbial activity in a liquidambar plantation unresponsive to CO_2- driven increases in primary production. Appl Soil Ecol 24:263–271. https://doi.org/10.1016/S0929-1393(03)00002-7

Soare R, Maria D, Babeanu C, Popescu M, Popescu A (2017) Nutritional value and antioxidant activities in fruit of some cultivars of pepper (*Capsicum annuum* L.). J Agroaliment Processes Technol 23:217–222

Spatafora JW, Chang Y, Benny GL, Lazarus K, Smith ME, Berbee ML, Bonito G, Corradi N, Grigoriev I, Gryganskyi A, James TY, O'Donnell K, Roberson RW, Taylor TN, Uehling J, Vilgalys R, White MM, Stajich JE (2016) A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. Mycologia 108:1028–1046. https://doi.org/10.3852/16-042

Suntornsuk L, Gritsanapun W, Nilkamhank S, Paochom A (2002) Quantitation of vitamin C content in herbal juice using direct titration. J Pharm Biomed Anal 28:849–855. https://doi.org/10.1016/S0731-7085(01)00661-6

Tang C, Sun B, Zeeshan M, Li J, Zhang X (2023) *Funneliformis mosseae*–induced changes of rhizosphere microbial community structure enhance *Capsicum annuum* L. plant growth and fruit yield. Soil Sci Soc Am 87:843–855. https://doi.org/10.1002/saj2.20528

Torres N, Goicoechea N, Carmen Antolín M (2018) Influence of irrigation strategy and mycorrhizal inoculation on fruit quality in different clones of Tempranillo grown under elevated temperatures. Agric Water Manag 202:285–298. https://doi.org/10.1016/j.agwat.2017.12.004

Trouvelot A (1986) Mesure du taux de mycorhization VA d'un systeme radiculaire. Recherche de methods d'estimation ayant une signification fonctionnelle. In: Gianinazzi-Pearson V, Gianinazzi S (eds) Physiological and genetical aspects of mycorrhizae. INRA, Paris, pp 217–221

Türkmen Ö, Sensoy S, Demir S, Erdinc C (2008) Effects of two different AMF species on growth and nutrient content of pepper seedlings grown under moderate salt stress. Afr J Biotechnol 7:392–396

Velikova V, Yordanov I, Edreva A (2000) Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. Plant Sci 151:59–66. https://doi.org/10.1016/S0168-9452(99)00197-1

Wang J, Fu W, Sun C, Cai S, Tang C (2022) *Funneliformis mosseae* inoculation enhances *Cucurbita pepo* L. plant growth and fruit yield by reshaping rhizosphere microbial community structure. Diversity 14:932. https://doi.org/10.3390/d14110932

Williams A, de Vries FT (2020) Plant root exudation under drought: implications for ecosystem functioning. New Phytol 225:1899–1905. https://doi.org/10.1111/nph.16223

Wu QS, Zou YN (2017) Arbuscular mycorrhizal fungi and tolerance of drought stress in plants. In QS Wu (Ed.), Arbuscular mycorrhizas and stress tolerance of plants. Springer 25–41. https://doi.org/10.1007/978-981-10-4115-0_2

Yasuda M, Dastogeer KMG, Sarkodee-Addo E, Tokiwa C, Isawa T, Shinozaki S, Okazaki S (2022) Impact of *Azospirillum* sp. B510 on the rhizosphere microbiome of rice under field conditions. Agronomy 12:1367. https://doi.org/10.3390/agronomy12061367

Zhang J, Cook J, Nearing JT, Zhang J, Raudonis R, Glick BR, Langille MGI, Cheng Z (2021) Harnessing the plant microbiome to promote the growth of agricultural crops. Microbiol Res 245:126690. https://doi.org/10.1016/j.micres.2020.126690

Zhang H, Sun X, Dai M (2022a) Improving crop drought resistance with plant growth regulators and rhizobacteria: mechanisms, applications, and perspectives. Plant Commun 3:100228. https://doi.org/10.1016/j.xplc.2021.100228

Zhang L, Zhou J, George TS, Limpens E, Feng G (2022b) Arbuscular mycorrhizal fungi conducting the hyphosphere bacterial orchestra. Trends Plant Sci 27:402–411. https://doi.org/10.1016/j.tplants.2021.10.008

Zheng FL, Liang SM, Chu XN, Yang YL, Wu QS (2020) Mycorrhizal fungi enhance flooding tolerance of peach through inducing proline accumulation and improving root architecture. Plant Soil Environ 66:624–631. https://doi.org/10.17221/520/2020-PSE

Zhishen J, Mengcheng T, Jianming W (1999) The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem 64:555–559. https://doi.org/10.1016/S0308-8146(98)00102-2

Zhou Y, Yi S, Zang Y, Yao Q, Zhu H (2021) The Predatory Myxobacterium *Citreicoccus inhibens* gen. nov. sp. nov. showed antifungal activity and bacteriolytic property against phytopathogens. Microorganisms 9:2137. https://doi.org/10.3390/microorganisms9102137

Zia R, Nawaz MS, Siddique MJ, Hakim S, Imran A (2021) Plant survival under drought stress: Implications, adaptive responses, and integrated rhizosphere management strategy for stress mitigation. Microbiol Res 242:126626. https://doi.org/10.1016/j.micres.2020.126626

Zou YN, Wu QS, Kuča K (2021) Unravelling the role of arbuscular mycorrhizal fungi in mitigating the oxidative burst of plants under drought stress. Plant Biol 23:50–57. https://doi.org/10.1111/plb.13161

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.