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Interactions of Arbuscular Mycorrhizal Fungi with Hyphosphere Microbial Communities in a Saline Soil: Impacts on Phosphorus Availability and Alkaline Phosphatase Gene Abundance

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Abstract: The limited availability of soil phosphorus to plants under salinity stress is a major constraint for crop production in saline soils, which could be alleviated by improving mycorrhizal and soil microbial interactions. This study investigated the effects of *Funneliformis mosseae* (*Fm*) inoculation on phosphorus (P) availability to *Sorghum bicolor*, and alkaline phosphatase (ALP) activity and gene abundance (*phoD*) in a P-deficient naturally saline soil. A greenhouse study was conducted in order to compare the experimental treatments of *Fm* inoculated vs. control plants grown in saline soil with and without (sterilized soil) native microbial community. A separate hyphosphere (root-free) compartment was constructed within the mycorrhizosphere and amended with phosphate. After four weeks of transplanting, shoot, roots, mycorrhizosphere, and hyphosphere samples were collected and analyzed for soil and plant P concentrations, root colonization, and abundance of ALP and *phoD*. The results showed significantly higher colonization in *Fm*-inoculated treatments compared to uninoculated. Plant available P concentrations, *phoD* gene abundance and ALP activity were significantly reduced ($p < 0.05$) in sterilized-hyphosphere as compared to unsterilized in both *Fm*-inoculated and uninoculated treatments. Inoculation with *Fm* significantly increased the plant P uptake ($p < 0.05$) when compared to uninoculated treatments, but only in the plants gown in unsterile mycorrhizosphere. It can be concluded that inoculation of *Fm* increased root colonization and the uptake of P by sorghum plant in saline soil and native microbial community interactions were critical for increasing bioavailable P concentrations. These beneficial interactions between plants, mycorrhizae, and native microbes should be considered for soil fertility management in saline soils.

Keywords: AMF inoculation; root colonization in salinity stress; AMF-microbe interactions; P availability

1. Introduction

Saline soils occur on more than 10% of Earth's land surface [1], impacting agriculture productivity worldwide [1,2]. Under salinity stress, phosphorus availability to plants is severely impacted due to the poor solubility of phosphate minerals and complexation and/or precipitation of phosphates with Ca^{+2} and Mg^{+2} [3]. An increasing number of studies have clearly demonstrated that plants depend on symbiotic associations with microflora to acquire sparingly soluble P-minerals [4]. Arbuscular mycorrhizal fungi are at the center of these symbiotic associations with most plants [5]. However,

salt stress has been noted to diminish these symbiotic associations, which may further contribute to lower P availability under saline conditions [6].

Applying AMF species to improve symbiotic interactions is one of the potential avenues for addressing P-availability problems in saline soils [7]. The application of plant beneficial microbes, such as AMF, have proven to be effective in improving plant growth [8,9], especially under salt stress where the abundance of AMF is generally low [10]. Such enhancement of plant growth has been attributed to the production of phytohormones [11], improvements in N-fixation [12], and P-solubilization [13,14] by plant growth promoting rhizobacteria (PGPR), and improved soil structure and water uptake [15], P uptake [16,17], and increased K^+/Na^+ plant ratio [17,18] by AMF. However, many reports suggest that AMF inoculation does not always result in beneficial effects and higher yields in crops [19]. It is argued that the beneficial effects of AMF may also depend on their interactions with plants and other microbes [20], which needs to be clearly understood to improve beneficial effects of AMF [21].

It is well known that AMF are obligate biotrophs [10], i.e., they depend on host plant roots for growth and multiplication. However, it is not clear to what extent the AMF-plant interactions also depend on their interactions with other microflora to influence a specific function, such as phosphorus availability. Recent reports suggest that the beneficial effects of AMF inoculants are dependent on tripartite symbiotic interactions between plants, mycorrhizae, and other microbes in the rhizosphere or hyphosphere [22]. The rhizosphere is defined as the soil zone that is influenced by plant roots, whereas hyphosphere refers to the soil zone under the influence of mycorrhizal hyphae (and other microbes), but not roots (together, these two zones are called mycorrhizosphere) [23]. An increasing number of studies provide evidence on the role of rhizosphere bacteria in plant-AMF symbiosis and the need for considering these tripartite associations for better understanding of their functions [24].

