Communications in Soil Science and Plant Analysis

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lcss20

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Available online: 07 Mar 2011

To cite this article: Santiago Schalamuk, Marta Noemí Cabello, Hugo Chidichimo & Silvina Golik (2011): Effects of Inoculation with Glomus mosseae in Conventionally Tilled and Nontilled Soils with Different Levels of Nitrogen Fertilization on Wheat Growth, Arbuscular Mycorrhizal Colonization, and Nitrogen Nutrition, Communications in Soil Science and Plant Analysis, 42:5, 586-598

To link to this article: http://dx.doi.org/10.1080/00103624.2011.546924

Communications in Soil Science and Plant Analysis, 42:586–598, 2011

ISSN: 0010-3624 print / 1532-2416 online
DOI: 10.1080/00103624.2011.546924

Taylor & Francis
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Effects of Inoculation with *Glomus mosseae* in Conventionally Tilled and Nontilled Soils with Different Levels of Nitrogen Fertilization on Wheat Growth, Arbuscular Mycorrhizal Colonization, and Nitrogen Nutrition

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Evaluation of the performance of inoculants in undisturbed and unsterilized soils, where diverse communities of microorganisms are present, is a necessary step before using arbuscular mycorrhizal fungi (AMF) in agricultural technology. The effects of inoculation with Glomus mosseae *on arbuscular mycorrhizal colonization, growth, and nitrogen (N) uptake of wheat plants in unsterilized tilled and untilled soils from the Argentinean Pampas with different levels of N fertilization were assessed. The fertilization and inoculation effects depended on the tillage treatments. In no-tillage, the colonization was greater than in conventional tillage, but it was reduced by the N fertilization. In conventional tillage, the inoculation with* G. mosseae *increased colonization. Both conventional tillage and N fertilization promoted wheat root growth. Inoculation did not affect root growth but enhanced N concentration in roots when fertilizer was not applied.*

Keywords Arbuscular mycorrhiza, *Glomus mosseae*, no tillage, nitrogen fertilization, wheat

Introduction

Arbuscular mycorrhizae (AM) are associations between fungi that belong to the phylum *Glomeromycota* (Schüβler, Schwarzott, and Walker 2001) and most plant species (Harley 1991). Arbuscular mycorrhizae are considered beneficial to plants, although their positive effects are variable because mycorrhizal symbioses reflect complex interactions among the plant, the fungi, and the environment (Brundrett et al. 1996; Johnson, Graham, and Smith 1997). In agriculture, research dealing with mycorrhizal fungi is valuable both for determining appropriate management strategies and as a background to achieve successful inoculations (Sieverding 1991).

Received 24 May 2009; accepted 13 May 2010.
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No-tillage belongs to the so-called "conservation tillage systems" because this practice keeps the soil surface covered by crop residues (Mannering and Fenster 1983), thus reducing soil erosion. Argentina is one of the countries with greater adoption of no-tillage practices, and most of its cropland is cultivated with this system (Giuffré et al. 2006). In conventional tillage, the soil is tilled two or three times prior to sowing. In no-tillage systems, there is less loss of soil organic matter because of the greater accumulation of crop residues, and less mineralization generates, in turn, nutrient deficiencies in the crops (Doran 1980; Alvarez et al. 1998). This is the main reason why the application of chemical fertilizers is necessary for achieving high yields in no-tillage systems (Fox and Bandel 1986).

Tillage is one of the agricultural practices with greater influence on the soil arbuscular mycorrhizal fungi (AMF) population (Kurle and Pfleger 1994). Soil disturbance directly affects all types of AMF propagules to a greater or lesser extent through different mechanisms acting together, such as the disruption of the hyphal network (McGonigle and Miller 1996), the dilution of the propagule-rich topsoil (Sieverding 1991), and the accelerated root decomposition. Therefore, no-tillage practice maintains an intact AMF hyphal network and the original distribution of the AM propagules, which may affect mycorrhizal infectivity.

