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

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Microbiological Research

DOI:
10.1016/j.micres.2023.127350

Published: 01/06/2023

Peer reviewed version

Supplementary Material

Supplementary Table 1. Chemical characterization of the soil used in the experiment.

Material	pH	O.M	P	S	K	Ca	Mg	Al	H+Al	SB	CEC	V	m
		g dm^{-3}	-mg dm^{-3}-		----------$mmol_c$ dm^{-3}----------							---- % ----	
Soil	4.0	8.0	<3.0	8.0	<0.9	<1.0	1.0	10.0	31.0	2.1	33.1	6.0	83.0

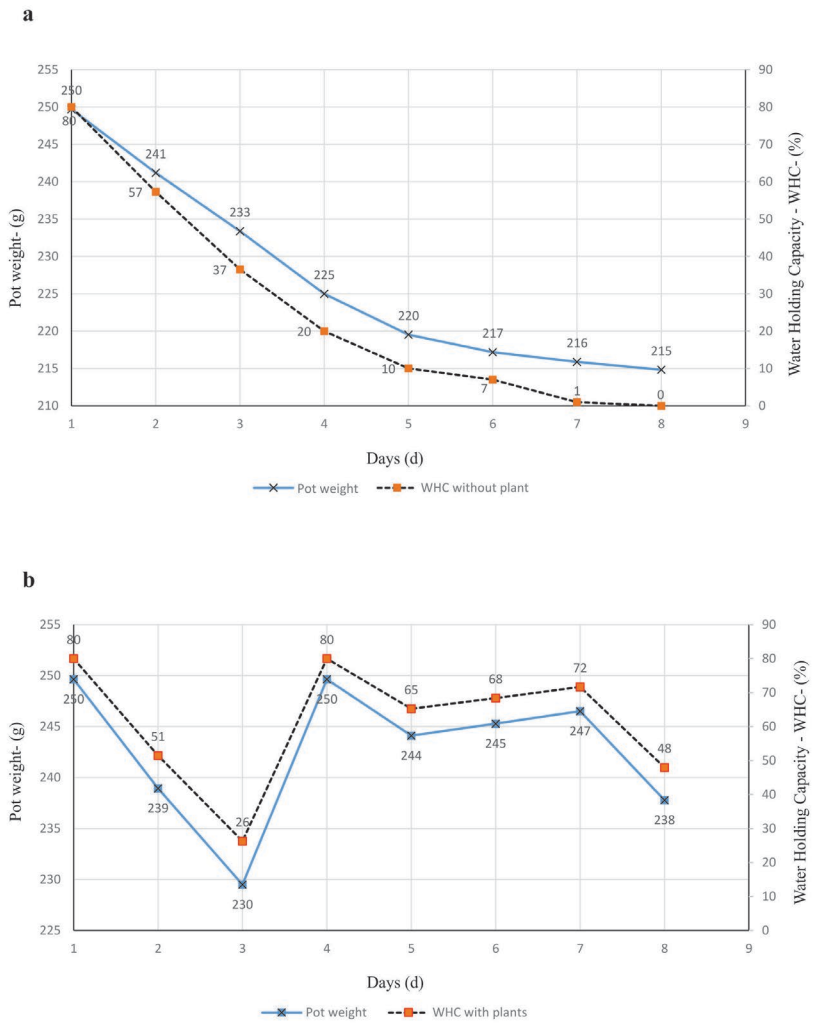
Soil sampled in ESALQ-USP, pH: measured in $CaCl_2$, O.M: organic matter - colorimetric method, P: phosphorus with anion exchange resin, S: sulfur - 0.01 mol L^{-1} calcium phosphate, K, Ca and Mg: potassium, calcium and magnesium measured in anion exchange resin, Al: aluminum in 1 mol KCl L^{-1}, H + Al: potential acidity in SMP buffer, SB: sum of bases (K + Ca + Mg), CEC: cation exchange capacity, V: base saturation and m: aluminum saturation. $mmol_c$ kg^{-1}: millimoles of charge per kilogram of soil according to SI unit (International Standard of Units).

Supplementary Note 1. Experiment 1: aims, design, and results.

