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Differential growth response to arbuscular mycorrhizal fungi and plant density in two wild plants belonging to contrasting functional types

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Abstract The effect of arbuscular mycorrhizal fungi (AMF) on plant growth was examined in two wild plant species belonging to contrasting functional types: an annual forb (*Bidens pilosa*, Asteraceae) and a deciduous shrub (*Acacia caven*, Fabaceae) at three contrasting plant densities (one, two, and three individuals *per* pot). AMF had a slightly negative effect on *B. pilosa* when the species grew in isolation while they positively affected *A. caven*. Positive effects of AMF on shoot mass of *A. caven* decreased at higher plant densities, while shoot mass of individuals of *B. pilosa* showed less marked differences between plant densities. When considering total biomass *per* pot, AMF positively affected *A. caven* growth while negatively affecting *B. pilosa*, at all three plant densities. Root/shoot ratio per pot was negatively affected by AMF but not plant density in both species. These findings highlight the importance of including plants belonging to different life forms and/or traits in research regarding the interaction between AMF and intraspecific plant competition.

Keywords Intraspecific plant competition · Mycorrhizal responsiveness · Plant traits · *Acacia caven* · *Bidens pilosa* · Planting density

Introduction

Arbuscular mycorrhizal fungi (AMF) are obligate symbionts that form associations with the roots of most terrestrial plants (Smith and Read 2008). Their effects on host plants can range from beneficial to antagonistic (Johnson et al. 1997; Jones and Smith 2004). The beneficial effects are mainly attributed to facilitation of nutrient acquisition, enhanced pathogen resistance, drought stress alleviation, and protection against herbivores (Newsham et al. 1995, Smith and Read 2008) and are frequently, but not exclusively, measured as biomass production. The negative effects are likely to be complex but seem related to the balance between carbon costs and phosphorous uptake via AMF (Li et al. 2008).

It is well known that responsiveness to mycorrhizal colonization depends on root system properties (Brundrett 1991). It has been shown that annual forbs exhibit higher specific root length, which is usually associated with rapid rates of root elongation, high relative growth rate, and high nutrient uptake capacities. In contrast, perennials are generally associated with traits that suggest a more conservative strategy like higher root tissue density, larger diameter, and lower root nutrient content, typical of long-lived roots (Roumet et al. 2006 and references therein). Thus, different plant functional types (PFTs), defined as groups of species that differ in their morpho-functional traits (Díaz and Cabido 1997; Lavorel et al. 1997), may show distinct patterns of growth response to mycorrhiza and plant density. For example, in the South African fynbos, it has been shown that an evergreen Fabaceae that has a cluster root system and is efficient in acquiring nutrients is less responsive to AMF than an evergreen species belonging to the same family but lacking cluster roots (Allsopp and Stock 1992). It has been proposed that annual ruderal plants will be either unresponsive to AMF or facultative mycotrophs while perennial competitors will be more responsive to AMF for growth (Gange et al. 1990; Peat and Fitter 1993). This mycorrhizal responsiveness

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(sensu Janos 2007) may have important consequences on interspecific competition and plant community structure (Urcelay and Díaz 2003).

Several studies have examined the role of AMF on plants at different densities (Koide 1991; Allsopp and Stock 1992; Hartnett et al. 1993; Facelli et al. 1999; Schroeder and Janos 2004, 2005; Ayres et al. 2006; Li et al. 2008). The results of some of these have led Koide and Dickie (2002) to propose a model of density-dependent responses to mycorrhizal colonization in which the degree of plant response to AMF decreases as plant density increases. However, the model was based mostly on studies that involved annual crops or grasses, and the observed patterns may not necessarily apply to other species with different growth forms that occur in natural communities (e.g., Allsopp and Stock 1992). Further experiments involving other growth forms would, therefore, aid in a better understanding on the relationship between mycorrhizas and interspecific competition in wild plants.

