Can arbuscular mycorrhizal fungi and rhizobacteria facilitate 33P uptake in maize plants under water stress?

Silva, Antonio M. M.; Jones, Davey L.; Chadwick, Dave R.; Qi, Xue; Cotta, Simone R.; Araujo, Victor L. V. P.; Matteoli, Filipe P.; Lacerda-Junior, Gileno, V; Pereira, Arthur P. A.; Fernandes-Junior, Paulo I.; Cardoso, Elke J. B. N.

Microbiological Research

DOI:
10.1016/j.micres.2023.127350

Published: 01/06/2023

Peer reviewed version

Can arbuscular mycorrhizal fungi and rhizobacteria facilitate ^{33}P uptake in maize plants under drought stress?

Antonio M.M. Silva[1,2*], Davey L. Jones[2,3], Dave R. Chadwick[2], Qi Xue[2,4], Simone R. Cotta[5], Arthur P.A. Pereira[6], Victor L.V.P. Araújo[1], Filipe P. Matteoli[1], Gileno V.L Júnior [7], Elke J.B.N. Cardoso[1]

Author affiliations

[1]University of São Paulo, "Luiz de Queiroz" College of Agriculture. Soil Science Department, Piracicaba, São Paulo, 13418-900, Brazil. E-mail: antoniomarcos@usp.br, victorlucas395@usp.br, matteolifilipe@gmail.com, ejbncard@usp.br

[2]School of Natural Sciences, Bangor University, Bangor, Gwynedd, LL57 2UW, United Kingdom. E-mail: d.jones@bangor.ac.uk, d.chadwick@bangor.ac.uk, x.qi@bangor.ac.uk

[3]SoilsWest, Centre for Sustainable Farming Systems, Food Futures Institute, Murdoch University, Murdoch, WA 6150, Australia. E-mail: davey.jones@uwa.edu.au

[4]College of Grassland, Resources, and Environment, Inner Mongolia Agricultural University, Hohhot 010018, Inner Mongolia Autonomous Region, China. E-mail: qixueer89@emails.imau.edu.cn

[5]University of São Paulo, Center for Nuclear Energy in Agriculture, Piracicaba, São Paulo, 13400-970, Brazil. E-mail: raposo.cotta@gmail.com

[6]Federal University of Ceará, Soil Science Department, Soil Microbiology Laboratory, Fortaleza, Ceará, Brazil. E-mail: arthur.prudencio@ufc.br

[7]Brazilian Agricultural Research Corporation – Embrapa, Jaguariúna, São Paulo, 13918-110, Brazil. E-mail: gilenolacerdajr@gmail.com

***Corresponding author**

Antonio Marcos Miranda Silva (antoniomarcos@usp.br)

HIGHLIGHTS

- Mycorrhizae and rhizobacteria led to a higher plant ^{33}P uptake under drought
- ^{33}P facilitation was modulated by the soil water content
- Consortium or only rhizobacteria were more efficient under severe drought
- Under moderate drought conditions, mycorrhizae alone stood out in plant ^{33}P uptake

ABSTRACT

The role of arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) in delivering important ecosystem services and protecting plants against biotic and abiotic stress is well recognized. Here, we hypothesized that a combination of AMF and PGPR could enhance P uptake in maize plants under drought stress. A microcosm experiment using mesh exclusion and a radiolabeled phosphorus tracer (^{33}P) was established using three types of inoculation: i) only AMF, ii) only PGPR, and iii) a consortium of AMF and PGPR, alongside a control treatment without inoculation. For all treatments, a gradient of three water-holding capacities (WHC) was considered i) 30 % (severe drought), ii) 50 % (moderate drought), and iii) 80 % (optimal condition, no water stress). Under severe drought conditions, the use of the consortium (AMF+PGPR) or PGPR alone both increased ^{33}P uptake by 2.4-fold compared to the uninoculated treatment. In contrast, under moderate drought the use of AMF promoted the highest ^{33}P uptake by plants, increasing it by 2.1-fold compared to the uninoculated treatment. Without drought stress, AMF showed the lowest ^{33}P uptake and, overall, plant P acquisition was lower for all inoculation types when compared to the severe and moderate drought treatments. Considering the water gradient, the plant physiological response, such as altering signalling networks and root exudation, may have been decisive in the success of the plant-microbial interaction investigated here. We found that AMF colonization, soil electrical conductivity, and the number of spores were the main drivers which explained plant ^{33}P uptake. In conclusion, this study demonstrates that a one-size-fits-all solution for plant bio-inoculants is an unexpected outcome, where microbial inoculants, whether constituted of purified strains or in consortia, are apt to vary in their ^{33}P uptake efficiency according to the soil water gradient.

Keywords: Phosphate nutrition, isotope tracer, water shortage, plant symbiosis, soil-dwelling microbes.

INTRODUCTION

According to the Food and Agriculture Organization of the United Nations (FAO), drought is now recognised as the primary reason for agricultural production losses globally, costing the sector USD 37 billion overall from 2008-2018. However, other extreme events caused by climate change, such as floods and heatwaves, are also contributing to ongoing issues with food security (FAO 2021). Therefore, improved management approaches are urgently required to improve agricultural sustainability. This

is particularly relevant to the supply and exploitation of soil nutrients which have a finite supply, such as phosphorus (P), especially given the dramatic rise in fertilizer prices over the past year (Smith 2022). To overcome the combined impact of drought stress and low nutrient use efficiency in cropping systems and the design of new management systems requires a greater fundamental understanding of plant-soil-microbial interactions. In this context, it has been highlighted that arbuscular mycorrhizal fungi (AMF) and rhizobacteria may provide an environmentally friendly solution to this combined problem (Mawarda et al. 2020).

In highly weathered soils where the exchange surfaces are dominated by aluminium and iron oxides/hydroxides, a large proportion of the applied phosphate fertilizer (ranging from 15-30 %) becomes rapidly immobilized on the solid phase by adsorption and precipitation processes (Dhillon et al. 2017; Zavaschi et al. 2020). AMF may provide a tool to exploit native soil P reservoirs or residual fertiliser-derived P ("legacy P"), that has accumulated over the past 50 years in these soils (Scrase et al. 2019; Pavinato et al. 2020a; Pavinato et al. 2020b). AMF is a key group of soil microorganisms that form symbiotic associations with more than 80 % of all land plants and play an important role in the acquisition of nutrients (Smith and Read 2010). For example, in maize (*Zea mays* L.), AMF is more important than root hairs for seedling growth under low P availability (Ma et al. 2021).

It is widely acknowledged that AMF and plant growth-promoting rhizobacteria (PGPR) can play an important role in the amelioration of a wide range of plants biotic and abiotic stresses such as drought, salinity, heavy metal exposure, and soil-borne pathogens (Pérez-de-Luque et al. 2017; Chukwuneme et al. 2020; Mishra et al. 2021; Santoyo et al. 2021; Chen et al. 2022). There is also evidence that co-inoculation with AMF and PGPR can increase plant growth and health through additive and/or synergistic effects between them (Saia et al. 2015; Battini et al. 2017; Dutta and Neog 2017; Nanjundappa et al. 2019). Understanding the plant-mycorrhizae-rhizobacteria interactions is crucial, as plants dedicate 5-30 % of all their photo-assimilate to supporting microbial growth in soil (Carvalhais et al. 2011; Almeida et al. 2020). A further 20 % of photo-assimilate is allocated to the maintenance of symbiotic AMF networks (Smith and Read 2008).

Some studies have demonstrated the ability of AMF or PGPR alone to promote plant growth under water shortage events. It has been postulated that AMF mechanisms, such as improvements in soil aggregation, photosynthetic efficiency, and nutrient uptake are

primarily responsible for this response (Ji et al. 2019; Quiroga et al. 2019; Al-Arjani et al. 2020). In the case of PGPR, the main purported mechanisms include direct (e.g., changes in hormonal signalling, P solubilisation, biological nitrogen fixation) and indirect mechanisms (e.g., antibiotic production, cell wall degrading enzymes, induced systemic resistance, osmotic adjustment, quorum quenching, and siderophores production) (Glick 2012; Olanrewaju et al. 2017; Naylor and Coleman-Derr 2018; Araújo et al. 2020).

Overall, the majority of crop plant species are responsive to mycorrhizal symbiosis and rhizobacteria inoculation. This discovery has subsequently led to the search for novel microbes with the potential to increase crop yields, especially maize, as this represents one of the most important global crops (Zhao et al. 2017; Li et al. 2021). Recently, the use of maize has become more inviting due to the current scenario of bio-economy, which has incentives for production of biofuels to reduce CO_2 emissions. Thus, there is the possibility to intensify the biofuels market, making it more prosperous and stable (Eckert et al. 2018).

