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Chemical alteration of the rhizosphere of the mycorrhizal-colonized wheat root

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Abstract Plexiglass pot growth chamber experiments were conducted to evaluate the chemical alterations in the rhizosphere of mycorrhizal wheat roots after inoculation with *Glomus intraradices* [arbuscular mycorrhizal fungus (AMF)]. Exchange resins were used as sinks for nutrients to determine whether the inoculated plant can increase the solubility and the uptake of P and micronutrients. Treatments included: (1) soil (bulk soil); (2) AMF inoculation 5no P addition (I−P); (3) no inoculation with no P addition (NI−P); (4) AMF inoculation with addition of 50 mg P (kg soil)$^{-1}$ (I+P), and (5) no inoculation with addition of 50 mg P (kg soil)$^{-1}$ (NI+P). The AMF inoculum was added at a rate of four spores of *G. intraradices* (g soil)$^{-1}$. The exchange resin membranes were inserted vertically 5 cm apart in the middle of Plexiglass pots. Spring wheat (*Triticum aestivum* cv. Len) was planted in each Plexiglass pot and grown for 2 weeks in a growth chamber where water was maintained at field capacity. Rhizosphere pH and redox potential (Eh), nutrient bioavailability indices and mycorrhizal colonization were determined. Mycorrhizal inoculation increased the colonization more when P was not added, but did not increase the shoot dry weight at either P level. The rhizosphere pH was lower in the inoculated plants compared to the noninoculated plants in the absence of added P, while the Eh did not change. The decrease in pH in the rhizosphere of inoculated plants could be responsible for the increased P and Zn uptake observed with inoculation. In contrast, Mn uptake was decreased by inoculation. The resin-adsorbed P was increased by inoculation, which, along with the bioavailability index data, may indicate that mycorrhizal roots were able to increase the solubility of soil P.

Keywords Wheat · Mycorrhiza · Rhizosphere pH and Eh · Exchange resins · Bioavailability index

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Introduction

It has been suggested that arbuscular mycorrhizal and non-mycorrhizal plants utilize the same sources of P (Gianinazzi-Pearson et al. 1981; Asea et al. 1988), and that mycorrhizal plants do not utilize unavailable P sources (Jakobsen et al. 1994). However, Bolan et al. (1984), in an experiment using ^{32}P, suggested the possibility of mycorrhizal plants taking up forms of P not accessible to non-mycorrhizal plants. In addition, Bago and Azcon-Aguilar (1997) reported that rhizosphere acidification by arbuscular mycorrhizal fungi (AMF) mobilizes P from sources that are unavailable to non mycorrhizal plants.

Investigating the mechanisms by which AMF enhances nutrient uptake is difficult. The use of routine soil testing is not always appropriate to predict plant responses to AMF inoculation (Abrams and Jarell 1992). Most soil tests extract a certain fraction of the nutrients from the soil. The correlation between this fraction and plant availability is influenced by the physical and chemical environment of the soil (Schoenau and Huang 1991). Thus, to evaluate whether AMF had access to, or can release, some of the unavailable sorbed P and micronutrients from the soil, one should use a method that takes into account all factors affecting nutrient availability. These factors include nutrient buffer capacity, diffusion characteristics of soil, amount of nutrients in the soil solution and on the solid phase, and other physical and chemical properties of the soil (Kamprath and Watson 1980).

Several mechanisms have been suggested by which the mycorrhizal plant can enhance growth and nutrient uptake (Miyasaka and Habte 2001). Mycorrhizal plants can (1) explore a greater volume of soil beyond the zone of P

depletion (Kothari et al. 1991a), (2) lower the threshold concentration for absorption from soil solution (O'Keefe and Sylvia 1991), (3) produce exudates that enhance the availability of P (Tawaraya and Saito 1994; Bethlenfalvay et al. 1997), (4) alter the rhizosphere pH as a result of anion and cation absorption by the mycorrhizal plants—which may affect the availability of P to the plant (Bago and Azcon-Aguilar 1997), (5) alter the microbial population that can solubilize P, and (6) solubilize organic P by the production of phosphatase (Tarafdar and Marschner 1994).

