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Mycorrhizal Effectiveness on Wheat Nutrient Acquisition in an Acidic Soil from Southern Chile as Affected by Nitrogen Sources

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ABSTRACT

Acidity, aluminium (Al) and manganese (Mn) phytotoxicity, and low levels of available phosphorus (P) are the main constraints for plants growing in Andisols from southern Chile. This study was carried out to evaluate the effect of arbuscular mycorrhizal (AM) fungus inoculation and two nitrogen (N)-sources on the growth and mineral uptake of wheat (*Triticum aestivum* L.) plants grown in an acidic Andisol at 120 and 150 days after sowing (DAS). The plants were grown in pots under greenhouse conditions with or without inoculation with the AM fungus *Glomus etunicatum* CH110 (Morton and Bentivenga) and fertilized with N-ammonium (NH_4^+) or N-nitrate (NO_3^-). The biomass production was not affected for any treatment used, but the inoculation with *G. etunicatum* increased the shoot contents and specific absorption rate (SAR) of P and zinc (Zn) at 120 and 150 DAS, especially when N-NH_4^+ was used. On the other hand, the use of N-NO_3^- increased the N (at 120 DAS), potassium (K) (120 and 150 DAS), and copper (Cu) (120 DAS) shoot content, reduced the Al shoot content and Mn SAR (both at 120 DAS). The combined use of *G. etunicatum* inoculation and N-NO_3^- use decreased significantly ($P<0.001$) the Mn shoot content and the Al SAR at 150 DAS. The results suggest the selection of NO_3^- as N-source to address practical and ecological fertilization management, which is important for acidic volcanic soils from southern Chile, with a natural tendency to produce Mn and Al phytotoxicity.

Keywords: arbuscular mycorrhiza, N fertilization, specific absorption rate, acidic volcanic soils

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INTRODUCTION

Arbuscular mycorrhizal (AM) symbiosis plays a critical role in plant nutrition, because the external mycorrhizal mycelium developed around the roots of host plants is able for enhancing mineral acquisition (Smith and Read, 1997), especially phosphorus (P) (Powell and Bagyaraj, 1984) and many other low mobile elements (Clark and Zeto, 2000). Studies have demonstrated that AM hyphae can transport nitrogen (N) to plant using ammonium (NH_4^+), nitrate (NO_3^-), or amino acids as N-source (Johansen et al., 1994; Hawkins and George, 1999; 2001; Johansen, 1999; Hawkins et al., 2000). In addition, AM association can decrease the plant uptake of some toxic elements from soil (Sieverding, 1991; Clark and Zeto, 2000; Vivas et al., 2003a; 2003b).

It was found that heavy fertilization of N and P reduced mycorrhizal colonization, which affected mycorrhizal functioning on plant nutrient acquisition (Azcón et al., 2003). If any soil treatment affects root colonization the acquisition of nutrients by mycorrhizal root could be also altered (Liu et al., 2000).

Acidic soil by definition has relatively high concentrations of H^+, and pH values are normally <5.5. Restriction of plant growth is not often a direct result of low pH, but usually associated with aluminium (Al) and manganese (Mn) toxicities and P, calcium (Ca), magnesium (Mg), and potassium (K) deficiencies (Marschner, 1991). Ability of some plants to grow in acidic soil has been associated with root colonization and mineral acquisition by AM-colonized plants (Koslowsky and Boener, 1989; Clark, 1997; Clark et al., 1999a; b).

The general interest is to reach a more sustainable form of agriculture in which plant growth and nutrition will depend on both the mycorrhizal efficiency and soil nutrients availability. Nevertheless, the goal is to know how to optimize AM/host plant development under agricultural conditions. However, to reach this objective more knowledge on the effect of agricultural practices on beneficial soil microbial activities are needed. Therefore, research was undertaken with the general objective of evaluating the effects of N-sources on root-soil interface activities, including AM development, on mineral acquisition by the plant, and on the yield of wheat growing in an Andisol from Southern Chile. Since chemical inputs affect mycorrhizal performance, this study was understood to ascertain the role of AM colonization under non-limiting N conditions as well as the behavior of *G. etunicatum* affecting macro- and micronutrient acquisition under each one of the N-sources applied.