The aim of Experiment 1 was i) to confirm whether the root was able to pass through a mesh with aperture of 45 µm; ii) to ascertain how many days the maize seeds took to germinate in soil (also in Petri dishes); iii) to verify how water-holding capacity decreases as a function of the soil volume; iv) to verify the contribution of plant weight, since the control of the water content was based on the pot weight.

Two groups of treatments were considered, i.e., with and without a plant (n = 3), in order to learn about the contribution of the plant weight. Each experimental unit comprised a plastic pot (8 cm internal diameter × 7 cm high), containing 200 g of soil (dry weight). Mesh exclusion (45 µm) was used to divide the pot into two compartments, each one receiving 100 g of sterilized soil. Seeds were sown in the planted compartment at 80 % of water-holding capacity. There was no daily maintenance of the water content for the treatment without plants in order to understand how the water-holding capacity decreases as a function of the soil volume. In the treatment with plants, there was rehydration to 80 % water-holding capacity (WHC) on the fourth day when the WHC was 26 %, followed by daily control to 80 % WHC using the addition of deionized and sterilized water (SN1 Figure 1A and 1B).

Overall, a seed germination rate of 98 % was found six days after sowing, and a high decrease in soil moisture after one day, requiring daily control of the water content in the next microcosm experiment. The plant weight contribution to pot weight followed the equation 1.47 ± 0.36 g (shoot, n = 3) and 2.49 ± 0.71 g (root, n = 3) on a wet basis.



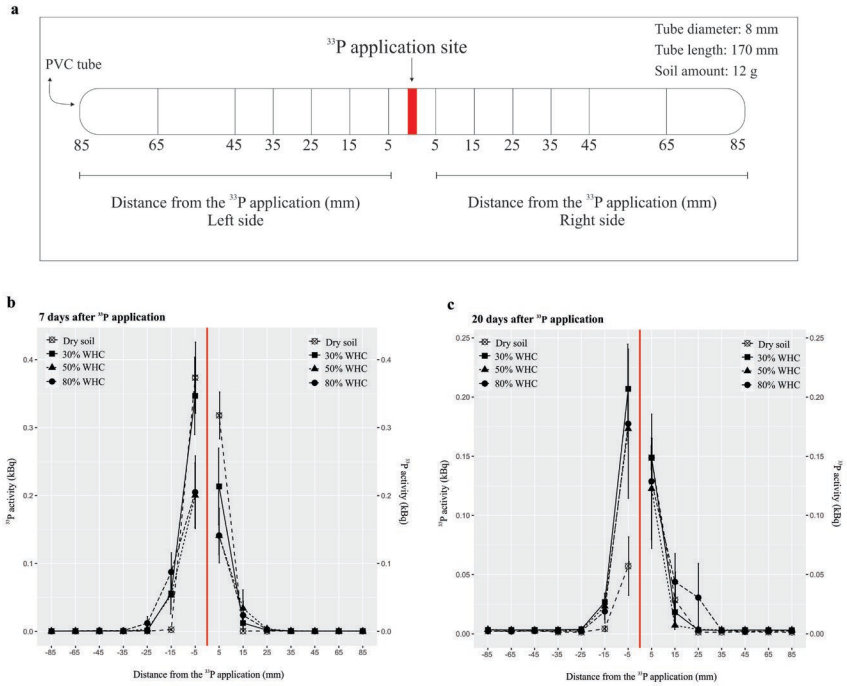
SN1 Fig 1. Representation of the pot weight (solid blue line) and the water-holding capacity (dashed black line) without plants (a) and with plants (b). Note: the increase in the water-holding capacity on the 4th day in b was due to the pot rehydration.

Supplementary Note 2. Experiment 2: aims, design, and results.