Some studies suggest that several hyphosphere microbes are responsible for producing phosphatases for solubilizing phosphate minerals [25–27]. However, it is not clear whether AMF can also solubilize phosphate minerals or organic-P complexes, independently or in association with hyphosphere microbes [28,29]. These knowledge gaps must be addressed in order to decipher mechanistic interactions of introduced AMF species with native microbes, and their implications on P bioavailability to plants. Current knowledge on phosphatases in the rhizosphere is mostly based on culture-dependent methods [30,31], which do not provide a comprehensive assessment. Recent advances in culture-independent methods for studying phosphatase-encoding genes can be applied for comprehensively assessing beneficial interactions in rhizosphere [32]. For example, alkaline phosphatase (ALP) activity has been shown to strongly correlate with *phoD* bacterial gene abundance in agricultural soil [33] and also in stressed conditions, such as in saline soils [34]. However, knowledge regarding the effects of AMF interactions with native microflora on plant growth in salt-stressed soils is limited. Some reports on co-inoculation with single or a few species of PGPR and AMF exist, but these have mostly been conducted under artificial salinity [35–37].

We hypothesized that supplementing an AMF species through inoculation would increase root colonization and P uptake by plants under salinity stress. We further hypothesized that the indigenous saline soil microbial community is essential for increasing P availability in the hyphosphere. The objectives of this study were to determine the AMF inoculation effects on root colonization and P concentrations in plant shoot, and to quantify plant-available P concentrations, phosphatase gene abundance and ALP activity in the hyphosphere, as affected by native microflora in a saline soil.

2. Materials and methods

2.1. Soil

Surface soil (top 0–30 cm) was collected near the Texas A&M AgriLife Research & Extension Center at Pecos in Reeves County, Texas, USA (Coordinates: 31°22′45.8″N 103°37′32.4″W). The soils in this region are naturally saline and moderately alkaline. The soil series was a Dalby clay and classified

as Fine, smectitic, frigid Oxyaquic Vertic Hapludalfs (NRCS, USDA. Web Soil Survey). No vegetative cover or recent agricultural practices were present at the site where the soil was collected. Table 1 lists the characteristics of the collected soil sample. The soil texture was determined by a hydrometer [38], and the percent organic matter content was determined by the wet oxidation method [39]. Soil pH (in H_2O), EC (saturated paste extract), and soil P (Mehlich-3) were determined by the Soil, Water, and Forage Testing Laboratory in the Department of Soil and Crop Sciences, Texas A&M University.

Table 1. Characteristics of the Dalby clay soil used in this study.

Parameter	Value
pH	8.5
EC (saturated extract, dS/m)	6.32
P (Mehlich-3, mg/kg)	45
Nitrate-N (mg/kg)	26
Potassium (mg/kg)	614
Organic matter content (%)	0.34
Clay (%)	46.7
Silt (%)	20.7
Sand (%)	32.6

2.2. AMF Inoculum and Plant Host

The AMF species used in this experiment was *Funneliformis mosseae* (*Fm*), (collected form an alkaline soil), obtained from INVAM (International Vesicular Arbuscular Mycorrhizal collection facility, University of West Virginia, accession code UT101) as whole inoculum containing different AMF propagules (soil with spores [average of 109 spores/gram inoculum], infected root pieces, and hyphae). We selected several AMF species, including *Fm* based on their performance in saline soils [40,41]. We used *Fm* for this study, as it produced higher root colonization when compared to other AMF species in our prior study [42]. *Sorghum bicolor* was used as the plant host in this experiment, since it is a moderately salt tolerant plant, which is commonly used as a mycorrhizal host [6], and it is suitable for the EC level of this experimental soil.

