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Influence of nitrogen source on the viability, functionality and persistence of *Glomus etunicatum* fungal propagules in an Andisol

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Abstract

This study investigated how two different N sources used as fertilizer (NO_3^- or NH_4^+) interact with an inoculated arbuscular mycorrhizal (AM) fungus (*Glomus etunicatum*) in an Andisol from southern Chile. The effect of NO_3^- or NH_4^+ on mycorrhizal and non-mycorrhizal wheat plants was measured on key root–soil interface activities: pH, acid phosphatase (P-ase) activity and P availability. Root AM colonization, extraradical mycelium length and spore number were also examined at three stages of AM symbiosis development (120, 150 and 240 days after sowing, DAS). The effect of N-source on AM propagule formation was used as an index of the quality and vigor of AM colonization. Mycorrhizal root length was greater with NO_3^- than with NH_4^+ at all times. The NO_3^- source also improved extraradical mycelium density, which reached its maximum at 150 DAS. At each harvest the spore number in the rhizosphere soil was also greater with NO_3^- fertilization. This NO_3^- effect on spore formation ranged from 20% at a 120 DAS to 287% at a 240 DAS increase, compared with NH_4^+. Extraradical mycelium and AM efficiency for P acquisition appeared to be related. The particular fungus/plant metabolism as affected by N sources (NO_3^- or NH_4^+) applied did not result in differential plant growth or in changes in N plant acquisition, but affected AM development and activity. Differences in soil pH, available P or P-ase activity in soil seems not to be responsible for the improved physiological status of mycorrhizal development in NO_3^- fed plants. Mycorrhizal propagule formation in this soil and the high persistence of extraradical mycelium are important factors which may have a strong influence on the next crop, and thus, this aspects should be considered when a cropping system is designed. The influence of N sources on AM performance is of ecological and practical interest in volcanic soils when conventional management is used.

Keywords: AM fungal propagules; Nitrogen source; Andisol

1. Introduction

The use of nitrogen fertilizers is a common practice in extensive agriculture systems such as volcanic soils of southern Chile (mainly Ultisols and Andisols) where they cover more than 5×10^4 km^2. Cereal–legume rotation is the main cropping system in these areas (Besoaín, 1985). These allophanic soils have serious limitations in fertility, due to low P-availability together with high exchangeable Al content, a pH range around 5.0–5.5 and high levels of highly condensed SOM. It is recognized that high aluminum activity is harmful for

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plant growth especially when P and bases are low (Baligar and Fageria, 1997). Previous studies (Rubio et al., 1989, 2002; Borie and Rubio, 1999; Borie et al., 2002) have demonstrated that the arbuscular mycorrhizal (AM) symbiosis plays a crucial role in P cycling and in the alleviation of Al toxicity in such soils. However, AM development and physiological status is affected by many management practices related to the previous crop, the type of fertilization used, and the crop system. It is known that agricultural practices are the primary determining factors affecting the quantity and functionality of AM fungi present in soils (Bethlenfalvay, 1992), especially in volcanic soils (Borie and Barea, 1983; Rubio et al., 1989; Mendoza and Borie, 1998; Borie and Rubio, 1999).

When the cereals are sown in the rotation, N fertilizers are typically applied as NO_3^--N or NH_4^+-N, thus affecting rhizospheric soil pH (Marschner, 1995). Different N sources modify the pH of the AM root environment (mycorrhizosphere) in different ways. Gianinazzi-Pearson and Smith (1993) associated a decrease in the pH of the hyphosphere with NH_4^+ uptake and an increase in pH with NO_3^- uptake. The pH changes may influence the solubility and mobilization of nutrients and the microbial activity in the rhizosphere.

Rhizosphere changes, caused by the N form supplied can modify the extent of AM propagule formation, root colonization and symbiosis functionality (Cuenca and Azcón, 1994; Ortas et al., 1996; Kabir et al., 1997). Previous studies in neutral and alkaline soils have found an increase in the plant growth response to mycorrhizal infection in the presence of NO_3^--N (Barea et al., 1989; Azcón et al., 1991, 1992). Other studies have demonstrated that mycorrhizal plants prefer NH_4^+ as N source (Cuenca and Azcón, 1994). However, the AM absorbing hyphae are able to take up and transport both NO_3^--N, NH_4^+-N (Johansen et al., 1993; Hawkins and George, 2001), and organic N (Hawkins et al., 2000) from soil.