MATERIALS AND METHODS

Experimental Design

A 2 × 2 full factorial randomized design, including two mycorrhizal treatments (with or without AM inoculation), and two N sources (NO_3^- or NH_4^+) were

Table 1
Selected chemical properties of soil used in this study

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

used. Each treatment combination had four replicates for each measurement stage (two stages).

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Soil Characteristics

The test soil used was collected from a 5- to 25-cm depth of an annual crop site in Experimental Agriculture Station Carillanca, Vilcún, Chile. The characteristics of the test soil, an Andisol (Entyc Dystrandept), are described in Table 1. Soil was air-dried, sieved through a 5 mm mesh, treated in a microwave oven (1000 W for 10 min) for three days to eliminate the native mycorrhizal propagules (Borie and Rubio, 1999), and re-inoculated with 10 mL of a filtrate containing the normal microbiota without AM popagules from a natural soil/water mixture (1/9, w/v). In addition, pots corresponding to uninoculated treatments were inoculated with 10 mL of a filtrate of AM inoculum/water as previously described. The soil was then supplied with 0.06 g P kg soil^{-1} as triple superphosphate and 6.3 g K kg soil^{-1} as potassium chloride (KCl) in solution. One-L pots were filled with 800 g of soil.

Biological Material

Soil containing spores, hyphae, and mycorrhizal root fragments from pot cultures of sudangrass (*Sorghum bicolor* L.) colonized by *Glomus etunicatum* CH110 (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 superior section of the soil in the pot as 6.25% of the total mix weight. *Triticum aestivum* L. cultivar 'Otto' was used as host plant. This specie and variety is currently cropped in the region under study. Seeds were surface sterilized with 2% Cloramin-T solution for 3 min and rinsed thoroughly with water. Four seeds were germinated between wet tissue paper and planted seven days later. The pots were thinned to two plants after establishment.

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Growth Conditions

Plants were grown under greenhouse conditions with temperatures ranging from $25 \pm 3^{\circ}$C day-time to $15 \pm 3^{\circ}$C night-time, a 16/8 h light/dark photoperiod, and a relative humidity of 80-90%. The plants were irrigated manually with distilled water as needed during the experiment (judged by weighing pots). Every 2 weeks, 10 mL of nutrient solution (Johnson et al., 1996) without P and N were added to each pot. The N was supplied in two applications, at establishment (30% total N in Zadocks 11 stage) and at 6 weeks into cultivation (70% total N in Zadoks 31 stage) (Zadocks et al., 1974) to an equivalent of 0.125 g N kg soil^{-1} that represents a normal fertilization rate. In the NO_3^- treatments, sodium nitrate ($NaNO_3$) was used, and urea [CO $(NH_2)_2$] in the NH_4^+ treatments. In both cases, the N was supplied in solution.

Measurements

Two harvest stages were considered. The first stage was at maturity (at 120 days after sowing, DAS, in Zadocks 71 stage), and the second stage was at dry grain (at 150 DAS, in Zadocks 99 stage). The harvested roots and shoots were dried at 65°C for 48 h in a forced-air oven and weighed. Shoots were crushed, ground, and a portion converted into ash in a furnace at 550°C. Ashes were further acid digested (Wolf, 1982). Before drying, a fraction of roots was collected and AM colonization was determined using the method described by Giovanetti and Mosse (1980) after clearing and staining (Phillips and Hayman, 1970). Phosphorus content was determined colorimetrically using the molybdate-blue method (Murphy and Riley, 1962), and Ca, Mg, K, Al, copper (Cu), zinc (Zn), and Mn were quantified by atomic absorption spectroscopy. One aliquot of crushed shoots was utilized to determine N content by the Kjeldhal method. Specific absorption rate (SAR) is defined as the amount of nutrient absorbed per unit of root mass (Gray and Schlesinger, 1983).