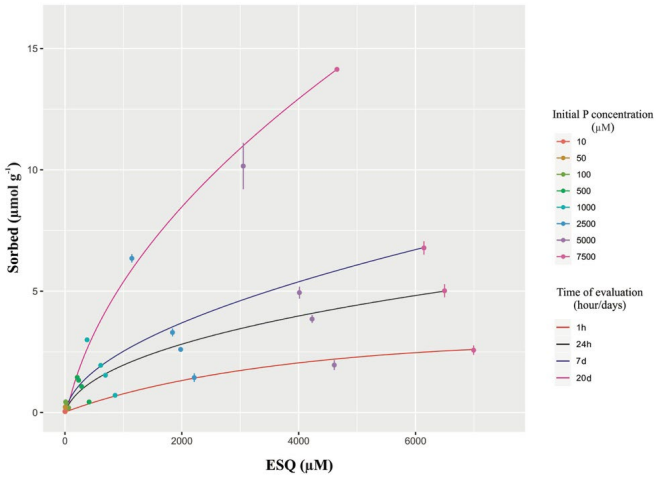
The soil diffusion microcosms were prepared by filling 8 mm inner diameter, 170 mm long polypropylene cylinders with air-dried soil (12 g of soil with a soil density of 1 g cm^{-3}). To retain the soil, one end of the cylinder was covered with gas-permeable polyvinyl chloride (PVC) film (Figure SN2 1A). Four types of treatment were considered, i) dry soil, ii) 30 % WHC, iii) 50 % WHC, and iv) 80 % WHC. Soil columns for each treatment were placed in a black box and then sealed (simulating the soil environment) and incubated in a growth chamber. This experiment was set up twice, as the first was evaluated at 7 days and the second at 20 days after ^{33}P application (100 µl).

According to the results, regardless of soil water content and evaluation time, P diffusion in soil was limited to 15 mm (SN2 Figure 1B and 1C).

The sorption of ~~phosphorus~~ P to the soil was also evaluated to ascertain the capacity of soil to remove phosphate from soil solution. Eight different target P concentrations were made from a 50 mM stock solution containing ^{33}P in a background of 0.01 M ionic strength buffer. The solid phase sorption of each substrate was determined by shaking 2.5 g of soil with 12.5 ml of each ^{33}P-labeled solution. After known shaking times (i.e., 1 h and 24 h, 7 days, and 20 days), 1.5 ml were removed from the soil suspensions. The soil suspension removed was centrifuged (18,000 g, 5 min) and the supernatant (1 ml) recovered for ^{33}P determination. In the P sorption process, initially, P from the soil solution sticks externally to the soil aggregate, and then gradually penetrates into the aggregate. However, it is important to take into account that after a long time (e.g., 7 and 20 days), P may be taken up by microbes. In other words, it looks like absorption, but it is actually an immobilization by the microbial community. In our results, we observed a high sorption of P, which would have been expected due to the high adsorption of P on iron and aluminium oxides/hydroxides, commonly found in Brazilian soils (SN2 Figure 2).



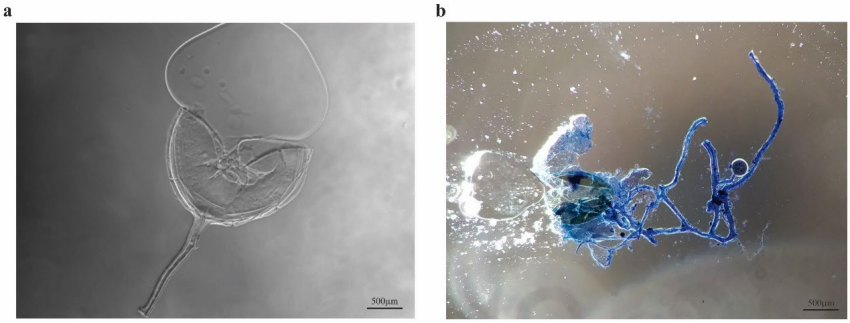
SN2 Fig 1. Soil diffusion microcosms representation used in the experiment of diffusion of P to soil (a). Diffusion of P to soil at 7 days (b) and 20 days after ^{33}P application (c). WHC: Water-holding capacity. Negative numbers represent the left side of the tube, while positive numbers represent the right side. The solid red line represents the ^{33}P application site. Standard errors are shown. *n*=3.