2.3. Experimental Design and Growth Conditions

The experimental design in this experiment was a 2 × 2 × 2 factorial completely randomized design with three replicates (three pots) for each treatment. This experiment was conducted using two compartment microcosms (inner (I) hyphosphere and outer (O) rhizosphere compartments) separated with 25 µm nylon mesh (LAB PACK, Sefar Inc., Buffalo, NY, USA) to allow hyphal penetration, but not roots (Figure 1). The hyphosphere compartment was a mini rectangular box (4.5-cm long, 2.5-cm wide, 1.5-cm height) (The Container Store Inc, Coppell, TX, USA) containing 12.5 g soil/box (2 boxes/pot, placed at depth of 5 cm, each box facing the root system). The rhizosphere compartment was a small square nursery pot (6.5-cm diameter, 9-cm long, 280-mL volume) containing 235 g soil. The soil in compartments had four sterilization treatments: both inner (hyphosphere) and outer (rhizosphere) compartments sterilized (IS-OS), inner sterilized and outer unsterilized (IS-OU), inner unsterilized and outer sterilized (IU-OS), and both unsterilized (IU-OU). The soils were sterilized by autoclaving for 1 hr at 121 °C three times, on three consecutive days. Soil in the rhizosphere (outer) compartment was amended with NH_4NO_3 at 50 mg N/kg soil. The hyphosphere compartments were amended with 200 mg P/kg soil as Na-phytate (Santa Cruz Biotechnology, Santa Cruz, CA, USA) as organic P (Po) and 200 mg P/kg soil rock phosphate as inorganic P (Pi). The plant seeds were sterilized with 10% sodium hypochlorite for 20 min., rinsed five times with sterile water, and the germinated in plug tray cells (cell size 7/8″ deep and 9/16″ wide, Harris Seeds Inc., Rochester, NY, USA) containing 2 g inoculum (either *Fm* or no-*Fm* control inoculum) and 2 g sterile low P sandy soil to promote AMF infection. After 12 days, seedlings with attached soils from the tray cells were transplanted to the designed pots of this experiment. The plants were grown for 42 days after transplanting in a growth

chamber at 25 °C day/21 °C night, 16 h/8 h light/dark, 60% humidity, and 500 µmol/m^2/s light intensity, and watered every other day to 85% water holding capacity (determined based on maximum water holding capacity) [43] while using sterilized distilled water.

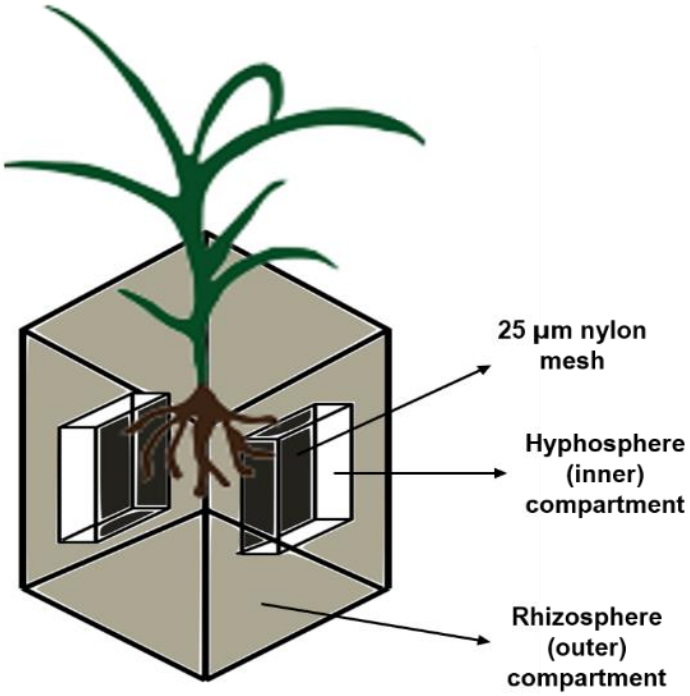

Figure 1. Diagram of the designed microcosm having rhizosphere and hyphosphere compartments separated by a nylon mesh.

2.4. Root Staining and AMF Colonization

Microcosms were terminated at approximately 42 days after transplanting. The plants were gently removed from the pots and shoot were separated from the root system. Shoots were placed in an oven at 60 °C for 48 h, and then stored for later analysis. The roots were gently removed from soil and washed under tap water, and then stained with trypan blue using a modified procedure of Phillips and Hayman [44]. Briefly, the roots were placed in tissue cassettes (Fischer Scientific Inc., Hampton, NH, USA) and then submerged in pre-boiled 10% KOH for 10 min. to remove cytoplasmic content of root cells. Cassettes were then washed 5X with tap water and submerged in 2% HCl for 30 min., followed by 5X washing with tap water. The cassettes were then submerged in pre-boiled 0.05% trypan blue solution (water, glycerin, lactic acid in 1:1:1 (v/v/v)) for 5 min. The cassettes were then washed 5X with tap water and stored at 4 °C for 3–5 days immersed in distilled water in order to remove excess stain. The percentage of AMF colonization was then determined while using the gridline intersect method [45].