AM propagules present in soil are an important biological factor to be considered when cropping systems are designed. Manipulation of AM fungi in the field to obtain an optimum by designing appropriate nutrient amendments is important. The effect of N-source on mycorrhizal propagule production (colonized roots, mycelium or spores) in rhizospheric soil was used here as a marker of the physiological state of AM colonization, as the potential of a soil as inoculum for subsequent cultures in a crop rotation system is related to the quantity and quality of AM propagules present in it.

The aims of the present study were: (1) to determine how use either NO_3^--N or NH_4^+-N fertilizer affects soil pH, acid phosphatase activity, P availability and P acquisition by mycorrhizal and non-mycorrhizal plants, (2) to assess whether any change in any of these plant–soil activities/responses is due to the effect of the NO_3^--N or NH_4^+-N fertilizer, or to the AM development, or the interactions between the two factors and (3) to study the effect of NO_3^--N or NH_4^+-N fertilizer on AM development and persistence during plant growth and even several weeks after grain harvest.

2. Materials and methods

2.1. Experimental design

A design 2 × 2 full factorial randomized experiment design was used, including two mycorrhizal treatments (with or without AM inoculation) and two N sources (NO_3^- or NH_4^+). Each treatment combination had four replicates for each of three measurement stage.

2.2. Soil characteristics

The test soil used was collected from a 5- to 25-cm depth of an annual crop site at the Agricultural Experimental Station Carillanca, Temuco, Chile. The characteristics of the test soil, an Andisol (Entyc Dystrandept), are described in Table 1. The soil was air-dried, sieved through a 5 mm mesh, treated in a microwave oven (1000 W for 10 min) for 3 days to eliminate the native mycorrhizal propagules (Borie and Rubio, 1999), and re-inoculated with 10 mL of a filtrate from a natural soil/water mixture (1/9 w/v) in Whatman® grade 40 filter paper containing the normal microbiota without AM propagules. In addition, half of the pots corresponding to non-mycorrhizal treatments were inoculated with 10 mL of a filtrate of AM inoculum/water as previously described for natural soil. Each 1-L pot was filled with 800 g of soil.

Table 1
Selected chemical properties of soil used in this study

Available P (mg kg^{-1})	4.0
pH (H_2O)	5.42
SOM (g kg^{-1})	180
K (cmol(+) kg^{-1})	0.70
Na (cmol(+) kg^{-1})	0.07
Ca (cmol(+) kg^{-1})	9.33
Mg (cmol(+) kg^{-1})	1.23
Al (cmol(+) kg^{-1})	0.07
CEC (cmol(+) kg^{-1})	11.33
Al sat (%)	0.61

2.3. Biological material

Soil containing spores, hyphae and mycorrhizal root fragments from pot cultures of sudangrass (*Sorghum bicolor* L.) colonized by *G. etunicatum* CH 110 (Morton and Bentivenga, INVAM culture collection) and grown in the tested Andisol was used as AM inoculum. In the inoculated treatments, the inoculum was mixed thoroughly with the upper section of the soil in the pot as 6.25% of the total mixed weight.

Triticum aestivum L. cultivar "Otto" was used as the host plant. This specie and cultivar is currently cropped in the region under study. Seeds were surface-sterilized with 2% Cloramin-T solution for 3 min and rinsed thoroughly. Four seeds were germinated between wet tissue paper and transplanted 7 days after seed germination. The pots were thinned to two plants after establishment.

2.4. Growth conditions

Plants were grown under greenhouse conditions with temperatures ranging from 25 ± 3 °C day to 15 ± 3 °C night, a 16/8 h light/dark photoperiod and a relative humidity of 80–90%. A photosynthetic photon flux density of 400–500 μmol m^{-2} s^{-1} was applied as supplementary light when necessary.

The plants were irrigated manually with distilled water as needed during the experiment (judged by weighing pots). Every 2 weeks, 10 mL nutrient solution (Johnson et al., 1996) without P and N were added to each pot.

