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Enhanced uptake of phosphorus by mycorrhizal sorghum plants as influenced by forms of nitrogen

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Abstract

The effects of NH_4^+-N and NO_3^--N applications on rhizosphere pH and P uptake were investigated with and without mycorrhizal inoculation. Sorghum (*Sorghum bicolor* L.) plants were grown in split pots and in different soils with different initial soil pH values and P contents. In both soils, ammonium treatment resulted in higher plant dry weight and P content than nitrate treatment at all P levels. Mycorrhizal inoculation enhanced the differences. Sorghum plants acidified the rhizosphere at low soil P status with both nitrogen forms, and with or without mycorrhiza. When N was supplied as NO_3^--N, rhizosphere pH increased gradually with increasing P addition.

The experiments showed that, although the zone of strong acidification coincided with the main zone of P depletion, the contribution of Arbuscular Mycorrhizal Fungi (AMF) to P uptake extended beyond the effect of rhizosphere pH change. There were no differences in pH change between mycorrhizal and non-mycorrhizal inoculated soils with the same N form, and it appears that pH change is an independent factor affecting P uptake regardless of whether the plant root is infected or not. The differences in soil pH change with N form do not however explain sufficiently the differences in dry matter increase and P uptake between mycorrhizal and non-mycorrhizal plants.

Introduction

Arbuscular Mycorrhizal Fungi (AMF) influence phosphorus (P) uptake and plant growth in P-deficient soils (Li et al., 1991a, b). Several mechanisms have been suggested for increases in the uptake of P (Sanders and Tinker, 1973; Tinker, 1975). One of the critical mechanisms is the modification of the rhizosphere (Bolan et al., 1987; Tinker, 1975) which may explain how AMF increase the uptake of P as a result of plant utilization of ammonium (NH_4^+-N). More efficient utilization of NH_4^+-N by mycorrhizal than nonmycorrhizal plants (Li et al., 1991c; Smith et al., 1985) may lead to increased H^+ secretion into the rhizosphere as a result (Bolan et al., 1991; Bolan, 1991). Rhizosphere pH has been reported to be altered by N forms both in the presence and absence of mycorrhizae (Bledsoe and Zasoski, 1983; Li et al., 1991c; Rygiewicz et al., 1984; Vaast and Zasoski, 1992). It has been reported that ecto-mycorrhizal inoculation enhanced significantly the capacity of plant roots to release H^+ in a medium which can increase the bioavailability of compounds not readily soluble under specific chemical conditions of the soil (Rigou et al., 1995). If a mycorrhizal plant induces a decrease in its rhizosphere pH, this effect may contribute to more P uptake by solubilising calcium-P and iron and aluminium phosphates, and thus increasing P availability to both the root and hyphae.

The addition of N usually stimulates uptake of P by the plant especially when NH_4^+-N is applied (Gahoonia et al., 1992; Hoffmann et al., 1994; Miller, 1974; Riley and Barber, 1971). Miller et al. (1970) demonstrated that the P content of the shoot was approximately doubled by the addition of the NH_4^+-N fertilizer. There is some evidence that under P-deprivation conditions there is a preference for excess cation over anion uptake

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and a lower pH in the rhizosphere which increases the availability of P (Gahoonia et al., 1992; Hedley et al., 1982a, b; Moorby et al., 1988). Under conditions of low P availability, acidification of the rhizosphere is of obvious ecological importance especially in calcareous soils in which the availability of P is limited due to ready precipitation of P as calcium phosphates.

The effect of pH change in the rhizosphere on P uptake in the presence of mycorrhizal infection has been speculated about by several workers who have generally concluded from their results that the rhizosphere pH must be considered a major variable to explain the inconsistent results (Bolan et al., 1987; Bolan, 1991; Koide, 1991; Stribley, 1987; Tinker, 1975). However there is no direct evidence of a quantitative change in pH in the rhizosphere due to mycorrhizal infection and also there is no quantitative evidence for the effect of pH change on P uptake under conditions of mycorrhizal infection.

The aim of the research is to investigate the effect of NH_4^+-N and nitrate (NO_3^--N) forms, rate of P and mycorrhizal inoculation on rhizosphere pH change and P uptake.

Materials and methods

Soils

Two soils (Hoosfield A and Hoosfield B) of low P status were collected fresh from IACR-Rothamsted Experimental Station (UK) and sieved (4 mm). The soils were partially sterilized with 1 *M* rad (10 K Gy) γ-irradiation and left to equilibrate for 30 days at 21 °C in order to re-establish a micro-organism population. Soil chemical and physical properties are presented at Table 1. The Hoosfield A soil is a calcareous soil, its pH is over 8 and the Hoosfield B soil is a slightly acidic and its pH is less than 7.