A myriad of early studies (Rhodes and Gerdemann 1975; Jakobsen et al. 1992; Pearson and Jakobsen 1993; Battini et al. 2017; Jongen et al. 2022) have demonstrated the ability of AMF hyphae to recover and translocate $^{32/33}P$ located beyond the immediate root zone. Nevertheless, these studies do not consider how the efficiency of AMF-mediated P absorption is affected under a gradient of soil water availability, especially when considering the presence of PGPR capable of tolerating low water activity. Here, our investigation was set up to test the hypothesis that the combined use of AMF and PGPR could enhance P uptake in maize plants under drought stress. For this, we evaluated ^{33}P uptake in maize plants, the response of soil phosphatase activity, and key soil chemical attributes, as well as monitoring the abundance and dynamics of soil mycorrhizal and bacterial communities.

MATERIAL AND METHODS

Experimental design

The microcosm experiment was set up in a completely randomized design, comprising a double factorial scheme (4 × 3) with three replicates. The first factor was the inoculation of microorganisms (either only AMF, only PGPR, or the consortium of AMF and PGPR, besides a control without any inoculation). The second factor was water stress (80, 50, and 30 % of the water-holding capacity, simulating no drought, moderate drought, and severe drought, respectively). Each experimental unit comprised a plastic pot (8 cm

131 internal diameter × 7 cm high), containing 200 g (dry weight) of sterilized soil (at 121 ºC
132 for 2 h) (Figure 1A and 1B). The soil was obtained from the Ah horizon (0-10 cm deep)
133 of a field site located in Brazil (22°42' S, 47°38' W) and classified as an Arenosol (WRB-
134 FAO 2015), with low P content (Supplementary Table 1).
135 Mesh exclusion (45 µm) was utilized to divide the pot into a fertilized compartment
136 and planted/inoculated compartment, each compartment receiving 100 g of sterilized soil.
137 This mesh allowed fungal hyphae to pass through and absorb nutrients but prevented the
138 ingrowth of roots from the planted/inoculated compartment. This approach has been used
139 to investigate the role of mycorrhizae on plant growth and water supply (Neumann and
140 Matzner 2013; Scrase et al. 2019; Kakouridis et al. 2020). Nevertheless, we set up a
141 preliminary experiment (Experiment 1) to confirm that roots were not able to pass through
142 the mesh, while simultaneously also determining seed germination rate in the soil and the
143 correction factor needed when calculating water-holding capacity over the course of
144 subsequent experiments (Supplementary Note 1).
145 The fertilized compartment received 2.8 mL of 6 mM KH_2PO_4 (equivalent to 30 mg P
146 kg^{-1} soil, as recommended for this type of weathered soil; van Raij et al. 1997) with a ^{33}P
147 activity of 185 kBq after the soil acclimatization, sowing of seeds and inoculation of
148 microbes in the planted/inoculated compartment (Figure 1C). Furthermore, we set up an
149 additional microcosm experiment to determine the distance that phosphorus can diffuse
150 in the soil to confirm that the presence of ^{33}P in the planted/inoculated compartment could
151 only occur via microbial transfer. In addition, we also measured P sorption to the soil to
152 characterize the P-dynamics in this soil (Supplementary Note 2).

153 **Plant material, cultivation, and water-holding capacity management**

154 Uniformly sized seeds of *Zea mays* L. (cv. BRS Gorotuba) were surface sterilized
155 twice in 2 % (v/v) sodium hypochlorite solution for 7 min, 70 % (v/v) ethanol for 1 min,
156 and rinsed thoroughly with sterile MilliQ water. The germination rate of seeds in soil and
157 Petri dishes was around 90 %. Seeds inoculated with PGPR or for consortium treatments
158 were soaked in the bacterial suspension for 2 h, whilst seeds for AMF and uninoculated
159 treatments were soaked in 0.85 % (w/v) saline solution for the same time (Kavamura et
160 al. 2013). Two seeds were sown in each pot and thinning was done when one of the
161 seedlings presented two true leaves. Soil bacterial inoculation was performed 21 days
162 after sowing, in PGPR or consortium treatments, when pots were reduced to 30 % of the
163 water-holding capacity according to the water content management described below. At

the same time, 0.85 % (w/v) saline solution was applied to the AMF, and uninoculated treatments

Plants were cultivated in a controlled environment chamber (Conviron Adaptis® CPM 6010) at the Environment Centre Wales, Bangor University, United Kingdom (53º13' N, 4º7' W), under a day/night cycle of 16/8 h, 25/20 °C, 70 % relative humidity, receiving artificial lighting at a photosynthetic photon flux density of 500 µmol m^{-2} sec^{-1}. Hoagland solution (without phosphorus) was applied 17 days after sowing, to keep nutritional balance of the plants. The final solution of pH 5.5 was composed of 4 mM $Ca(NO_3)_2$, 6 mM KNO_3, 2 mM $MgSO_4$, 1 mM Fe-EDTA, and 1 mM trace elements (Hoagland and Arnon 1950).

The microcosms were randomized daily to ensure equal growth conditions and were weighed for 35 days, and the desired moisture was maintained with the addition of deionized and sterilized water when needed. At the beginning of the experiment, all microcosms (n = 36) were kept at 80 % water-holding capacity to ensure seed germination. Ten days after sowing, $^2/_3$ of the microcosms (n = 24) were reduced to 50 % water-holding capacity. Finally, twenty days after sowing, $^1/_3$ of the microcosms (n = 12) were reduced to 30 % water-holding capacity (Figure 1C). This approach was used to better understand the potential of inoculated microbes in the context of a decreasing gradient of soil water content (Ahmad et al. 2018; Czarnes et al. 2020; Lopes et al. 2021).

After 35 days of growth, the plants were harvested and separated into above and belowground material, whilst the soil was separated into fertilized and planted/inoculated compartments. In addition, soil was sampled in the planted/inoculated compartment five days after changing the water holding capacity (i.e., on the 15th and 25th day after sowing) to monitor soil bacteria and mycorrhiza total abundance via quantitative polymerase chain reaction (qPCR).

Fungal and bacterial inoculum

Fungal and bacterial strains were previously isolated from Serra do Ouricuri, Petrolina, Pernambuco, Brazil (39°3' S, 8°28 W) in the Caatinga Biome, where a bio-prospecting program was developed to find microbes with the potential of helping crop plants to tolerate drought stress (Kavamura et al. 2013). Initially, a pool of fungi and bacteria was isolated from the rhizosphere of *Tripogonella spicata* (Nees) plants, the so-called resurrection grass, due to its surprising rehydration capacity (Fernandes-Júnior et al. 2015; Aidar et al. 2017). Then, they were selected for their plant growth-promoting

properties, such as the ability to grow under reduced water availability (ca. 0.9 Aw) (Hallsworth et al. 1998), indole-3-acetic acid (IAA) production (Bric et al. 1991; Kuss et al. 2007) and calcium phosphate solubilization (Verma et al. 2001).

Bacillus sp. was grown in 10 % (w/v) TSB (trypticase soy broth) culture medium at 30 °C and 150 rpm for 48 h. The inoculum was homogenized at OD_{550} = 0.2 and washed twice in a 0.85 % (w/v) saline solution to obtain the bacterial suspensions (at 10^8 CFU mL^{-1}). A second bacterial inoculation (3 mL pot^{-1} at 10^8 CFU mL^{-1}) was done when the water-holding capacity was changed to 30 % (i.e., twenty days after sowing). The same amount of saline solution was provided to the AMF and control treatments (Figure 1C).

Rhizophagus clarus spores were obtained from the pure trap culture, using maize as host plant, and transferred to the soil after disinfection, directly under the seeds, in the form of 5 mL of sterile water containing 50 spores at the time of sowing. The germination rate of *R. clarus* spores in the soil was around 85 % according to our third additional microcosm experiment, as reported in Supplementary Note 3.

Analytical procedures

At the end of the experiment (i.e., 35 days after sowing), the shoot was cut off at the soil surface and dried at 80 ºC in paper bags for 16 h to quantify dry weight. The roots were separated from soil by gently shaking them and rinsing them with water. Then, 5 g of roots were placed in a Falcon tube with 70 % ethanol for analysis of mycorrhizal colonization percentage, and the remainder was dried to obtain the dry weight. For phosphorimager analysis to visualize the ^{33}P, dried plant tissue was placed in a 20 × 25 cm cassette for 1 h, and then analyzed in a Bio-Rad Molecular Imager® FX.