Many different approaches, such as glass beads (Chen and Christie 2001), anion and cation exchange resins (Abrams and Jarrell 1992), and double pot systems (Ness and Vlek 2000), have been used to study plant/nutrient/microbe interactions. Anion and cation exchange resins have been shown to mimic the root system in terms of absorbing nutrients from the soil. Therefore, they have the potential to be used in studying the dynamics of nutrient cycling in the soil-plant system and the mechanisms by which AMF enhance nutrient uptake. In addition, exchange resins have been used to determine a bioavailability index, which can be used to predict plant responses to fertilizers and to mycorrhizal fungi (Abrams and Jarell 1992). Mycorrhizae increase the uptake of the nutrients that move to plant roots mainly by diffusion, particularly in dry soil when nutrient diffusion rates are most limited. Exchange resins have been used to predict responses and dependencies of crops to mycorrhizae (Ojala et al. 1983).

Little research has been conducted to investigate chemical alterations in the rhizosphere caused by AMF inoculation (Clark and Zeto 2000). In non-wetland species grown in aerated soil, reliable data on the redox potentials in the rhizosphere and in the bulk soil are quite rare. The objectives of this study were (1) to determine whether AMF inoculation of wheat alters rhizosphere pH and redox potential (Eh), (2) to use the exchange resin as a sink for nutrients to evaluate AMF inoculation effects on soil nutrient availability, and (3) relate these potential mycosphere effects to wheat uptake of P and micronutrients.

Materials and methods

Growth chamber experiment

Surface soil (top 30 cm) from the Palouse silt loam (fine-silty, mixed, mesic Pachic Ultic Haploxerolls) was collected from the sideslope of an eroded Palouse toposequence near Pullman (Wash.). The sideslope was highly eroded compared with the toeslope position. This erosion has resulted in the depletion of essential plant nutrients such as P, low organic matter, decreased soil productivity (Pan and Hopkins 1991), and a possible decrease in AMF population (Day et al. 1987). The soil was air-dried and sieved through a 5 mm screen. Water content at −0.03 MPa was estimated for each soil by the pressure plate method (Richards 1965). Soil samples were analyzed for pH in a saturated paste, P by extraction with sodium acetate (Peech and English 1944), and organic matter by rapid oxidation (Nelson and Sommers 1982). Soil fertility characteristics, including P, are provided in Table 1.

Wheat seedlings were grown in Plexiglass pots with 5×15×20 cm dimensions. The soil treatments comprised: (1) soil only with low level of P (bulk soil), (2) AMF inoculation and no added P (I−P), (3) no AMF inoculation and no added P (NI−P), (4) AMF inoculation with addition of 50 mg P (kg soil)$^{-1}$ (I+P), and (5) no AMF inoculation with addition of 50 mg P (kg soil)$^{-1}$ (NI+P). Each treatment received 250 mg N (kg soil)$^{-1}$ as ammonium nitrate and 40 mg S (kg soil)$^{-1}$ as ammonium sulfate. Each pot received 1,900 g soil sieved to pass 2 mm. The AMF inoculum was added at a rate of four spores of *Glomus intraradices* (g soil)$^{-1}$ using a commercial inoculum (Nutri-Link, Salt Lake City, Utah). The bacterial count found in the inoculum was low [$<1.2\times10^3$ colony forming units (cfu) g^{-1} on tryptic soy agar]. Less than 1% of individual bacterial isolates exhibited phosphatase activity. We added less than five bacterial cells per gram of soil to the soil in the Plexiglass pots. The addition of bacteria from the inoculum was extremely low and the inoculum had little if any phosphatase activity due to bacteria. The inoculum was thoroughly mixed with the soil. The treatments were replicated three times. Non-inoculated pots received no spores. The exchange resin membranes were inserted vertically 5 cm apart in the middle of the Plexiglass pot. Pots were then watered to the soil moisture at which free drainage occurred, approximating field capacity, and placed in the growth chamber to allow the soil water to equilibrate before planting.