Pots

The experiment was conducted using split cylindrical pots, 23 cm long and 7.5 cm in diameter. Each split pot was sealed with plastic and a nylon gauze. The pots were made with PVC pipe cut lengthways which could be separated easily to remove the entire core. The cylinders facilitated root separation and collection of rhizosphere soil.

Basal nutrient treatments

Monocalcium phosphate ($Ca(H_2PO_4)_2$) was applied at the rates of 0, 20, 40 and 60 mg P kg^{-1} soil. After equilibration for 30 days, soil mineral NH_4^+-N and NO_3^--N was determined (Table 1) and N was added as either ammonium sulphate or calcium nitrate to yield a final concentration of mineral N of 120 mg N kg^{-1} soil. Additionally, 50 mg K kg^{-1} soil as KCl and 25 mg Mg kg^{-1} soil as Mg $SO_4.7H_2O$ were added. To prevent nitrification, N-serve (2 mg kg^{-1}) was added at this stage. Fertilized soil (750 g soil and 250 g sand mix) was packed into pots. Inoculum of *Glomus mosseae* (Nicol. and Gerd.) consisting of 18 g of a mix of Terragreen, chopped roots and mycorrhizal spores was placed 30 mm below the seeds. The nonmycorrhizal plants received the same amount of mycorrhiza free inoculum (containing the same microbes). The total number of treatment combinations are 4 P levels × 2 forms of N × plus and minus *G. mosseae* × 3 replicates.

Plants

Sorghum (*Sorghum bicolor* (L) SSV2 genotype) was grown on the two Rothamsted soils. Seeds were sieved on a 5 mm sieve, to remove small seeds. Four sorghum seeds were sown per pot and thinned to one seedling per pot after 5 days. Distilled water was added daily to maintain moisture near field capacity. The plants were grown in a growth room at 21 °C and a relative humidity of 60-70%, with a 16 h day and 8 h dark photoperiod under a light intensity of 350 μmol m^{-2} s^{-1}.

Plants were harvested 40 days after sowing. The soil core was removed from the split PVC tubes. After subsamples of rhizosphere and non-rhizosphere soil had been taken (described below), the roots were carefully washed from the soil and cut into 10 mm segments,

Rhizosphere soil sample collection for pH measurement

Due to the difficulty in separating rhizosphere soil, the method of Riley and Barber (1969) was modified. Rhizosphere and nonrhizosphere soil fractions were separated manually. The soil that was easily shaken from the root surface was assumed to be nonrhizosphere soil, and the soil adhering to the roots after a gentle shake (usually 5 times) was considered to be rhi-

Table 1. Some physical and chemical properties of the soils

Soil	Site use	Plot No	Clay (%)	Silt (%)	Sand (%)	Initial soil pH (2:5 in water)	Soil bulk density ($g\ cm^{-3}$)	Extractable ($mg\ P\ kg^{-1}$ soil)		Olsen extractable P ($mg\ P\ kg^{-1}$ soil)
								NH_4^+	NO_3^-	
Hoosfield A	Continuous Barley	114	36	47	17	8.23	1.20	18.09	12.59	2.69
Hoosfield B	Exhaustion land	North Side Discard	32	56	12	6.33	1.24	20.17	17.76	5.12

Means (three replicates).

zosphere soil. Both categories of soil were sieved on a 540 μm sieve in order to collect the roots and hyphae. After measuring rhizosphere pH in a 2:5 soil:water suspension, the suspension was dropped into a plastic bag. Any remaining soil adhering to the plant roots was washed off with a few mL of distilled water into the same bag. The plastic bags were then exposed to the open air for evaporation of excess water for 7 days. When the excess water had evaporated, the root-free rhizosphere soil was crushed, and then sieved to 4 mm for P measurement.

Extractable inorganic N and P measurement

The 1 *M* KCl extractable inorganic N (NH_4^+ and NO_3^-) was determined by the method of Keeney and Nelson (1982) using an auto-analyser. Extractable soil phosphorus was determined according to Olsen (Olsen and Sommers, 1982).