2.5. Soil Extractable P and Plant Shoot P Concentration

The top surface layer (~2 mm) of the hyphosphere compartments was removed and discarded in order to reduce biases and possible exchange of microbes and nutrients between the rhizosphere and hyphosphere compartments. The remaining soil from the hyphosphere compartments of each pot (two compartments) were then mixed to have one homogenized hyphosphere soil sample/pot and stored at −80 °C for later molecular and enzyme assays. A portion of the soil samples (all three replicates for individual treatments) from the hyphosphere compartments (stored at −80 °C) and dried plant shoots were submitted to the Soil, Water, and Forage Testing laboratory at Texas A&M University (College Station, TX, USA) in order to measure extractable P in soil (Mehlich-III) and determine P concentration

in plant shoot tissue while using inductively coupled plasma mass spectrometry equipped with a charge coupled device (SPECTRO Analytical Instruments, Kleve, Germany).

2.6. Phosphatase Gene Quantitation in Hyphosphere Soil

Soil DNA was extracted from 0.5 g of the frozen hyphosphere soil samples while using a PowerSoil DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA) following the manufacturer's instructions. After extraction, all of the DNA samples were quantified to detect DNA quality while using a Nanodrop ND-1000 spectrophotometer (Thermo-Fisher Scientific Inc., Wilmington, DE, USA).

Quantitative real-time PCR (qPCR) was used to quantify the abundances of microbial *phoD* (alkaline phosphatase), total bacterial 16S rRNA, total AMF 18S rRNA, and total fungal internal transcribed spacer (ITS) gene targets in hyphosphere soil (root-free soil). Each qPCR run was setup to include appropriate quality controls (positive, negative, no template controls, check gBlock standards, and spikes). The gBlock standards and quality control details are outlined in Table S1 and Table S2, respectively. Table S3 outlines the primers (obtained from Integrated DNA Technologies Inc. Collierville, IA, USA), qPCR conditions, and references used. Amplifications of DNA was performed while using Rotor-Gene SYBR® Green qPCR kit, with gene abundance measured using Rotor-Gene Q Software version 2.3.1.49 (QIAGEN, Hilden, Germany).

2.7. Alkaline Phosphatase Enzyme Assay

Potential soil alkaline phosphatase (ALP) activity was measured from the frozen hyphosphere soil (−80 °C) using a modified assay of Tabatabai and Bremner [46]. Briefly, 0.5 g soil in duplicate was incubated in 0.0625 M *p*-nitrophenyl phosphate substrate (Sigma–Aldrich, St. Louis, MO, USA) along with modified universal buffer solution (pH 11) at 28 °C in 2 mL deep-well plates. After 2 h, the reactions were stopped with 2.5 M $CaCl_2$ and 2.5 M NaOH. The plates were then shaken for 5 min. and centrifuged for 5 min. at 500 rpm. Using 96-well plates, the formation of *p*-nitrophenol was determined colorimetrically using a Biolog Microstation Elx808BLG (BIO-TEK Instruments Inc., Winooski, VT, USA) spectrophotometer at 405 nm.

2.8. Statistical Analysis

All of the treatment effects were statistically analyzed using Three-Way ANOVA in SAS software (version 9.4), while using PROC GLM procedure. Differences between treatments were obtained using Fisher's least-significant-difference (LSD) test at a *p*-value of <0.05.

3. Results

3.1. Root Colonization Effects of Experimental Treatments

As expected, the percentages of AMF root colonization (Table 2) were significantly higher ($p < 0.0001$) in *Fm*-inoculated IS-OS and IU-OU treatments as compared to all uninoculated treatments, while *Fm*-inoculated IS-OU and IU-OS were significantly higher only compared to uninoculated IS-OS and IU-OS. However, within *Fm*-inoculated treatments, root colonization was significantly lower in IS-OU and IU-OS treatments when compared to the IU-OU treatment. The highest colonization was detected in IU-OU treatment, where none of the compartments were sterilized.

Table 2. Impact of soil compartment sterilization on AMF colonization.