Spring wheat (*Triticum aestivum* cv. Len) seeds were germinated on germination paper. Three seedlings were then transplanted into each Plexiglass pot. The moisture content of the pot was maintained daily by weighing the pot and replenishing water lost to evapotranspiration. The pots were positioned at a 25-degree angle with a removable Plexiglass cover facing downward to force the root to grow against it. This facilitated the in situ measurement of the rhizosphere pH and Eh. A sheet of plastic film was mounted between the soil surface and the removable Plexiglass cover to ensure easy removal of the Plexiglass cover without disturbing the soil when measuring pH and Eh. The outside of the removable Plexiglass cover was covered by a

Table 1 Chemical characteristics of the the eroded Palouse silt loam used in the experiment

pH	5.5
EC (dS m^{-1})	0.6
OM (%)	1.5
Ca [meq (100 g soil)$^{-1}$]	13.0
Mg [meq 100 g soil)$^{-1}$]	5.8
NaOAc-P (mg kg^{-1})	1.4
NaOAc-K (mg kg^{-1})	82.0
Zn (mg kg^{-1})	0.3
Cu (mg kg^{-1})	1.8
Mn (mg kg^{-1})	18.0
Fe (mg kg^{-1})	35.0

black cover sheet to prevent light penetration. The plants were grown in a growth chamber at 22°C with 16 h day-length, 45% relative humidity and a light intensity of 600–650 μE m^{-2} s^{-1}. The light source was provided by alternating sodium vapor and metal halide lamps.

Exchange resins

The exchange resins were a membrane type with the resins uniformly embedded at the membrane surface. The anion exchange resin (AER) used in this experiment consisted of cross-linked copolymers of vinyl monomers with quaternary NH_4 anion exchange groups (204-U-386; Ionics, Watertown, Mass.). The minimum adsorption capacity of the anion resin membrane was 2.8 meq (dry g resin)$^{-1}$. The chelating cation exchange resin (CER) membrane consisted of styrene divinylbenzene copolymers containing paired iminodiacetate ions, which act as chelating groups in binding polyvalent metal ions and had an adsorption capacity of 0.03 meq cm^2 (Chelex 100 resin, Bio-Rad, Richmond, Calif.). Chelex chelating resin is classified as a weakly acidic cation exchange resin by virtue of its carboxylic acid groups, but it differs from ordinary exchangers because of its high selectivity for metal ions and its higher bond strength. The resins operate in basic, neutral and weakly acidic solutions. The resin has higher selectivity for divalent cations. The selectivity for various cations in aqueous solution at pH 4 is:

$$Cu^{+2} > Pb^{+2} >>> Ni^{+2} > Zn^{+2} > Cd^{+2} > Fe^{+2} > Mn^{+2} > Ca^{+2} >>> Na^{+} \quad (1)$$

pH and Eh measurement

Glass membrane microelectrodes can be used for measurements of in situ soil pH (Hauter and Mengel 1988; Conkling and Blanchar 1989). In this study a micro-combination pH electrode, model MI-410, and a redox combination microelectrode of the MI-800-XXX series (Microelectrodes, Londonderry, N.H.) were used for pH and Eh measurements. The outer diameter of the tip for both the pH and Eh combination microelectrodes was 0.75 mm. After 2 weeks, the removable Plexiglass cover was removed, leaving a 'Handiwrap' sheet attached to the soil surface to prevent soil contact with the atmosphere. The pH and the Eh of soil were measured at ten points along the lateral root surfaces. These lateral roots were exposed to the soil surface in each pot while the plants were growing. The pH and Eh readings for the bulk soil were taken from pots where no plants were growing. Thirty Eh readings were collected by computer to calculate a composite soil Eh at each sampling point. The rhizosphere pH was measured by inserting the microelectrode in the vicinity of the root surface and by recording the most stable reading at each point.