Soil available phosphorous was extracted using 0.5 M acetic acid (1:5 w/v) according to Fisher et al. (1998). Samples were extracted by shaking (200 rpm for 30 min at room temperature), centrifuging for 15 min (18,000 *g*), filtering and the supernatant was recovered for analysis. ^{33}P activity of the samples was determined in counts per minute (CPM) of ^{33}P using 1 mL of soil extract and 4 mL of HiSafe 3 Scintillation cocktail (PerkinElmer Inc., Waltham, MA, USA) and analyzing in a liquid scintillation counter with an automated quench correction (Wallac 1404 liquid scintillation counter, PerkinElmer Inc). For plant tissue, the extract was obtained by placing 0.2 g in a muffle furnace and ashing at 500°C overnight. Later, this was dissolved in 1 mL of 20 % HCl and 9 mL of deionized water was added, according to Adrian (1973). The colorimetric P

determination in soil and plant tissue was determined according to Murphy and Riley (1962).

The soil pH and electrical conductivity were determined according to Thomas (1996), whereby 10 g of 2 mm-mesh sieved soil was mixed with 25 ml of water, placing it on an orbital shaker for 10 min at 200 rpm, and then allowing the sample to settle for 10 min before taking measurements with standard electrodes.

The acid and alkaline phosphatase activities (EC 3.1.3.2 and EC 3.1.3.21, respectively) were measured using the methodology described by Marx et al. (2001). For evaluation of AMF root colonization, the roots were prepared according to Vierheilig et al. (1998), with the roots dispersed in a Petri dish with a grid background and scored using a stereomicroscope according to Giovannetti and Mosse (1980).

Molecular analysis

Soil (0.25 g) was utilized for DNA extraction using DNeasy® PowerSoil® Pro Kit (QIAGEN Inc., Germany) according to the manufacturer's protocol. Extracted DNA was stored at -80 °C before quantitative PCR analysis. DNA concentrations were determined using the Qubit quantification platform with Qubit 1X dsDNA HS Assay Kit (Invitrogen, Carlsbad, CA, USA).

The quantitative PCR (qPCR) was used to determine gene copy number per gram of soil for bacteria (16S rRNA) and AMF (LSU rDNA region), using the StepOnePlus™ Real-Time PCR System (Applied Biosystems Inc., Carlsbad, CA, USA) with the fluorescent marker GoTaq® qPCR Master Mix (Promega, Madison, WI, USA). All samples were analysed in triplicate.

16S rRNA reactions were run in 10 µL comprising 5 µL of GoTaq® qPCR Master Mix, received 1 µL (5 µM) of each primer (Eub338 5'-CCTACGGGAGGCAGCAG-3' and Eub518 5'-ATTACCGCGGCTGCTGG-3'), 0.1 µL de CXR Reference Dye, 2 µL of DNA template, and 0.9 µL nuclease free sterile water in the same conditions as described by Muyzer et al. (1993). Standard curves were obtained using 7-fold serial dilutions of purified PCR (10^2 to 10^8 copies) containing the targeted gene. The reliability of the standard curves was controlled by verifying reproducibility of the Ct values, the quality of the dilution series, and the efficiency (101.42 %, $R^2 = 0.993$). The specificity of the primers was confirmed by melting curves analysis.

AMF reactions were run in 10 µL comprising 5 µL of GoTaq® qPCR Master Mix, received 1 µL (5 µM) of each primer (FLR3 5'-TTGAAAGGGAAACGATTGAAG T-

3' and FLR4 5'-TAC GTCAACATCCTTAACGAA-3'), 0.1 µL de CXR Reference Dye, 2 µL of DNA template, and 0.9 µL free sterile water. FLR3 is localized between the D1 and D2 domains of LSU rRNA, whilst FLR4 is in the D2 domain (Gollotte et al. 2004). Standard curves were obtained using 7-fold serial dilutions of purified PCR (10^2 to 10^8 copies) containing the targeted gene. The reliability of the standard curves was controlled by verifying reproducibility of the Ct values, the quality of the dilution series and the efficiency (101.46 %, R^2 = 0.975). The specificity of the primers was confirmed by melting curves analysis.

Data analyses

Data were tested for normal distribution using the Shapiro-Wilk test, followed by the homogeneity of variances tests, using the Bartlett test. Having met the criteria (residuals normality and variance homoscedasticity), a two-way analysis of variance (ANOVA) was performed and, when appropriate, Tukey's posthoc pairwise comparison (cut-off significance at $p < 0.05$) was applied to determine individual differences between means.

Principal components analysis (PCA) was performed using the statistical packages *FactoMineR* and *factoextra* in the R® program (R Core Team 2017). In PCA, to meet the premise of multivariate normality, the data were transformed into log (x+1) and the attributes subject to collinearity were removed (Ramette 2007). Additionally, using k-means clustering algorithm, an unsupervised machine learning method of identifying and grouping similar data points, we classified our variables into groups (Jansson et al. 2022).

RESULTS

In both soil compartments (i.e., planted/inoculated and fertilized compartments) we determined their ^{31}P and ^{33}P content alongside soil pH, electrical conductivity, and soil acid and alkaline phosphatase activity. In addition to plant P content, AMF root colonization and number of spores for the planted/inoculated compartment were determined. Here, we primarily concentrate on the results from the planted/inoculated compartment, while results from the fertilized compartment are presented in the supplementary material. Data is reported on P uptake by arbuscular mycorrhizal hyphae, as revealed by the use of radioactive P (^{33}P), added to fertilized compartment.

^{33}P uptake by plants, biomass, and soil P content

Soil water content greatly affected ^{33}P uptake by the plants, with the highest ^{33}P uptake (0.4 kBq.plant^{-1}, on average) observed in moderate drought (50 % WHC) with the lowest uptake (0.2 kBq.plant^{-1}, on average) observed in the absence of drought (80 % WHC) (Figure 2A and 2B). Overall, for the planted/inoculated compartment, the highest levels of ^{33}P activity in soil were observed in the presence of mycorrhizal inoculum under severe or moderate water stress, but this was not observed in the fertilized compartment (Supplementary Figures 1A and 1B). In addition, for shoot biomass, the main difference among the inoculum types occurred in severe drought, with the highest biomass found in the presence of bacterial inoculum. This same pattern could be detected for morphological traits, such as height and diameter (Supplementary Figure 2).

Under severe drought (30 % WHC), ^{33}P uptake in shoot was 2.4-fold greater ($p \leq 0.05$) in the PGPR and AMF+PGPR treatments than in uninoculated control. Whilst under moderate drought (50 % WHC), ^{33}P uptake in shoot of AMF treatment was 2.1-fold greater ($p \leq 0.05$) than uninoculated control, outperforming the other inoculation types. On the other hand, under optimal conditions (80 % WHC), the highest ($p \leq 0.05$) ^{33}P uptake in shoot was found in AMF+PGPR and the lowest ($p \leq 0.05$) in AMF treatment (Figure 2A, Supplementary Figure 3). The same pattern of ^{33}P uptake was observed in the root (Figure 2B, Supplementary Figure 4).

The total shoot P content was modulated by the water-holding capacity and inoculation type, with the lowest values ($p \leq 0.05$) observed under severe drought (18.6 µg P plant^{-1}, on average) and the highest values ($p \leq 0.05$) under moderate drought (28.3 µg P plant^{-1}, on average), almost reflecting the results of those for ^{33}P uptake (Figure 2C). However, this did not occur in the roots, in which there was an increase in P content with the increase of water content (Figure 2D). Under severe drought, the highest shoot P content was observed in the PGPR treatment ($p \leq 0.05$), whilst under moderate drought the uninoculated control was, in general, superior to the other inoculation types. Under no water stress, shoot P content in AMF+PGPR and PGPR was 1.7 and 1.5-fold greater than in the AMF treatment (Figure 2C). Overall, soil P contents were higher in the planted/inoculated compartment than in the fertilized compartment (Supplementary Figures 1C and 1D).

Mycorrhizal root colonization and number of spores in soil

AMF root colonization was higher under severe (20.9 %, on average) and moderate water stress (21.8 %, on average) than in no drought (6.4 %, on average). Under severe

drought, the AMF treatment showed a higher ($p \leq 0.05$) AMF root colonization percentage (56.7 ± 7.4) than the AMF+PGPR treatment (26.7 ± 16.1). Whilst under moderate and no drought, there was no difference ($p > 0.05$) between them (Figure 3A). The number of spores differed only between the AMF and AMF+PGPR treatments under severe drought ($p \leq 0.05$), where the highest value was found in the AMF treatment (18 ± 6). Overall, the water-holding capacity did not influence ($p > 0.05$) the number of spores (Figure 3B).

According to the microscopy results, a different pattern in WHC response occurred in the presence of AMF structures inside the roots. Considering a severe drought in the AMF treatment, there was a large presence of hyphae (60 %), whilst in the AMF+PGPR treatment, we observed only about 40 % of hyphae and 20 % of vesicles (Figure 3C and 3D, respectively). On the other hand, under a moderate drought, for AMF treatment, there was a higher presence of vesicles (50 %), hyphae (35 %), and arbuscules (10 %) (Figure 3E). Considering the AMF+PGPR treatment, there was the presence of spores inside the root (20 %), as well as hyphae (20 %) and vesicles (30 %) (Figure 3F). Without water stress, in the AMF treatment, there was a higher presence of arbuscules (25 %), while in the AMF+PGPR we only noticed the presence of hyphae (20 %) (Figure 3G and 3H, respectively).

