Differential contribution of arbuscular mycorrhizal fungi to plant nitrate uptake (^{15}N) under increasing N supply to the soil

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Abstract: The objective of this study was to determine how the uptake and transport of nitrate by two species of arbuscular mycorrhizal (AM) fungi is affected by its concentration in the medium and by the age of the AM symbiosis. Tracer amounts of ^{15}N nitrate were applied at two plant growth periods to mycorrhizal or nonmycorrhizal lettuce plants, which had been grown in soil supplied with nitrate to provide a total of 84, 168, or 252 mg N/kg. At both injection times, *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe and *Glomus fasciculatum* (Thaxter sensu Gerd.) Gerd. and Trappe reached the highest values of nitrogen derived from the fertilizer (NdfF) at 84 mg N/kg. *Glomus mosseae* also reached the highest values of labeled fertilizer N utilization at 84 mg N/kg, whereas *G. fasciculatum* reached the highest values at 168 mg N/kg in the medium. The highest N level in the medium (252 mg N/kg) had a negative effect on % NdfF and % labeled fertilizer utilization for all mycorrhizal plants. Regarding the time of ^{15}N fertilizer application, *G. fasciculatum*-colonized plants had a minimum change in % NdfF and % labeled fertilizer utilization during the growth period (60 days application vs. 30 days application). In contrast, *G. mosseae*-colonized plants growing at 168 mg N/kg in the medium, decreased these two values in the latest application. The present results confirm that mycorrhizal symbiosis may be particularly important for nitrogen nutrition in plants growing in neutral–alkaline soils.

Key words: arbuscular mycorrhizae, nitrate assimilation, nitrate uptake, ^{15}N-labeled fertilizer.

Résumé : L'objectif de cette étude était de déterminer comment l'absorption et le transport du nitrate par deux espèces de champignon mycorhizien arbusculaire, est affecté par sa concentration dans le milieu et par l'âge de la symbiose mycorhizienne. Afin de suivre son parcours, les auteurs ont appliqué de petites quantités de nitrate ^{15}N à deux stades de la croissance de plants de laitue mycorhizés ou non-mycorhizés, lesquels avaient été préalablement cultivés dans un sol additionné de nitrate pour fournir un total de 84, 168 ou 252 mg N/kg. Aux deux étapes d'injection, le *Glomus mosseae* (Nicol. et Gerd.) Gerd. et Trappe et le *Glomus fasciculatum* (Thaxter sensu Gerd.) Gerd. et Trappe atteignent leurs valeurs maximales pour l'azote provenant du fertilisant (NdfF) à 84 mg N/kg. *G. mosseae* atteint également les valuers les plus élevées d'utilisation du fertilisant N marqué à 84 mg N/kg alors que le *G. fasciculatum* atteint son maximum avec 168 mg N/kg dans le milieu. La concentration d'azote la plus élevée dans le milieu (252 mg N/kg) exerce un effet négatif sur le % NdfF et le % d'utilisation du fertilisant marqué chez toutes les plantes mycorhizées. Pour ce qui a trait au moment de l'application du fertilisant marqué au ^{15}N, les plants colonisés par le *G. fasciculatum* montrent un minimum de changement du % NdfF et du % d'utilisation du fertilisant marqué au cours de la période de croissance (application au jour 60 vs application au jour 30). Au contraire, les plantes colonisées par le *G. mosseae* poussant en présence de 168 mg N/kg dans le milieu, voient ces deux valeurs diminuer avec la dernière application. Les résultats obtenus confirment que la symbiose mycorhizienne peut être particulièrement importante pour la nutrition azotée des plantes poussant dans des sols neutres ou alcalins.

Mots clés : mycorhizes arbusculaires, assimilation des nitrates, absorption des nitrates, fertilisant marqué au ^{15}N.