In the present study, we examined the effect of AMF and plant density on plant growth and root/shoot ratio in two individual species belonging to contrasting PFTs: an annual forb (*Bidens pilosa*, Asteraceae) and a deciduous shrub (*Acacia caven*, Fabaceae) at three contrasting plant densities. Due to the fact that *A. caven* is a long-lived nitrogen fixing shrub with a conservative strategy that depends on efficient P acquisition to fix N, it is possible that this plant species is strongly dependent on mycorrhizal fungi for growth. In contrast, *B. pilosa* is a ruderal annual forb with high specific root length, which is usually associated with rapid rates of root elongation, high relative growth rate, and high nutrient uptake capacity; therefore, it might not be dependent on mycorrhiza for growth (Brundrett 1991; Peat and Fitter 1993). Accordingly, we hypothesized that these species belonging to different PFTs will differ in their response to AMF and plant density. We predicted that both species would be negatively affected by plant density. In addition, we also predicted that *A. caven* would be positively affected at the single plant level by mycorrhizal fungi and that this effect would decrease at higher densities while *B. pilosa* would be not affected by AMF at any density.

Material and methods

Plant species and experimental design

We selected two abundant co-occurring species, each dominant of two contrasting functional types of the secondary woodland–shrubland vegetation of the mountains of central Argentina (31º30′ S, 64º35′ W). These were the slow growing deciduous shrub *Acacia caven* (Fabaceae) and the fast growing annual forb *Bidens pilosa* (Asteraceae). Our species selection was not phylogenetically-based but rather community-based. This appeared the most appropriate strategy considering that the posed questions are more relevant to the community ecology than to evolutionary ecology (Westoby et al. 2002).

Seeds collected in this woodland–shrubland were germinated in a greenhouse in an autoclaved mix of sand and native soil (2:1 *v*/*v*). All seedlings were transplanted at the same time to 150-cm^3 pots. Plants were grown in the greenhouse under temperatures ranging from 15°C to 25°C and without water stress (daily watering with tap water).

Each species was individually grown at contrasting densities: in isolation, two and three individuals, with and without AMF. Each combination of species treatment (one, two, or three individuals) × AMF treatment (with or without mycorrhizal fungi) consisted of eight replicates. Due to pot size, strong competition at densities of two and three individuals was ensured. The pots were rotated twice per week to avoid any potential artifacts related to their position in the greenhouse. Plants of the same age were grown with and without AMF following the method of Koide and Li (1989). Pots from AMF treatments were filled with a mixture of autoclaved sandy soil (130 g) and field soil (50 g) from the natural habitat of the species, which was the source of mycorrhizal inoculum (AMF spores and roots). We chose this method because it has been widely demonstrated that different AMF taxa have different effects on plant growth (e.g., Van der Heijden et al. 1998; Maherali and Klironomos 2007) and more than one AMF taxon can colonize plant roots in the field (e.g., Vandenkoornhuyse et al. 2002). In the non-mycorrhizal treatment, the fraction of field soil was autoclaved and received 12.5 ml of microbial slurry to equalize microbial community. This slurry was prepared by filtering a soil suspension through a 38-μm mesh to remove AMF spores and mycorrhizal root fragments but allowing other soil organisms, including soil pathogens (bacteria and fungi) to pass through the mesh to correct for possible differences in microbial communities between mycorrhizal and non-mycorrhizal soil (Koide and Li 1989). The mycorrhizal-inoculated plants received 12.5 ml water to ensure that each pot received the same amount of liquid. To assess AMF spore composition in this inoculum, spores were extracted from 50 g of soil from each sample using a wet sieving and centrifugal flotation technique (Daniels and Skipper 1982). The extracted spores were observed under a light microscope and identified to the species level using current morpho-taxonomic criteria (http://invam.caf.wvu.edu/).