The N was supplied in two portions, at establishment (30% of total N at Zadocks 11 stage) and at 6 weeks of cultivation (70% of total N at Zadocks 31 stage) (Zadocks et al., 1974) to an equivalent amount of 0.125 g N kg $soil^{-1}$, and this represented a normal fertilization rate. In the NO_3^- treatments, $NaNO_3$ was used, and $CO(NH_2)_2$ in the NH_4^+ treatments. In both cases, the N was applied in solution, supplied with 0.06 g P kg $soil^{-1}$ as TSP and 0.063 g K kg $soil^{-1}$ as KC1 in solution.

2.5. Harvests and analyses

Three harvest stages were considered. The first stage was at maturity (120 days after sowing (DAS) at Zadocks 71 stage), the second stage was at dry grain (150 DAS, at Zadocks 99 stage; Zadocks et al., 1974), and the last stage was at 240 DAS (3 months after grain harvest). Only the soil and roots were present in the pots in the last stage, and they had been watered once a week since grain harvest.

The harvested roots and shoots were dried at 65 °C and weighed. Before drying, a portion of roots was separated and AM colonization was estimated by the method described by Giovanetti and Mosse (1980) after clearing and staining (Philips and Hayman, 1970). Root length was determined by Tennant's gridline intersect method (1975).

The total extraradical mycorrhizal hyphae were determined by the method described in Borie et al. (2000) and the active hyphae were determined using dehydrogenase activity (Sylvia, 1988; Kabir et al., 1997). To quantify the total and active hyphal density, we used Newman's intersect gridline method (1966).

Mycorrhizal spores were separated from soil by wet sieving and decanting (Gerdemann and Nicholson, 1963) and quantified under stereoscopic microscope at 50×.

Soil pH was measured in a soil/water mix (2/5) and available P in soil by the method described by Olsen and Sommers (1982) after extraction with 0.5 M $NaHCO_3$ (pH 8.5). Acid phosphatase (P-ase) activity in the root-associated soil was estimated as described by Tabatabai and Bremner (1969).

2.6. Statistical analyses

The main effects of inoculation/uninoculation, N source and their interaction were tested by means of a two-way analysis of variance using the general linear model procedures of the SPSS software, version 11.0 (SPSS Inc., 1989–2001) (Visauta, 1998). Means were compared by the orthogonal contrast test (Petersen, 1977). A *t*-test for paired samples was used to determine the level of significance between the two N sources in the case of AM propagules. Data sets not meeting assumptions for ANOVA were transformed as required, but the results are presented in their original scale of measurement. Statistical significance was determined at $P < 0.05$

3. Results

Root length was differently influenced by the AM colonization, in relation to the N source applied (NO_3^- or NH_4^+) (Fig. 1). The AM effect in increasing root length was highest with the application of NO_3^- after 120 DAS of wheat growth while NH_4^+ appears to be the best N source after 150 DAS. Nevertheless, at whatever growth period, the mycorrhizal root length was greatest with NO_3^- as N source. The percentage of infection development and of mycorrhizal roots increased with the time irrespective of N source applied.

After 120 or 150 DAS, the AM extraradical hyphal length showed differences according to the N source

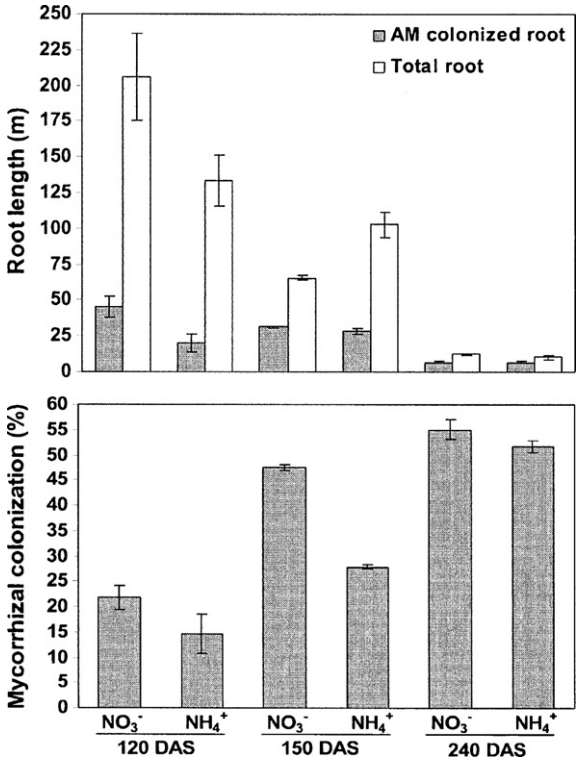

Fig. 1. Total and AM colonized root length (m pot^{-1}) of wheat plants inoculated with *Glomus etunicatum* and level of mycorrhizal colonization (%) as influenced by the N source (NH_4^+ or NO_3^-) at 120, 150 and 240 DAS. Bars denote mean ± S.E. ($n = 4$).