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Introduction

The process of nitrate assimilation by plants has a fundamental biological importance because N is an essential element for life. Nitrate is the main nitrogen source for many higher plants growing in wide geographic areas (Schmidt 1982). The global rate of nitrate assimilation by plants is roughly 2×10^{13} kg N/year, which is approximately 10-fold greater than the rate of biological N_2 fixation (Guerrero et al. 1981). Nitrate assimilation may be as important for plant life as the CO_2 assimilation in photosynthesis (Marschner 1986).

It has been reported that arbuscular mycorrhizal (AM) fungi can reduce nitrate by the assimilatory reduction pathway, indicating that AM fungi have the gene set for assimilatory nitrate reduction (Ho and Trappe 1975; Kaldorf et al. 1994, 1998; Azcón and Tobar 1998). In many soils,

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such as those from the mediterranean area, nitrate is the main ion constituent of the mineral N pool. Mass flow is recognized as the process by which it is taken up by plant roots. Because of the high mobility of nitrate, its uptake generally has not been considered to be affected by AM fungi (Read et al. 1989). However, in recent years, several studies have demonstrated that nitrate can be mobilized from soils and transferred to the root cells by AM external hyphae, improving the inflow of N to the mycorrhizal plant (Johansen et al. 1993; Tobar et al. 1994*a*; Azcón et al. 1996). Most of the evidence concerning the effect of AM fungi on nitrate acquisition has been attributed to an indirect P-mediated mechanism since the enzymatic system for nitrate reduction requires phosphate (Hoff et al. 1992). But the direct uptake and transport of nitrate by extraradical mycorrhizal mycelium have been confirmed using ^{15}N under non-stressed (Johansen et al. 1993) and water-stressed conditions (Tobar et al. 1994*a*). In fact, isotopes provide the only direct method for measuring the amount or proportion of a given nutrient that is taken up by the plant from a given fertilizer. These studies were carried out under N-limiting conditions (i.e., native soil N or low N supply). However, it is known that growth and activity of AM fungi are influenced by the concentration of N in both soil and plants (Chambers et al. 1980; Azcón et al. 1982). In addition, it has been suggested that the transport of a nutrient by the AM hyphae may be related to the concentration of nutrients in the roots (Johansen et al. 1994). Hence, more information is needed about the effects of external AM hyphae on the uptake of nitrate by mycorrhizal plants under nonlimiting N conditions, as well as on the behavior of different AM fungi under such conditions.

The main objective of this study was to investigate, under a gradient of N supply in the soil, how mycorrhizal colonization by two species of AM fungi affects the uptake of nitrate by the host plant. An additional objective was to determine if the age of the plant and AM symbiosis have an effect on the uptake and transport of nitrate by the AM mycelium. For that, tracer amounts of ^{15}N-labeled nitrate were applied at two plant growth periods. *Lactuca sativa* L. plants, which have been shown to respond well to nitrate nutrition (Azcón et al. 1992; Tobar et al. 1994*a*), and two AM fungi (i.e., *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe and *Glomus fasciculatum* (Thaxter sensu Gerd.) Gerd. and Trappe) were selected for this study. Previously, these two AM fungi exhibited different symbiotic efficiencies for N acquisition and plant growth promotion in lettuce plants (Tobar et al. 1994*a*, 1994*b*; Ruiz-Lozano et al. 1995), thus becoming a useful model for comparison of mycorrhizal responses with increasing soil nitrate levels.

Materials and methods

Experimental design

The experiment consisted of a randomized block in a factorial arrangement, with three factors: (*i*) microbial treatments composed of two *Glomus* species and two uninoculated controls, one P-fertilized and the other unfertilized; (*ii*) three N levels supplied to the growth medium as nitrate (84, 168, or 252 mg N/kg); and (*iii*) time of tracer ^{15}N-fertilizer application, 30 or 60 days after transplanting. Five replications per treatment were made to give a total of 120 pots (one plant per pot).

