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## Short Communication

## Effects of arbuscular mycorrhizal colonisation on shoot and root decomposition of different plant species and species mixtures

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## ABSTRACT

We studied the decomposition of shoot and root tissues of four plant species from central Argentina belonging to contrasting functional types: a deciduous shrub (*Acacia caven*), a perennial forb (*Hyptis mutabilis*), an annual forb (*Bidens pilosa*) and a tussock grass (*Jarava pseudoichu*). They were grown from seed in a greenhouse in isolation or in 2- or 4-species mixtures, with and without arbuscular mycorrhizal fungi (AMF), and then placed to decompose under natural conditions in the field. AMF significantly enhanced decomposition of shoots, but not that of roots, independently of species identity and species-mixture composition. Our results suggest that AMF may be significantly affecting ecosystem functioning through the observed plant-mediated effects on decomposition.

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Arbuscular mycorrhizal fungi (AMF) are obligate symbionts associated with most terrestrial plants (Smith and Read, 2008). AMF acquire carbon from their host plants (Jakobsen et al., 2002) and improve plant access to soil nutrients, mainly phosphorous and nitrogen (Govindarajulu et al., 2005; Sikes et al., 2010; Smith and Read, 2008) thus leading to changes in plant nutrient concentration and carbon-allocation strategy (Cornelissen et al., 2001; Smith and Read, 2008). However, whether these effects are translated to ecosystem processes such as decomposition is poorly known (Langley and Hungate, 2003). Schädler et al. (2010) recently found that decomposition rates of aboveground mass of seven herbaceous species common in Central Europe was higher in plants colonised by AMF than in controls without mycorrhizas. It is unknown, however, how widespread these effects are beyond their particular set of species and experimental conditions. On the basis of the available evidence, these effects are likely to vary according to the nature of the individual species involved, and their combination in assemblages. This is because plant functional types differ in their leaf physical and chemical properties and decomposition rate (Cornelissen et al., 1999; Díaz et al., 2004; Pérez Harguindeguy

et al., 2000) and their response to mycorrhizal colonisation (e.g. Pérez and Urcelay, 2009). Moreover, decomposition rates of mixtures of species or plant functional types sometimes are not the additive result of individual plant species decomposition rates (Wardle et al., 1997; Anderson and Hetherington, 1999; Pérez Harguindeguy et al., 2008). In order to examine whether recent novel findings regarding the “afterlife” effects of AMF on decomposition of plant mass (Schädler et al., 2010) extend to other growth forms and species mixtures, including above and belowground organs, we evaluated the effects of AMF colonisation on decomposition of shoot and root dry tissue of four native species of central Argentina, belonging to different functional types, grown in isolation and in mixtures, with and without AMF colonisation.

We selected the most abundant species of each of the four dominant functional types in the central Argentina lower mountain vegetation belt (31° 30' S, 64° 35' W) (Urcelay et al., 2009). The species selected were the deciduous shrub *Acacia caven* (Molina) Molina (Fabaceae), the perennial forb *Hyptis mutabilis* (Rich.) Briq. (Lamiaceae), the annual forb *Bidens pilosa* L. (Asteraceae) and the perennial tussock grass *Jarava pseudoichu* Ruiz & Pav. (Poaceae). All four species are frequently associated with AMF in the field (Pérez and Urcelay, 2009; Urcelay and Batistella, 2007). We collected seeds that were germinated in a greenhouse in an autoclaved mix of sand and native soil (2:1 v/v). We transplanted all seedlings at the same time to 150 ml pots and grown at temperatures ranging from 15 to 25 °C, with no water stress. We grew each species in isolation and in

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combinations of two and four species, with and without AMF. Each combination of species treatment  $\times$  AMF treatment was applied to six replicates, consisting of 132 pots. The pots were rotated twice per week to avoid any potential artifact related to their position in the greenhouse. Following the method of Koide and Li (1989), we filled pots from both AM treatments with 2/3 of autoclaved sandy soil and 1/3 (v/v) of field soil from the natural habitat of the species, which was also the source of mycorrhizal inoculum (AMF spores). We filled pots from non-mycorrhizal treatments with 2/3 of autoclaved sandy soil and 1/3 (v/v) of autoclaved field soil that received 10 ml/pot of a microbial non-AMF filtrate produced from the inoculum to control for differences in soil flora. After 180 days, plants were harvested, washed, separated into shoots and roots, dried at 60 °C for 72 h, and weighed. Examination of randomly-selected root samples belonging to the AMF treatment showed that they had been successfully colonised. Roots from non-mycorrhizal treatments were not colonised by AMF (see Pérez and Urcelay, 2009 for further details).