Mycorrhizal infection

Before drying the roots, small samples were taken and preserved in a mixture of ethanol, acetic acid and formalin (ratio 20:1:3 V), for determination of root length and mycorrhizal infection. A proportion of the preserved root was stained and examined for the presence and degree of mycorrhizal infection. The root clearing and staining procedure followed the method of Koske and Gemma (1989). The percentage mycorrhizal infection was calculated as the number of 10 mm long root segments out of 100 identified as infected under a stereo microscope at a magnification of × 20 (Giovannetti and Mosse, 1980).

Plant analysis

After drying the plants at 75 °C, the plant material from each pot was ground using a Tema mill. Plant material was digested with a acid catalyst mixture (84 mL of H_2SO_4, 70 mL of H_2O_2, 2.8 g of $LiSO_4.H_2O$ and 0.084 g of Se powder). After the digestion of the plant material, the concentration of phosphorus in dry matter in this solution was determined by an auto-analyzer.

Statistical analysis

All statistical analyses were performed using the Statistical Analysis System (SAS).

Results

Plant measurements

The effect of N form and P rate of application on total dry matter production at harvest varied with mycorrhizal inoculation. In both soils, and in all P treatments, NH_4^+-N as the N source resulted in higher dry matter than NO_3^--N as the N source. Inoculation with *G. mosseae* enhanced differences between N sources. Sorghum plants infected with mycorrhizae had nearly 3 times higher shoot dry matter yields than noninoculated control plants (Figure 1). Mycorrhizal inoculation and increased P application significantly increased the tiller dry weight production. In both soils the differences in tiller weight between N forms was enhanced by the mycorrhizal inoculation (Figure 2).

Root lengths were slightly different between N forms and there were small differences between mycorrhizal inoculated and nonmycorrhizal plants (Table

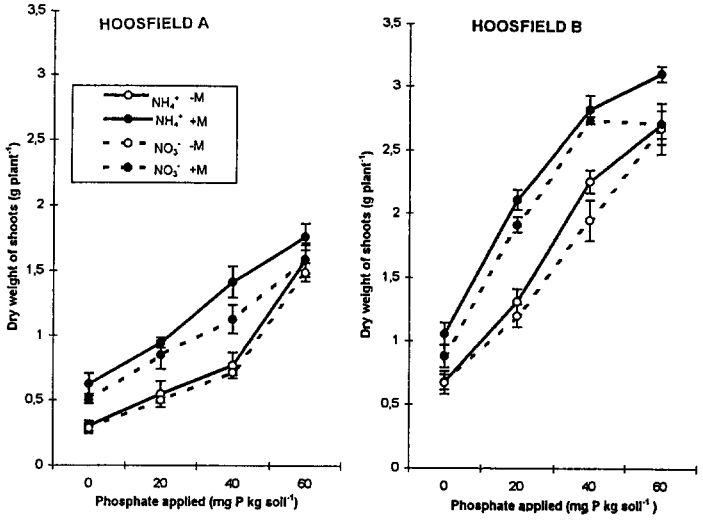

Figure 1. The effect of nitrogen form, rate of phosphate and mycorrhizal inoculation on the shoot dry weight of sorghum plants at 40 days. (+M = inoculated with *Glomus mosseae*, -M = noninoculated). Vertical bars indicate standard error.

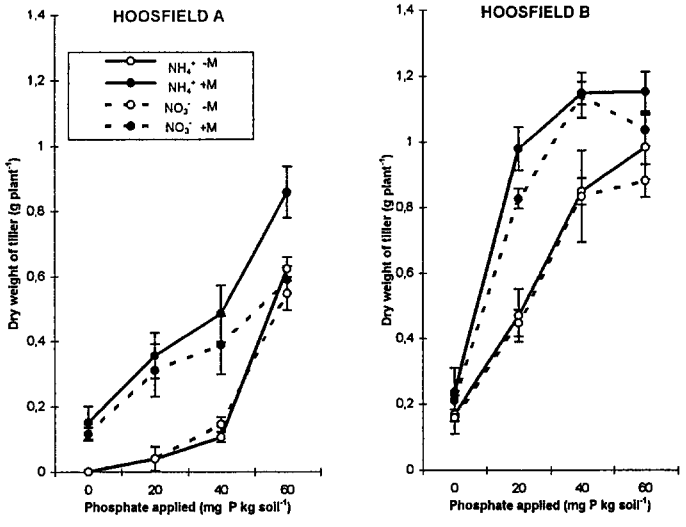

Figure 2. The effect of nitrogen form, rate of phosphate and mycorrhizal inoculation on the dry weight of tillers of sorghum plants at 40 days. (+M = inoculated with *Glomus mosseae*, -M = noninoculated). Vertical bars indicate standard error.