Shoot sampling and analysis

The aboveground portion of each plant was harvested 4 weeks after planting and oven-dried at 65°C. The dry weight was determined, then samples were ground to pass through a 2 mm sieve for further chemical analysis. The ground material was dried, ashed and an acid extract was analyzed for P colorometrically, using a vanadate-molybdate-yellow method (Chapman and Pratt 1961) and for micronutrients by atomic absorption spectrophotometry.

Root sampling and analysis

Plant roots were washed with tap water and subsamples were randomly taken for the estimation of AMF colonization. The roots were fixed in a 90:5:5 (by volume) formaldehyde:acetic acid:ethanol solution. Using a modified version of the method of Phillips and Hayman (1970), root samples were cleared in 2% KOH, stained in Trypan Blue, and cut into 1 cm pieces. From each root sample, ten 1-cm pieces were randomly selected and arranged parallel to each other on a microscope slide. The mycorrhizal root colonization was determined microscopically at 100× (Bierman and Linderman 1981). Five vision fields were examined in each 1-cm root section; therefore, 50 field visions were examined for each sample. Colonization was determined when hyphae, vesicles, or arbuscules were observed. The percent colonization was calculated as the ratio of the colonized sections to the total sections examined.

Bioavailability index

Immediately after harvest the ion exchange resin membranes were removed and rinsed with deionized water. The AER membranes were placed in a 50 ml plastic centrifuge tube containing 25 ml 0.1 *N* HCl. The CER membranes were handled similarly but placed in 1 *N* HCl. All resins were shaken in these desorbing solutions for 24 h on a horizontal shaker. After shaking, the desorbing solutions were removed and stored in glass bottles for further analysis. An additional fresh 25 ml of the desorbing solutions was added to the resin membrane tubes and shaken again for an additional 24 h. Thereafter, the desorbing solutions were removed and stored for further analysis. The P in the desorbing solution was determined colorimetrically, using a vanadate-molybdate-yellow method (Chapman and Pratt 1961). Since bioavailability is related to both the diffusion rate in soil and the solution concentration of the element, the bioavailability index (C_2D_e) for each element was calculated as suggested by Abrams and Jarrell (1992). They derived their equation from that proposed by Nye and Tinker (1977) for measuring effective diffusion rates in soils using ion exchange resin using the following equation:

$$M_t = 2(c_1 - c_2)\left(D_e \frac{t}{\pi}\right)^{1/2} \quad (2)$$

where c_1 is the initial uniform concentration in the soil matrix (solution and desorbable nutrient) and c_2 the concentration at the soil-resin interface (μmol cm^{-3}), D_e is the effective diffusion coefficient (cm^2 s^{-1}), M_t the mass sorbed to the resin membrane at time t (μmol cm^{-2}), and t the time (seconds) after replacement. The bioavailability index (C^2D_e) as proposed by Abrams and Jarrell (1992) for an element in the soil was calculated by the following equation:

$$(c^2 D_e) = \left(\frac{M_t^2}{t}\right)\left(\frac{\pi}{4}\right) \quad (3)$$

The assumption was made that over short time periods, the interface concentration remains near zero.

Soil sampling and analysis

The soils from the Plexiglass pots were thoroughly mixed and two subsamples equivalent to 200 g air-dry soil each were taken. The soil subsamples were placed in glass jars and brought up to saturation. After 1 day of equilibration, fresh 5×5 cm AER and CER membranes were inserted vertically into the saturation paste 3 cm apart. The jars were covered with handiwrap and incubated for 1 week to ensure equilibrium. At the end of the incubation period the membranes were removed, treated and analyzed as described above.