The most well-known benefit of AMF to the host plant is the promotion of phosphorus (P) uptake (e.g., Smith and Read 1997); however, positive effects for other nutrients have been documented (e.g., Sieverding 1991). Evidence of the contribution of AMF to the nitrogen (N) nutrition of plants remains contradictory. In some experiments, mycorrhizal plants had greater N concentrations than nonmycorrhizal controls (Frey and Schüepp 1993; Johansen, Jakobsen, and Jensen 1994; Tobar, Azcón, and Barea 1994), whereas in other studies no differences were detected (Johansen, Jakobsen, and Jensen 1992; Johansen 1999; Hawkins, Johansen, and George 2000). The AMF effects on N uptake have been difficult to study (Govindarajulu et al. 2005), because this element presents different forms in the soil, and various N losses can occur in soils by volatilization, denitrification, or lixiviation (Fox and Bandel 1986). Therefore, sophisticated experiments have become necessary to elucidate the mechanism of N uptake and transport by AM associations. In most of these experiments, pure AMF strains were inoculated in sterilized soils, generating less *Glomeromycota* diversity than in field soils, N was applied as labeled forms in hydroponic or semihydroponic systems, and the N sources were controlled in the systems, for example, by using nitrification inhibitors (e.g., Hawkins and George 2001; Subramanian and Charest 1998). Valuable knowledge was acquired, since it was demonstrated that AMF contributes to the uptake of ammonium (Johansen, Jakobsen, and Jensen 1992, 1994; Tobar, Azcón, and Barea 1994), nitrate (Hawkins and George 2001), and even organic forms from the soil (Govindarajulu et al. 2005).

Sterilized soil controls allow the comparison of mycorrhizal and nonmycorrhizal plants (Smith and Read 1997), but sterilized soils may not be representative of the field conditions because the presence of other microorganisms, including other AMF, in nonsterile soils may influence the physiology of the plant and may compete and interact with the inoculated AMF (Wilso, Daniels Hetrick, and Gerschefske Kilt 1987, 1988; Daniels Hetrick et al. 1988). Therefore, testing the success of microbial inoculants by "re-inoculating" soils where diverse AMF communities are present, without altering their physical distribution in soil, is a necessary step for using AMF in agricultural technology. In addition, N fertilization has been proved to reduce the effects of AMF on the host and to inhibit root colonization, although the results are often not consistent (Treseder and Allen 2002). Hence, the assessment of the effects of inoculation with AMF in soils fertilized with the amount of N normally applied for wheat cultivation in Argentina is valuable technological

information. On the other hand, in no-tillage systems, although the organic-matter accumulation generates benefits to the soil physical fertility, shallow compaction effects have been associated with these systems in the Argiudoll soils from the Pampa region (Chagas, Marelli, and Santanatoglia 1994; Balbuena et al. 1996; Taboada et al. 1998; Soza et al. 2003). As AM have been found to alleviate the stress of soil compaction (Yano et al. 1998; Miransari et al. 2008), the role of these associations in untilled soils is an interesting point to explore.

The objective of this investigation was to evaluate the effects of inoculation with the AMF *Glomus mosseae* in unsterilized soils from conventional tillage and no-tillage systems with different levels of N fertilization on AMF colonization, growth, and N uptake of wheat plants. The influence of indigenous or inoculated *Glomeromycota* on wheat shoot and root growth under the physical and chemical limitations related to both soil management systems is discussed in this article.

Materials and Methods

Intact soil cores were collected from a field experiment conducted in the agricultural experimental station J. Hirshhorn, Los Hornos, Argentina, (35° LS), Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata. Details of the field experiment can be seen in Schalamuk et al. (2006). The soil cores were extracted from conventional tillage (CT) and no-tillage (NT) plots following the methodology of Bellgard (1993), without physical disturbance in order to maintain the integrity of the AMF hyphal network and the distribution of the propagules in the soil. Each entire soil block was carefully placed in a 3000-cm^3 plastic pot and then taken to the Instituto Spegazzini, where the present experiment was conducted. The composition of the top 20-cm layer of the soil was 3.9% organic matter, 0.21% N, 11.3 ppm phosphorus (P), and 76.6 ppm NO^{3-} for CT and 4.1% organic matter, 0.19% N, 10.1 ppm P, and 42.6 ppm nitrate (NO_3^-) for NT.