Soil pH and electrical conductivity, soil phosphatases activity, and microbial monitoring over sampling time

The water-holding capacity did not influence the soil pH ($p > 0.05$), but it did influence ($p \leq 0.05$) the soil electrical conductivity (EC). The highest soil EC values were found under severe drought (86.1 $\mu S.m^{-1}$, on average), and the lowest for moderate (64.7 $\mu S.m^{-1}$, on average) and no drought (61.4 $\mu S.m^{-1}$, on average). A subtle difference was found in soil pH between inoculum types, within the same soil water-holding capacity. Compared with the other treatments, the AMF treatment showed the lowest pH value under severe and moderate stress ($p \leq 0.05$), whilst non-water restriction showed the highest pH value ($p \leq 0.05$). The main difference in electrical conductivity (EC) was found under severe drought, where the AMF+PGPR treatment presented the lowest EC (Table 1).

The highest soil acid phosphatase activity was found in the uninoculated treatment without any water restriction, which was on average 3-fold higher than the other treatments. There was no difference between treatments under severe drought, whilst

under moderate drought, the uninoculated and PGPR treatments showed higher values compared to other inoculation types. For soil alkaline phosphatase activity, the highest values were found both under moderate and non-stress conditions. An opposite behaviour was observed in the AMF+PGPR treatment, in which, under moderate stress, there was higher phosphatase activity, whereas in non-stress was the lowest compared to the others treatment with the same water-holding capacity (Table 1). The results for pH, EC, acid, and alkaline phosphatase in the fertilized compartment are presented in Supplementary Table S2.

Overall, water-holding capacity influenced the total soil bacterial and mycorrhizal abundance over time (Supplementary Table S3 and Table S4). Soil bacterial abundance increased from 15 days after sowing (DAS) to 25 DAS and decreased from 25 DAS to 35DAS (Supplementary Table S3). Whilst mycorrhizal fungal abundance decreased from 15 DAS to 25 DAS (only in severe and moderate drought) and increased substantially from 25 DAS to 35DAS (Supplementary Table S4). For both, bacterial and mycorrhizal inoculum, the highest abundances were found under severe and moderate drought.

Principal components analysis (PCA)

Principal component analysis (PCA) was conducted to address relationships between water-holding capacity and inoculation type and to determine the major trait components that explain the variation in the original data.

The water-holding capacity and inoculum type influenced the attribute dynamics according to the visualization of residuals in the PCA, which explained about 48 % in the first two components (Figure 4A). Overall, fungal parameters (AMF root colonisation and number of spores), and ^{33}P uptake, besides soil EC and pH, were more correlated to the inoculum type under severe drought (square dots). Whilst plant parameters (biomass, diameter, and height), phosphatase dynamics, and phosphorus contents were more correlated with moderate stress and non-drought (triangular and circular dots, respectively). Despite the dispersion within the replicates, a subtle difference was evident in relation to the inoculum type, where the AMF+PGPR, only PGPR, and uninoculated treatments showed better clustering than AMF treatment.

According to the results of the k-means clustering algorithm, three clusters of variables were identified, the first being composed of AMF root colonization, soil electrical conductivity, number of spores and ^{33}P activity (in soil and plant), which was more correlated to AMF+PGPR treatment under severe drought. The second was composed of

plant parameters (diameter and height) and plant P content, while the third was composed of phosphatases activity and soil pH (Figure 4B). We assessed the most important variables in explaining the variability in our data set according to the contribution level. The variables that contributed the most to the definition of the principal component 1 were AMF root colonisation (12.81 %), plant height (12.75 %), root P content (10.65 %), soil ^{33}P activity (10.31 %), and number of spores (10.24 %) (Figure 4C). Whilst for the principal component 2, the most important variables were root and shoot ^{33}P activity (26.53 % and 25.57 %, respectively) (Figure 4D).

DISCUSSION

Inoculated plants outperformed the uninoculated plants in terms of ^{33}P uptake, especially under drought stress. The enhanced drought tolerance promoted by AMF inoculated plants can be ascribed to several mechanisms. For instance, Quiroga et al. (2019), when utilizing maize inoculated with *Rhizophagus irregularis*, under drought conditions, demonstrated an increase in stomatal conductance in the shoots and, thereby, enhanced photosynthesis. Other mechanisms were extensively detailed by Li et al. (2019), Ji et al. (2019), Al-Arjani et al. (2020), Chen et al. (2021), and Zhang et al. (2021). Also, we showed a synergistic effect of the consortium (AMF+PGPR), which significantly increased ^{33}P uptake under severe drought. More importantly, we provide evidence that AMF responsiveness in a hostile environment may be due to the presence of PGPR. Therefore, our results demonstrated that the presence of AMF-helper bacteria (i.e., *Bacillus* sp. screened from an extreme environment), contributes to ^{33}P uptake and maize growth. Thus, we confirmed our initial hypothesis that inoculation of AMF and PGPR enhances plant nutrient acquisition in treatments with increased frequency of drought.

We found that either bacterial (*Bacillus* sp.) or microbial consortia (*Rhizophagus clarus* and *Bacillus* sp.), under severe drought, enhanced ^{33}P uptake 2.4-fold more intensely, than *R. clarus* alone. In a similar approach, Battini et al. (2017) found that AMF inoculated plants showed higher ^{33}P uptake when also co-inoculated with bacteria. According to Jiang et al. (2021), mycorrhizas can control the interaction with the bacteria and actively recruit, transport and stimulate it to mineralize organic nutrients with benefit to the fungi, mainly in a region called mycorrhizosphere (a microhabitat in soil where plant roots are surrounded by fungal hyphae (Johansson et al. 2004)). Conversely, the bacteria benefit from the released of carbon in hyphal exudates, representing cooperation between them, which is allied with the symbiosis between the AMF and plant. However,

this cooperation seems to be closely dependent on the soil water level since, under moderate drought, *R. clarus* alone showed higher ^{33}P uptake as compared to co-inoculated treatment. Thus, this efficiency may vary substantially with species identity of both fungi and plants, host phenology, soil nutrients or root exudation, which significantly impacts rhizosphere/mycorrhizosphere microbial community (Pauwels et al. 2020; Jongen et al. 2022; Pérez Castro et al. 2019; Ulrich et al. 2019).

Although we have evidence of the ability of inoculation to increase ^{33}P uptake, this may not be related to the potential of mitigation of drought effects on maize growth, since we did not observe great differences in biomass production. Therefore, higher ^{33}P plant uptake did not necessarily translate into higher growth. Perhaps, under highly stress conditions more P is utilized by the plant to support the given conditions, such as supporting the mycorrhizae-rhizobacteria interaction, whilst under less stress conditions the plant can utilize the more absorbed P for growth.

We observed higher AMF colonization under severe and moderate water stress. However, the prevalence of arbuscules was observed under no drought. Our results may indicate a close association between the *R. clarus* and the maize genotype (*Zea mays* L. cv. BRS Gorotuba), with the determination of C allocation to the different fungal structures driven by the severity of drought stress. The effectiveness of plant–AMF interaction can lead to a plant physiologic improvement and, consequently, to a higher C supply to the fungi even under drought stress, with severe drought increasing the C allocation to hyphae, and moderate drought to vesicles, and no stress investing in arbuscules (Kiers et al. 2011; Jongen et al. 2022). Overall, arbuscules have a rapid turnover and are the exchange structure in mycorrhizal symbiosis (Smith and Read 2008). Interestingly, although the highest presence of arbuscules was found under no drought stress, the highest ^{33}P uptake occurred under drought stress conditions (both severe and moderate). However, the arbuscules in the treatments under drought would probably be formed and functional during earlier stages of the plant–AMF interaction, mainly due to the moment of water shortage experienced, explaining the observed AMF effects on the ^{33}P uptake.

The assessment of soil enzymes is crucial to understand the potential functioning response of the plant-microbe system since they are involved in nutrient cycling. Thus, soil phosphatase activities (e.g., acid and alkaline) strongly control phosphorus biotic pathways (Margalef et al. 2017). Here, we observed an inverse relationship between

457 enzyme activity and ^{33}P uptake by plants, which was somehow expected, since the P
458 source considered in our study (mono-potassium phosphate [KH_2PO_4]) is an inorganic
459 compound, and the production of these enzymes in the soil is used to perform the
460 acquisition of phosphate ions from organic molecules (Margalef et al. 2021). Indeed, the
461 high presence of inorganic P can repress the expression of *pho* genes, inhibiting soil
462 phosphatase activities (Nannipieri et al. 2011).