Analysis of variance

Analysis of variance (ANOVA) was used to determine the effect of each factor. The experiment was analyzed as a randomized complete block design. The multiple mean comparison was performed using Fisher's least significant test at the 0.05 level of probability. Statistical analyses were performed with the statistical program Systat (Wilkinson 1990).

Results and discussion

AMF colonization and shoot dry weight

The AMF colonization was 9–12% in non-inoculated plants and was not affected by added P (Table 2). AMF inoculation (I−P) increased AMF colonization 4-fold compared to the non-inoculated treatment (NI−P) when P was not added (Table 2), and by more than 2-fold when P was added, (I+P) vs (NI+P). Other experiments on wheat (Mohammad et al. 1995, 1998; Clark and Zeto 2000) have demonstrated that colonization decreased with the addition of P. This decrease in colonization at high levels of soil P has been attributed to a decrease in root exudation (Graham et al. 1981).

Shoot dry weight doubled with the addition of P regardless of the level of inoculation (Table 2), confirming that the soil was P deficient. However, AMF inoculation did not increase the shoot dry weight at either level of P (Table 2). This may be attributed to the fact that the plants were not grown to maturity but were harvested at tillering.

Table 2 Shoot dry weight and root colonization as affected by no inoculation (NI), arbuscular mycorrhizal fungi (AMF) inoculation (I), no P addition (−P), or addition of 50 mg P (kg soil)$^{-1}$ (+P)

Treatment	Shoot dry weight (g plant^{-1})	AMF colonization (%)
NI−P	0.39 b[d]	12.0 c
I−P	0.40 b	46.6 a
NI+P	0.82 a	8.7 c
I+P	0.81 a	20.0 b

[d]In all tables, means in each column that have different letters are significantly different at the 0.05 level of probability

Nutrient concentration and uptake

Shoot P concentration and total uptake increased with AMF inoculation compared to the non-inoculated plants when P was not added; however, AMF inoculation had no effect on shoot P when P was added (Tables 3, 4). Similarly, shoot Zn concentration and total accumulation were increased only by AMF inoculation in the absence of added P (Tables 3, 4). The addition of P increased shoot P concentration compared to non-P treatments regardless of the AMF levels. Zn uptake was also increased in mycorrhizal plants. Shoot Fe concentration and uptake were not affected by AMF inoculation at either level of P (Tables 3, 4). However, shoot Mn concentration was decreased by AMF inoculation compared to non inoculated, in either the presence or absence of added P (Table 3). Total shoot Mn was also decreased by AMF inoculation compared to non-inoculated when P was not added, but was not affected by inoculation when P was added (Table 4). In contrast, shoot Cu concentration and uptake were not affected by AMF inoculation, but were higher with P application (Tables 3, 4).

The increase in P uptake by mycorrhizal plants is well documented (Clark and Zeto 2000) and can be as much as 3–4 times that of non-mycorrhizal plants (Bolan 1991; Al-Karaki 2002). The enhancement of both P and Zn concentration and uptake by AMF inoculation in the present study may be attributed to the solubilizing effect of the mycorrhizal root exudations and decreased soil pH on P solubility, and to the extended AMF hyphae, which ex-

Table 3 Shoot P and micronutrient concentration as affected by NI, I, −P, or +P treatments as in Table 2

Treatment	P	Fe	Mn	Zn	Cu
	mg (kg dry shoot wt)$^{-1}$				
NI−P	1,859 c	76.9 a	182.7 a	29.9 b	3.9 b
I−P	3,035 b	71.4 ab	133.9 bc	47.4 a	5.2 b
NI+P	5,220 a	64.7 b	137.7 b	24.0 c	13.1 a
I+P	5,458 a	65.3 b	125.3 c	25.7 bc	10.1 a

Table 4 Plant P and micronutrient uptake as affected by NI, I, −P, or +P treatments as in Table 2