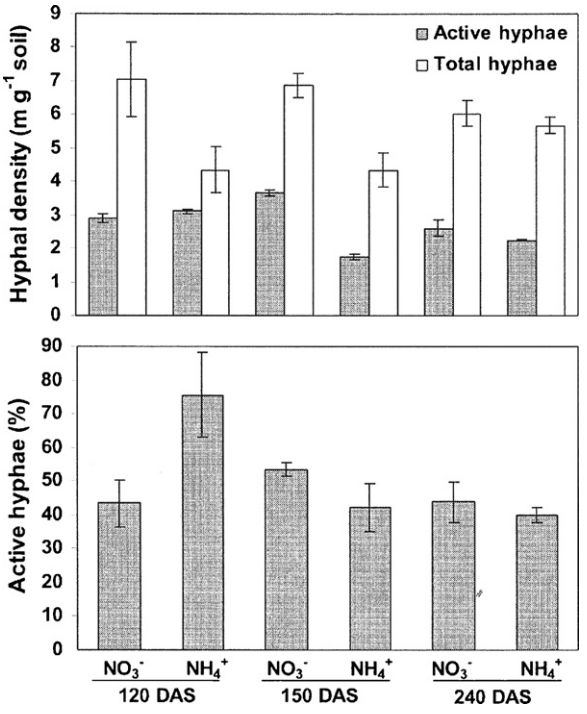

Fig. 2. Density of active and non-active (mg^{-1} soil) extraradical mycorrhizal hyphae and proportion of active hypha from AM-colonized wheat plants as influenced by the N source (NH_4^+ or NO_3^-) at 120, 150 and 240 DAS. Bars denote mean ± S.E. ($n = 4$).

applied (Fig. 2). In both growth periods the NO_3^- produced a greater hyphal length than the NH_4^+-N source. The length of active mycelium was similar (120 DAS) or greater (150 or 240 DAS) in soils where N was supplied as NO_3^-.

In soil colonized by *G etunicatum* more spores were present when N was supplied as NO_3^- (Fig. 3). This effect was maintained over the two harvest periods and in the post-harvested soil. The greatest spore number was found in the soil supplied with N-nitrate at the 150 DAS harvesting. Comparing spore production according to the N-source applied differences found ranges from 20% (120 DAS) to 287% (240 DAS).

Soil–root interface traits studied were significantly affected by the different treatments used, especially at 120 DAS (Table 2). Root colonization by *G. etunicatum* increased available P in soil receiving N-NO_3^- or N-NH_4^+ (Tables 2 and 3) after 120 or 150 DAS of wheat growth. At the first harvest (120 DAS) NO_3^- application significantly depleted available P in soil from non-mycorrhizal treatment, but this effect was not maintained at the second harvest (150 DAS). In addition, increases of available P in soil by AM colonization after 120 DAS was greatest with NH_4^+ supply (30% increase) compared to NO_3^- (21% of increase). After 150 DAS, the available P in the soil was affected more by the interaction of *G. etunicatum* with NO_3^- (24% increase) than with NH_4^+ (18% increase) (Table 3).

AM colonization did not significantly change the rhizosphere pH irrespective of the N source applied. In

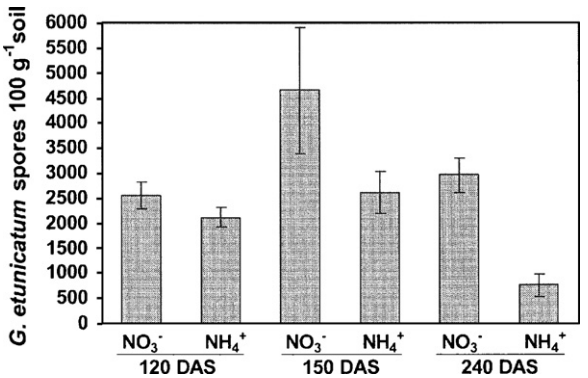

Fig. 3. *G. etunicatum* spores number in 100 g of soil as influenced by the N source (NH_4^+ or NO_3^-) at 120, 150 and 240 DAS. Bars denote mean ± S.E. ($n = 4$).