Field soil characteristics were: pH 6.59, 2.85 mg N/g soil, 0.17 mg P/g soil, N/P 16.78% and 7.01% organic matter. After 150 and 210 days, respectively, plants of *B. pilosa* and *A. caven* were harvested, washed, separated into shoots and roots, dried at 60°C for 72 h, and weighed. *B. pilosa* individuals were harvested before *A. caven* in order

Table 1 *T* test on mean dry mass (standard error) of the annual *Bidens pilosa* and the shrub *Acacia caven* with and without arbuscular mycorrhizal fungi

	Mean dry mass (g)		*T* test	*p* Value
	With AM	Without AM		
B. pilosa	0.33 (0.04)	0.42 (0.03)	−1.88	0.0833
A. caven	0.65 (0.07)	0.40 (0.04)	3.13	0.0073

to prevent blooming. In the case of *B. pilosa*, the harvested plants were at an adult stage while they were at a seedling stage for *A. caven*. Because roots of *B. pilosa* at two or three plant densities were difficult to separate, measurements and analyses at the individual level were performed on shoot dry mass. We examined randomly selected root subsamples to check for mycorrhizal colonization among treatments. The root subsamples were rehydrated and lateral fine roots (<2 mm, no obvious tannins) were cleared in 20% potassium hydroxide solution, acidified with 10% hydrochloric acid and stained with 0.05% aniline blue solution according to the method of Grace and Stribley (1991). The examination of these randomly selected root subsamples from the different treatments showed typical AMF structures (hypha, vesicles, and arbuscules) in mycorrhizal treatments while no colonization was observed in non-mycorrhizal treatments.

Data analysis

Mycorrhizal responsiveness of each plant species was evaluated using *T* test comparing total plant dry biomass in single individuals with and without AMF.

The shoot biomass, total biomass per pot, root/shoot ratio per pot, and root nodules (only in *A. caven*) were analyzed with two-way ANOVA with mycorrhiza and density as main effects and mycorrhiza × density as the interaction effect. In treatments with two or three individuals, an individual per pot was randomly selected at the beginning of the experiment in which shoot biomass and root nodules were measured. Prior to the analyses, data normality was tested with the modified test of Shapiro–Wilks (Mahibbur and Govindarajulu 1997). Fisher tests (LSD) were applied a posteriori to locate differences among treatment means (Sokal and Rohlf 1998). All statistical analyses were performed with *Infostat* Version 1.1 (Di Rienzo et al. 2001).

Results

Spore composition in soil inoculum

Nine AMF morphotypes were identified (average AMF spore number in 100 g of dry soil indicated in brackets): *Glomus aggregatum-microaggregatum* (40.5±25.9), *Glomus constrictum* (52.9±17.3), *Glomus* sp.3 (5.2±6.2), *Glomus* sp.4 (5.7±6.8), *Acaulospora mellea* (2.6±2.2), *Acaulospora scrobiculata* (6.4±2.3), *Entrophospora infrequens* (1.2±2.1), *Scutellospora biornata* (1.2±2.1), and *Gigaspora* sp. (1.2±2.1).

Mycorrhizal responsiveness

Opposite patterns of mycorrhizal responsiveness were observed when the two plant species were grown in

Table 2 Two-way ANOVA on growth of the annual *Bidens pilosa* and the shrub *Acacia caven* with and without arbuscular mycorrhizal fungi at three plant densities

	Source of variation					
	Arbuscular mycorrhizas		Plant density		AM x PD	
	F	*p* Value	*F*	*p* Value	*F*	*p* Value
B. pilosa						
Individual shoot dry mass (gr)	0.49	0.4868	27.10	<0.0001	1.78	0.1807
Total dry mass *per* pot	14.09	0.0005	9.20	0.0005	0.15	0.8589
Root/shoot ratio *per* pot	31.24	<0.0001	0.38	0.6852	0.42	0.6610
A. caven						
Individual shoot dry mass (gr)	21.07	<0.0001	15.60	<0.0001	3.59	0.0367
Total dry mass *per* pot	22.70	<0.0001	13.97	<0.0001	0.65	0.5298
Root/shoot ratio *per* pot	13.11	0.0008	0.68	0.5114	0.88	0.4227
Root nodules production *per* individual	8.38	0.0061	0.80	0.4568	1.12	0.3353
Root nodules per shoot dry mass *per* individual	0.00015	0.9904	2.28	0.1149	0.43	0.6551