2) but the effect of inoculation was not statistically significant (Table 3).

Plant P content

Total (shoot and root) P content was greater in mycorrhizal plants for all P levels for both soils (Figure 3). In Hoosfield A soil there was a small difference between NH_4^+-N and NO_3^--N treatments for both inoculated and noninoculated plants. However, in Hoosfield B, soil in mycorrhizal plants showed large differences between ammonium and nitrate application in shoot P content for all P levels. Nevertheless, in nonmycorrhizal plants the differences between ammonium and nitrate in shoot P content were lower than for mycorrhizal-infected plants.

Mycorrhizal infection

The effect of N form and rate of P application on mycorrhizal infection was estimated in both soils. In both soils without inoculation, no mycorrhizal infection was

Table 2. The effect of mineral N supply as either ammonium or nitrate and rates of P on mycorrhizal infection and rhizosphere and non-rhizosphere soil pH of sorghum plants

AMF	P levels (mg kg^{-1})	Mycorrhizal infection (%)		Root length (m)		Rhizosphere soil pH		Non-rhizosphere soil pH	
		NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-
Hoosfield A soil									
-M	0	0.8(0.8)	0.8(0.8)	17.3(0.4)	17.9(0.97)	7.62(.04	7.86((.04)	7.93(.03)	8.01(.05)
+M	0	63.3(14.6)	60.8(9.2)	19.22(1.41)	19.9(41.43)	7.55(.02)	7.83(.01)	7.92 (.03)	7.99(.03)
-M	20	0(0)	0(0)	36.28(1.22)	39.95(4.47)	7.67(.02)	7.88(.03)	7.87(.02)	8.08(.02)
+M	20	73.3(2.7)	70.8(8.0)	38.53(6.53)	44.89(1.83)	7.66(.03)	7.95(.07)	7.87 (.01)	8.12(.01)
-M	40	0(0)	0(0)	38.48(6.2)	43.5(7)	7.87(.01)	8.06(.01)	8.10(.01)	8.15 (.02)
+M	40	83.3(5.2)	70.0(5.0)	34.94(2.78)	44.27(1.4)	7.80(0.1)	8.08(.01)	7.99 (.02)	8.17(.01)
-M	60	0(0)	0(0)	56.2(12.26)	48.2(4.91)	7.81(.04)	8.29(.01)	7.99(.02)	8.13 (.01)
+M	60	69.2(9.4)	63.3(8.1)	49.7(3.1)	52.1(1.8)	7.75(.02)	8.31(.02)	8.02(.02)	8.17(.01)
Hoosfield B soil									
-M	0	0(0)	1.1(1.1)	42.2(6.97)	35.4(1.62	5.27(.05)	6.21(.03)	5.89(.03)	6.27 (.05)
+M	0	56.9(11)	56.5(4.4)	47.2(4.39)	39.5(4.42)	5.20(.01)	6.24(0.2)	5.78(.03)	6.35(.03)
-M	20	0(0)	0(0)	57.6(6.9)	49.4(3.9)	5.29(.07)	6.58(.01)	5.84(.01)	6.48 (.05)
+M	20	63.3(3.3)	71.1(6.5)	65.03(3.19)	59.36(3.13)	5.26(.02)	6.67(.01)	5.82(.02)	6.51(.01)
-M	40	0(0)	0(0)	64.1(10)	58.0(3.7)	5.39(.03)	6.71(.02)	5.99(.06)	6.61(.03)
+M	40	46.3(3.4)	45.8(3.3)	70.52(3.1)	62.48(2.1)	5.41(.03)	6.80(.07)	5.89(.07)	6.47(.05)
-M	60	0(0)	0(0)	65.7(3)	64.8(3.4)	5.47(.03)	6.72(.08)	5.89(.02)	6.49(.03)
+M	60	33.3(1)	36.2(0.8)	69.4(4)	62.2(7.3)	5.45(0.4)	6.84(0.4)	6.14(0.4)	6.50(.03)

Mean (three replicates). Bracket is SE (Standard error) -M–noninoculated; +M–inoculated with *Glomus mosseae*.