Treatment	P	Fe	Mn	Zn	Cu
	mg (plant dry wt)$^{-1}$	μg (plant dry wt)$^{-1}$			
NI−P	0.6 c	30.1 b	71.1 ab	9.2 b	1.6 c
I−P	1.2 b	28.9 b	54.0 c	19.1 a	2.2 c
NI+P	4.2 a	52.0 a	110.4 a	19.2 a	10.5 a
I+P	4.4 a	53.0 a	101.3 a	20.8 a	8.0 a

plore a larger volume of soil than non mycorrhizal plants (Kothari et al. 1991a). However, both shoot Mn concentration and Mn uptake were decreased by AMF inoculation (Table 3, 4), as observed in other studies (Mohammad et al. 1998, 2003; Kothari et al. 1991a). Both shoot concentration and plant uptake of Fe and Cu were unaffected by AMF inoculation at both levels of P. This disagrees with an earlier study that found that AMF inoculation decreased shoot Fe and increased Cu concentration (Mohammad et al. 1995). This may be due to the fact that we harvested the plants at tillering. The increased Fe and Cu uptake upon addition of P to the soil is attributable to the higher shoot dry weight obtained with the addition of P (Table 2).

Soil rhizosphere pH and Eh

The soil pH in the root rhizosphere was lower than the bulk soil in all treatments (Table 5). AMF inoculation further decreased rhizosphere pH, but only when P was not added. Mycorrhizal plants have been shown to produce more CO_2 in the rhizosphere (Knight et al. 1989), which promotes higher microbial populations and a higher rate of nitrification (Li et al. 1991), all of which can contribute to increased rhizosphere acidity. The rhizosphere Eh was also lower than the bulk soil, but was not affected by P addition or AMF inoculation (Table 5). The decrease in rhizosphere Eh may be attributed to the root exudation of reductants to the rhizosphere (O'Keefe and Sylvia 1991).

Exchange resin and bioavailability index of P, Fe, Mn, Zn and Cu

The exchange resins (ER) inserted into the soil with plant roots acted as a sink for P (using the AER) and for micronutrients (using the CER). The adsorption of nutrients onto

Table 5 Soil redox potential (Eh) and pH of the bulk soil and rhizosphere as affected by NI, I, −P, or +P treatments as in Table 2

Treatment	pH	Eh
Bulk soil	5.10 a	312 a
NI−P	4.79 b	278 b
I−P	4.53 c	277 b
NI+P	4.64 bc	308 ab
I+P	4.75 b	278 b

Table 6 Exchange resin P and micronutrients after 4 weeks of plant growth at field capacity soil moisture content as affected by NI, I, −P, or +P treatments as in Table 2. *ER* Exchange resin

Treatment	P	Fe	Mn	Zn	Cu
	mg 25 cm^2 ER				
Bulk soil	0.13 a	1.7 b	10.9 b	0.48 b	0.11 a
NI−P	0.23 a	1.5 b	10.9 b	1.37 a	0.08 b
I−P	0.45 a	1.6 b	4.2 c	0.50 b	0.08 b
NI+P	0.42 a	2.4 ab	25.5 a	0.37 b	0.10 ab
I+P	0.30 a	3.1 a	8.7 bc	0.36 b	0.11 a

the resin simulates the absorption by the plant roots, thereby providing insights into the mechanisms by which mycorrhizal plants influence P and micronutrient availability. The exchange resin P (ER-P) at field capacity was not affected by the different treatments (Table 6). At saturation, the ER-P was increased by AMF inoculation when P was not added, compared to all other treatments (Table 7). The ER-Fe at field capacity was increased by AMF inoculation when P was added compared to all other cases except for non inoculated when P was added (Table 6). The ER was successfully used under saturation conditions by Abrams and Jarrell (1992). Based on theoretical considerations, they suggested the possible use of ER under field capacity conditions. However, in the current study, competition with plant roots at field capacity water content may have limited the P diffusion to the resin.