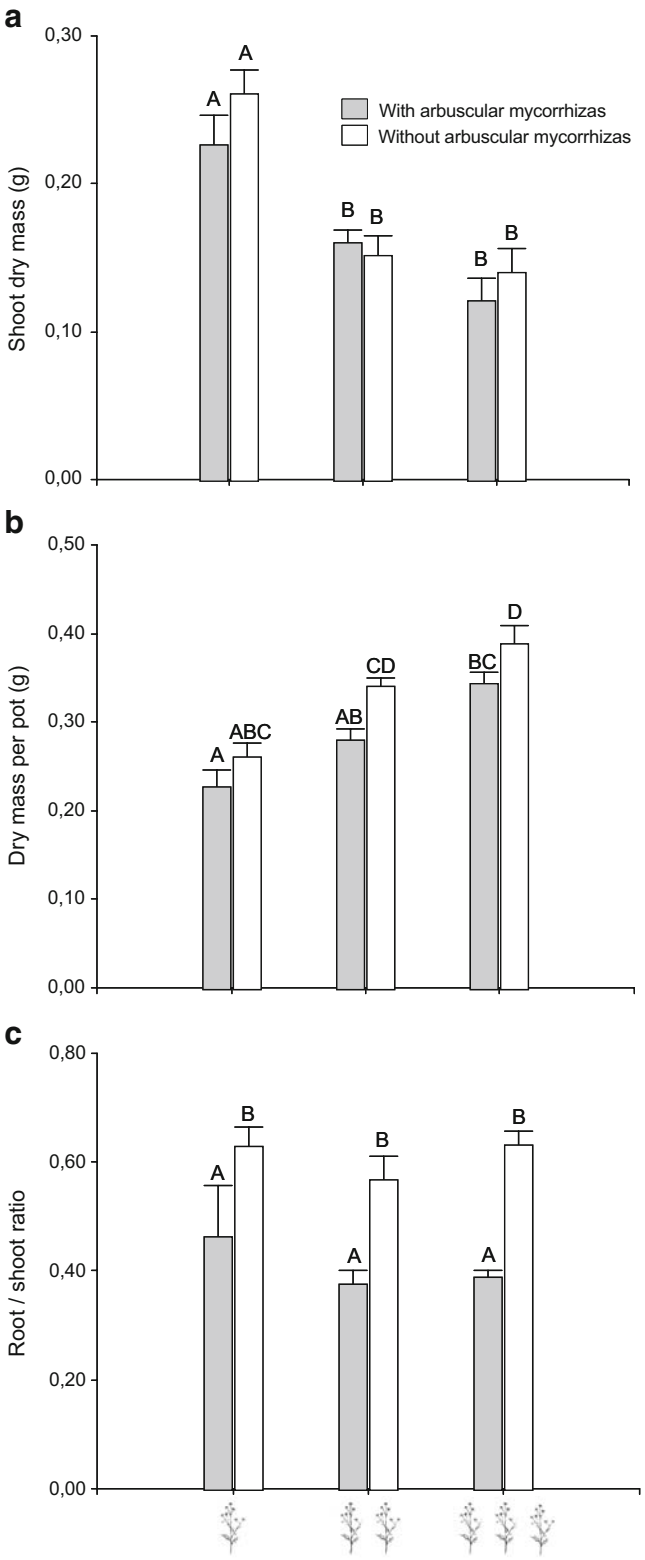

Fig. 1 Effects of arbuscular mycorrhizal fungi and plant density on *Bidens pilosa* (annual forb): **a** shoot dry mass, **b** total dry mass per pot, and **c** root/shoot ratio. Plant schemes indicate number of individuals per pot. *Letters* indicate significant differences among *bars* (Test LSD Fisher test, $P<0.05$)

isolation. AMF had only a slightly negative effect on *B. pilosa* while they significantly and positively affected total plant dry mass of *A. caven* (Table 1).