Table 2
F-values and probabilities of significance for the main effects and factor interaction for the variables measured and analysed by means of a two-way ANOVA at three crop stages

	120 DAS			150 DAS			240 DAS		
	AM	N	AM × N[a]	AM	N	AM × N	AM	N	AM × N
Available P (μg g^{-}1)	14.86**	2.18 NS	0.59 NS	32.37**	6.64 NS	0.93 NS	0.75 NS	2.46 NS	0.46 NS
Soil pH (water)	7.81***	23.04***	0.00 NS	0.07 NS	4.70 NS	3.32 NS	0.50 NS	2.54 NS	0.01 NS
P-ase (mg PNF g^{-1} h^{-1})	1.19 NS	7.77*	12.91**	0.17 NS	0.94 NS	0.85 NS	0.04 NS	0.00 NS	0.11 NS
P content (mg)	21.47***	26.17***	0.39 NS	6.40*	0.20 NS	0.24 NS	_		_

Significance conventions: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.
[a] Interaction between the factors AM inoculation and N source.

mycorrhizal and non-mycorrhizal soils (120 DAS) N-NH_4^+ application resulted in a lower pH than when NO_3^- was supplied to soil (Table 3).

P-ase activity was enhanced only in mycorrhizal soil where N was supplied as NO_3^- and at 120 DAS. A large reduction in P-ase activity at 240 DAS was found in all the treatments (Table 3).

AM root length was affected significantly by N source at 120 DAS, and hyphal density at 120 and 150 DAS (Table 4). AM colonization (%) and the length of active hyphae were affected at 150 DAS while spore production was significantly affected by applied treatments only at 240 DAS.

The magnitude of P plant assimilation differed between mycorrhizal and non-mycorrhizal plants and changed with the N-source applied. AM colonization increased P uptake by 25% irrespective of N source applied (Table 5). Differences in P acquisition between AM colonized and non-colonized plants were statistically significant irrespective of the N-source applied at the two growing periods (120 and 150 DAS). Nevertheless, at 150 DAS the effectiveness of AM colonization in increasing P uptake was more reduced in NH_4^+ treated plants. In the case of NO_3^- fertilized plants, the AM effectiveness on P uptake was maintained at the same level (25% of increase) at 120 and 150 DAS (Table 5).

Table 3
Soil–root interface traits as influenced by the AM inoculation (−M or +M) and N source (NH_4^+ or NO_3^-) at 120, 150 and 240 DAS

AM inoculation	N source	P-Olsen (μg g^{-1})		
		120 DAS	150 DAS	240 DAS
−M	NH_4^+	11.37 (0.95) b	11.39 (1.11) c	8.83 (2.77) a
	NO_3^-	10.85 (1.10) c	12.09 (0.06) bc	8.58 (1.77) a
+M	NH_4^+	14.74 (1.46) a	13.43 (0.31) ab	11.72 (0.13) a
	NO_3^-	13.10 (2.07) ab	14.95 (0.39) a	9.73 (1.56) a
AM inoculation	N source	pH (water)		
		120 DAS	150 DAS	240 DAS
−M	NH_4^+	5.44 (0.04) c	5.47 (0.06) a	5.64 (0.05) a
	NO_3^-	5.64 (0.13) ab	5.58 (0.01) a	5.74 (0.11) a
+M	NH_4^+	5.56 (0.04) bc	5.51 (0.01) a	5.69 (0.10) a
	NO_3^-	5.76 (0.09) a	5.52 (0.06) a	5.77 (0.02) a
AM inoculation	N source	P-ase activity (mg PNF g^{-1} h^{-1})		
		120 DAS	150 DAS	240 DAS
−M	NH_4^+	0.93 (0.10) b	1.03 (0.07) a	0.43 (0.21) a
	NO_3^-	0.89 (0.16) b	0.93 (0.03) a	0.47 (0.16) a
+M	NH_4^+	0.80 (0.08) b	1.00 (0.03) a	0.45 (0.04) a
	NO_3^-	1.13 (0.10) a	1.00 (0.14) a	0.41 (0.20) a

For each stage cultivation, means (S.E.) followed by the same letter in a column are not significantly different using orthogonal contrasts test ($P < 0.05$; $n = 4$).