There were no differences in the P bioavailability index (P-BI) among different treatments at field capacity (Table 8). The P-BI was increased by AMF inoculation when P was not added compared to all other treatments at saturation (Table 9). There were no differences in the P-BI among other treatments. Under saturation conditions, as with the ER-, the P-BI was increased by AMF inoculation when P was not added. ER placed in inoculated and in non inoculated soil are both exposed to the same pool of soil P; however, the saturated ER-P and the P-BI were increased by inoculation, suggesting that the bioavailable P level was higher. This may also suggest that the inoculated roots were able to release to the soil solution some of the unavailable P, which was adsorbed by the ER.

It was reported by Cress et al. (1979) and Howeler et al. (1981) that mycorrhizal plants have lower K_m (higher

Table 7 Exchange resin P and micronutrients after 1 week incubation with soil taken from the experimental pots and brought to saturation. Treatments include NI, I, −P, or +P as in Table 2

Treatment	P	Fe	Mn	Zn	Cu
	mg 25 cm^2 ER				
Bulk soil	0.05 b	2.8 b	46.7 a	0.20 a	0.07 b
NI−P	0.10 b	2.5 b	48.3 a	0.35 a	0.07 b
I−P	0.61 a	4.2 a	33.1 b	0.16 a	0.07 b
NI+P	0.18 b	2.6 b	45.3 a	0.17 a	0.08 a
I+P	0.11 b	2.4 b	34.3 a	0.15 a	0.07 b

Table 8 Phosphorus and micronutrient bioavailability as affected by different treatments after 4 weeks of plant growth at field capacity soil moisture content. Treatments include NI, I, −P, or +P as in Table 2

Treatments	P	Fe	Mn	Zn	Cu
	$\mu mol^2 cm^{-4} s^{-1}$				
	(10^{-8})	(10^{-5})	(10^{-3})	(10^{-8})	(10^{-8})
Bulk soil	0.8 a	0.15 c	0.06 b	12 b	0.61 a
NI−P	2.7 a	0.11 c	0.06 b	57 a	0.30 d
I−P	10.2 a	0.13 c	0.01 c	13 b	0.35 c
NI+P	8.8 a	0.29 b	0.33 a	7 b	0.51 b
I+P	4.6 a	0.50 a	0.04 b	7 b	0.61 a

affinity) so are able to absorb P from a lower level of soil solution P. Given this, the inoculated roots would be expected to absorb P from a lower soil solution P level rather than increasing solution P from unavailable forms. This would explain why ER placed with inoculated roots did not adsorb significantly more P than ER incubated with non-inoculated roots (Table 6). However, the post-harvest increase in ER-P in the saturated soil (Table 7) may reflect a shift in P solubility, in the absence of active roots, in response to rhizosphere acidification (Table 5).

Similar to the treatment effects on ER-P, inoculation had no effect on ER-Fe during plant growth (Table 6), but post-experiment ER-Fe under saturation was increased by AMF inoculation when P was not added (Table 7). This suggests that the mycorrhizal roots may have been releasing more available Fe, but at the field capacity water content, Fe diffusion to the ER was limiting.

Resin results did not support the hypothesis that AMF inoculation increases Zn and Cu solubility. While the roots of non-inoculated plants with no P added (NI−P) increased ER-Zn over the non-plant bulk soil (Table 6), AMF inoculation had no effect on post-experiment ER-Zn at saturation (Table 7). This suggests that low P plants can increase Zn solubility and movement into the ER, but that AMF inoculation has no apparent additional effect on Zn solubility that would help to explain the higher plant Zn concentration and Zn uptake of I−P plants (Tables 3, 4). Similarly, inoculation had no influence on ER-Cu at either P levels during plant growth (Table 6), and at saturation, non inoculated with added P had slightly higher ER-Cu than inoculated (Table 7). Other mechanisms of enhanced Zn uptake by Zn-polyphosphate transport through mycorrhizal hyphae (Kothari et al. 1991a) would be consistent with these results.