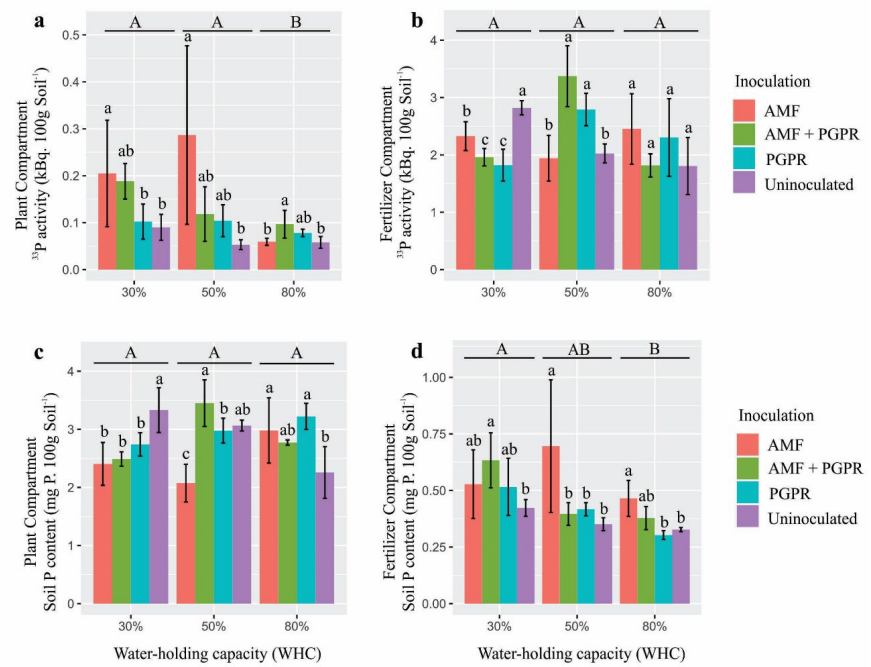
SN2 Fig 2. Sorption of P to soil as a function of the equilibrium of P solution concentrations (ESQ), considering the different times of evaluation. Standard errors are shown. *n*=3.

Supplementary Note 3. Experiment 3: aims, design, and results.

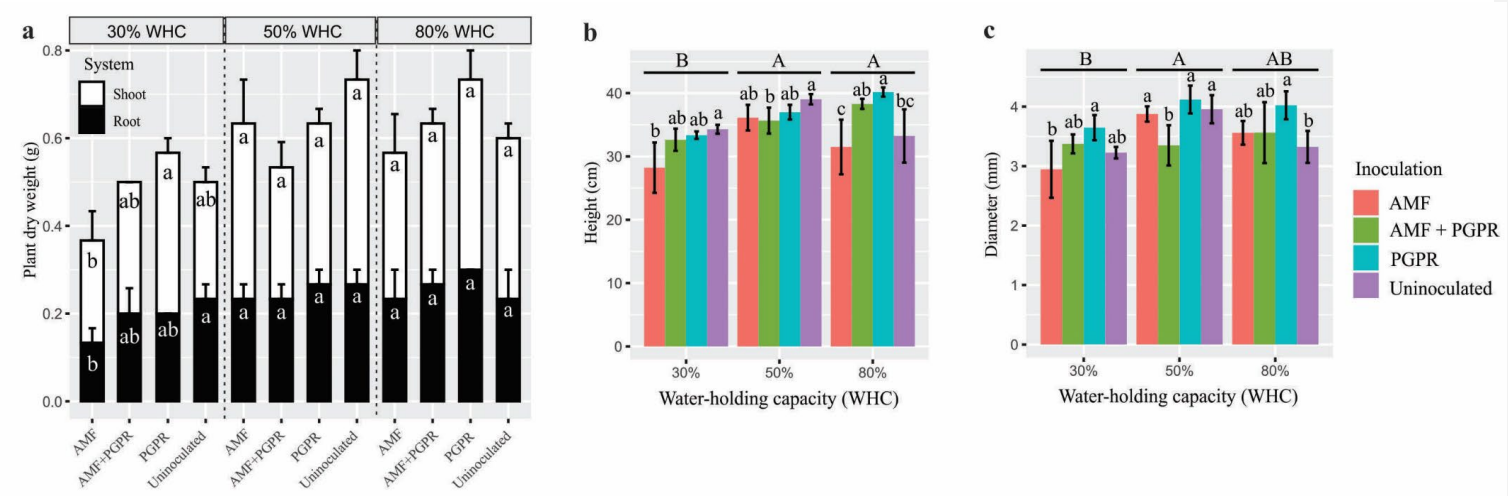
Arbuscular Mycorrhizal fungi spore germination in soil was taken according to INVAM (International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi). For this, *Rhizophagus clarus* spores were extracted from pure culture, washed repeatedly in tap water, and agitated in a bead beater for 4-5 minutes at 4800 rpm to remove the debris surface. A membrane filter (0.45 µm pores) was premoistened, and 25 spores were transferred to the filter under a stereomicroscope. Then, spores were redistributed, in a way that none were touching. Microcosms were filled with 200 g of sterile soil used in the previous experiment. Filters were folded in half and then in half again (with the spores inside). They were then buried in the soil mix of the microcosm, moistened with sterile distilled water, covered with foil, and then placed in a rack in growth chamber. Filters were removed after 3 weeks, opened, and placed in a glass Petri dish containing hot 0.05 % direct blue stain. After immersion for 30 seconds, the filter was transferred to a clean Petri dish and spores transferred to glass slides for permanent mounts in PVLG (Polyvinyl Lacto Glycerol) and examined. Overall, a spore germination rate of 80 % was seen after three weeks, including new spore formation, (SN3 Figure 1).