Mycorrhiza and plant density

When we considered mycorrhiza and plant density in the model (Table 2), shoot mass of *B. pilosa* was not significantly affected by AMF (Fig. 1a) and was negatively affected by plant density. Total dry mass per pot was higher with increasing plant density but negatively affected by AMF (Fig 1b). Root/shoot ratio was negatively affected by AMF while not affected by plant density (Fig. 1c). No significant interaction between factors was observed.

In contrast to the annual forb, shoot dry mass of *A. caven* was positively affected by AMF when grown alone, and the effect decreased at higher densities. In addition, it was negatively affected by plant density (Table 2, Fig. 2a, b). There was a significant interaction effect on shoot dry mass because AMF had a strong positive effect on *A. caven* when grown alone that decreased at higher densities (Fig. 2a). Total dry mass per pot was positively affected by AMF, mainly at one and three plant densities. Root/shoot ratio per pot was negatively affected by AMF (Fig. 2c) while not affected by plant density.

Root nodule production per individual *A. caven* plant was positively affected by AMF but not by plant density (Fig. 3a), while root nodule production per gram of dry mass was not affected by either variable (Fig. 3b).

Discussion

In the present study, plant species belonging to different functional types differed in their response to AMF. As predicted, when grown alone, the deciduous shrub *A. caven* was positively affected while the annual ruderal forb *B. pilosa* was not significantly affected, although a slight negative effect on shoot mass was observed. It has been suggested that woody species may derive more benefits from mycorrhizal fungi than plants with shorter life spans and that this benefit could be related to root system structure (Brundrett 1991; Peat and Fitter 1993). For example, species with lower root diameter and suberization, shorter lifespan, higher branching order, and hair development would derive less benefit for mycorrhizal fungi than species with contrasting root traits (Brundrett 1991). Accordingly, it has been shown in central Argentina that annual forbs have higher specific root length (ratio of total root length to root dry mass) and lower root tissue density (ratio of root dry mass to root volume) than perennials (Roumet et al. 2006).