At the same moment of core extraction, nearby soil samples were collected from the same plots to assess the number of AMF spores in CT and NT at the beginning of the experiment by wet sieving and centrifugation techniques (Gerdemann and Nicolson 1963; Walker, Mize, and McNabb 1982). The number of spores (three replications) was significantly greater ($P \leq 0.01$) in NT than in CT (321 vs.155 AMF spores 100 g^{-1} dry soil respectively).

Treatments consisted of a $2 \times 3 \times 2$ factorial combination of tillage, N fertilization, and AMF inoculation. The tillage treatments were CT and NT. The N fertilization treatments consisted of the application of amounts of N equivalent to 0, 80, and 160 kg N ha^{-1} (0, 80 and 160). The mycorrhizal inoculation treatments were composed of the noninoculated treatment (AM–) and the treatment inoculated with *Glomus mosseae* (AM+). The *Glomeromycota* species *G. mosseae* was selected because numerous studies have demonstrated its beneficial effects, for example, on P uptake and plant growth (e.g., McGonigle, Yano, and Shinhama 2003).

Therefore, the 12 combinations of the treatments were named as follows: CT0AM–, CT0AM+, CT80AM–, CT80AM+, CT160AM–, CT160AM+, NT0AM–, NT0AM+, NT80AM–, NT80AM+, NT160AM–, and NT160AM+. Six replications of each treatment were performed. The AMF colonization, wheat shoot biomass, and density of spores were determined in half of the replications, and root biomass and root N content were assessed in the other half.

In the inoculated treatments, 1 week before planting wheat, 40 g of inoculant composed of *G. mosseae* in a perlite–vermiculite 50% v/v substrate was placed in the center of

each pot with a 1-in. corer, without disturbing the nearby soil. The concentration of AMF spores in the inoculant was 20,120 spores in 100 g of dry substrate (mean spore density of three replications). In the noninoculated pots, autoclaved perlite–vermiculite (1 h at 120 °C, three times after an interval of 24 h) was placed in the same way as in the inoculated ones to provide the same conditions. Six wheat seeds of the cultivar Buck Pronto were sown in each pot at the normal sowing date of the region (July), and N fertilizer was applied as urea at the same time. Before sowing, the wheat seeds were surface-sterilized in 3% sodium hypochlorite solution for 10 min and then rinsed with deionized water.

After wheat emergence, plants were thinned to two per pot, and they grew in open benches at air temperature. Wheat plants were irrigated with purified water, and light was supplemented by incandescent and cool-white lamps. After 80 days from emergence, wheat reached the 3.1 growth stage (Zadoks, Chang, and Konzak 1974), and plants were harvested for the determination of shoot and root biomass, AMF colonization, and root N content. At this time, soil penetration resistance was measured with a standardized cone penetrometer, ASAE S 313.2 (American Society of Agricultural Engineers 1992), and water soil content was measured by Hidrosense soil moisture method. Roots were extracted from the soil of the pots through successive washing and sieving, using 450-μ meshes. Harvested shoots and roots were dried at 65 °C for 48 h for dry mass determination and tissue analysis. Root N content was evaluated by the micro-Kjeldahl method.

The AM colonization was determined by clearing and staining the roots (Phillips and Hayman 1970). The different intraradical fungal structures such as hyphae, arbuscules, and vesicles were observed under a microscope, and the percentages of each structure were determined using the methodology proposed by McGonigle et al. (1990). Spores belonging to native species and *G. mosseae* (inoculated) were extracted by wet sieving (Gerdemann and Nicolson 1963) and centrifugation in 80% sucrose (Walker, Mize, and McNabb 1982). The soil was dried at 70 °C to express the result as spores 100 g^{-1} dry soil. The *G. mosseae* spores were also counted after plant growth and expressed as percentage of total spores. The percentages were arcsin-transformed, and the number of spores log (X + 1) transformed according to Sieverding (1991). The statistical analysis was performed by analysis of variance that included main effects of fertilization, tillage, and inoculation, as well as their interactions, and least significant differences were calculated for mean separation.

Results and Discussion

In the intact soil cores, penetration resistance measured at the moment when wheat was sampled was 0.39 Mpa and 1.06 Mpa for CT for NT, respectively, at 5 cm deep, and 0.45 and 1.21 Mpa for CT and NT, respectively, at 10 cm deep. The values are the average of six replications. Significant differences were found for penetration resistance, with greater levels in NT than in CT ($P \leq 0.05$) for both depths.