Table 3. Significant of F values (probability) from analysis of variance for different soil and plant parameters

Sources	DF	Shoot weight	Tiller weight	Root length	Total P content	Non-rhizosphere soil P	Rhizosphere soil P	Rhizosphere soil pH
Soil	1	0.0001	0.0001	0.0001	0.0001	0.1761	0.3777	0.0001
P level	3	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Soil × P level	3	0.0001	0.0001	0.0469	0.0001	0.0001	0.0001	0.0250
N form	1	0.0001	0.0128	0.3310	0.0001	0.0597	0.1092	0.0001
Soil × N form	1	0.5041	0.8451	0.0.140	0.0489	0.3898	0.8072	0.0001
P level × N form	3	0.6139	0.1837	0.6648	0.0447	0.1914	0.8932	0.0001
Soil × P × N form	3	0.9877	0.8489	0.2157	0.7518	0.7753	0.6373	0.0001
Mycorrhiza(M)	1	0.0001	0.0001	0.0578	0.0001	0.0001	0.0001	0.4328
Soil × M	1	0.0176	0.6303	0.5285	0.0380	0.1566	0.0642	0.0463
P × M	3	0.0001	0.0001	0.2086	0.0001	0.0046	0.0326	0.5538
Soil × P × M	3	0.2793	0.4291	0.8127	0.1817	0.0020	0.3581	0.6951
N × M	1	0.0983	0.0995	0.4930	0.0956	0.4289	0.0528	0.0002
Soil × N × M	1	0.8241	0.5069	0.4615	0.3623	0.7056	0.3939	0.3420
P × N × M	3	0.6436	0.9111	0.9521	0.9556	0.2423	0.6416	0.9789
Soil P × N × M	3	0.3365	0.6637	0.8440	0.7853	0.7546	0.6326	0.8298

observed. The roots of inoculated plants were well infected with *G. mosseae* when soil P concentration was low (Table 2). In Hoosfield A soil, mycorrhizal infection increased with increasing P application up to 40 mg P kg^{-1} soil then slightly decreased. With Hoosfield B soil, mycorrhizal infection was stimulated

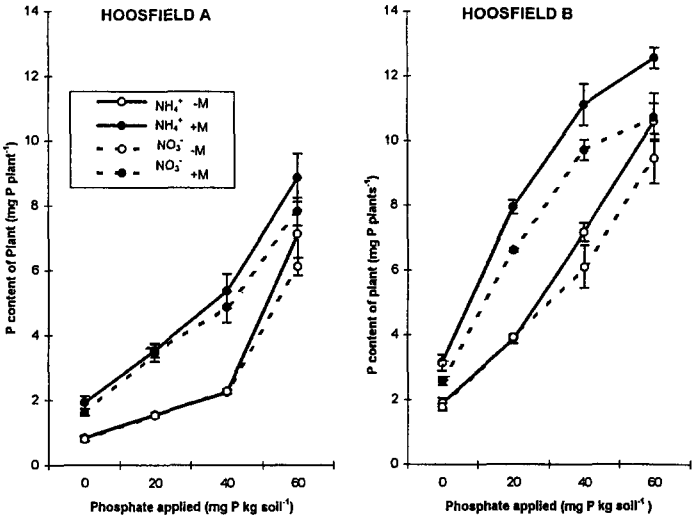

Figure 3. The effect of nitrogen form, rate of phosphate and mycorrhizal inoculation on the total plant phosphorus uptake by sorghum plants at 40 days. (-M = inoculated with *Glomus mosseae*, -M = noninoculated). Vertical bars indicate standard error.

up to 20 mg P kg^{-1} soil then decreased with increasing P supply. In Hoosfield A soil, NH_4^+-N resulted in a slightly higher percentage of mycorrhizal infection than the NO_3^--N, whereas in Hoosfield B soil, the nitrate N source resulted in a slightly higher percentage of mycorrhizal infected root than the ammonium N source.

pH of the rhizosphere

The effect of N form, P rate and mycorrhizal inoculation on rhizosphere pH was determined (Table 2). In both soils, plants acidified the rhizosphere at low P status with both N forms, irrespective of mycorrhizal inoculation. However, mycorrhizal infection did not significantly change the rhizosphere pH. In those treatments where N was supplied as NO_3^-, rhizosphere pH increased gradually with increasing P application. In all P treatments, NH_4^+-N resulted in a lower pH than NO_3^--N application.