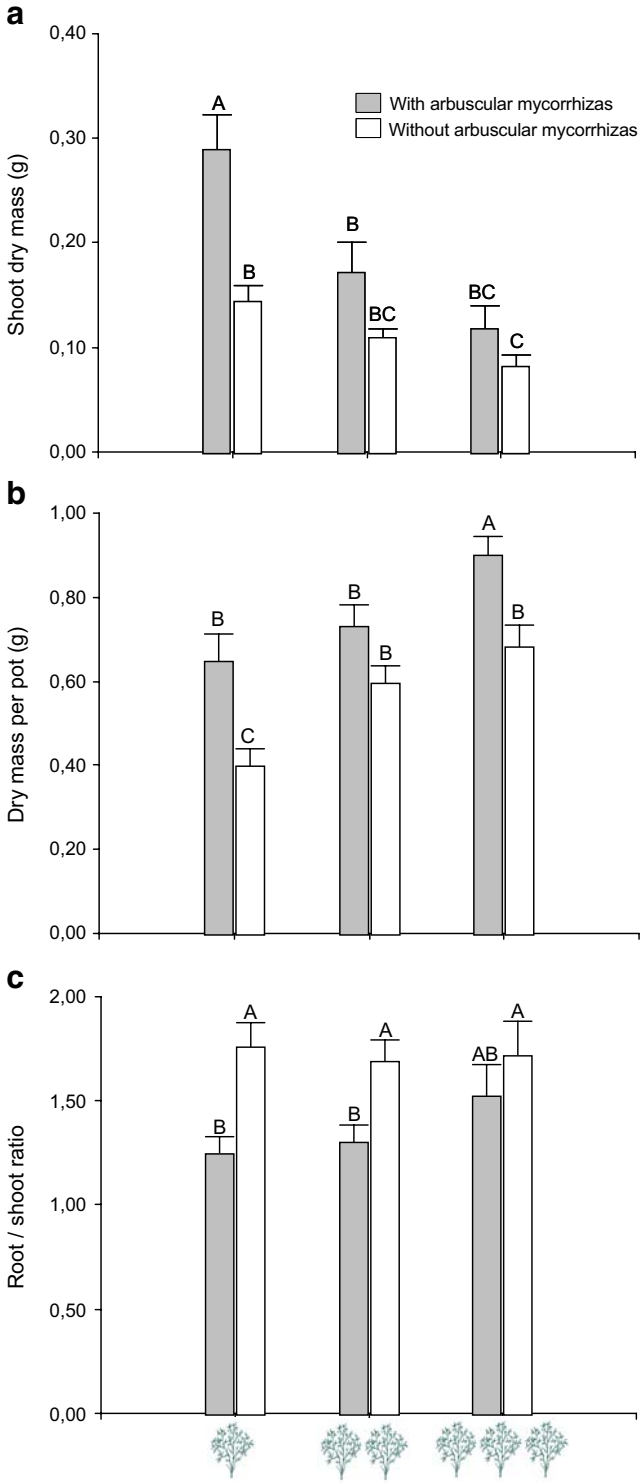

Fig. 2 Effects of arbuscular mycorrhizal fungi and plant density on *Acacia caven* (deciduous shrub): **a** shoot dry mass, **b** total dry mass per pot, and **c** root/shoot ratio. Plant schemes indicate number of individuals per pot. *Letters* indicate significant differences among *bars* (Test LSD Fisher test, $P<0.05$)

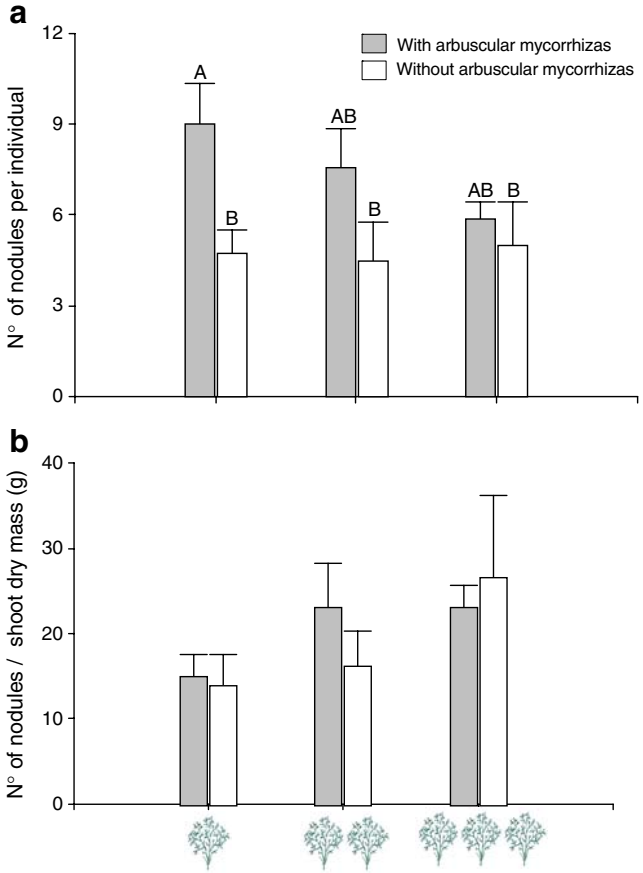

Fig. 3 Effects of arbuscular mycorrhizal fungi and plant density on *Acacia caven* (deciduous shrub): **a** number of *N* fixing nodules in roots and **b** number of *N* fixing nodules in roots/shoot dry mass. Plant schemes indicate number of individuals per pot. *Letters* indicate significant differences among *bars* (Test LSD Fisher test, $P<0.05$)