463 In general, we observed that there was an increase in soil acid phosphatase activity
464 with increasing soil water content, especially for the uninoculated treatment, which may
465 be due to the high demand for P by plants since there was no microbial inoculation to
466 facilitate the ^{33}P uptake. This result is interesting, considering that plants, although
467 developing their adaptation to alleviate most biotic and abiotic stresses in nature, also rely
468 on their microbial partners when they are present to absorb nutrients such as P (Hassani
469 et al. 2018). Overall, for soil alkaline phosphatase, the lowest activity was found in the
470 presence of microbial inoculation with AMF alone, which may be related to the
471 facilitation of phosphorus nutrition promoted by AMF via hyphal network, which reflects
472 the ^{33}P uptake results obtained in our experiments.

473 Regarding changes in soil pH and electrical conductivity (EC), our results showed that
474 the EC was dependent on water content, whereas pH was not affected. Soil EC increased
475 with decreases in the soil water content, which may be due to other factors, since soil EC
476 is also modulated by a combination of soluble salts, and soil temperature, for example
477 (Bai et al. 2013). Pankaj et al. (2020), using PGPR to improve plant growth and crop yield
478 of *Bacopa monnieri* (L.), observed that soil EC and pH decreased in inoculated soils.
479 Likewise, Al-Enazy et al. (2018) demonstrated the inoculation of maize plants with
480 *Azotobacter chroococcoccum*, *Bacillus megaterium* and *Pseudomonas fluorescens* also
481 decreased soil EC and pH.

482 Over time, we observed an increase in the bacterial abundance from 15 DAS to 25
483 DAS, which may be attributed to the soil bacterial inoculation that occurred at 21 DAS
484 to assist the plants when the water-holding capacity decreased during a severe drought.
485 After that, the bacteria abundance decreased from 25 DAS to 35 DAS, indicating a
486 transient rather than persistent effect. At the same time, i.e., 25 DAS and 35 DAS, we
487 observed an increase in AMF abundance, evidencing the complementary and synergistic
488 effects provided by *R. clarus* and *Bacillus* sp. According to Mawarda et al. (2020), when
489 inoculants are delivered into soil, they have low persistence due to a combination of high

levels of competition from the native microbial community and a lack of available resources (e.g., C, water, nutrients). This transient effect, however, does not necessarily imply a lack of lasting legacy on plant growth. Therefore, the inoculant effects may not necessarily be due to the size of the inoculant populations, since various changes in soil community structure and functioning can be found, even though the number of inoculant cells declined following introduction into the soil. Florio et al. (2017) using rhizosphere soil from an experiment with maize seeds inoculated with *Azospirillum lipoferum* CRT1, under field conditions, could not detect the inoculated strain by qPCR at 37 DAS. Indeed, these authors maintain that, in general, bacterial inoculants stimulate root growth and modify plant metabolism at very early stages, and generate lasting effects on the root system, disappearing quickly, usually after a few weeks. Likewise, Silva et al. (2021), using bulk soil from an experiment with sugarcane, inoculated with a bacteria consortium, observed that bacterial abundance remains constant over time, whilst changes occur in its composition and functions.

In our study, a higher bacteria abundance was detected in the AMF treatments, even though the spores were previously disinfected, suggesting that the bacteria were located inside the spore walls. Indeed, bacteria belonging to the order *Bacillales*, which include the *Bacillus* strain used here, are found to be intimately associated with AMF spores, increasing the AMF activity. Furthermore, they are often embedded in the outer or inner of the spore wall layers or the microniches formed by the peridial hyphae interwoven around the spores of various *Glomus* species, now assigned as *Rhizophagus* (Walley and Germida, 1995; Filippi et al. 1998; Rouphael et al. 2015; Selvakumar et al. 2016). On the other hand, other authors maintain that bacteria belonging to the order *Acidobacteriales* can suppress AMF activity (Svenningsen et al. 2018). In addition, it is important to take into account that seeds have their microbiota, which comes from the flower microbiota (so-called anthosphere) and, therefore, bacteria can reside in and on seeds (Nelson, 2018; Johnston-Monje et al. 2021). In our investigation, as the seeds were sterilized, the bacteriome that lives inside the seeds may have contributed in some way to our results. However, there is still insufficient knowledge allowing us to determine which specific bacterial species would be helping AMF, as it will strongly depend on the associated soil and plant microbiome.

Our investigation is a breakthrough in the topic of dual microbe inoculation, shedding light on the beneficial use of *Rhizophagus clarus* and *Bacillus* sp. (potential new

523 species) to increase the ^{33}P uptake by maize plants under drought stress. Furthermore,
524 given that our obtained dataset was composed of three clusters of variables according to
525 the k-means algorithm, we concluded that AMF root colonization, soil EC, and the
526 number of spores (first cluster) were the main drivers to explain the ^{33}P uptake, especially
527 using AMF+PGPR under severe drought and, therefore, reinforcing the synergism
528 between mycorrhizae and bacteria.

529 **CONCLUSIONS**

530 We conclude that *Rhizophagus clarus* and *Bacillus* sp. inoculation offers one potential
531 strategy to promote nutrient acquisition by plants in the context of the increasing
532 frequency of drought seen in most cropping regions of the world. This is supported by the
533 enhanced uptake of ^{33}P in all inoculated plants at all moisture regimes in comparison to
534 the uninoculated plants.

535 The synergistic response of *Rhizophagus clarus* and *Bacillus* sp. increased under drought
536 conditions, representing greater stress tolerance. This supports the future use of microbial
537 consortia as an inoculation technology to alleviate drought stress under future climate
538 change scenarios, as expected in the coming future, resulting in longer dry periods and a
539 decrease in rains in certain areas of the world.

540 Thus, future investigations should be carried out to test whether the microbes that have
541 emerged here under water scarcity events can respond in the same way considering the
542 presence of indigenous microbial communities. In addition, recent research has revealed
543 the potential of microbes, especially bacteria, associated with Brazilian Caatinga biome
544 plants as an inoculant in promoting plant growth under drought conditions. Therefore, the
545 microbes tested in our investigation are potential candidates for a microbial inoculant in
546 the near future, combining both bacteria and mycorrhizae.

547 **ACKNOWLEDGMENTS**

548 We thank the São Paulo Research Foundation (FAPESP) (#2020/12751-4, #2019/13436-
549 8, #2019/27682-0, and 2016/18944-3) for funding this study. In addition, Luís Fernando
550 Baldesin and Denise Mescolotti collaborated with technicians of the Microbiology Lab
551 of the Department of Soil Science at ESALQ/USP, Brazil. Thanks are also due to Sarah
552 Chesworth and Jonathan Roberts for laboratory supervision at the Environment Centre
553 Wales (ECW), United Kingdom. We would like to thank Emily Cooledge, Lucy

554 Greenfield, Nickolas Welsby, Luke Hillary, and Usman Hussain for all laboratory
555 assistance at ECW, and Samuel E. Jones for critically reading the text and for all
556 contributions.

557 **REFERENCES**

558 Adrian WJ (1973) A comparison of a wet pressure digestion method with other commonly
559 used wet and dry-ashing methods. Analyst 98:213–216.
560 https://doi.org/10.1039/an9739800213

561 Ahmad M, Pataczek L, Hilger TH, Zahir ZA, Hussain A, Rasche F, Schafleitner R,
562 Solberg S (2018) Perspectives of microbial inoculation for sustainable development
563 and environmental management. Front Microbiol 9:.
564 https://doi.org/10.3389/fmicb.2018.02992

565 Aidar SDT, Chaves ARDM, Fernandes Júnior PI, Oliveira MS, Costa Neto BP, Junior
566 TC, Morgante CV (2017) Vegetative desiccation tolerance of Tripogon spicatus
567 (Poaceae) from the tropical semiarid region of northeastern Brazil. Funct Plant Biol
568 44:1124–1133. https://doi.org/10.1071/FP17066

569 Al-Arjani ABF, Hashem A, Abd_Allah EF (2020) Arbuscular mycorrhizal fungi
570 modulates dynamics tolerance expression to mitigate drought stress in Ephedra
571 foliata Boiss. Saudi J Biol Sci 27:380–394.
572 https://doi.org/10.1016/j.sjbs.2019.10.008

573 Al-Enazy AA, Al-Barakah F, Al-Oud S, Usman A (2018) Effect of phosphogypsum
574 application and bacteria co-inoculation on biochemical properties and nutrient
575 availability to maize plants in a saline soil. Arch Agron Soil Sci 64:1394–1406.
576 https://doi.org/10.1080/03650340.2018.1437909