Statistical Analyses

The data of main effects of AM inoculation/uninoculation, N-source and factor 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.; Pérez, 2001). 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-colonization data. 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 $P < 0.05$.

RESULTS

At 120 DAS several characteristics were affected by the different analyzed factors (12 by the inoculation, 8 by the N-source, and 6 by their interaction), being less affected at 150 DAS (7, 2 and 2, respectively) (Table 2). An effect of some of the treatments used on the phytomass production in any considered stage was not registered, even though a global increase of 8.3% at 150 DAS relative to the previous stage was observed. On the other hand, changes in root

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Table 2
$F-$values and significance for the main effects and factor interactions for biomass production, shoot element content and Specific Absorption Rate (SAR) analyzed by means of a two-way ANOVA at two crop stages (120 and 150 DAS)

	120 DAS			150 DAS		
Experimental variable	AM	N	AM × N[a]	AM	N source	AM × N
Shoot dry weight (g)	1.19^{ns}	1.11^{ns}	1.74^{ns}	0.28^{ns}	0.01^{ns}	0.09^{ns}
Root dry weight (g)	0.25^{ns}	0.08^{ns}	0.25^{ns}	0.19^{ns}	0.42^{ns}	0.09^{ns}
Shoot: Root ratio	0.02^{ns}	0.10^{ns}	2.42^{ns}	1.64^{ns}	1.19^{ns}	1.02^{ns}
Shoot N content (mg)[b]	0.12^{ns}	7.68^{*}	0.02^{ns}	0.37^{ns}	0.16^{ns}	0.29^{ns}
Shoot P content (mg)	21.47^{***}	26.17^{***}	0.39^{ns}	6.40^{*}	0.21^{ns}	0.24^{ns}
Shoot K content (mg)	0.23^{ns}	68.69^{***}	0.16^{ns}	5.16^{*}	13.61^{**}	2.67^{ns}
Shoot Ca content (mg)	0.02^{ns}	0.20^{ns}	0.46^{ns}	0.66^{ns}	0.53^{ns}	0.77^{ns}
Shoot Mg content (mg)	1.47^{ns}	0.61^{ns}	0.95^{ns}	0.02^{ns}	2.22^{ns}	0.36^{ns}
Shoot Zn content (μg)	78.48^{***}	75.44^{***}	26.05^{***}	12.01^{**}	0.01^{ns}	0.14^{ns}
Shoot Cu content (μg)	3.39^{ns}	16.57^{**}	5.88^{*}	3.26^{ns}	0.45^{ns}	2.47^{ns}
Shoot Al content (μg)	0.71^{ns}	76.54^{***}	3.76^{ns}	45.18^{***}	1.92^{ns}	3.70^{ns}
Shoot Mn content (μg)	66.78^{***}	1.36^{ns}	9.18^{**}	0.62^{ns}	0.04^{ns}	49.73^{***}
SAR N (μg mg^{-1})[c]	2.20^{ns}	3.27^{ns}	2.21^{ns}	0.01^{ns}	0.10^{ns}	0.71^{ns}
SAR P (μg mg^{-1})	30.89^{***}	36.10^{***}	0.22^{ns}	9.03^{*}	0.25^{ns}	0.56^{ns}
SAR K (μg mg^{-1})	0.66^{ns}	56.01^{***}	3.69^{ns}	2.90^{ns}	12.62^{**}	1.60^{ns}
SAR Ca (μg mg^{-1})	0.80^{ns}	2.17^{ns}	0.36^{ns}	1.71^{ns}	0.66^{ns}	0.37^{ns}
SAR Mg (μg mg^{-1})	4.94^{*}	2.99^{ns}	4.95^{*}	0.14^{ns}	2.43^{ns}	0.74^{ns}
SAR Zn (μg mg^{-1})	96.07^{***}	91.80^{***}	17.06^{***}	15.44^{**}	0.00^{ns}	0.01^{ns}
SAR Cu (μg mg^{-1})	8.95^{*}	10.29^{**}	14.56^{**}	1.65^{ns}	0.65^{ns}	1.65^{ns}
SAR Mn (μg mg^{-1})	0.00^{ns}	95.18^{***}	0.81^{ns}	38.65^{***}	2.51^{ns}	2.66^{ns}
SAR Al (μg mg^{-1})	54.10^{***}	5.88^{*}	3.10^{ns}	1.60^{ns}	0.15^{ns}	46.08^{***}