AMF Inoculum	Treatment	% Root Colonized by AMF
None	IS-OS	0 ± 0 e
	IS-OU	2.91 ± 2.6 ecd
	IU-OS	0.05 ± 0.08 e
	IU-OU	1.95 ± 1.5 ed
Fm	IS-OS	25.9 ± 15.1 ba
	IS-OU	15.30 ± 6.2 bc
	IU-OS	14.47 ± 7.6 bcd
	IU-OU	30.82 ± 10.7 a

Note: IS-OS: soil in inner (hyphosphere) and outer (rhizosphere) compartments sterilized; IS-OU: inner sterilized and outer unsterilized; IU-OS: inner unsterilized and outer sterilized; IU-OU: both unsterilized. "None": control AMF inoculum. "Fm": inoculated with Funneliformis mosseae. Data are presented as the mean ± s.d (n = 3). Different letters indicate significant difference ($p < 0.05$).

3.2. Plant Available P in Hyphosphere Soil and Its Uptake by Plants

Figure 2A presents plant available-P concentrations in hyphosphere soils. Treatments with unsterilized soil in the hyphosphere (IU-OS and IU-OU) had significantly higher P concentrations as compared to sterilized soils (IS-OU and IS-OS) in both *Fm*-inoculated and uninoculated treatments. In IS-OS treatments of both *Fm*-inoculated and uninoculated, P concentrations in hyphosphere compartments were reduced by 20% and 18.7%, respectively, when compared to IU-OU. Similarly, in IS-OU treatments of both *Fm*-inoculated and uninoculated, extractable P in hyphosphere compartments was reduced by 11.8% and 10%, respectively, as compared to IU-OS. On the other hand, inoculation with *Fm* significantly increased P concentrations in plant shoots as compared to uninoculated ones in IS-OU and IU-OU treatments (Figure 2B). In contrast, inoculation with *Fm* did not significantly impact plant-P uptake in IS-OS and IU-OS treatments when compared to uninoculated ones.

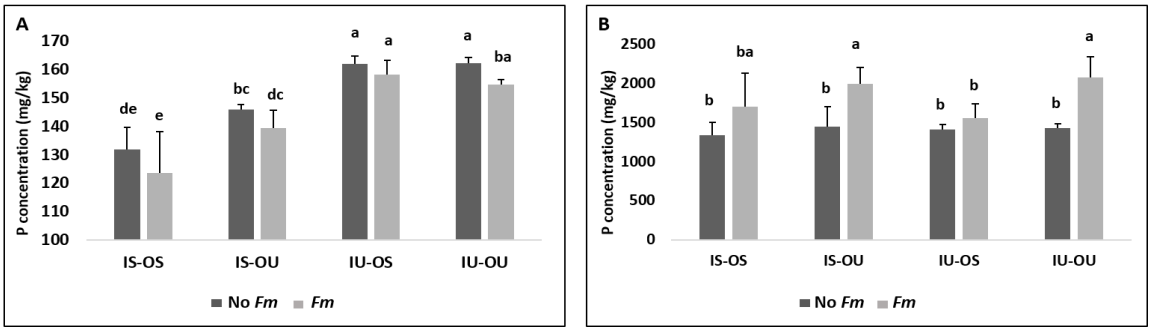

Figure 2. Impact of AMF inoculation and soil sterilization on extractable soil P and its uptake by plants. (**A**): Extractable P (Mehlich-3) in hyphosphere soil. (**B**): P concentrations in plant shoot. inner (hyphosphere) and outer (rhizosphere) compartments sterilized (IS-OS): soil in inner (hyphosphere) and outer (rhizosphere) compartments sterilized; inner sterilized and outer unsterilized (IS-OU): inner sterilized and outer unsterilized; IU-OS: inner unsterilized and outer sterilized; IU-OU: both unsterilized. "*Fm*": inoculated with *Funneliformis mosseae*. Data presented are mean with ± standard deviation (n = 3). Different letters above the bars indicate significant difference between the treatments ($p < 0.05$).