577 Albornoz FE, Dixon KW, Lambers H (2021) Revisiting mycorrhizal dogmas: Are
578 mycorrhizas really functioning as they are widely believed to do? Soil Ecol Lett
579 3:73–82. https://doi.org/10.1007/s42832-020-0070-2

580 Almeida DS, Delai LB, Sawaya ACHF, Rosolem CA (2020) Exudation of organic acid
581 anions by tropical grasses in response to low phosphorus availability. Sci Rep 10:1–
582 8. https://doi.org/10.1038/s41598-020-73398-1

583 Bai W, Kong L, Guo A (2013) Effects of physical properties on electrical conductivity of
584 compacted lateritic soil. J Rock Mech Geotech Eng 5:406–411.
585 https://doi.org/10.1016/j.jrmge.2013.07.003

586 Battini F, Grønlund M, Agnolucci M, Giovannetti M, Jakobsen I (2017) Facilitation of
587 phosphorus uptake in maize plants by mycorrhizosphere bacteria article. Sci Rep
588 7:1–11. https://doi.org/10.1038/s41598-017-04959-0

589 Beltrano J, Ronco MG (2008) Improved tolerance of wheat plants (Triticum aestivum L.)
590 to drought stress and rewatering by the arbuscular mycorrhizal fungus Glomus
591 claroideum: Effect on growth and cell membrane stability. Brazilian J Plant Physiol
592 20:29–37. https://doi.org/10.1590/S1677-04202008000100004

593 Boucher A, Dalpé Y, Charest C (1999) Effect of arbuscular mycorrhizal colonization of
594 four species of Glomus on physiological responses of maize. J Plant Nutr 22:783–
595 797. https://doi.org/10.1080/01904169909365671

596 Bric JM, Bostock RM, Silverstone SE (1991) Rapid in situ assay for indoleacetic acid
597 production by bacteria immobilized on a nitrocellulose membrane. Appl Environ
598 Microbiol 57:535–538. https://doi.org/10.1128/aem.57.2.535-538.1991

599 Cardoso IM, Boddington CL, Janssen BH, Oenema O, Kuyper TW (2004) Double pot
600 and double compartment: Integrating two approaches to study nutrient uptake by
601 arbuscular mycorrhizal fungi. Plant Soil 260:301–310.
602 https://doi.org/10.1023/B:PLSO.0000030180.95607.0b

603 Carvalhais LC, Dennis PG, Fedoseyenko D, Hajirezaei MR, Borriss R, Wirén NV (2011)
604 Root exudation of sugars, amino acids, and organic acids by maize as affected by
605 nitrogen, phosphorus, potassium, and iron deficiency. J Plant Nutr Soil Sci 174:3–
606 11. https://doi.org/10.1002/jpln.201000085

607 Chen Q, Deng X, Elzenga JTM, Dirk J (2022) Effect of soil bacteriomes on mycorrhizal
608 colonization by Rhizophagus irregularis — interactive effects on maize (Zea mays
609 L .) growth under salt stress. Biol Fertil Soils. https://doi.org/10.1007/s00374-022-
610 01636-x

611 Chen W, Ye T, Sun Q, Niu T, Zhang J (2021) Arbuscular Mycorrhizal Fungus Alters
612 Root System Architecture in Camellia sinensis L. as Revealed by RNA-Seq
613 Analysis. Front Plant Sci 12:1–15. https://doi.org/10.3389/fpls.2021.777357

614 Czarnes S, Mercier PE, Lemoine DG, Hamzaoui J, Legendre L (2020) Impact of soil
615 water content on maize responses to the plant growth-promoting rhizobacterium
616 Azospirillum lipoferum CRT1. J Agron Crop Sci 206:505–516.
617 https://doi.org/10.1111/jac.12399

618 Dhillon J, Torres G, Driver E, Figueiredo B, Raun WR (2017) World phosphorus use
619 efficiency in cereal crops. Agron J 109:1670–1677.
620 https://doi.org/10.2134/agronj2016.08.0483

621 Dutta SC, Neog B (2017) Inoculation of Arbuscular Mycorrhizal Fungi and Plant Growth
622 Promoting Rhizobacteria in Modulating Phosphorus Dynamics in Turmeric
623 Rhizosphere. Natl Acad Sci Lett 40:445–449. https://doi.org/10.1007/s40009-017-
624 0582-1

625 Eckert CT, Frigo EP, Albrecht LP, Albrecht AJP, Christ D, Santos WG, Berkembrock E,
626 Egewarth VA (2018) Maize ethanol production in Brazil: Characteristics and
627 perspectives. Renew Sustain Energy Rev 82:3907–3912.
628 https://doi.org/10.1016/j.rser.2017.10.082

629 FAO - The Food and Agriculture Organization of the United Nations (2021) The impact
630 of disasters and crises on agriculture and food security

631 Fernandes-Júnior PI, Aidar S de T, Morgante CV, Gava CAT, Zilli JE, Souza LSB,
632 Marinho RCN, Nóbrega RSA, Brasil MS, Seido SL, Martins LMV (2015) The
633 resurrection plant Tripogon spicatus (Poaceae) harbors a diversity of plant growth
634 promoting bacteria in northeastern Brazilian caatinga. Rev Bras Cienc do Solo
635 39:993–1002. https://doi.org/10.1590/01000683rbcs20140646

636 Fisher E, Thornton B, Hudson G, Edwards AC (1998) The variability in total and
637 extractable soil phosphorus under a grazed pasture. Plant Soil 203:249–255.
638 https://doi.org/10.1023/A:1004375221616

639 Filippi C, Bagnoli G, Citernesi AS, Giovannetti M (1998) Ultrastructural spatial
640 distribution of bacteria associated with sporocarps of Glomus mosseae. Symbiosis
641 24:1–12

642 Florio A, Pommier T, Gervaix J, Bérard A, Roux XL (2017) Soil C and N statuses
643 determine the effect of maize inoculation by plant growth-promoting rhizobacteria
644 on nitrifying and denitrifying communities. Sci Rep 7:1–12.
645 https://doi.org/10.1038/s41598-017-08589-4

646 Giovannetti M, Mosse B (1980) Giovannetti and mosse 1980.pdf. New Phytol 84:489–
647 500

648 Glick BR (2012) Plant Growth-Promoting Bacteria : Mechanisms and Applications.
649 Scientifica (Cairo) 2012. https://doi.org/10.6064/2012/963401

650 Gollotte A, Van Tuinen D, Atkinson D (2004) Diversity of arbuscular mycorrhizal fungi
651 colonising roots of the grass species Agrostis capillaris and Lolium perenne in a field
652 experiment. Mycorrhiza 14:111–117. https://doi.org/10.1007/s00572-003-0244-7

653 Hallsworth JE, Nomura Y, Iwahara M (1998) Ethanol-induced water stress and fungal
654 growth. J Ferment Bioeng 86:451–456. https://doi.org/10.1016/S0922-
655 338X(98)80150-5

656 Hassani MA, Durán P, Hacquard S (2018) Microbial interactions within the plant
657 holobiont. Microbiome 6:1–17. https://doi.org/10.1007/978-1-4419-9863-7_100630

658 Haq IU, Zhang M, Yang P, Van Elsas JD (2014) The interactions of bacteria with fungi
659 in soilP: Emerging concepts, 1st edn. Elsevier Inc.

660 Hestrin R, Hammer EC, Mueller CW, Lehmann J (2019) Synergies between mycorrhizal
661 fungi and soil microbial communities increase plant nitrogen acquisition. Commun
662 Biol 2. https://doi.org/10.1038/s42003-019-0481-8

663 Hoagland DR, Arnon DI (1950) The water-culture method for growing plants without
664 soil. Calif Agric Exp Stn Circ 347 Berkeley

665 Jakobsen I, Abbott LK, Robson AD (1992) External hyphae of vesicular—arbuscular
666 mycorrhizal fungi associated with Trifolium subterraneum L. 2. Hyphal transport of
667 32P over defined distances. New Phytol 120:509–516.
668 https://doi.org/10.1111/j.1469-8137.1992.tb01800.x

669 Jansa J, Mozafar A, Frossard E (2005) Phosphorus acquisition strategies within
670 arbuscular mycorrhizal fungal community of a single field site. Plant Soil 276:163–
671 176. https://doi.org/10.1007/s11104-005-4274-0

672 Jansson NF, Allen RL, Skogsmo G, Tavakoli S (2022) Principal component analysis and
673 K-means clustering as tools during exploration for Zn skarn deposits and industrial
674 carbonates, Sala area, Sweden. J Geochemical Explor 233.
675 https://doi.org/10.1016/j.gexplo.2021.106909

676 Ji L, Tan W, Chen X (2019) Arbuscular mycorrhizal mycelial networks and glomalin-
677 related soil protein increase soil aggregation in Calcaric Regosol under well-watered
678 and drought stress conditions. Soil Tillage Res 185:1–8.
679 https://doi.org/10.1016/j.still.2018.08.010