DAS = days after sowing.
*P<0.05, **P <0.01, ***P <0.001, ns = no significant differences.
[a]Interaction between the factors AM inoculation and N source.
[b]Element content = amount in plant shoot (two plants).
[c]μg of element per mg of root dry matter.

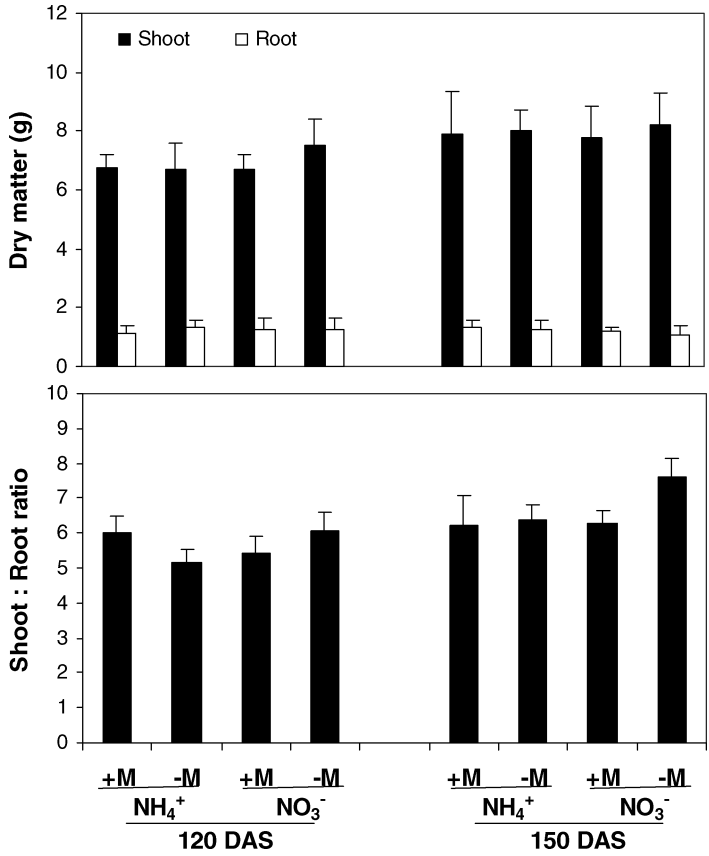

Figure 1. Shoot and root dry weight of wheat plants and Shoot:Root ratio as influenced by the AM inoculation (+M or −M) and N-source fertilizer (NH_4^+ or NO_3^-) at 120 and 150 DAS. Bars denote mean + S.E., $n = 4$.

weight between both stages were not observed, this could explain why the shoot: root relationships were greater at 150 DAS (Figure 1).

The AM inoculation increased 25% the P acquisition in both stages, although at 120 DAS greater P shoot content in the NH_4^+-fed treatments was observed (Figure 2). The other macronutrient contents were not affected at 120 DAS. However, the NO_3^- treatment produced greater N (at 120 DAS) and K (in both stages) shoot content than the NH_4^+ treatment. The interaction between the analyzed factors did not affect the total shoot content of any macronutrient.