We estimated the decomposition rate of shoot and root tissue separately through the widely-used litterbag technique (Cornelissen, 1996). Samples of each treatment ( $n = 252$ ) were weighted and then sealed into nylon bags of 0.3 mm mesh size. Due to an operational error, shoot tissue of *H. mutabilis* grown alone was lost before the decomposition experiment, therefore we are unable to report decomposition rate for this species in isolation. Shoot and root tissue samples were incubated during the 3 summer months (January–March) in a decomposition bed located in the same shrubland from which the seeds were initially collected. We placed the litterbags at random positions, buried them at 5 cm below-ground and covered them with soil and litter mixture in order to homogenise physical conditions, reduce the effect of the unpredictable environment close to the surface, and avoid damage by vertebrates. On the basis of previous work, (Pérez Harguindeguy et al., 2000; Vaieretti et al., 2005), we retrieved the samples 30 days after burying. After retrieval, samples were cleaned, dried for 48 h at 60 °C and weighed. Decomposition was defined as the percentage of dry weight loss after the incubation period (Cornelissen, 1996). We analyzed shoot and root dry weight loss of species and species mixtures using ANCOVA, with the AMF and species composition treatments as factors and initial shoot or root dry weight as covariate in order to account for effects related to initial weight. For species mixtures, we rank-transformed data on shoot dry weight loss because they were not normally distributed (Zar, 1999).

Shoot decomposition was higher in samples with AMF than in those without them (Table 1, Fig. 1A and B), both for plants grown in isolation and for plants grown in mixtures. As expected, decomposition rate also varied significantly among species (Table 1). The highest decomposition rates corresponded to the annual forb *B. pilosa* and the lowest to tussock grass *J. pseudoichu* (Fig. 1A, B). There was no significant interaction term between factors and no initial dry weight effect on dry weight loss was observed (Table 1). Root decomposition of plants grown in isolation and in mixtures was also affected by the identity of the plant species (Table 1). However, root decomposition was not significantly different between samples corresponding to AMF and non-AMF treatments (Table 1).

In the experimental set up to prepare the material for decomposition for this study, growth of *A. caven* and *H. mutabilis* were mostly positively affected by AMF while *B. pilosa* and *J. pseudoichu* negatively affected. Despite these differences in mycorrhizal response and in vegetative traits (i.e. chemical and physical) between species (Vaieretti et al., 2005), AMF consistently enhanced shoot decomposition in most species and species mixtures. These findings agree with Schädler et al. (2010) and suggest that they