Table 4
Probabilities of significance for N source on AM propagules development analysed by means of a Student's *t*-test at three crop stages

Experiment variable	Crop stage		
	120 DAS	150 DAS	240 DAS
Total root length (m)	−2.07*	4.08*	−1.60 NS
AM root length (m)	−2.51*	−1.19 NS	−2.99*
AM colonization (%)	−1.55 NS	−29.88***	−1.48 NS
Total hyphae (mg^{-1})	−2.04*	−3.95*	−0.82 NS
Active hyphae (mg^{-1})	1.55 NS	−15.51***	−1.50 NS
Hyphal activity (%)	−2.26 NS	−1.56 NS	−0.59 NS
G. etunicatum spores	−1.32 NS	−1.52 NS	−5.35**

Significance conventions: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 5
P content in shoot wheat plants growing in an Andisol as influenced by the AM inoculation (+M or −M) and N form fertilizer (NH_4^+ or NO_3^-) at 120 and 150 DAS

AM inoculation	N source	P content (mg)	
		120 DAS	150 DAS
−M	NH_4^+	3.15 (0.20) b	4.17 (0.19) b
	NO_3^-	2.48 (0.15) c	3.87 (0.24) b
+M	NH_4^+	3.94 (0.12) a	4.81 (0.45) a
	NO_3^-	3.08 (0.11) b	4.83 (0.33) a

Means (S.E.) followed by the same letter in a column are not significantly different using orthogonal contrasts test ($P < 0.05$; $n = 4$).

4. Discussion

The effect of N sources on plant metabolism and physiology has been well documented (Barea, 1991; Azcón et al., 1992). Nevertheless, the effect of this main nutrient (N) when applied as N-NO_3^- or N-NH_4^+ on the physiology and performance of AM-colonization and propagule formation (spores, mycelium and mycorrhiza) remains poorly understood (Villegas et al., 1996). Results from Johansen et al. (1993) suggest that extraradical mycelium can assimilate NO_3^- and Smith et al. (1985) reported that AM fungi are efficient as assimilating NH_4^+. But different species of AM fungi can show preferences for uptake of N-NO_3^- or N-NH_4^+ (Tobar et al., 1994; George et al., 1995; Azcón et al., 1996).

G. etunicatum is known to assimilate both N forms (NO_3^- and NH_4^+) (Cuenca and Azcón, 1994). In this study we tested the hypothesis that a change in the N source applied will lead to a change in the mycorrhizal performance. Results showed that mycorrhizal colonization decreased differences between N sources on plant P assimilation, particularly at 150 DAS. In this moderately acidic soil, *G. etunicatum*-colonized plants utilize N-NO_3^- more efficiently than non-mycorrhizal plants. This AM effect was previously demonstrated only in neutral-alkaline soil (Azcón et al., 1992, 1996; Cuenca and Azcón, 1994; Tobar et al., 1994). The enhancement of mycorrhizal roots and extraradical hyphal length by NO_3^- fertilization seem to be responsible for such effectiveness (Bago et al., 1996).

For wheat, an increase in AM colonization with NO_3^- application causes an increase in P uptake, but not better plant growth or a better N nutrition. Results on the enhancement of hyphal length observed in AM-NO_3^--fed plants after 150 DAS suggest that it is the enhanced functional capacity of the AM system when supplied with NO_3^- that caused the higher plant P uptake. In fact, the development of AM mycelium is linked to the P plant nutrition and the physiological and practical significance of AM fungi are closed related to the development and activity of AM extraradical mycelium formed by AM roots (Kabir et al., 1997). Mycorrhizal colonization increased extractable P, but we do not know if such P enhancement is the result of mycorrhizal metabolism or other mechanisms that might increase P solubility in mycorrhizal soil. Factors other than pH may influence P mobilization in N amended soils. The results presented show that in the low available P soil used, N forms slightly increased soil pH (by 0.12 units) as a consequence of AM-colonization. Thus, any change in P uptake by plants or mycorrhizal development cannot be attributed to this chemical aspect particularly after 150 days of plant growth.