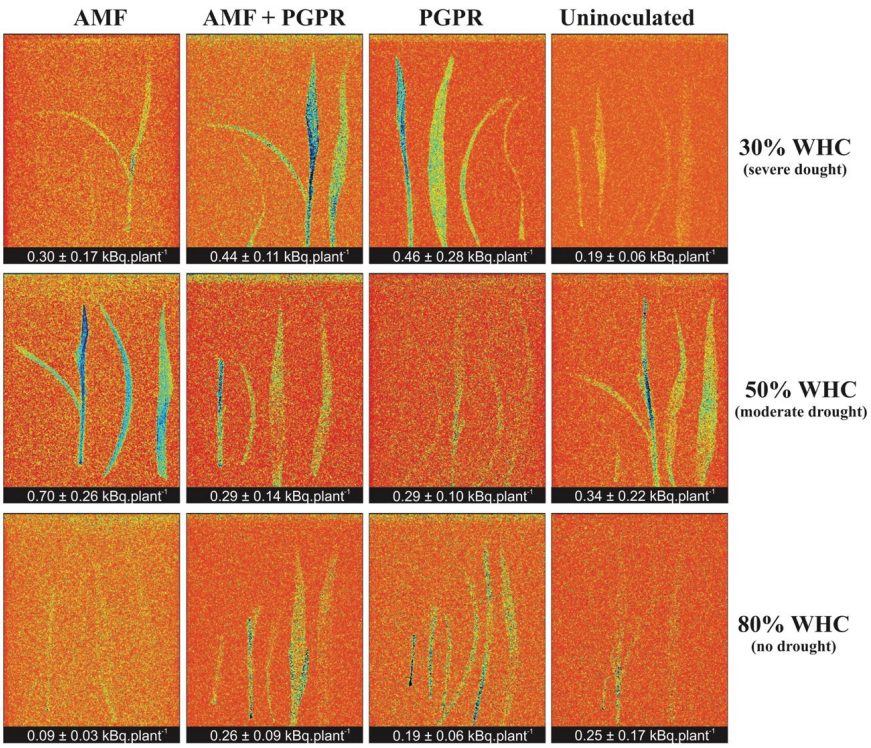
SN3 Fig 1.*Rhizophagus clarus* spore extracted from pure culture (a) and germinated spore after three weeks of incubation in soil (b).