The *G. etunicatum* colonization increased the Zn shoot contents in both stages, although at 120 DAS the inoculation combined with N-NH_4^+ use presented a Zn shoot content 1.7–2.1 fold relative to the other treatments (Figure 3). The combined use of inoculation and N-NO_3^- produced greater Cu shoot content at 120 DAS, but did not produce differences at 150 DAS. A remarkable

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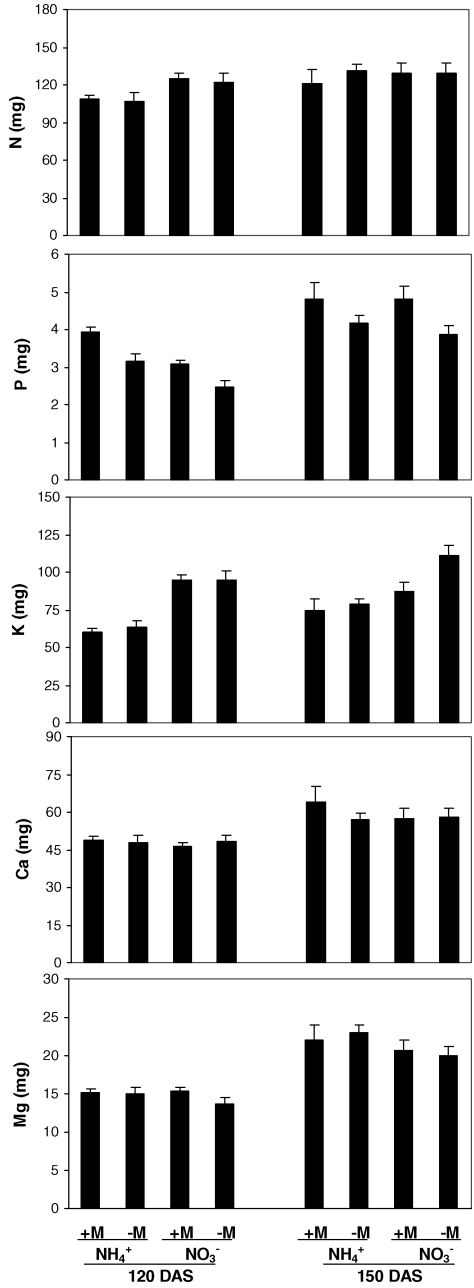

Figure 2. Macroelement (N, P, K, Ca and Mg) content in wheat plants shoot (two plants) growing in an acidic soil as influenced by the AM inoculation (+M or −M) and N-source fertilizer (NH_4^+ or NO_3^-) at 120 and 150 DAS. Bars denote mean + S.E., $n = 4$.

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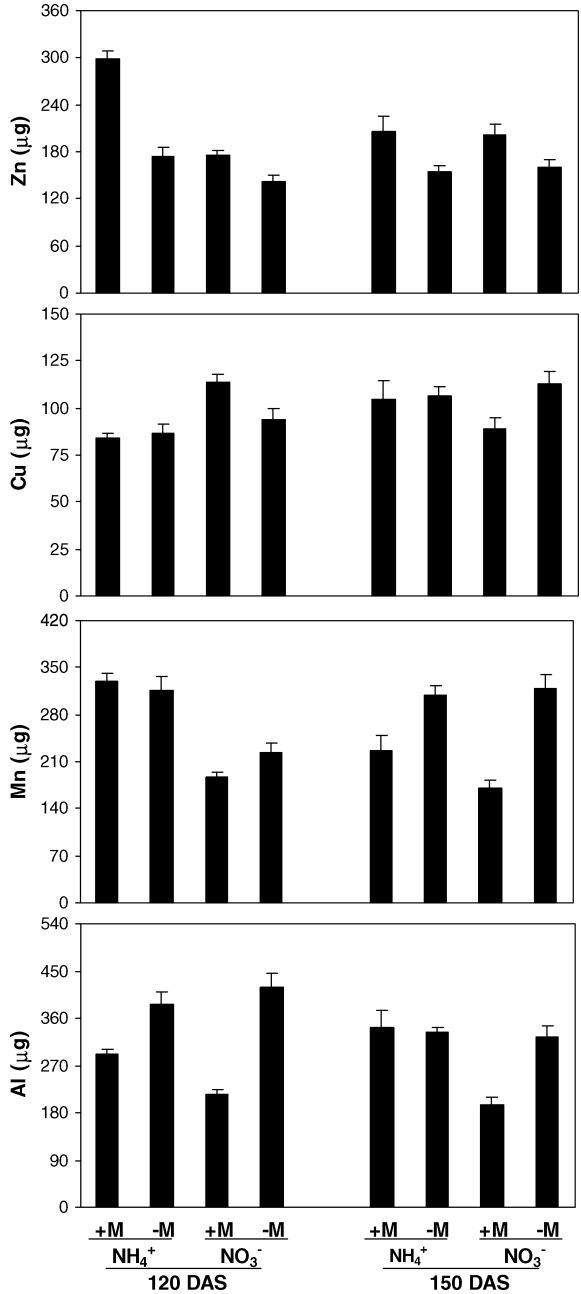