Soil and biological material

A loamy agricultural soil was collected from Granada (Spain), sieved (2 mm), diluted with quartz sand (4:6 v/v), and sterilized (100°C, 1 h for 3 consecutive days). The main characteristics of the undiluted soil used were the following: pH (water) 7.8; 2.07% organic matter; 0.1% total N, 4.6 μg NO_3^--N/g, 1.8 μg NH_4^+-N/g, 32 μg P/g ($NaHCO_3$-extractable P), 311. 2 μg K/g. The soil texture was made up of 35.8% sand, 43.6% silt, and 20.5% clay. Seeds of *Lactuca sativa* cv. Romana were sown in sterilized sand. One week after emergence, uniform seedlings were transplanted to pots containing 550 g of the sterilized mixture of soil and sand. The mycorrhizal treatments were inoculated with either *G. mosseae* (BEG 122) or *G. fasciculatum* (LPA 30). The mycorrhizal inoculum, with similar numbers of viable propagules (MPN) for each fungus, consisted of spores, mycelia, and mycorrhizal root fragments from a stock culture of each fungus with *Allium cepa* L. The inocula (5 g/pot) were placed directly below the seedling in the planting hole. Uninoculated control treatments were provided with 5 ml of an inoculum filtrate containing its own microbiota except arbuscular mycorrhizal propagules. This filtrate was obtained by suspending 100 g of the inocula in 1 L of sterile water. After shaking and decanting, the suspension was filtered (Whatman n° 1) twice.

Nitrogen and phosphorus application

During the experiment, plants were fertilized (10 mL/week) with P-free Hewitt's nutrient solution (Hewitt 1952), modified to provide a total supply of 84, 168, or 252 mg N/kg, according to the treatment. Nitrogen was added as $Ca(NO_3)_2$. Tracer amounts of ^{15}N-labeled fertilizer were applied as an aqueous solution (10 mL of $(^{15}NO_3)_2Ca$, 10 atom % ^{15}N excess) to each pot, providing a total of 2.5 mg N/kg soil. Timing for tracer ^{15}N supply was selected taking into account the establishment and dynamics of AM colonization by these endophytes in lettuce plants (data not shown).

Phosphorus as KH_2PO_4 (100 μg/g) was only supplied to the P-fertilized uninoculated plants. This rate was selected in an attempt to match the effect of the fungus on plant growth (Azcón et al. 1992; Azcón and Tobar 1998).

Growth conditions

The experiment was carried out under greenhouse conditions with temperatures ranging from 19 to 25°C, 16:8 h light:dark photoperiod and a relative humidity of 70–90%. A photosynthetic photon flux density of 800 $\mu E \cdot m^{-2} \cdot s^{-1}$, measured with a lightmeter (LICOR LI-188B), was used. The plants were grown for 75 days in a controlled greenhouse, maintaining the moisture in the soil–sand mixture at 100% of the water holding capacity (Azcón et al. 1992).

Determinations

Plants were harvested at 75 days after transplanting, dried for 48 h at 70°C, and the shoot dry weight (SDW) was recorded. The shoot tissues were analysed for N and P contents (Lachica et al. 1973). The extent of root colonization by the mycorrhizal fungi was determined by the staining method described by Phillips and Hayman (1970), modified to avoid using phenol. The percentage of total root length that became mycorrhizal was calculated by a gridline-intersect technique (Giovannetti and Mosse 1980).

The N isotopic composition of plants shoots was determined by mass spectrometry (Fiedler and Proksch 1975). The % atoms ^{15}N in excess was calculated by subtracting 0.366 (^{15}N natural abundance) from the measured % atoms ^{15}N. The ^{15}N enrichment of the plant sample is related to the amount of N taken up from the ^{15}N-labeled fertilizer. The percentage of plant N derived from the labeled fertilizer (NdfF) was calculated according to Zapata (1990) by the following equation:

Table 1. Shoot dry weight (SDW; g/plant) and mycorrhizal root colonization (% AM and total AM root length mycorrhizal; cm) in control, P-fertilized, *G. mosseae*- or *G. fasciculatum*-colonized lettuce plants grown in soil supplemented with three N levels.