Comparing rhizosphere pH change for NH_4^+-N and NO_3^--N treatments, NH_4^+-N caused a decrease of up to 0.55 pH units from the initial Hoosfield A soil level and 1.17 units in Hoosfield B soil. Nitrate-N resulted in a pH decrease up to 0.27 pH units in Hoosfield A soil and 0.16 units in Hoosfield B soil. In Hoosfield A soil, NH_4^+-N supply with mycorrhizal inoculation resulted in a lower rhizosphere pH than the noninoculated treatment. However, in the case of NO_3^--N treatment with no P application, mycorrhizal inoculated plants had a lower rhizosphere pH than noninoculated plants. With nil P application, rhizosphere pH was lower than initial soil pH in Hoosfield B soil. In Hoosfield B soil when N was supplied as NO_3^-, mycorrhizal inoculation resulted in a higher pH than without inoculation. However, when N was supplied as NH_4^+ and mycorrhizal infection was present, rhizosphere pH was slightly lower, but the differences were not significant. Nonplanted and nonrhizosphere soil pH was also measured.

The effect of N forms and VA mycorrhizal inoculum on P depletion

After harvest, in order to examine the effect of pH change and mycorrhizal inoculation on P depletion in rhizosphere and nonrhizosphere soil, samples were collected and 0.5 *M* $NaHCO_3$ extractable P was measured. The magnitude of P depletion between rhizosphere and non-rhizosphere P differed with mycorrhizal inoculation and P rate application. In rhizosphere soil inoculated with *G. mosseae*, more extractable P was measured when N was supplied as NH_4^+-N, whereas in noninoculated soil more extractable P was measured in the rhizosphere supplied with NO_3^--N (Figure 4). Differences between inoculated and noninoculated treatments were statistically significant. When plant roots were not infected with mycorrhizae, extractable P in the rhizosphere was reduced more than for mycorrhizal plants (Figure 4).