Figure 3. Microelement (Cu, Zn, Mn and Al) content in wheat plants shoot (two plants) growing in an acidic soil as influenced by the AM inoculation (+M or −M) and N-source fertilizer (NH_4^+ or NO_3^-) at 120 and 150 DAS. Bars denote mean + S.E., $n = 4$.

result is that the AM inoculation reduced Mn and Al shoot contents, especially at 120 DAS for Al and at 150 DAS for Mn. This mycorrhizal effect was especially evident when N-NO_3^- was used. Particularly, in the NO_3^- treatments, *G. etunicatum* inoculation reduced the Mn shoot contents by 17% at 120 DAS and 47% at 150 DAS. In NH_4^+ treatments, the inoculation reduced the Mn shoot content by 27% at 150 DAS. On the other hand, Al shoot contents were significantly reduced by the AM colonization, especially when NO_3^- was used, registering reductions from 49 and 40% at 120 and 150 DAS, respectively, relative to the inoculated and NH_4^+ treatment. The effect of the inoculation was smaller in NH_4^+ treatments, and a reduction (24%) in the Al shoot content only was observed at 120 DAS.

In spite no significant differences in the biomass production, particularly in roots, differences in nutrient uptake efficiency among the different treatments were present (Table 3). The N and Ca SAR were not affected by the studied factors; nevertheless, increments of P SAR were observed in the inoculated treatments in both stages, although at 120 DAS a considerable increase in P SAR in the NH_4^+ treatments was also observed. On the other hand, greater K SAR values were registered in both stages in the NO_3^- treatments. The Mg SAR values were greater in the *G. etunicatum* inoculated treatments only at 120 DAS.

The *G. etunicatum* colonization produced increases in Zn and Cu SAR values, registering large differences between the different treatments at 120 DAS. In particular, Zn SAR showed an important increase in treatment inoculated and NH_4^+treatments (1.7–2.3 folds relative to the other treatments), whereas in

Table 3
Specific absorption rate (SAR; μg element mg^{-1} root) of macroelements (N, P, K, Ca and Mg) and microelements (Cu, Zn, Mn and Al) in mycorrhizal (+M) and non mycorrhizal (−M) wheat plants as influenced by N source fertilizer (NH_4^+ or NO_3^-) at two crop stages (120 and 150 DAS)*

Crop stage	AM	N source	Macroelement					Microelement			
			N	P	K	Ca	Mg	Zn	Cu	Mn	Al
120 DAS	−M	NH_4^+	15.97 a	0.47 b	9.52 b	7.10 a	2.22 b	0.026 b	0.013 b	0.047 a	0.058 a
		NO_3^-	16.23 a	0.33 c	12.63 a	6.40 a	1.82 b	0.019 c	0.012 b	0.030 b	0.056 a
	+M	NH_4^+	15.97 a	0.58 a	8.90 b	7.20 a	2.24 a	0.044 a	0.012 b	0.049 a	0.043 b
		NO_3^-	18.64 a	0.46 b	14.16 a	6.90 a	2.28 a	0.026 b	0.017 a	0.028 b	0.032 c
150 DAS	−M	NH_4^+	16.30 a	0.52 ab	9.83 b	7.12 a	2.87 a	0.019 b	0.013 a	0.039 a	0.039 a
		NO_3^-	15.73 a	0.47 b	13.45 a	7.01 a	2.42 a	0.020 b	0.014 a	0.039 a	0.039 a
	+M	NH_4^+	15.30 a	0.60 a	9.50 b	8.13 a	2.78 a	0.026 a	0.013 a	0.029 b	0.044 a
		NO_3^-	16.57 a	0.62 a	11.22 ab	7.38 a	2.64 a	0.026 a	0.011 a	0.022 c	0.025 b

DAS = Days after sowing.
*For each crop stage, means followed by the same letter in a column are not significantly different using orthogonal contrasts test ($P < 0.05$, $n = 4$).