	84 mg N/kg			168 mg N/kg			252 mg N/kg		
Treatment	SDW	% AM	Total AM length	SDW	% AM	Total AM length	SDW	% AM	Total AM length
Control	1.27*ef*	0.0*d*	0.0*e*	1.02*f*	0.0*d*	0.0*e*	0.76*g*	0.0*d*	0.0*e*
P-fertilized	1.50*d*	0.0*d*	0.0*e*	1.44*de*	0.0*d*	0.0*e*	1.52*d*	0.0*d*	0.0*e*
G. mosseae	1.80*c*	56.6*b*	2.829*c*	1.62*cd*	41.2*c*	1.884*d*	1.76*cd*	43.1*c*	2.672*c*
G. fasciculatum	2.80*a*	66.9*a*	5.909*a*	2.89*a*	62.1*a*	4.840*b*	2.31*b*	67.7*a*	4.418*b*

Note: Within each parameter means followed by the same letter are not significantly different ($P < 0.05$) as determined by Duncan's multiple range test ($n = 10$).

[1] % NdfF = % ^{15}N atoms in excess in plant sample / % ^{15}N atoms in excess in labeled fertilizer × 100

The percentage of labeled fertilizer N utilization was calculated as the fraction of the fertilizer taken up by the plant in relation to the rate of fertilizer applied, as shown in the following equation:

[2] % labeled fertilizer N utilization = Total N in plant X (% ^{15}N atoms in excess in plant sample) / Total N in fertilizer X (% ^{15}N atoms in excess in labeled fertilizer) × 100

The N-use efficiency was calculated as the dry biomass produced per milligram of N taken up by the plant.

SDW and mycorrhizal colonization were not significantly affected by the time of ^{15}N fertilizer application. Hence, the values shown in Table 1 are means of 10 replicates. In the same way, data of N and P contents (Table 2) and those of N-use efficiency (Table 3) are also means of 10 replicates, since they are calculated on the basis of SDW. Data were subjected to analysis of variance (ANOVA) with microbial treatment, N level, and time of fertilizer application (when appropriate) as factors. When the main effect was significant ($P \leq 0.05$), differences among means were evaluated by Duncan's multiple range test (Duncan 1955). For the percentage values, arcsin square root transformation was made before the statistical analysis.

Results

The positive effect of P-fertilization and mycorrhizal inoculation on plant biomass production was evident at each of the three N levels (Table 1). In general, *G. mosseae* matched the effect of P-fertilization, except at 84 mg N/kg, where its positive effect on plant growth was greater than P-fertilization. Increasing N application in the growth medium to 252 mg N/kg had a negative effect on plant growth in unfertilized control plants and in *G. fasciculatum*-colonized plants. In any case, inoculation with *G. fasciculatum* was always the more effective treatment in terms of plant biomass production.

Roots of inoculated plants became well colonized by AM fungi, while control and P-fertilized roots remained nonmycorrhizal. The percentage of root-length colonized was unaffected (*G. fasciculatum*-colonized roots) or little affected (*G. mosseae*-colonized roots) by increasing the N level in the medium. Regarding the total mycorrhizal root length, inoculation with *G. fasciculatum* resulted in a longer mycorrhizal system, which was negatively affected by increasing N over 84 mg N/kg. Length of root colonized by *G. mosseae* decreased at 168 mg N/kg in the medium.

P-fertilized and *G. mosseae*-colonized plants absorbed similar quantities of N and P. The N and P contents were the highest in plants colonized by *G. fasciculatum* under the three N levels (Table 2). In general, both nutrients were acquired as efficiently by the chemical treatment as by the biological treatment. The overall results show the positive impact of *G. fasciculatum*, which, at the lowest N level, induced a similar (N) or greater (P) nutrient uptake than *G. mosseae* colonization or P fertilization at the two higher N levels supplied in the soil.