Table 1 shows the combined analysis of CT and NT, the three levels of fertilization, and the effect of inoculation with *Glomus mosseae* for AMF colonization and arbuscules, spores 100 g^{-1} soil, wheat shoot and root biomass (g $plant^{-1}$), and root N (%).

The AMF colonization showed significant interactions between tillage and fertilization and between tillage and inoculation (Table 1). *Glomeromycota* vesicles were not observed in the samples. In CT, fertilization did not significantly affect AM colonization. In NT the colonization was greater than in CT in the unfertilized treatment and was reduced by the application of N (Figure 1A).

Table 1

Mean squares and significance levels for the combined analysis of two tillage systems: conventional tillage (CT) and no tillage (NT), three levels of fertilization (0, 80, 160 kg ha^{-1}), and with and without inoculation with *Glomus mosseae* (AMF+ and AMF– respectively) for AMF colonization and arbuscules (% root length), spores 100 g^{-1} soil, wheat shoot and root biomass (g $plant^{--1}$), and root N (%)

		AMF colonization (%)		AMF arbuscules (%)		AMF spores (100 g^{-1} soil)		Wheat shoot biomass (g $plant^{-1}$)		Wheat root biomass (g $plant^{-1}$)		Wheat root N (%)	
Main effect	df	Mean square	P > F	Mean square	P > F	Mean square	P > F	Mean square	P > F	Mean square	P > F	Mean square	P > F
Tillage (T)	1	394.22	0.019*	399.7	0.050*	134088	0.030*	0.015	0.324ns	0.134	0.000**	0.566	0.028*
Fertilization (F)	2	371.33	0.008**	412.7	0.030*	21507	0.438ns	0.377	0.000**	0.174	0.000**	1.649	0.000**
Inoculation (I)	1	254.45	0.047*	62.6	0.438ns	72227	0.103ns	0.001	0.729ns	0.016	0.156ns	0.878	0.008*
Interaction													
T × F	2	175.25	0.043*	27.8	0.760ns	736	0.971ns	0.001	0.892ns	0.003	0.615ns	0.020	0.822ns
T × I	1	251.06	0.046*	14.5	0.707ns	40947	0.214ns	0.001	0.761ns	0.001	0.703ns	0.091	0.355ns
F × I	2	22.38	0.698*ns*	61.6	0.550ns	3327	0.876ns	0.004	0.762ns	0.004	0.563ns	0.420	0.030*

*Significant at the 0.05 probability level.
**Significant at the 0.01 probability level.
ns, not significant.