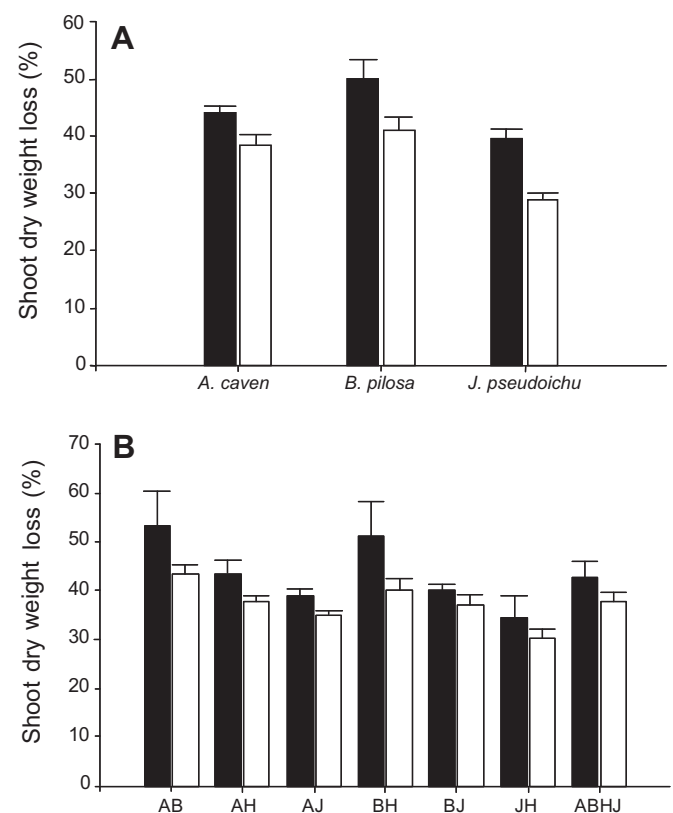
**Table 1**

Results of ANCOVA on shoot and root plant tissue decomposition of species grown in isolation and in mixtures. Plant species and arbuscular mycorrhizal fungus (AMF) colonisation main effects, Sp  $\times$  AMF species-by-arbuscular mycorrhizal colonisation interaction term. Initial dry weight was used as covariate in the ANCOVA. \* $P < 0.05$ ; \*\* $P < 0.01$  and \*\*\* $P < 0.001$ .

Dry weight loss	Source of variation			
	Plant species	AMF colonisation	Sp. $\times$ AMF interaction	Initial dry weight
	$F_{(df)}$	$F_{(df)}$	$F_{(df)}$	$F_{(df)}$
Shoot tissue				
Species	12.04 <sub>(3, 35)</sub> ***	20.78 <sub>(1, 35)</sub> ***	0.71 <sub>(2, 35)</sub>	1.17 <sub>(1, 35)</sub>
Species mixtures	4.52 <sub>(6, 67)</sub> ***	10.10 <sub>(1, 67)</sub> **	0.35 <sub>(6, 67)</sub>	0.00 <sub>(1, 67)</sub>
Root tissue				
Species	27.68 <sub>(3, 35)</sub> ***	2.38 <sub>(1, 35)</sub>	1.22 <sub>(2, 35)</sub>	2.53 <sub>(1, 35)</sub>
Species mixtures	30.25 <sub>(6, 67)</sub> ***	1.72 <sub>(1, 67)</sub>	2.26 <sub>(6, 67)</sub> *	0.89 <sub>(1, 67)</sub>

extend to other growth forms and species mixtures independently from growth response to mycorrhizal fungi and initial biomass. The positive effects of AMF treatment on growth on *A. caven* and *H. mutabilis* and on decomposition of all species combinations preclude any important fertilization effect of autoclaving the small soil fraction applied of non-mycorrhizal treatment.

We also examined whether AMF colonisation affected the relationship between observed and expected decomposition rates of mixtures (Pérez Harguindeguy et al., 2008). We observed that only *A. caven*/J. *pseudoichu* and *B. pilosa*/J. *pseudoichu* species



**Fig. 1.** Mean shoot dry weight loss (%) of (A) species grown in isolation (*Acacia caven*, *Bidens pilosa* and *Jarava pseudoichu*); and (B) species mixture (*A. caven*; *B. pilosa*; *H. mutabilis* and *J. pseudoichu*) grown with (black bars) and without (white bars) arbuscular mycorrhizal fungi (AMF) ( $n = 6$ ). Error bars indicate  $\pm 1$  SE. Decomposition values of *Hyptis mutabilis* grown in isolation with AMF were not available.

mixtures grown with AMF showed decomposition rates significantly lower than those expected based on the averages of component species grown in isolation. No difference was observed in the other species mixtures (Supplementary material, Fig. 1).

In agreement with Langley and Hungate (2003), root decomposition was not affected by AMF colonisation. Despite the fact that, as Schädler et al. (2010) we used green tissues instead of litter, it has been previously shown that the proportion of nutrients and C:N ratio can be maintained in senesced litter (Bradford et al., 2002) and green tissue has been successfully used in a number of other studies (Smith and Bradford, 2003; McLaren and Turkington, 2010; Moore and Fairweather, 2006). Additionally, in order to examine for comparability between our results and decomposition of litter, we correlated shoot decomposition rates (with AMF) from our experiment with those on litter decomposition of the same species and species mixtures reported by Pérez Harguindeguy et al. (2008) (field-grown plants). We found a positive and significant correlation ( $r = 0.712$ ;  $P = 0.021$ ). These results suggest that the patterns we have found in green tissue are likely to be maintained in litter, as found elsewhere (Bradford et al., 2002; McLaren and Turkington, 2010). Our results extend the evidence on the positive effects of AMF on decomposition to other growth forms, species mixtures and ecosystems suggesting that these root symbioses may be playing a significant role in ecosystem-level nutrient cycling via the decomposition pathway.

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## Appendix Supplementary material

Supplementary material related to this article can be found at doi:10.1016/j.soilbio.2010.11.006.

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