The N-use efficiency (Table 3) was considerably increased by mycorrhizal colonization, with the highest values found in plants colonized by *G. fasciculatum* at all N levels.

The NdfF values were stimulated by P-fertilization and by mycorrhizal colonization at 84 mg N/kg (Table 4). At 168 mg N/kg the increase of % NdfF by P fertilization and *G. fasciculatum* colonization was only evident when ^{15}N was applied at 60 days. Finally at 252 mg N/kg, only P-fertilized plants, fertilized at 60 days, showed stimulation in NdfF as compared with control plants. The percentage of labeled fertilizer N utilization by the plant was also increased by P fertilization and by mycorrhizal colonization, although there were some exceptions, mainly for *G. mosseae*-colonized plants at 168 mg N/kg in the medium. Regardless of the time of ^{15}N application, at the lowest N level in the soil, NdfF was maximal with *G. mosseae*, while % of fertilizer N utilization was similar in both mycorrhizal treatments. Plants colonized by *G. fasciculatum* showed the highest values of labeled N utilization at 168 mg N/kg in the medium. Under such conditions, the time of ^{15}N application did not affect fertilizer utilization in *G. fasciculatum*-colonized plants (Table 4). In contrast, *G. mosseae*-colonized plants, growing at 168 mg N/kg in the medium, decreased NdfF and % fertilizer utilization when ^{15}N was applied at 60 days. *Glomus mosseae* showed its maximum activity and increased plant fertilizer N utilization (more than 100% of increase over unfertilized controls) at the lowest N amount in the medium (84 mg N/kg). For this treatment, when the N availability increased, plant fertilizer N utilization decreased. The highest N content in the growth medium considerably reduced both % NdfF and % of fertilizer N utilization, particularly in AM plants at any growing stage.

Discussion

In this study, the ^{15}N isotope was used as a tracer, not only for evaluation of the quantities of N acquired by

Table 2. Shoot N and P contents (mg/plant) in control, P-fertilized, *G. mosseae*- or *G. fasciculatum*-colonized lettuce plants grown in soil supplemented with three N levels.

	84 mg N/kg		168 mg N/kg		252 mg N/kg	
Treatment	N	P	N	P	N	P
Control	43.4*de*	1.93*d*	43.6*de*	1.60*d*	30.3*e*	1.16*e*
P-fertilized	41.5*de*	3.50*b*	48.9*cd*	3.10*bc*	52.0*bc*	3.10*bc*
G. mosseae	45.8*d*	3.25*bc*	47.1*cd*	2.75*c*	59.8*bc*	2.95*c*
G. fasciculatum	58.8*bc*	4.42*a*	74.2*a*	4.55*a*	61.1*ab*	3.30*b*

Note: Within each parameter means followed by the same letter are not significantly different ($P < 0.05$) as determined by Duncan's multiple range test ($n = 10$).

Table 3. Shoot N-use efficiency (mg biomass/mg N recovered) in control, P-fertilized, *G. mosseae*- or *G. fasciculatum*-colonized lettuce plants grown in soil supplemented with three N levels.

Treatment	84 mg N/kg	168 mg N/kg	252 mg N/kg
Control	30.0*ef*	25.3*fg*	25.5*fg*
P-fertilized	34.6*de*	30.5*ef*	23.1*g*
G. mosseae	41.0*bc*	37.2*cd*	29.3*f*
G. fasciculatum	52.0*a*	42.0*b*	39.0*bc*

Note: Means followed by the same letter are not significantly different ($P < 0.05$) as determined by Duncan's multiple range test ($n = 10$).