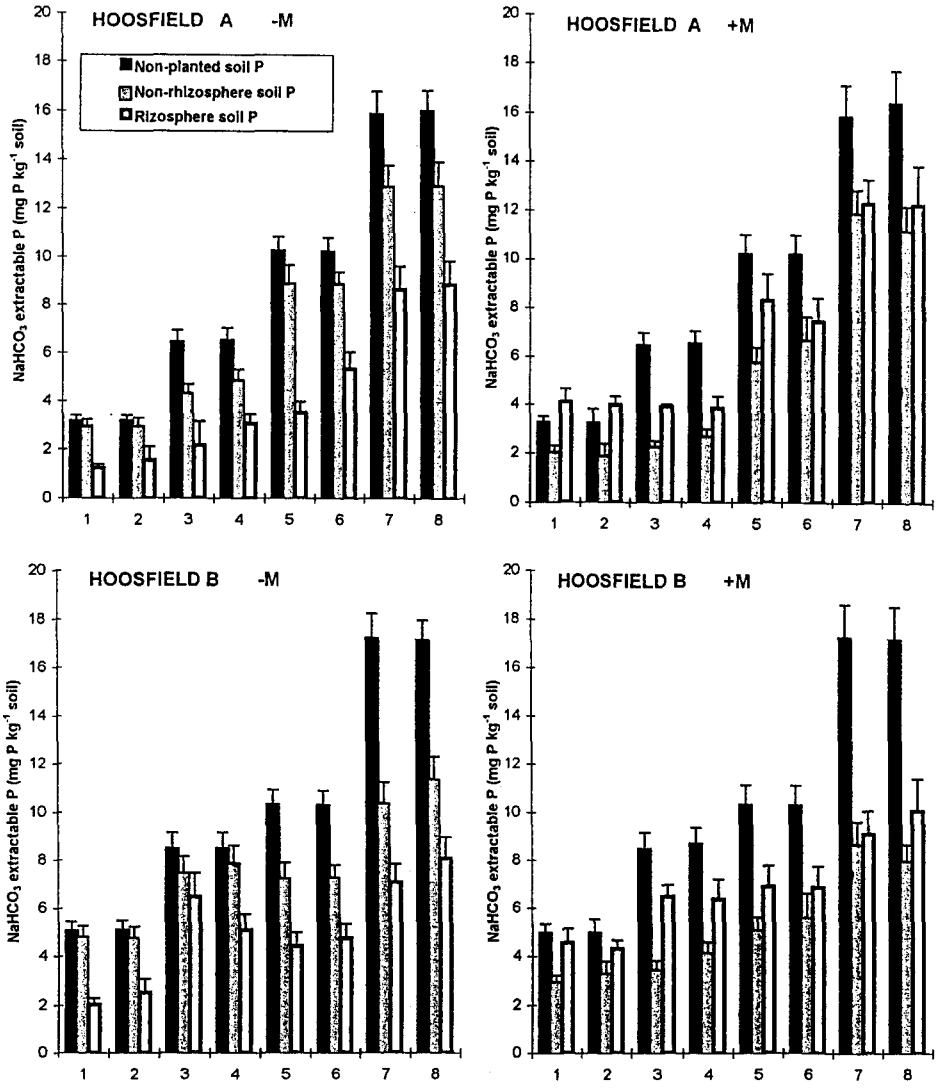

Figure 4. The effect of nitrogen form, rate of phosphate and mycorrhizal inoculation on extractable P from different soil fractions. (+M = inoculated with *Glomus mosseae*, -M = noninoculated). Vertical bars indicate standard error. 1. 0 (mg P kg^{-1} soil) NH_4^+. 2. 0 (mg P kg^{-1} soil) NO_3^-. 3. 20 (mg P kg^{-1} soil) NH_4^+. 4. 20 (mg P kg^{-1} soil) NO_3^-. 5. 40 (mg P kg^{-1} soil) NH_4^+. 6. 40 (mg P kg^{-1} soil) NO_3^-. 7. 60 (mg P kg^{-1} soil) NH_4^+. 8. 60 (mg P kg^{-1} soil) NO_3^-.

Discussion

Rhizosphere pH change

The results show that in a calcareous soil with low P availability, plant roots decrease their rhizosphere pH under both nitrogen forms, with and without mycorrhizal infection. When N was supplied as NH_4^+-N, the decrease in the rhizosphere pH was always greater than with NO_3^--N supply. With the NO_3^--N amendments in Hoosfield A soil, plants acidified their rhizosphere at all rates of P supply, whereas in Hoosfield B soil, plants acidified their rhizosphere at the low and intermediate rates of P supply.

Usually soil acidification in the rhizosphere has been related to NH_4^+-N uptake, but the uptake of NO_3^--N results in a pH increase in the rhizosphere as a result of OH^-/HCO_3^- released from the root (Gahoonia et al., 1992; Marschner et al., 1986; Muranyi et al., 1994; Nye, 1986; Riley and Barber, 1969). For both soils, a high level of P application did not acidify the rhizosphere soil (Table 2); however, under P deficiency

conditions plants tended to acidify the rhizosphere. The only literature reports of a decrease in rhizosphere pH with nitrate nitrogen come from Gahoonia et al. (1992) and Moorby et al. (1985). In order to achieve sufficient P uptake the plants acidify their rhizosphere under low available P conditions whether they are provided with NH_4^+-N or NO_3^--N. Possible reasons for the general acidification effect under low P supply conditions are: (1) although plant roots received the same amount of N, plant roots did not utilise the two N forms with equal efficiency, and (2) rhizosphere acidification was possibly caused by P deficiency where enhanced root excretion of H^+ was a result of more cation uptake than anion. In low P soils, once the available P has been depleted and the plants become P deficient, the plant produces H^+ ions at the root-soil interface and as a result this decreases the pH of the soil. It has been emphasised that root-induced pH change can be related to sources of N (NH_4^+ versus NO_3^-) and to P deficiency (Gahoonia et al., 1992).

Glomus mosseae infected sorghum roots substantially when soil P concentration was low (Table 2). In Hoosfield A soil, mycorrhizal infection increased with increasing P application up to 40 mg P kg^{-1} soil then slightly decreased but in Hoosfield B soil, mycorrhizal infection was stimulated up to 20 mg P kg^{-1} soil then decreased with increasing P supply. The Olsen P is slightly less in the Hoosfield A soil than the Hoosfield B soil at all levels of P application which may be one of the reasons for the higher mycorrhizal infection in the former soil. According to Stribley et al. (1980) the plant available P in soil solution is more important for mycorrhizal infection than the total P content of the soil.

Mycorrhizal and nonmycorrhizal plants acidified their rhizospheres, but the mycorrhizal infected roots extended their acidification through to the hyphal section. Li et al. (1991c) related the acidification of the hyphal section to the nitrification of ammonium as a result of higher microbial populations, but in the present case there were no significant differences between inoculated and noninoculated plants with respect to pH change. Differences between mycorrhizal and nonmycorrhizal plants in absorption of anion and cations may lead to differences in rhizosphere pH (Bolan et al., 1991; Buwalda et al., 1983). The results of Bledsoe and Zasoski (1983) and Vaast and Zasoski (1992) show that with both NH_4^+ and NO_3^- forms, ectomycorrhizal inoculation slightly increased the rhizosphere soil pH. Conversely, the results of Li et al. (1991c) show that when N is supplied as a NH_4^+-N, AMF infected plants slightly reduced rhizosphere soil pH. The results are hence consistent with the findings of Li et al. (1991c) for AMF but contrast with those for ectomycorrhizal fungi.