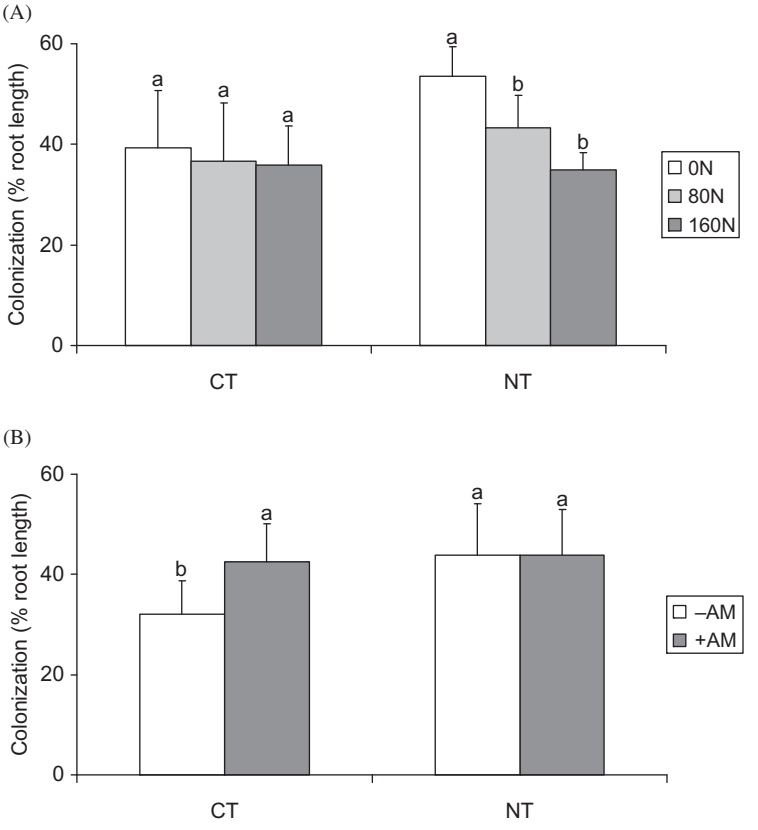

Figure 1. Root length colonization (%) of wheat roots for the interactions Tillage × Fertilization (A) and Tillage × Inoculation (B). Error bars: SE. The same letter above bars indicates that the values did not differ significantly as determined by LSD ($P \leq 0.05$). Same notations as in Table 1 and 2.

The most colonized plants were those from NT without N fertilizer, whereas the less colonized were those from CT or those highly fertilized with N. Inoculation with *G. mosseae* increased AMF colonization in CT (Figure 1B), mainly in the unfertilized treatments (data not shown), whereas it had no effect in NT soils. Spore density was only significantly influenced by the tillage system (Table 1). In the inoculated treatments, *G. mosseae* spores were found in the range of 22–39% from the total spores (data not shown), but neither the tillage system nor the fertilization showed statistically significant differences between the treatments.

Table 2 shows the mean values for the parameters analyzed in the different systems of tillage, fertilization, and AMF inoculation. The AMF colonization, arbuscule percentage, and spore density were greater in NT.

In the fertilized treatments, wheat plants showed the greatest shoot biomass, and N160 shoot dry weight almost doubled that of the treatments without fertilizer. Tillage did not affect wheat shoot biomass. Root biomass increased with fertilization but was also influenced by tillage, showing significantly lower values in NT. Root N content (%) increased with N fertilization and also with inoculation. The effect of inoculation was particularly important in the treatments without N fertilization, where percentage N increased almost to the level of those fertilized with N (Figure 2).

Table 2
Mean values for AMF colonization and arbuscules (% root length), spores 100 g^{-1} soil, shoot and root biomass (g $plant^{-1}$), and root N (%) colonization for two tillage systems: conventional tillage (CT) and no tillage (NT), three levels of fertilization (0, 80, 160 kg ha^{-1}), and with and without inoculation with *Glomus mosseae* (AMF+ and AMF– respectively)

Parameter	AMF colonization (%)	AMF arbuscules (%)	AMF spores (100 g^{-1} soil)	Wheat shoot biomass (g $plant^{-1}$)	Wheat root biomass (g $plant^{-1}$)	Wheat root N (%)
Tillage						
CT	37.31 b	15.33 b	347.75 b	0.599 a	0.396 a	1.93 a
NT	43.93 a	22.00 a	469.81 a	0.558 a	0.274b	1.68 b
Fertilization						
0N	46.47 a	25.23 a	447.09 a	0.434 c	0.201 c	1.74 c
80N	39.99 b	16.83 b	415.92 a	0.525 b	0.361 b	1.87 a
160N	35.40 c	13.94 c	363.33 a	0.777 a	0.440 a	1.81 b
Inoculation						
–AM	37.96 b	17.35 a	363.99 a	0.571 a	0.314 a	1.65 b
+AM	43.28 a	19.99 a	453.57 a	0.586 a	0.356 a	1.96 a

Note. Mean values followed by the same letter in the same row are not statistically different. LSD ($P \leq 0.05$).

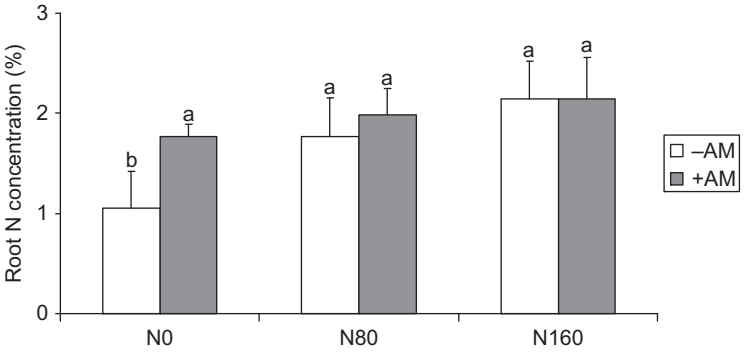

Figure 2. Root nitrogen concentration (%) in wheat plants for the Fertilization × Inoculation interaction. Same notations as in Fig. 1.

