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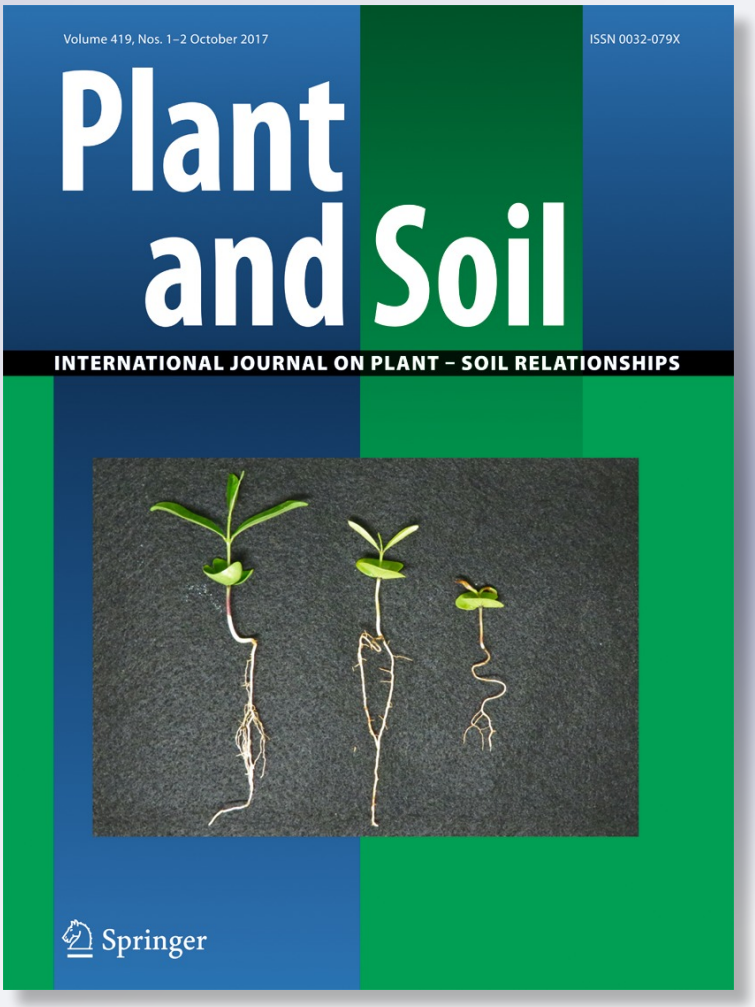

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REGULAR ARTICLE

Arbuscular mycorrhizal fungi can shift plant-soil feedback of grass-endophyte symbiosis from negative to positive

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Abstract
Aims Plants affect each other by modifying soils conditions in plant-soil feedbacks, where associated microbes have an integral role. Since epichloid endophytes and arbuscular mycorrhizal fungi (AMF) are highly widespread grass symbionts, here we explore the role of AMF and endophyte in plant-soil feedback within the same grass population.
Methods Through a manipulative experiment, we evaluated the performance of endophyte-free and endophyte-associated *Lolium multiflorum* plants grown in soils previously conditioned by endophyte-free and endophyte-associated plants and inoculated or not with three AMF species.
Results The biomass of endophyte-free and endophyte-associated plants was increased by AMF inoculation, when growing in soils conditioned by equal endophytic status plants (i.e. home soils). When growing in soils conditioned by plants with different endophytic status, plant biomass was higher than in home soil only in absence of AMF. The content of P and the arbuscular colonization also increased in plants growing in home soils.
Conclusion We demonstrated that AMF shift the intraspecific feedback effects between E+ and E- conspecific plants from negative to positive. Furthermore, we found that the outcome of simultaneous occurrence of foliar and root symbionts on grass performance depends on the matching with the endophytic status of the previous plant.

Keywords Aboveground-belowground interactions · Epichloë · Foliar symbionts · Home – away soils · *Lolium multiflorum* · Multisymbiotic systems · Mutualisms · Mycorrhiza · Root symbionts