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Table 4
AM-colonization (%) of wheat plants in relation to N source fertilizer at two crop stages (120 and 150 DAS)

	N source fertilizer	
Crop stage*	NH_4^+	NO_3^-
120 DAS	15 a	22 a
150 DAS	28 b	47 a

DAS = Days after sowing.
*For each crop stage, means followed by the same letter are not significantly different using *t*-student test ($P < 0.05$, $n = 4$).

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the case of Cu SAR, a significant increase (30–42%) in the treatment that combined inoculation and NO_3^- use was registered, both at 120 DAS. At 150 DAS, only Zn SAR presented greater values from the effect of the *G. etunicatum* colonization (33% more relative to the uninoculated treatments).

Potentially phytotoxic elements, Al and Mn, presented a significant reduction due to the N-source used and the *G. etunicatum* inoculation. Smaller Al SAR values in the inoculated and NO_3^- treatment at both stages were observed, whereas the Mn SAR values were smaller in the NO_3^- treatments at 120 DAS, and in the inoculated treatment at 150 DAS, especially when NO_3^- was used.

On the other hand, the mycorrhizal colonization was greater in the NO_3^- treatments (Table 4). This difference was significantly greater at 150 DAS, when the colonization reached 1.7 fold relative to the NH_4^+ treatment.

DISCUSSION

This study was carried out to investigate the impact of the use of contrasting N-sources on the mycorrhizal efficiency and its interactive effect on the growth and nutrition of wheat plants grown in an acidic soil. The obtained results did not show an effect of any inoculation treatment and N-source combination on the wheat growth; however, this result is expected since the applied N and P doses were not limiting. On the other hand, the increase of the shoot: root ratio at 150 DAS relative to the previous stage is explained by a smaller vegetative activity, since the plant allocated most of the formed compounds to the grain's growth, resulting simultaneously in root senescence. In this study, a small increase in the shoot biomass was observed from 120 at 150 DAS (about 15%), that would corroborate the previous appreciation. Also, an increase in the N, P, K, Ca, and Mg contents was observed. These nutrients would constitute some compounds allocated to their storage in the grain.

Nitrogen content and N SAR were similar in all the studied combinations and stages, which can be explained by the nonlimiting status of N in the soil (Hawkins and George, 1999). In contrast, K content and K SAR were affected mainly by the source of N used, being greater when NO_3^- was applied, which can be explained by the cations absorption altogether with anions (NO_3^-) to maintain root electroneutrality (Gerendás et al., 1997). The other analyzed macroelements, Ca and Mg, did not present differences in their contents, which is expected since their levels are usually nonlimiting in Andisols. This agrees with results of previous studies in similar conditions (Azcón et al., 1996; Liu et al., 2000; Borie et al., 2002; Rubio et al., 2002). However, higher Mg SAR in the inoculated treatments (at 120 DAS) was determined, which would suggest a greater efficiency in the acquisition of this element in mycorrhizal plants.