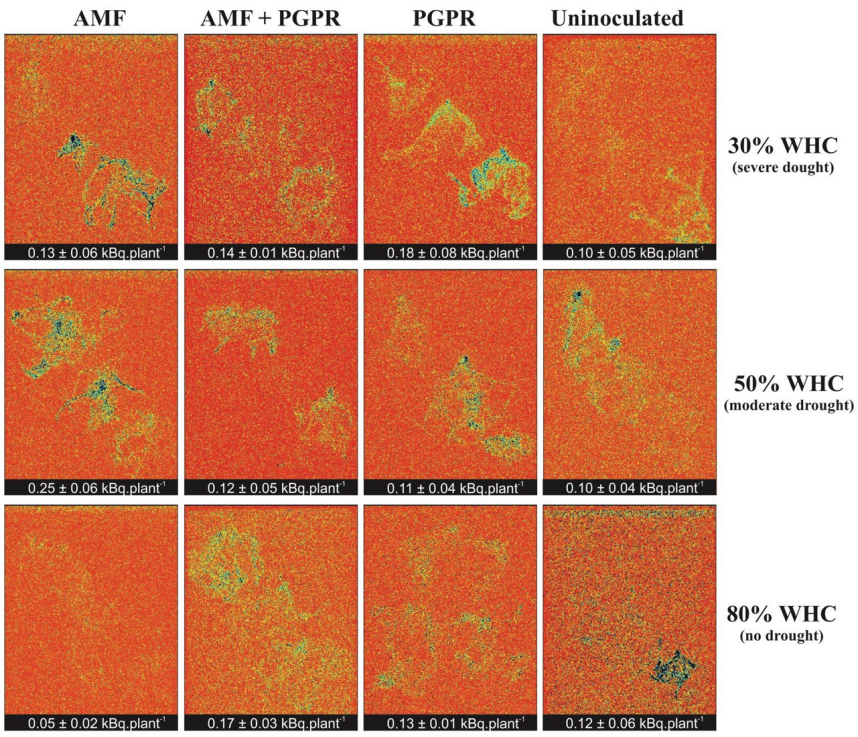
Supplementary Fig 1. Soil ^{33}P activity in the plant compartment (a) and fertilizer compartment (b). Soil P content in plant (c) and fertilizer compartments (d). Uppercase letters compare differences in water-holding capacity, while lowercase letters compare differences according to inoculant types by Tukey's test at 5 % ($p \leq 0.05$). Standard errors are shown ($n = 3$).



Supplementary Fig 2. Plant dry weight, considering root (black bar) and shoot (white bar) systems (a). Plant height (b) and diameter (c). Uppercase letters compare differences in water-holding capacity, while lowercase letters compare differences in inoculant types by Tukey's test at 5 % ($p \leq 0.05$). Standard errors are shown ($n = 3$).



Supplementary Fig 3. Phosphorimager results of shoots, considering type of inoculum (in the column) and the water-holding capacity (in the row), where the intensity of the blue colour indicates the presence of ^{33}P activity. The mean values are followed by the standard error (n = 3).



Supplementary Fig 4. Phosphorimager results of roots, considering type of inoculum (in the column) and the water-holding capacity (in the row), where the intensity of the blue colour indicates the presence of ^{33}P activity. The mean values are followed by the standard error (n= 3).

Supplementary Table S2. Soil pH, electrical conductivity (EC), acid phosphatase activity (Ac. Phosphatase) and alkaline phosphatase activity (Alk. Phosphatase) in fertilized compartment at three contrasting soil water-holding capacities (WHC) and with different types of inoculum (AMF and PGPR).

WHC	Inoculum	pH	EC ($\mu S.cm^{-1}$)	Ac. Phosphatase (nmol g^{-1} soil h^{-1})	Alk. Phosphatase (nmol g^{-1} soil h^{-1})
30%	AMF	7.1 ± 0.1 bA	88.0 ± 4.0 aA	37.7 ± 6.8 aB	7.5 ± 1.0 aA
	AMF+PGPR	7.3 ± 0.04 abA	94.0 ± 9.0 aA	17.2 ± 2.6 bB	5.6 ± 0.7 cA
	PGPR	7.4 ± 0.1 aA	92.0 ± 6.0 aA	13.4 ± 1.6 bB	5.9 ± 0.6 bcA
	Uninoculated	7.4 ± 0.01 aA	71.0 ± 2.0 bA	14.9 ± 0.5 bB	7.4 ± 0.5 abA
50%	AMF	7.2 ± 0.04 cA	88.0 ± 8.0 aA	22.7 ± 4.3 bB	6.2 ± 0.4 bA
	AMF+PGPR	7.4 ± 0.1 aA	79.0 ± 14.0 abA	21.7 ± 3.7 bB	7.0 ± 0.5 bA
	PGPR	7.2 ± 0.03 bcA	75.0 ± 6.0 abA	36.1 ± 4.5 aB	8.4 ± 0.8 aA
	Uninoculated	7.3 ± 0.01 abA	65.0 ± 3.0 bA	35.0 ± 3.0 aB	6.8 ± 0.2 bA
80%	AMF	7.4 ± 0.04 aA	67.0 ± 8.0 abB	37.0 ± 0.8 bA	7.5 ± 0.8 bA
	AMF+PGPR	7.2 ± 0.04 bcA	55.0 ± 6.0 bB	47.4 ± 9.6 aA	7.4 ± 0.3 bA
	PGPR	7.4 ± 0.05 abA	56.0 ± 1.0 bB	49.5 ± 6.9 aA	8.8 ± 0.6 bA
	Uninoculated	7.2 ± 0.05 cA	74.0 ± 4.0 aB	51.6 ± 3.8 aA	12.0 ± 1.9 aA