3.3. Alkaline Phosphatase Gene (phoD) and Microbial Community Abundance in the Hyphosphere

Alkaline phosphatase gene (*phoD*) abundances were significantly reduced in sterilized hyphosphere when compared to unsterilized in both *Fm* and non-*Fm*-inoculated treatments (Figure 3A). In the *Fm*-inoculated treatment, sterilization reduced *phoD* gene abundance by 78.3%, while, in the uninoculated treatment, the abundance was reduced by 77.7%. Within unsterilized soils, *phoD* gene abundance was also significantly higher in the *Fm*-inoculated treatments as compared to uninoculated

treatments. No significant differences were found in the abundance of 16S rRNA and AMF 18S rRNA genes between the sterilized and unsterilized treatments (Figure 3B,C, respectively). However, hyphosphere fungal ITS abundance was significantly higher in unsterilized soils when compared to sterilized ones (Figure 3D). Moreover, when comparing *phoD* relative proportions among the total microbial community abundance (total of 16S rRNA and fungal ITS gene abundances), the *phoD* proportions ranged from 0.30 in the uninoculated IS-OS up to 0.71 in the *Fm*-inoculated IU-OU (Figure 4).

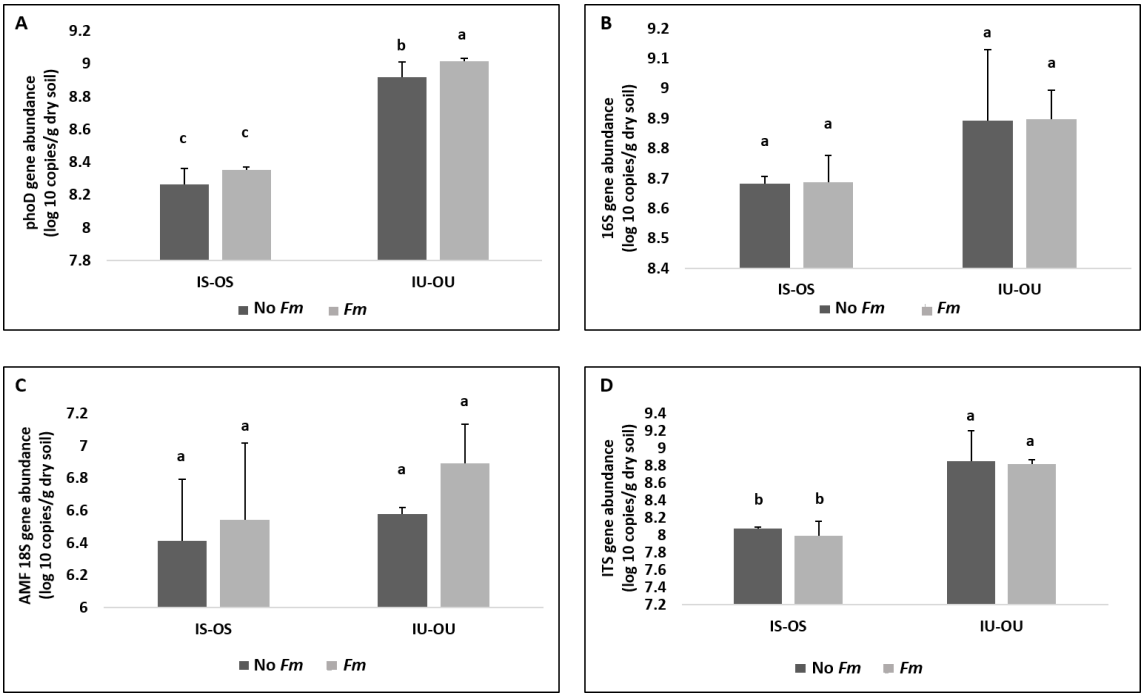

Figure 3. Abundances of targeted genes in hyphosphere soil. (**A**): *phoD* gene. (**B**):16S rRNA gene. (**C**): AMF 18S rRNA. (**D**): fungal ITS. IS-OS: soil in inner (hyphosphere) and outer (rhizosphere) compartments sterilized; IU-OU: both soils unsterilized. "*Fm*": inoculated with *Funneliformis mosseae*. Data presented are mean with ± standard deviation (n = 3). Different letters above the bars indicate significant difference between the treatments ($p < 0.05$).