Effect of rhizosphere pH on P uptake

There is more extractable P in nonrhizosphere and rhizosphere soils with NO_3^--N application compared to NH_4^+-N application which is in agreement with the plant absorbing more P with NH_4^+-N supply than with NO_3^--N supply (Figure 4). Lowering of soil pH due to NH_4^+-N nutrition has resulted in a higher P content of plants (Gahoonia et al., 1992; Miller et al., 1970; Riley and Barber, 1971; Sarker and Wyn Jones, 1982). Sarkar and Wyn Jone (1982) and Hoffmann et al. (1994) demonstrated that with NH_4^+-N application rhizosphere pH decreased and P uptake increased, whereas NO_3^--N application resulted in a reverse trend. Decrease in rhizosphere pH by roots under low P conditions could be a beneficial plant adaptation. In neutral and alkaline soils, with decreases in rhizosphere pH, the mobilization of sparingly soluble calcium phosphate can increase (Gahoonia et al., 1992; Hedley et al., 1982 a). According to Marschner (1995), the association between pH decreases and P depletion with both mycorrhizal and nonmycorrhizal plants in the rhizosphere and in the 'hyphosphere' of NH_4^+ -fed plants contributed to the P acquisition of both roots and hyphae.

Effect of mycorrhiza infection on P uptake

Mycorrhizal infection increased the accessibility of P as shown by the greater magnitude of P depletion in the nonrhizosphere soil section. The magnitude of P depletion depends on mycorrhizal infection and in part depends on N form which caused soil pH changes. All the observations about P depletion patterns which have been presented here show that the P depletion pattern in soil around a mycorrhizal root system is wider than in a nonmycorrhizal system. Depletion of P in soil around the non-mycorrhizal root is more intensive but is limited in extent. This is an interesting indication of the location of P uptake by the external hyphae. In the present experiment hyphal length was not measured, but its contribution can be deduced. It can be suggested that mycorrhizal hyphae in the soil can increase nutrient uptake by increasing absorbing areas and by enhancing the solubility of P in the rhizosphere through altering the pH of the surrounding soil. With all treat-

ments, NH_4^+-N supply resulted in higher P uptake than NO_3^--N. Mycorrhizal inoculation enhanced the differences between the forms of N, It has been shown that mycorrhizal infected plants utilize NH_4^+-N more efficiently than nonmycorrhizal plants (Smith et al., 1985).

A soil P depletion pattern was used as a measure of soil P mobilization which was markedly affected by N nutrition, the decrease of pH as a result of NH_4^+ nutrition has been related to a marked increase in depth and width of the P depletion zone compared to NO_3^--N sources (Gahoonia et al., 1992). When mycorrhizal inoculum was introduced to the soil, the rhizosphere soil had more extractable P than did noninoculated rhizosphere soil. It is not known whether the increase in extractable P in rhizosphere soil is as a result of mycorrhizal mechanisms or not. It has been reported that mycorrhizal plants deplete $NaHCO_3^-$ extractable P (Kothari et al., 1991; Li et al., 1991a, b). More extractable P in mycorrhizal inoculated rhizosphere soil can be achieved by increasing the solubility of soil P. Gahaonia et al. (1992) compared the effects of NH_4^+-N and H_2SO_4 on soil acidification and P depletion in the root-soil interface, and they indicated that factors other than pH also influenced P mobilization of NH_4^+-N-fed plants. The higher P uptake and better plant growth of NH_4^+-N supplied plants may be ascribed to other mechanisms. The extra enhancement of P uptake by NH_4^+-N compared to NO_3^--N in mycorrhizal plants may be due to NH_4^+-N stimulated root exudates which release P from the soil phase and thus increase P acquisition. However, there is no evidence on quantitative and qualitative changes in exudation of organic acids by roots as affected by NH_4^+-N supply.

Conclusion

The results give support to the hypothesis that mycorrhizal and non mycorrhizal plants reduce their rhizosphere soil pH when P supply is limited with both ammonium and nitrate nitrogen sources. Under the conditions of very low available P another acidification process operates which can overcome the effect of NO_3^--N. In the present experiment, when AMF inoculum was used, P depletion extended further than soil acidification. The high P in non-AMF rhizosphere soil suggests a "sparing" effect. It is concluded that rhizosphere pH change is unlikely to have an appreciable contribution to the differences in P supply between mycorrhizal and non-mycorrhizal infected plants, because there were only small differences between inoculated and non-inoculated rhizosphere soil pH when the same form of N was used.

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