680 Jiang F, Zhang L, Zhou J, George TS, Feng G (2021) Arbuscular mycorrhizal fungi

Field Code Changed

enhance mineralisation of organic phosphorus by carrying bacteria along their extraradical hyphae. New Phytol 230:304–315. https://doi.org/10.1111/nph.17081

Jongen M, Albadran B, Beyschlag W, Unger S (2022) Can arbuscular mycorrhizal fungi mitigate drought stress in annual pasture legumes? Plant Soil 472:295–310. https://doi.org/10.1007/s11104-021-05233-z

Johansson JF, Paul LR, Finlay RD (2004) Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. FEMS Microbiol Ecol 48:1–13. https://doi.org/10.1016/j.femsec.2003.11.012

Johnston-Monje D, Gutiérrez JP, Lopez-Lavalle LAB (2021) Seed-Transmitted Bacteria and Fungi Dominate Juvenile Plant Microbiomes. Front Microbiol 12:. https://doi.org/10.3389/fmicb.2021.737616

Kakouridis A, Hagen JA, Kan MP, Mambelli S, Feldman LJ, Herman DJ, Pett-Ridge J, Firestone MK (2020) Routes to Roots: Direct Evidence of Water Transport by Arbuscular Mycorrhizal Fungi to Host Plants. bioRxiv 4:1–23. https://doi.org/https://doi.org/10.1101/2020.09.21.305409

Kiers ET, Duhamel M, Beesetty Y, Mensah JA, Franken O, Verbruggen E, Fellbaum CR, Kowalchuk GA, Hart MM, Bago A, Palmer TM, West SA, Vandenkoornhuyse P, Jansa J, Buet al (2011) Reciprocal Rewards Stabilize Cooperation in the Mycorrhizal Symbiosis. Science (80) 333:880–883

Kuss AV, Kuss VV, Lovato T, Flôres ML (2007) Nitrogen fixation and in vitro production of indolacetic acid by endophytic diazotrophic bacteria. Pesqui Agropecu Bras 42:1459–1465. https://doi.org/10.1590/s0100-204x2007001000013

Larimer AL, Clay K, Bever JD (2014) Synergism and context dependency of interactions between arbuscular mycorrhizal fungi and rhizobia with a prairie legume. Ecology 95:1045–1054. https://doi.org/10.1890/13-0025.1

Li J, Meng B, Chai H, Yang X, Song W, Li S, Lu A, Zhang T, Sun W (2019) Arbuscular mycorrhizal fungi alleviate drought stress in C3 (Leymus chinensis) and C4 (hemarthria altissima) grasses via altering antioxidant enzyme activities and photosynthesis. Front Plant Sci 10:1–12. https://doi.org/10.3389/fpls.2019.00499

Li X, Quan X, Mang M, Neumann G, Melchinger A, Ludewig U (2021) Flint maize root mycorrhization and organic acid exudates under phosphorus deficiency: Trends in breeding lines and doubled haploid lines from landraces. J Plant Nutr Soil Sci 184:346–359. https://doi.org/10.1002/jpln.202000471

Lopes MJ dos S, Dias-Filho MB, Gurgel ESC (2021) Successful Plant Growth-Promoting Microbes: Inoculation Methods and Abiotic Factors. Front Sustain Food Syst 5:1–13. https://doi.org/10.3389/fsufs.2021.606454

Ma X, Li X, Ludewig U (2021) Arbuscular mycorrhizal colonization outcompetes root hairs in maize under low phosphorus availability. Ann Bot 127:155–166. https://doi.org/10.1093/aob/mcaa159

Margalef O, Sardans J, Fernández-Martínez M, Molowny-Horas R, Janssens IA, Ciais P, Goll D, Richter A, Obersteiner M, Asensio D, Peñuelas J (2017) Global patterns of phosphatase activity in natural soils. Sci Rep 7:1–13. https://doi.org/10.1038/s41598-017-01418-8

724 Marx MC, Wood M, Jarvis SC (2001) A microplate fluorimetric assay for the study of
725 enzyme diversity in soils. Soil Biol Biochem 33:1633–1640.
726 https://doi.org/10.1016/S0038-0717(01)00079-7

727 Mawarda PC, Le Roux X, Dirk van Elsas J, Salles JF (2020) Deliberate introduction of
728 invisible invaders: A critical appraisal of the impact of microbial inoculants on soil
729 microbial communities. Soil Biol Biochem 148:107874.
730 https://doi.org/10.1016/j.soilbio.2020.107874

731 Murphy J, Riley JP (1962) A modified single solution method for the determination of
732 phosphate in natural waters. Eurasian J Soil Sci 27:31–36.
733 https://doi.org/10.18393/ejss.477560

734 Muyzer G, Waal EC De, Uitterlinden DAG (1993) Profiling of Complex Microbial
735 Populations by Denaturing Gradient Gel Electrophoresis Analysis of Polymerase
736 Chain Reaction-Amplified Genes Coding for 16S rRNA. Appl Environ Microbiol
737 59:695–700

738 Nanjundappa A, Bagyaraj DJ, Saxena AK, Kumar M, Chakdar H (2019) Interaction
739 between arbuscular mycorrhizal fungi and Bacillus spp. In soil enhancing growth of
740 crop plants. Fungal Biol Biotechnol 6:1–10. https://doi.org/10.1186/s40694-019-
741 0086-5

742 Nannipieri P, Giagnoni L, Landi L, Renella G (2011) Chapter 9 Role of Phosphatase
743 Enzymes in Soil. In: Phosphorus in Action. pp 215–243

744 Naylor D, Coleman-Derr D (2018) Drought stress and root-associated bacterial
745 communities. Front Plant Sci 8:1–16. https://doi.org/10.3389/fpls.2017.02223

746 Neumann J, Matzner E (2013) Biomass of extramatrical ectomycorrhizal mycelium and
747 fine roots in a young Norway spruce stand - a study using ingrowth bags with
748 different substrates. Plant Soil 371:435–446. https://doi.org/10.1007/s11104-013-
749 1701-5

750 Nelson EB (2018) The seed microbiome: Origins, interactions, and impacts. Plant Soil
751 422:7–34. https://doi.org/10.1007/s11104-017-3289-7

752 Olanrewaju OS, Glick BR, Babalola OO (2017) Mechanisms of action of plant growth
753 promoting bacteria. World J Microbiol Biotechnol 33:1–16.
754 https://doi.org/10.1007/s11274-017-2364-9

755 Pankaj U, Singh DN, Mishra P, Gaur P, Vivek Babu CS, Shanker K, Verma RK (2020)
756 Autochthonous halotolerant plant growth-promoting rhizobacteria promote bacoside
757 A yield of Bacopa monnieri (L.) Nash and phytoextraction of salt-affected soil.
758 Pedosphere 30:671–683. https://doi.org/10.1016/S1002-0160(20)60029-7

759 Pauwels R, Jansa J, Püschel D, Müller A, Graefe J, Kolb S, Bitterlich M (2020) Root
760 growth and presence of Rhizophagus irregularis distinctly alter substrate hydraulic
761 properties in a model system with Medicago truncatula. Plant Soil 457:131–151.
762 https://doi.org/10.1007/s11104-020-04723-w

763 Pavinato PS, Cherubin MR, Soltangheisi A, Rocha GC, Chadwick DR, Jones DL (2020a)
764 Revealing soil legacy phosphorus to promote sustainable agriculture in Brazil. Sci
765 Rep 10:1–11. https://doi.org/10.1038/s41598-020-72302-1

766 Pavinato PS, Rocha GC, Cherubin MR, Harris I, Jones DL, Withers PJA (2020b) Map of

767 total phosphorus content in native soils of Brazil. Sci Agric 78:1–5.
768 https://doi.org/10.1590/1678-992x-2020-0077

769 Pearson JN, Jakobsen I (1993) The relative contribution of hyphae and roots to
770 phosphorus uptake by arbuscular mycorrhizal plants, measured by dual labelling
771 with 32P and 33P. New Phytol 124:489–494. https://doi.org/10.1111/j.1469-
772 8137.1993.tb03840.x

773 Pérez-De-Luque A, Tille S, Johnson I, Pascual-Pardo D, Ton J, Cameron DD (2017) The
774 interactive effects of arbuscular mycorrhiza and plant growth-promoting
775 rhizobacteria synergistically enhance host plant defences against pathogen. Sci Rep
776 7:1–10. https://doi.org/10.1038/s41598-017-16697-4

777 Pérez Castro S, Cleland EE, Wagner R, Sawad RA, Lipson DA (2019) Soil microbial
778 responses to drought and exotic plants shift carbon metabolism. ISME J 13:1776–
779 1787. https://doi.org/10.1038/s41396-019-0389-9