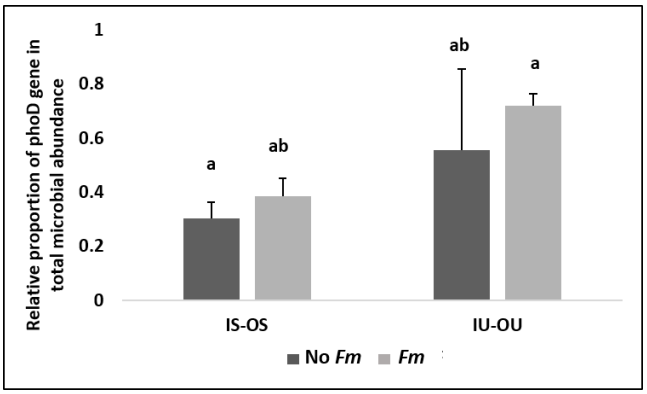

Figure 4. Relative proportion of *phoD* gene among the total microbial community abundances (as total 16S rRNA and ITS) in hyphosphere soil. 1 = 100%. IS-OS: soil in inner (hyphosphere) and outer (rhizosphere) compartments sterilized; IU-OU: both soils unsterilized. "*Fm*": inoculated with *Funneliformis mosseae*. Data presented are mean with ± standard deviation (n = 3). Different letters above the bars indicate significant difference between the treatments ($p < 0.05$).

3.4. Alkaline Phosphatase Enzyme Assay

Figure 5 shows the potential activity of soil alkaline phosphatase (ALP) from the hyphosphere soils. The activity of ALP showed significant differences between all treatments. Soil sterilization significantly reduced ALP activity when compared to unsterilized soils in both *Fm*-inoculated (reduction by 78%) and uninoculated (reduction by 70%) treatments. Moreover, *Fm* inoculation resulted in significantly less ALP activity for both unsterile and sterile soils as compared to uninoculated ones. In sterilized soils, *Fm* inoculation reduced ALP activity by 76% compared to uninoculated treatment. Similarly, in unsterilized soils, *Fm* inoculation reduced ALP activity by 23.8% as compared to uninoculated treatment.

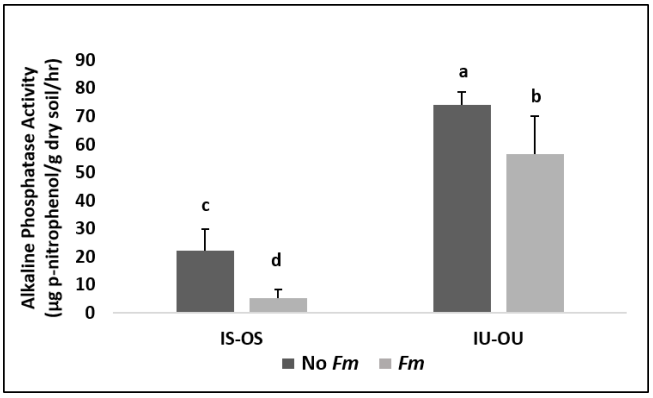

Figure 5. Potential soil alkaline phosphatase (ALP) activity in the hyphosphere soils. IS-OS: soil in inner (hyphosphere) and outer (rhizosphere) compartments sterilized; IU-OU: both soils unsterilized. "*Fm*": inoculated with *Funneliformis mosseae*. Data presented are mean with ± standard deviation ($n = 3$). Different letters above the bars indicate significant difference between the treatments ($p < 0.05$).

4. Discussion

The results of this study indicated that inoculation of sorghum using a potentially salt-tolerant AMF species, such as *Fm*, was effective in increasing the root colonization in a saline soil. Several reports have indicated a similar response under artificial inoculation in saline soils [40,41,47]. One reason for lower root colonization by native AMF may be due to lack of host compatible and competitive AMF species [10,48]. In addition, the absence of vegetative cover where the soil was collected may have further contributed to the lower abundance of native AMF. The results also indicated the potential synergistic interactions between AMF and native microflora and their role in affecting percentage of colonization, since the highest colonization under *Fm* inoculation was noted when both of the compartments of rhizosphere and hyphosphere were not sterilized (although this percentage was not significant when compared to IS-OS treatment). Studies have shown that specific soil microbes, such as mycorrhizal helper bacteria, can promote hyphal growth and root colonization [49], and that suppression or stimulation of AMF growth and colonization is related to microbial composition in soils [50]. A recent report by Ordoñez et al. [51] also found that some bacterial strains strongly affect AMF colonization inside roots and hyphae growth outside roots, and that soil microbial community might have a role in limiting or increasing this effect, depending on the P-solubilizing microbial species [51].