Table 9 Phosphorus and micronutrient bioavailability as affected by different treatments after 1 week incubation with soil taken from the experimental pots and brought to saturation. Treatments include NI, I, −P, or +P as in Table 2

Treatments	P	Fe	Mn	Zn	Cu
	$\mu mol^2\ cm^{-2}\ s^{-1}$				
	(10^{-8})	(10^{-5})	(10^{-3})	(10^{-8})	(10^{-8})
Bulk soil	0.5 b	1.7 b	4.7 a	8.9 b	1.17 b
NI−P	2.3 b	1.3 c	5.1 a	26.1 a	1.06 c
I−P	34.2 a	3.9 a	2.4 c	5.8 b	1.17 b
NI+P	6.7 b	1.5 bc	4.5 b	6.0 b	1.27 a
I+P	2.7 b	1.3 c	2.6 c	5.1 b	1.17 b

The Fe-BI was increased by inoculation compared to the non inoculated when P was added (NI+P), and by these two treatments compared to all others at field capacity (Table 8). At saturation, the Fe-BI was increased by inoculation when P was not added compared to all others (Table 9). The inoculated and non inoculated treatments had similar effects on the Fe-BI when P was added. The Mn-BI at field capacity was decreased by the inoculated compared to the non-inoculated at both levels of P (Table 8). At saturation, the Mn-BI was also decreased by inoculation treatments compared to non-inoculated treatments at both levels of P (Table 9). The Zn-BI at both field capacity and saturation was higher for the non-inoculated when P was not added compared to all other treatments (Tables 8, 9). There were no differences in the Zn-BI among the other treatments at both field capacity and saturation. The Cu-BI at field capacity was increased by the inoculated treatments compared to the non inoculation treatments at both levels of P (Table 9). At saturation, Cu-BI was increased by the inoculated treatment compared to non-inoculation when P was not added (Table 9).

The ER-Mn and the Mn-BI were decreased by the inoculated roots at both levels of P at field capacity during plant growth (Tables 6, 8) and post-harvest under saturation (Tables 7, 9), and this apparent decrease in Mn availability was correlated to decreased Mn uptake (Tables 3, 4). Since the ER placed with inoculated roots adsorbed less Mn than the ER placed with non-inoculated roots, we may conclude that the Mn level in the soil solution was actually lower than the Mn level in the soil solution in which the non-inoculated plants were growing. If the inoculated plants were able to increase P and Fe solubility by decreasing the rhizosphere pH, why was Mn not affected in similar fashion? Kothari et al. (1991b) suggested that microbial competition in the rhizosphere during mycorrhizal colonization results in a decrease in Mn-reducing bacteria and a lower concentration of exchangeable Mn. Pacovsky (1986) suggested that AMF hyphae accumulate lower concentrations of Mn to avoid precipitation of insoluble mineral phosphates or the complexation and inactivation of organic phosphates.

Conclusion

AMF inoculation enhanced P and Zn uptake compared to non-inoculated plants when P was not added to spring wheat growing in a low P, low organic matter silt loam soil. In contrast, Mn uptake was decreased by AMF inoculation regardless of P levels, as also demonstrated by other researchers. The rhizosphere pH was decreased in AMF inoculated plants grown in soil unamended with P fertilizer, compared to non-inoculated plants, possibly due to modifications in root exudation, hyphal activity, or shifts in

microbial populations. This, along with hyphal nutrient absorption capacity, could be responsible in part for the increase in P availability to plants, which may in turn have stimulated Zn uptake. The ER and BI data indicate that mycorrhizal roots may be increasing P chemical availability. The potential ability of AM roots to use forms of P unavailable to uninfected plants has economical and environmental ramifications (Kucey et al. 1989).

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