780 Prudêncio de Araújo VLV, Lira Junior MA, Souza Júnior VS de, Araújo Filho JC,
781 Fracetto FJC, Andreote FD, Pereira APA, Mendes Júnior JP, Barros FMR, Fracetto
782 GGM (2020) Bacteria from tropical semiarid temporary ponds promote maize
783 growth under hydric stress. Microbiol Res 240:126564.
784 https://doi.org/10.1016/j.micres.2020.126564

785 Quiroga G, Erice G, Ding L, Chaumont F, Aroca R, Ruiz-Lozano JM (2019) The
786 arbuscular mycorrhizal symbiosis regulates aquaporins activity and improves root
787 cell water permeability in maize plants subjected to water stress. Plant Cell Environ
788 42:2274–2290. https://doi.org/10.1111/pce.13551

789 R Core Team (2017) R: a language and environment for statistical computing. R
790 Foundation for Statistical Computing Vienna, Austria. https:// www.r- proje ct. org/

791 Ramette A (2007) Multivariate analyses in microbial ecology. FEMS Microbiol Ecol
792 62:142–160. https://doi.org/10.1111/j.1574-6941.2007.00375.x

793 Rhodes LH, Gerdemann JW (1975) Phosphate Uptake Zones of Mycorrhizal and Non-
794 Mycorrhizal Onions Author (s): L . H . Rhodes and J . W . Gerdemann Published
795 by : Wiley on behalf of the New Phytologist Trust Stable URL :
796 https://www.jstor.org/stable/2431598 PHOSPHATE UPTAKE ZONES OF MYC.
797 New Phytol 75:555–561

798 Rolfe SA, Griffiths J, Ton J (2019) Crying out for help with root exudates: adaptive
799 mechanisms by which stressed plants assemble health-promoting soil microbiomes.
800 Curr Opin Microbiol 49:73–82. https://doi.org/10.1016/j.mib.2019.10.003

801 Rouphael Y, Franken P, Schneider C, Schwarz D, Giovannetti M, Agnolucci M, Pascale
802 S, Bonini P, Colla G (2015) Arbuscular mycorrhizal fungi act as biostimulants in
803 horticultural crops. Sci Hortic (Amsterdam) 196:91–108.
804 https://doi.org/10.1016/j.scienta.2015.09.002

805 Saia S, Rappa V, Ruisi P, Abenavoli MR, Sunseri F, Giambalvo D, Frenda AS, Martinelli
806 F (2015) Soil inoculation with symbiotic microorganisms promotes plant growth and
807 nutrient transporter genes expression in durum wheat. Front Plant Sci 6:1–10.
808 https://doi.org/10.3389/fpls.2015.00815

809 Santoyo G, Gamalero E, Glick BR (2021) Mycorrhizal-Bacterial Amelioration of Plant

Abiotic and Biotic Stress. Front Sustain Food Syst 5:. https://doi.org/10.3389/fsufs.2021.672881

Scrase FM, Sinclair FL, Farrar JF, Pavinato PS, Jones DL (2019) Mycorrhizas improve the absorption of non-available phosphorus by the green manure Tithonia diversifolia in poor soils. Rhizosphere 9:27–33. https://doi.org/10.1016/j.rhisph.2018.11.001

Selvakumar G, Krishnamoorthy R, Kim K, Sa TM (2016) Genetic diversity and association characters of bacteria isolated from arbuscular mycorrhizal fungal spore walls. PLoS One 11:1–16. https://doi.org/10.1371/journal.pone.0160356

Sendek A, Karakoç C, Wagg C, Domínguez-Begines J, Couto GM, Van der Heijden MGA, Naz AA, Lochner A, Chatzinotas A, Klotz S, Gómez-Aparicio L, Eisenhauer N (2019) Drought modulates interactions between arbuscular mycorrhizal fungal diversity and barley genotype diversity. Sci Rep 9:1–15. https://doi.org/10.1038/s41598-019-45702-1

Silva AMM, Estrada-Bonilla GA, Lopes CM, Matteoli FP, Cotta SR, Feiler HP, Rodrigues YF, Cardoso EJBN (2021) Does Organomineral Fertilizer Combined with Phosphate-Solubilizing Bacteria in Sugarcane Modulate Soil Microbial Community and Functions? Microb Ecol. https://doi.org/10.1007/s00248-021-01855-z

Smith SE, Read DJ (2008) Mycorrhizal Symbiosis

Smith A (2022) The Story of Rising Fertilizer Prices. Agric Resour Econ 25. https://asmith.ucdavis.edu/news/story-rising-fertilizer-prices#:~:text=US%20nitrogen%20fertilizer%20use%20increased,crops%20and%20increased%20fertilizer%20applications (Accessed: 30th May 2022).

Svenningsen NB, Watts-Williams SJ, Joner EJ, Battini F, Efthymiou A, Cruz-Paredes C, Nybroe O, Jakobsen I (2018) Suppression of the activity of arbuscular mycorrhizal fungi by the soil microbiota. ISME J 12:1296–1307. https://doi.org/10.1038/s41396-018-0059-3

Thomas GW (1996) Soil pH and soil acidity. Methods Soil Anal Part 3 Chem Methods 475–490. https://doi.org/10.2136/sssabookser5.3.c16

Timmusk S, Abd El-Daim IA, Copolovici L, Tanilas T, Känneste A, Behers L, Nevo E, Seisenbaeva G, Stenström E, Niinemets U (2014) Drought-tolerance of wheat improved by rhizosphere bacteria from harsh environments: Enhanced biomass production and reduced emissions of stress volatiles. PLoS One 9:. https://doi.org/10.1371/journal.pone.0096086

Ulrich DEM, Sevanto S, Ryan M, Albright MBN, Johansen RB, Dunbar JM (2019) Plant-microbe interactions before drought influence plant physiological responses to subsequent severe drought. Sci Rep 9:1–10. https://doi.org/10.1038/s41598-018-36971-3

Verma SC, Ladha JK, Tripathi AK (2001) Evaluation of plant growth promoting and colonization ability of endophytic diazotrophs from deep water rice. J Biotechnol 91:127–141. https://doi.org/10.1016/S0168-1656(01)00333-9

Vierheilig H, Coughlan AP, Wyss U, Piché Y (1998) Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. Appl Environ Microbiol 64:5004–5007.

https://doi.org/10.1128/aem.64.12.5004-5007.1998

Wang L, Wu J, Ma F, Yang J, Li S, Li Z, Zhang X (2015) Response of arbuscular mycorrhizal fungi to hydrologic gradients in the rhizosphere of phragmites australis (Cav.) Trin ex. steudel growing in the Sun Island Wetland. Biomed Res Int 2015:. https://doi.org/10.1155/2015/810124

Walley FL, Germida JJ (1995) Failure to decontaminate Glomus clarum NT4 spores is due to spore wall- associated bacteria. Mycorrhiza 6:43–49. https://doi.org/10.1007/s005720050104

WRB-FAO (2015) International soil classification system for naming soils and creating legends for soil maps. FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS, Rome.

Yang P, van Elsas JD (2018) Mechanisms and ecological implications of the movement of bacteria in soil. Appl Soil Ecol 129:112–120. https://doi.org/10.1016/j.apsoil.2018.04.014

Zavaschi E, de Abreu Faria L, Ferraz-Almeida R, Nascimento CAC, Pavinato RO, Vitti AC, Vitti GC (2020) Dynamic of P Flux in Tropical Acid Soils Fertilized with Humic Acid–Complexed Phosphate. J Soil Sci Plant Nutr 20:1937–1948. https://doi.org/10.1007/s42729-020-00265-3

Zhang J, Bi Y, Song Z, Xiao L, Christie P (2021) Arbuscular mycorrhizal fungi alter root and foliar responses to fissure-induced root damage stress. Ecol Indic 127:107800. https://doi.org/10.1016/j.ecolind.2021.107800

Zhao C, Liu B, Piao S, Wang X, Lobell DB, Huang Y, Huang M, Yao Y, Bassu S, Ciais P, Durand JL, Elliott J, Ewert F, Janssens IA, Li T, Lin E, Liu Q, Martre P, Müller C, Peng S, Peñuelas J, Ruane AC, Wallach D, Wang T, Wu D, Liu Z, Zhu Y, Zhu Z, Asseng J (2017) Temperature increase reduces global yields of major crops in four independent estimates. Proc Natl Acad Sci U S A 114:9326–9331. https://doi.org/10.1073/pnas.1701762114

Zhou J, Wilson GWT, Cobb AB, Zhang Y, Liu L, Zhang X, Sun F (2022) Mycorrhizal and rhizobial interactions influence model grassland plant community structure and productivity. Mycorrhiza 32:15–32. https://doi.org/10.1007/s00572-021-01061-2