The results also indicated that native microbial communities play a critical role in improving plant available P concentrations in the hyphopshere. This is based on the results that sterilization significantly reduced plant available-P concentrations in hyphosphere soils (IS-OS and IS-OU) compared to unsterilized treatments (IU-OS and IU-OU) in both *Fm*-inoculated and uninoculated treatments. This finding supports our hypothesis that native communities are important for improving plant available-P concentrations, whereas AMF was mostly responsible for transferring solubilized P to plants, as suggested by several studies [7,27,41].

Potential ALP enzyme activity and its gene (*phoD*) abundance results further validated the role of native communities in increasing plant-available P concentrations in the hyphosphere. The relative abundance of *phoD* was significantly reduced in sterilized treatments (IS-OS) in both *Fm*-inoculated (by 78.3%) and uninoculated (by 77.7%) treatments when compared to unsterilized soils (IU-OU). Within unsterilized treatments, inoculation with *Fm* led to significantly higher *phoD* gene abundance compared to uninoculated. Subsequently, higher plant tissue P concentrations noted in the *Fm*-inoculated IU-OU treatment compared to uninoculated IU-OU could be mostly due to *phoD*- community (bacteria and fungi) solubilizing and mineralizing Pi/Po complexes, which was then transported by *Fm* to plant roots. However, there were no differences found between *Fm* inoculated IS-OS and IU-OU in terms of plant tissue P concentrations. This could be related to the one-timepoint sampling that we used in this study. Perhaps, differences in tissue P concentrations could have been more apparent if plants were growing for a longer period. These results support our hypothesis that AMF and indigenous microbe interactions were synergistic and increased P availability and plant uptake. These are novel findings suggesting that synergistic interactions between native bacteria and AMF were essential to increase P solubilization and uptake in saline soils. It was also clear that synergistic interactions were not limited to native AMF, but they extended to exogenously introduced AMF, which appeared to be more efficient in colonizing and transporting solubilized P.

Similar trends were observed for ALP activity in the hyphosphere, as soil sterilization significantly reduced ALP activity when compared to unsterilized soils in both *Fm*-inoculated (reduction by 78%) and uninoculated (reduction by 70%) treatments. However, inoculation with *Fm* significantly reduced ALP activity in the hyphosphere soils, contrary to the trends observed for *phoD* gene abundance. It is not clear why ALP activity was higher in *Fm* uninoculated treatment as compared to inoculated treatment. One reason could be root induced ALP activity in treatments without *Fm* inoculation that were in need of more P uptake (due to lower tissue P concentrations). Yet, plants still need AMF to transport P (P concentration was higher in plants with *Fm* inoculation). Several studies in saline soils have demonstrated root ALP activity in response to P availability and demand by plants [35,36,52]. Furthermore, some discrepancies between gene abundance and enzyme activity is anticipated, as it is known that some microbial species induce higher transcription rates [33]. Additionally, our qPCR assays did not include other ALP encoded genes that have been identified in the Pho regulon, such as *phoA* and *phoX*, as 32% of sequenced prokaryotic genomes contain at least one of these three genes [53], although, the *phoD* gene has been identified as the key ALP encoded gene in soils [54]. These results indicate the possibility of microorganisms (and/or factors) were responsible for inducing ALP activity in the absence of extensive inoculation by AMF. Further exploration of these factors could be valuable for inducing ALP activity when AMF inoculation is not feasible.

5. Conclusions

It can be concluded from this study that artificial inoculation of AMF significantly increased the root colonization under saline stress. The hyphosphere microbial community was mostly responsible for increasing plant available-P concentrations in the hyphosphere, whereas *Fm* inoculation was mostly responsible for increasing P uptake. Soil extractable P, *phoD* gene abundance, and ALP activity were reduced in sterile soil lacking native microflora. The results clearly showed that synergistic interactions between AMF and the naive community can potentially increase P availability in saline soils and could be a promising tool for soil fertility management and sustainable agriculture production in saline soils.

Supplementary Materials: The following are available online at http://www.mdpi.com/2571-8789/4/4/63/s1: Table S1: Details of gBlock qPCR standards, dilution range had 1 order of magnitude apart between each of 5 standards; Table S2: Quality control details of the qPCR runs; Table S3: Primers and conditions used for the qPCR assays in this study.

Author Contributions: Conceptualization, A.M.; Methodology, A.M., A.S. and T.G.; Writing—original draft, A.M.; Writing—review & editing, A.S. and T.G. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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