Studies have shown that AM fungi can modify the mycorrhizosphere pH as well as the ionic surrounding environment (Vaast and Zasoski, 1992; Bago et al., 1996; Bago and Azcón-Aguilar, 1997; Borie et al., 2002; Rubio et al., 2002). Results from this study support the view that AM colonization produced a similar effect on the pH of the sterile soil used independent of the N source applied. These results disagree with those reported by Villegas et al. (1996) who found a different effect on the pH of the media by the extraradical mycelium when developed on NO_3^- or NH_4^+ supplied media. In Villegas et al. (1996) study only NO_3^- supply significantly increased pH of the medium.

The ions mobilization from soil, as HPO_4^{2-}, by AM mycelium is generally accepted and in this study such an AM effect was present at all growth stages and with either N-form. Previous studies by Hoffmann et al. (1994) and others have demonstrated that with NH_4^+ the soil pH decreased and plant P uptake increased whereas the NO_3^- supply resulted in a reversed pH trend.

In our study, the association between pH and soil P enhancement in AM colonized NO_3^- or NH_4^+ fed plants contributed to a better P acquisition by

mycorrhizal roots. Previous studies using different AM isolates have shown that a pH increase in the rhizosphere by an enhancement of OH^- root excretion, which is the result of more anion uptake than cation, improved AM colonization (Medeiros et al., 1994). According to these results, as Borie et al. (2002) and Rubio et al. (2002) also reported, there is more available P in *G. etunicatum*-colonized soil.

AM spore abundance and diversity may be reduced with high N deposition (Egerton-Warburton and Allen, 2000). In this study, N-NO_3^- produced a better source than NH_4^+ regarding mycorrhizal root length, extraradical mycelium and number of spores produced.

It is, therefore, likely that the effect of N-fertilization of one crop may affect the growth and nutrition of the succeeding crop by affecting the soil's biological properties. One of the causes is the abundance of AM colonization in the root and AM propagules in soil, particularly if available soil P limits growth (Thompson, 1991). In this study, spore formation after 240 DAS was 287% higher in NO_3^- than in NH_4^+-fed soil. No differences between N source on the AM colonized roots and hyphal density were shown at 240 DAS; nevertheless, it is interesting to highlight the high extraradical hyphal density determined at this time. These results demonstrated for the first time in this kind of soil the high persistence of extraradical mycelium, that together with the elevated number of spore remainder in the NO_3^- fertilized treatment can represents an important AM inoculum source for the next crop in the rotation (Jasper et al., 1989; Klironomos and Hart, 2002). Results from this study suggest that the source of N fertilization as NO_3^- may improve the growth and yield of the succeeding crop modifying the AM status in the soil (Karasawa et al., 2001) more than other plant attributes.

In fact, in the present study similar shoot and root growth were found in AM inoculated and non-inoculated wheat plants at both harvest times (120 or 150 DAS) irrespective of the N source applied. In addition, N acquisition did not change as a result of the chemical (N source) and/or biological (AM colonization) treatments applied. Thus, these results are not presented here, but they are important to understand the direct N-source effect on AM fungal performance.

AM status in soil was greater when NO_3^- was applied as N source. The extraradical mycelium developed in the soil appears to be a major determinant of the efficiency of AM fungi to P uptake since it is linked to the plant P nutrition. The use of NO_3^- enhanced the vital cycle of the AM fungus assayed and in the first period (120 DAS) increased AM root length and hyphal development (being 75% higher in NO_3^- fed plants than in NH_4^+ fed plants) and in a subsequent period (150 DAS) the active mycelium was particularly increased in NO_3^- fed soil, as Bago et al. (1996) also observed. The last step in the AM development is the spore formation and thus, only at the last observation (240 DAS) NO_3^- was nearly four times more effective than NH_4^+ in stimulating spore production. This aspect is essential for understanding the ecology and function of AM fungi. Results show that the effectiveness of NO_3^- application was not due to the changes in soil chemical properties but rather, it acted directly by favoring and improving AM development.

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