mycorrhizal plants, but also to determine if the mycorrhizal ability to acquire N compounds from the soil is maintained throughout the plant growth period. Results show that ^{15}N uptake was considerably increased in the plants colonized by *G. fasciculatum*, and to a lesser extent in those colonized by *G. mosseae*. Nitrate is transported towards the root by mass flow of nitrate ions, which are the predominant inorganic N-form in many agricultural soils and are readily mobile. According to conventional models, it was expected that AM fungi would be effective only for the acquisition of slowly diffusing nutrients by plants (Harley and Smith 1983). However, our results demonstrate the effectiveness of mycorrhizal roots to promote nitrate uptake, even when nitrate is present in the soil in nonlimiting amounts, as well as a higher efficient use of this nutrient in AM plants than in control ones. In addition, the present data demonstrate that AM symbiosis helps the host plant to acquire nutrients with a ready mobility in soil since extraradical mycelium can exploit a large volume of soil. Recent studies (Azcón et al. 1992; Johansen et al. 1993; Cuenca and Azcón 1994; Azcón et al. 1996) have also shown nutritive responses to AM colonization in the presence of NO_3^-. Some of these studies demonstrated that AM mycelia were able to transport N directly as nitrate, while others have reported that AM fungi increase nitrate reductase (NR) activity (Ruiz-Lozano and Azcón, 1996; Azcón and Tobar, 1998).

Previous experience on the effect of mycorrhizal endophytes on hosts supplied with NO_3^- fertilization also shows that growth, NR activity, and protein content were increased in *G. fasciculatum*-colonized plants (Azcón and Tobar 1998). In a comparative study of AM response to N forms (Azcón et al. 1992, 1996), nitrate was a better N source for AM plants, and *G. fasciculatum*-colonized plants grew faster when they were only fed with nitrate. The particular ability of each AM endophyte to increase % fertilizer utilization was best observed by comparing data corresponding to 84 and 168 mg N/kg in the growing medium.

In general, the application of increased amounts of nitrate to the soil did not improve plant growth or N accumulation. Plants colonized by *G. fasciculatum* showed the highest fertilizer N utilization at 168 mg N/kg and the lowest at 252 mg N/kg. Also, in plants colonized by *G. mosseae*, the amount of labeled fertilizer N utilization decreased with increasing soil N fertilization rate. These findings agree with previous results obtained by Mäder et al. (2000) in *G. mosseae*-inoculated tomato plants, which took more ^{15}N from a hyphal compartment when the root compartment had a low N fertilizer concentration. Our data suggest that the plants could have been growing under P deficiency, and that the observed mycorrhizal effect on ^{15}N uptake could be due to a better P nutrition of the host plants. However, plant N and P contents were not significantly different between *G. mosseae*-colonized plants and P-fertilized plants at all N levels. Nevertheless, the effect of *G. mosseae* on % NdfF and % of fertilizer utilization did not match with those of P-fertilized plants since, at 84 mg N/kg, these values were higher for the mycorrhizal plants, while at 168 and 252 mg N/kg, P-fertilization enhanced such parameters. In the same way, at the highest N level applied, the effect of *G. fasciculatum* on % NdfF and % fertilizer utilization cannot be attributed to a higher P content of *G. fasciculatum*-colonized plants than that of P-fertilized plants, since the effect was similar in both treatments. Hence, all results suggest that better N utilization was not due to better P nutrition of mycorrhizal plants (i.e. via P requirements for nitrate reductase activity; Hoff et al. 1992) but rather to a direct fungal effect, as was also suggested by Faure et al. (1998).