One of the most relevant results of this study was the reduction of the Mn and Al shoot contents by effect of the mycorrhizal establishment, particularly when NO_3^- was used. It is well known that both elements are potentially phytotoxic elements, being in abundance in acidic soils (Baligar and Fageria, 1997), especially those of volcanic origin as they are the Andisols from southern Chile (Borie and Rubio, 1999; Borie et al., 2002; Rubio et al., 2002). The reduction observed in the Mn and Al translocation in mycorrhizal plants can be attributed to a series of symbiotic mechanisms based on the metal binding of AM extraradical mycelium developed around the roots (Joner et al., 2000; Gaur and Adholeya, 2004; Janoušková et al. 2006). On the other hand, high Al exchangeable contents in the soil are related to a smaller P use and availability, especially in soil of volcanic origin with high allophane content (Van Ranst et al., 2004). In addition to a positive effect of reducing the Mn an Al contents, *G. etunicatum* played an important role improving the P and Zn acquisition. The greater reduction of the Mn and Al contents in the inoculated and NO_3^- treatments can be related indirectly to an increase of rhizospheric pH, due to the physiological alkalinization associated to the NO_3^- absorption by the root. In addition, the greater P absorption by mycorrhizal plants can enhance the alkalinization, since P is absorbed by the roots of the plants and by the fungal hyphae [like phosphate anion ($H_2PO_4^-$)], which would be related to an OH^- exudation to the rhizosphere, which would act as an additional source of physiological alkalinization. It is important to remark this mycorrhizal effect, which produces, by additive mechanisms, an important decrease in the absorption in phytotoxic elements.

According to these results, the AM fungus used showed different abilities to immobilize Al and Mn within or near the root depending on the N source applied and these direct and/or indirect fungal activities are able to reduce the available Al or Mn in soil and the further translocation from soil to the shoot as results show.

In contrast, AM-colonization increased Zn and P values of specific absorption rate in plants, which evidenced that the uptake of these elements by units

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of roots is improved. The AM colonization provides a large surface area for the absorption of the immobile nutrients (Clark and Zeto, 2000). These AM nutritional effects improving nutrients (P and Zn) and reducing phytotoxic elements (Al and Mn) uptake were greatest when NO_3^- was the amended source. This probably occurred because it promoted a better developed AM-colonization and represented the most relevant results of this study since in the soil used Mn and Al toxicities are common factors limiting plant growth. On the other side, from an applied point of view, these results provide supplementary tools to the farmers for a better choice of N-source, since the Andisols from southern Chile are used mainly in annual cereal-legume rotations (Besoaín, 1985).

These results are in agreement with the existence of a regulatory mechanism for hyphal-element transport which can be affected by the N-source in the medium and the particular ability of AM mycelium development and function under the different N-sources. The fact that P and Zn increased in mycorrhizal plants, while particular elements, such as Mn and Al, were decreased by AM symbiosis, suggests that the availability of an element for a mycorrhizal plant is not necessarily increased since binding and/or immobilization capacity of AM roots for some microelements can be carried out concomitantly. These mycorrhizal behaviours have been also described in heavy metals contaminated soils (Leyval et al., 2002; Vivas et al., 2003a; 2003b). The highest nutritional effects provided by the AM colonization were exhibited under NO_3^- fertilization. This could be explained because both N-sources displayed different degrees on fungal infection being the AM development was more limited in NH_4^+ treated plants than in NO_3^- treated plants. The longest AM colonized root length was observed under NO_3^- fertilization may have accounted for the highest P and Zn uptake and the less efficient Mn and Al acquisition concomitantly exhibited.

An important result is the increased ability of *G. etunicatum* colonized plants for P acquisition and the AM depressing ability for Al acquisition observed in NO_3^- treated plants, which was maintained for a longer period than in NH_4^+ treated plants (i.e., 150 DAS vs. 120 DAS). Previous studies using a neutral alkaline soil (Azcón et al., 1992; 1996; 2001) have also shown the particular compatibilities between AM fungal species and N sources in the medium. Such studies suggest the selection of NO_3^- as N-source to address practical and ecological fertilization management.

The present study can lead to practical agronomic application since AM fungi resulted very important to maximize plant nutrition as a biotechnological approach to manage infertile environments having important limitations for plant growth. These results also emphasize that AM-colonized plants may behave differently depending on the N-source involved. Here, N-sources did not affect plant growth and N acquisition, thus a possible explanation for the different fungal behavior in NO_3^- or NH_4^+ treated plants could be related to the particular AM development under fertilized situations.

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