*Uppercase letters compare differences in water-holding capacity, while lowercase letters compare differences according to inoculant types by Tukey's test at 5 % ($p \leq 0.05$). Means values are followed by standard errors ($n = 3$).

Commented [DJ1]: Just checking these are not uS cm^{-1}

Commented [AMMS2R1]: I just checked the EC unit and It is uS cm^{-1}, indeed.

Supplementary Table S3. Total soil bacterial abundance (gene copy number g $soil^{-1}$) over sampling time based on the qPCR of 16S rRNA gene, considering the water-holding capacities (WHC), type of inoculum, and soil sampling taken 15, 25, and 35 days after sowing (DAS).

WHC	Inoculum	Sampling		
		15 DAS	25 DAS	35 DAS
30% SD	AMF	$3.2 \times 10^9 \pm 7.3 \times 10^8$ abA	$2.9 \times 10^9 \pm 1.4 \times 10^9$ aA	$2.0 \times 10^9 \pm 3.1 \times 10^8$ aA
	AMF+PGPR	$3.3 \times 10^9 \pm 7.8 \times 10^8$ abAB	$3.1 \times 10^9 \pm 1.6 \times 10^9$ aA	$1.4 \times 10^9 \pm 2.0 \times 10^8$ aB
	PGPR	$4.1 \times 10^9 \pm 1.2 \times 10^9$ aA	$4.5 \times 10^9 \pm 4.6 \times 10^7$ aB	$1.6 \times 10^9 \pm 5.1 \times 10^8$ aA
	Uninoculated	$1.9 \times 10^9 \pm 4.5 \times 10^7$ bA	$3.2 \times 10^9 \pm 4.4 \times 10^8$ aA	$2.0 \times 10^9 \pm 6.3 \times 10^8$ aAB
50% MD	AMF	$3.1 \times 10^9 \pm 9.6 \times 10^8$ abA	$4.1 \times 10^9 \pm 8.6 \times 10^7$ bA	$1.3 \times 10^9 \pm 2.3 \times 10^8$ aAB
	AMF+PGPR	$3.8 \times 10^9 \pm 1.7 \times 10^9$ aA	$2.9 \times 10^9 \pm 8.6 \times 10^8$ bA	$1.5 \times 10^9 \pm 3.1 \times 10^8$ aB
	PGPR	$2.0 \times 10^9 \pm 2.6 \times 10^7$ abB	$8.0 \times 10^9 \pm 5.0 \times 10^9$ aA	$1.5 \times 10^9 \pm 1.4 \times 10^8$ aA
	Uninoculated	$1.9 \times 10^9 \pm 2.2 \times 10^8$ bA	$2.1 \times 10^9 \pm 1.8 \times 10^8$ bA	$1.3 \times 10^9 \pm 1.8 \times 10^8$ aB
80% ND	AMF	$1.4 \times 10^5 \pm 6.3 \times 10^4$ bB	$2.9 \times 10^9 \pm 1.4 \times 10^8$ aA	$1.2 \times 10^9 \pm 2.1 \times 10^8$ cB
	AMF+PGPR	$2.2 \times 10^9 \pm 4.3 \times 10^8$ aB	$2.7 \times 10^9 \pm 3.3 \times 10^8$ aA	$2.9 \times 10^9 \pm 2.3 \times 10^8$ aA
	PGPR	$1.5 \times 10^9 \pm 7.4 \times 10^8$ abB	$2.2 \times 10^9 \pm 4.3 \times 10^8$ aB	$1.9 \times 10^9 \pm 6.7 \times 10^8$ bcA
	Uninoculated	$1.5 \times 10^9 \pm 6.0 \times 10^8$ abA	$4.8 \times 10^9 \pm 2.8 \times 10^9$ aA	$2.5 \times 10^9 \pm 7.5 \times 10^8$ abA