Our results are in agreement with the existence of a possible regulatory mechanism for hyphal N transport, which can be affected by the amount of N in the medium and the differential ability of AM mycelia from various fungal species to function under increasing N supply. The application of 168 mg N/kg produced a "supraoptimal N level" for *G. mosseae*-colonized plants, while for *G. fasciculatum*-colonized plants, this N application increased both % NdfF and % of fertilizer utilization, requiring 252 mg N/kg to reach the "supraoptimal N level". Although mycorrhizal symbiosis is not considered to be functionally specific among plants and fungi, different fungi show differences in their symbiotic effectiveness in specific situations (Ruiz-Lozano et al. 1995). Our previous results emphasized that AM-colonized plants may behave differently depending on the fungal species involved, affecting the host plant physiology and the dynamic of nitrogen acquisition, according to particular environmental conditions. A possible explanation for the different behav-

Table 4. Percentage of plant N derived from the labeled fertilizer (% NdfF) and percentage of labeled fertilizer N utilization (% Fert. N Util.) in control, P-fertilized, *G. mosseae*- or *G. fasciculatum*-colonized lettuce plants grown in soil supplemented with three N levels.

	% NdfF			% Fert. N Util.		
	84 mg N/kg	168 mg N/kg	252 mg N/kg	84 mg N/kg	168 mg N/kg	252 mg N/kg
^{15}N applied at 30 days						
Control	3.6*gh*	7.4*a*	5.9*cd*	12.7*e*	20.5*cd*	13.6*e*
P-fertilized	6.4*c*	7.1*ab*	5.3*de*	21.3*cd*	29.0*ab*	21.6*c*
G. mosseae	7.3*a*	4.5*f*	3.3*hi*	25.7*b*	17.0*d*	15.6*de*
G. fasciculatum	6.0*c*	5.6*d*	3.2*hi*	30.6*ab*	35.4*a*	15.5*de*
^{15}N applied at 60 days						
Control	3.1*hi*	4.6*ef*	2.5*i*	10.4*f*	17.0*d*	7.1*f*
P-fertilized	5.9*cd*	6.5*bc*	4.8*ef*	19.9*d*	21.4*c*	17.9*d*
G. mosseae	6.6*bc*	3.6*gh*	2.5*i*	25.4*b*	10.4*e*	12.1*de*
G. fasciculatum	6.0*cd*	6.3*bc*	3.0*hi*	25.8*b*	35.6*a*	15.3*de*

Note: ^{15}N was applied at two plant growth stages (30 or 60 days). Within each parameter means followed by the same letter are not significantly different ($P < 0.05$) as determined by Duncan's multiple range test ($n = 5$).

ior exhibited by both fungi is that *G. mosseae*- and *G. fasciculatum*-colonized plants displayed different degrees of fungal infection, colonization was slightly repressed by increased N levels in *G. mosseae*-colonized roots and unaffected in the case of *G. fasciculatum*. The longer mycorrhizal root length (and, probably, also the more extensive hyphal growth) of *G. fasciculatum*-colonized plants found in this study may have accounted for the more efficient nitrate uptake and translocation to the host plant exhibited by this fungus. A similar effect was previously observed by Hawkins and George (1999) in mycorrhizal wheat plants grown at two N levels in the medium. They found that the higher rate of hyphal N uptake was related to the more extensive hyphal growth. Another remarkable result is that *G. fasciculatum*-colonized plants maintained the increased ability for N-fertilizer uptake for a longer period than did *G. mosseae*-colonized plants (i.e., 60 days application vs. 30 days application), regardless of the amount of N supplied to the soil. Studies using several fungal species belonging to the genus *Glomus* have also shown that different traits affect nitrogen acquisition (Azcón et al. 1992, 1996). Thus, selection of AM fungi and plant species to address specific environmental situations is a promising concept for sustainable agriculture.

Our results are a further confirmation of the significance of AM fungal selection with respect to nitrate uptake and metabolism in colonized plants. Our results show that mycorrhizal colonization by specific endophytes is particularly important for nitrogen nutrition and fertilizer utilization by plants growing in neutral–alkaline soils, where nitrate is the predominant nitrogen form. Regulation of nitrogen uptake and transfer in the AM symbiosis seems to depend on the environmental nitrogen availability and the endophyte involved.

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