In our experiment, AMF colonization of wheat was low both when AMF propagule density was reduced, that is, in CT, and when N fertilizer was applied. No-tillage provided adequate conditions for the establishment of the symbiosis between wheat and AMF when N availability was low, as plants from this system showed greater colonization and arbuscule percentages than those from CT. It has been reported that tillage affects mycorrhizal colonization negatively (Douds et al. 1995; McGonigle and Miller 1996; Kabir 2005; Kabir, O'Halloran, and Hamel 1997; Kabir et al. 1998; Schalamuk et al. 2003, 2004). The infectivity of the mycorrhizal propagules in the soil has a marked effect on the rate of AM

colonization (e.g., Vierheilig and Ocampo 1991; Plenchette, Perrin, and Duvert 1989), particularly at the early stages of the crop, as in our experiment, when the colonization process starts. When the soil is not disturbed, a well-preserved hyphal network can serve as a high infective inocula (e.g., Evans and Miller 1990; Jasper, Abbot, and Robson 1989a, 1989b). Thus, the physical disruption of the hyphae probably could have been one of the factors that reduced soil mycorrhizal infectivity. A salient point was the significant interaction between tillage and inoculation observed in AM colonization. In CT, where the original AM density is considered to be low, AMF colonization increased with inoculation. Nevertheless, no differences were found between the inoculated and the noninoculated treatments in NT. It is known that the response to inoculation decreases with increasing AMF propagules (e.g., Sieverding 1991). In addition, we propose that the physical integrity of the hyphal network in NT was also an important factor in reducing the response of inoculation to AMF colonization.

Nitrogen fertilization reduced AMF colonization and arbuscular formation, in accordance with previous studies (Hayman 1970; Kruckelmann 1975; Jensen and Jacobsen 1980; Land, von Alten, and Schonbeck 1993). The reductions of AMF colonization with fertilization occurred both in NT treatments and in inoculated plants; nevertheless, N fertilization did not affect colonization in CT without inoculation. In the latter, we consider the inoculum density as the limiting factor for AMF growth, and thus the negative effect of N fertilization on colonization was not evident. On the other hand, vesicles were not observed in this experiment, because they are often associated with plant senescence (Bonfante-Fasolo 1984).

The AMF spore population was greater in NT than in CT; however, no consistent effects of fertilization or inoculation were found. Before wheat was planted, the soil cores from NT presented greater spore densities than those from CT. It is known that spore production increases as the plant matures (Hayman 1970; Sutton and Barron 1972; Koske and Halvorson 1981; Giovannetti 1985) and as the colonization process advances in the roots (Gazey, Abbot, and Robson 1992; Abbott and Gazey 1994). Sieverding (1991) indicated that spore formation can start from 3–4 weeks to up to 6 months after root infection. Therefore, in the wheat plants from our experiment, AMF colonization was probably too early to initiate spore formation, and the lack of significant effects of fertilization and inoculation on the number of spores, in spite of their influence on AMF colonization, may indicate that differences after wheat growth were probably related only to residual effects of the greater number of spores in the NT field soil.

As expected, N fertilizer increased wheat shoot and root biomass. Shoot biomass was solely affected by fertilization; however, root growth was also negatively influenced by NT. Root/shoot ratios were in accordance with Gregory (1991) for the phenological stage assessed and showed some variability in concidence with Hamblin and Tennant (1979). As penetration resistance was greater in NT, root growth could have been limited by soil compaction. Greater levels of penetration resistance in NT have been previously found in Argiudoll soils from the Argentinean pampas (Chagas, Marelli, and Santanatoglia 1994; Balbuena et al. 1996; Taboada et al. 1998; Soza et al. 2003). Although in our experiment penetration resistance was significantly greater in NT than in CT, the observed reduction in root growth in NT did not affect shoot biomass. According to Gupta and Allmaras (1987), the critical penetration resistances for plant growth are those greater than 2 Mpa. Although the levels found in NT (1.06 Mpa and 1.21 Mpa for 5 and 10 cm, respectively) affected root growth, they were probably not severe enough to limit shoot growth. The mechanical impedance in NT was not high enough to allow us to observe any alleviation of compaction stress by AMF, as found by Yano et al. (1998) and Miransari et al. (2008) in other crops.