*Uppercase letters compare differences in water-holding capacity, while lowercase letters compare differences according to inoculant types by Tukey's test at 5 % ($p \leq 0.05$). Means values are followed by standard errors ($n = 3$). SD: severe drought, MD: moderate drought, ND: non drought

Supplementary Table S4. Total soil mycorrhizal abundance (gene copy number g soil^{-1}) over sampling time based on the qPCR using FLR3 and FLR4 primers, considering the water-holding capacities (WHC), type of inoculum, and soil sampling taken 15, 25, and 35 days after sowing (DAS).

WHC	Inoculum	Sampling		
		15 DAS	25 DAS	35 DAS
30% SD	AMF	$3.78 \times 10^3 \pm 2.20 \times 10^3$ aA	$1.75 \times 10^3 \pm 1.04 \times 10^2$ aB	$1.04 \times 10^5 \pm 9.15 \times 10^4$ aB
	AMF+PGPR	$2.89 \times 10^3 \pm 9.07 \times 10^2$ abA	$2.52 \times 10^3 \pm 6.82 \times 10^2$ aB	$1.20 \times 10^5 \pm 5.30 \times 10^4$ aA
	PGPR	$1.84 \times 10^3 \pm 4.01 \times 10^2$ abA	$1.55 \times 10^3 \pm 5.57 \times 10^2$ aB	$2.15 \times 10^4 \pm 1.20 \times 10^4$ aA
	Uninoculated	$1.12 \times 10^3 \pm 1.65 \times 10^2$ bA	$1.49 \times 10^3 \pm 1.70 \times 10^2$ aA	$1.02 \times 10^4 \pm 2.98 \times 10^3$ aA
50% MD	AMF	$5.63 \times 10^3 \pm 4.14 \times 10^3$ aA	$2.59 \times 10^3 \pm 7.75 \times 10^2$ aB	$2.36 \times 10^5 \pm 1.82 \times 10^5$ aA
	AMF+PGPR	$2.22 \times 10^3 \pm 1.90 \times 10^2$ bAB	$1.86 \times 10^3 \pm 3.64 \times 10^2$ aB	$3.65 \times 10^4 \pm 1.52 \times 10^4$ bAB
	PGPR	$1.10 \times 10^3 \pm 1.35 \times 10^2$ bA	$1.42 \times 10^3 \pm 3.32 \times 10^2$ aB	$1.50 \times 10^4 \pm 2.65 \times 10^3$ bA
	Uninoculated	$1.35 \times 10^3 \pm 7.21 \times 10^1$ bA	$1.78 \times 10^3 \pm 2.51 \times 10^2$ aA	$6.83 \times 10^3 \pm 5.49 \times 10^2$ bA
80% ND	AMF	$8.49 \times 10^2 \pm 2.86 \times 10^2$ aB	$9.71 \times 10^3 \pm 1.83 \times 10^3$ bA	$4.77 \times 10^4 \pm 3.97 \times 10^4$ aB
	AMF+PGPR	$7.28 \times 10^2 \pm 4.33 \times 10^1$ aB	$1.87 \times 10^4 \pm 4.42 \times 10^3$ aA	$1.05 \times 10^4 \pm 6.01 \times 10^2$ aB
	PGPR	$1.48 \times 10^3 \pm 1.94 \times 10^2$ aA	$7.26 \times 10^3 \pm 1.75 \times 10^3$ bA	$6.83 \times 10^3 \pm 5.52 \times 10^2$ aA
	Uninoculated	$2.00 \times 10^3 \pm 5.64 \times 10^2$ aA	$3.46 \times 10^3 \pm 1.52 \times 10^3$ cA	$5.66 \times 10^3 \pm 2.48 \times 10^2$ aA

*Uppercase letters compare differences in water-holding capacity, while lowercase letters compare differences according to inoculant types by Tukey's test at 5% ($p \leq 0.05$). Means values are followed by standard errors ($n = 3$). SD: severe drought, MD: moderate drought, ND: non drought.