We hypothesized that the differential benefits derived from mycorrhizal fungi would affect the outcome of the relationship between arbuscular mycorrhiza and intraspecific competition on plant growth. The present results support this hypothesis. The shoot mass of the target *B. pilosa* was not significantly affected while total biomass *per* pot was negatively affected by AMF, mainly at higher densities. In contrast, the deciduous shrub *A. caven* was positively affected at both levels of analysis. Nevertheless, these positive effects tended to decrease at higher densities. A previous model on the interaction between AMF and plant density (Koide and Dickie 2002) predicted that the effect of mycorrhiza will decrease with increasing plant density. In our study, the effects of AMF on growth of both species in isolation, either significantly positive or slightly negative, decreased with plant density supporting the model. However, the negative effect of density on *A. caven* growing with AMF was markedly stronger than the effect of density when growing without AMF. In contrast, the effect of AMF on *B. pilosa* showed less marked differences

between densities. These findings and others (e.g., Allsopp and Stock 1992) suggest that the outcome of the interaction between AMF and intraspecific plant competition may be related to the life form and the vegetative traits of individual plant species. Certainly, this point deserves elucidation in future studies.

In both the forb and shrub species studied here, root/shoot ratio was consistently decreased by arbuscular mycorrhizal colonization but not affected by plant density. These results contrast with other reports that competition, but not AMF, positively affected root/shoot ratio (Ayres et al. 2006). Our results suggest that AMF affect carbon partitioning in these species or, alternatively, that an important fraction of the carbon allocated to build roots is taken up by the fungi in colonized roots. Albeit, these findings support the idea that AMF are important in the carbon economy of plants (Smith and Read 2008) although the mechanisms are not well known yet. It is striking that AMF appeared to affect carbon allocation of both the forb and shrub species in the same direction, independently of their response to mycorrhizal fungi or plant density.

Previous studies found positive (e.g., Smith et al. 1979; Kawai and Yamamoto 1986; Lekberg and Koide 2005) or negative (Bethlenfalvay et al. 1982) relationships between AMF and root nodulation. Here, root nodulation in *A. caven* was increased in mycorrhizal plants when grown alone. This effect is attributed to a biomass-mediated effect rather than to an effect of AMF colonization per se, since no differences in nodule production were observed when it was calculated on a per gram basis. These differences in nodulation, either total production or on a per gram basis disappear as plant density increased following the same pattern observed for shoot biomass.

It is well known that different AMF species promote differential effects depending on the host plant species. Moreover, the net effect of various fungal species colonizing the same host cannot be predicted by the sum of the individual effects of each fungal species (van der Heijden et al. 1998). For this reason, we chose to inoculate with a community assemblage of AMF present in the natural soil instead artificially with some selected species. In this way, we assured that each host plant species had the possibility to be colonized by the fungi that naturally colonized them in the field. On the other hand, we were not able to analyze which AMF species actually colonized the host species in our experiment. The interaction between fungal composition in roots and plant density is an issue that certainly deserves more attention in order to disentangle the mechanisms by which AMF communities affect the outcome on plant intraspecific competition.

The experiment performed here to maximize competition between plants of a same species under contrasting densities shows that plants belonging to different life forms have different mycorrhizal responsiveness; therefore, the outcome of the interaction between AMF colonization and plant density also differ among them. Because it is now widely recognized that the arbuscular-mycorrhizal symbiosis ranges along a mutualism–parasitism continuum (Johnson et al. 1997; Jones and Smith 2004), the results presented here suggest that if we want to disentangle the mechanisms behind the role of AMF in structuring natural plant communities, more research about the interaction between AMF and intraspecific competition in wild plants, with different life forms and/or traits, is necessary.

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