Neither the shoot nor the root biomass was affected by AMF inoculation. Wheat is considered a crop with low mycorrhizal dependency (Jeffries and Dodd 1991; Li 2005), as it has a relatively large, finely branched root system with dense root hairs, and has thus a greater capacity to absorb nutrients than other crops. Although positive effects of AMF on wheat have been previously found (Kucey and Janzen 1987; Thompson 1990; Tarafdar and Marschner 1994; Al-Karaki and Clark 1999; Al-Karaki and Al-Omoush 2002), other studies have shown no responses of AMF inoculation in shoot biomass (Jensen and Jakobsen 1980; Vierheilig and Ocampo 1991, Ryan and Graham 2002) or even negative growth responses (Graham and Abbott 2000; Zhu et al. 2001). In our experiment, no positive effects of AMF inoculation on root biomass were found, in agreement with that observed by Mohammad and Malkawi (2004). The responses of plants to AMF inoculation are usually greater in sterilized than in unsterilized soil (Smith and Smith 1981; Daniels Hetrick et al. 1988; Ortas 2003). It is important to highlight that because we did not compare the inoculated treatment with a sterilized nonmycorrhizal control, differences might be difficult to find. Because, in our experiment, arbuscules were observed, the symbiosis between the plant and fungi was active and we can thus consider that the cost–benefit relationship for carbon was not positive for the wheat symbiont. In addition, taking into account that AMF colonization levels were similar in NT without N and in the inoculated plants, we can state that AMF colonization of wheat as a result of either indigenous AMF or inoculation with *Glomus mosseae* was not reflected in a greater shoot or root biomass.

Nitrogen concentrations increased following inoculation with *G. mosseae*, especially in the unfertilized plants from CT. This is a remarkable point, because inoculation with *G. mosseae* increased N root concentration in nonsterile soils where other AMF fungi were already present. This fact supports the results of Hawkins and George (2001), who found that inoculation with *G. mosseae* improved N concentration in wheat. However, their experiments were carried out in greatly controlled conditions using an inert sterilized substrate, semihydroponics, nitrification inhibitors, and labeled N, whereas in our experiment the positive effect of inoculation in N uptake took place with the presence of indigenous AMF and other microorganisms from the rhizosphere, which could have competed and interacted with the inoculated strains. Interestingly, plants from NT0NAM– and CT0NAM+ showed similar levels of AMF colonization but different N concentrations. In a study of AMF diversity in the site where the soil cores were taken, we found 24 *Glomeromycota* species (Schalamuk et al. 2006). Differences in functional diversity and effectiveness among *Glomeromycota* species have been demonstrated (e.g. Sieverding 1991; Johnson, Graham, and Smith 1992). In our experiment, considering the success in improving N concentration by the inoculation with *Glomus mosseae*, we can speculate that this species may show a greater efficiency than the indigenous species for the promotion of N uptake of wheat plants and that that could be the reason why colonization in $CTAM^{+}$ was more effective than that generated by indigenous AMF in NT.

Conclusions

The AM colonization and their positive effects depend on N fertilization, and hence the benefits resulting either from indigenous or inoculated AMF are low in high-input arable lands, in agreement with other studies (e.g., Sieverding 1991). In the unfertilized soils, inoculation with *G. mosseae* did not increase wheat biomass but enhanced N concentration in roots. The reduced AM colonization in CT and the positive effect of inoculation in root N concentration indicates that tillage decreases soil AM inoculum level and NT

favors *Glomeromycota* presence in the soils. However, in certain conditions, the AMF that predominate in NT may not necessarily be beneficial to the crops.

Acknowledgments

This research is part of the 11/N527 UNLP Project. S. Schalamuk is a recipient of a scholarship from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), and M. Cabello and H. Chidichimo are researchers from Comisión de Investigaciones Científicas de la provincia de Buenos Aires (CIC). A grant from CIC supported this research.

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