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Introduction

Understanding how plant shoot and root microsym bionts interact and affect plant-soil feedbacks is attracting special attention (van der Putten et al. 2013; van der Putten et al. 2016). Positive or negative feedbacks occur when a plant modifies biotic or abiotic soil conditions which, in turn, benefit or impair the performance of the next generation of plants, compared with the performance of those growing in other soils (Bever et al. 1997; Bever 2002; van der Putten et al. 2013). In

particular, root symbionts such as arbuscular mycorr hizal fungi (AMF) or rhizobia can be affected by these changes in soil conditions, with an impact on response-plants (García-Parisi et al. 2017; Klironomos 2002; Bever 2002). Shoot microbes can also influence these processes through two different mechanisms. On the one hand, during the conditioning phase, symbionts such as fungal endophyte of grasses can modify the effect that host plants have on soil conditions (García-Parisi et al. 2017; Matthews and Clay 2001; Cripps et al. 2013). On the other hand, during the response phase, these shoot symbionts can modify the host plant response to changes in soil conditions (Matthews and Clay 2001). Although the role of belowground microbes mediating plant-soil feedbacks have been thoroughly studied (Klironomos 2002; Bever 2002), the interactive effects with shoot microbes on feedbacks have been scarcely studied.

Asexual *Epichloë* endophytes of grasses (Ascomycota, Clavicipitaceae), common shoot symbionts, can exert a great influence on soil components and processes through different pathways (Omacini et al. 2012), even when these fungi are restricted to growing inside aboveground tissues. Endophyte association may change root chemistry, including the quantity and quality of secondary metabolites like alkaloids or flavonoids (Malinowski et al. 2000; Malinowski et al. 2008; Rasmussen et al. 2009; Ponce et al. 2009), and increase their exudation (Van Hecke et al. 2005). Furthermore, endophytes modify soil communities (Franzluebbers 2006; Jenkins et al. 2006; Buyer et al. 2011; Casas et al. 2011; Bowatte et al. 2011), by affecting a multitude of interactions in the rhizosphere (Breen 1994; Omacini et al. 2012; García Parisi et al. 2015; Arrieta et al. 2015; Pérez et al. 2016; Vignale et al. 2016). All these changes are thought to be responsible for the effects that endophyte occurrence imposes on host neighbourhood and community dynamics (Rudgers et al. 2004; Omacini et al. 2005; Rudgers and Orr 2009).

The simultaneous occurrence of both AMF and epichloid endophytes is highly likely in the tissues of C3 grasses and several studies have been developed in an attempt to understand the outcome of this tripartite symbiosis. The simultaneous association with both symbionts can result positive, negative or neutral for host growth according to plant, AMF and endophyte genotypes and depending on environmental context (Omacini et al. 2006; Mack and Rudgers 2008; Liu et al. 2011; Larimer et al. 2012; Vignale et al. 2016; Zhou et al. 2016). Similarly, there is also a lack of consensus about the effect of endophyte on host AMF colonization: colonization of AMF can be impaired by endophyte occurrence when sharing the host grass (Guo et al. 1992; Chu-Chou et al. 1992; Omacini et al. 2006; Mack and Rudgers 2008; Liu et al. 2011; Zhou et al. 2016), but increased root colonization of non-agronomic grasses associated with endophytes was detected (Novas et al. 2005; Vignale et al. 2016). The underlying mechanisms are poorly understood but it has been observed that root exudates of endophyte-associated plants can improve AMF growth (Novas et al. 2011) while aqueous extracts from live or dead tissues of endophyte-associated plants can reduce spore germination and non-host plant colonization (Antunes et al. 2008). Furthermore, as far as we know, the role of AMF in the effect that endophyte has on the next generation of the host grass via changes in soil has never been tested.

Our objective was to assess the role of AMF in plant-soil feedback effects within grass-endophyte symbiosis. To achieve this objective, either endophyte-free or endophyte-associated plants were grown in soils with legacy of (i.e. previously conditioned by) plants with the same or different endophytic status (home and away soils, respectively), inoculated or not with three species of AMF (Fig. 1). Considering that endophyte-associated plants (E+) may suppress AMF (García-Parisi et al. 2017; Guo et al. 1992; Chu-Chou et al. 1992; Antunes et al. 2008), we hypothesized that the legacy of E+ plants reduces the benefits conferred by these root symbionts to endophyte-free conspecific plants (E-). Then, the ratio between biomass of plants growing in home and away soils (i.e. the feedback effect) would be positive only in endophyte-free plants inoculated with AMF, and not in E+ plants if they present lower mycorrhizal colonization than E- plants. Without AMF inoculation, this feedback effect would be similar on plants with either endophytic status. Here, we present the results of a factorial experiment in which we evaluated biomass, P content and AMF colonization of endophyte-free and endophyte-associated *Lolium multiflorum* plants growing in soils with legacy of (i.e. previously conditioned by) plants with the same or different endophytic status, inoculated or not with three species of AMF.

Materials and methods

Study system

We developed a two-phase experiment. In the conditioning phase of our experiment, plants of the annual grass

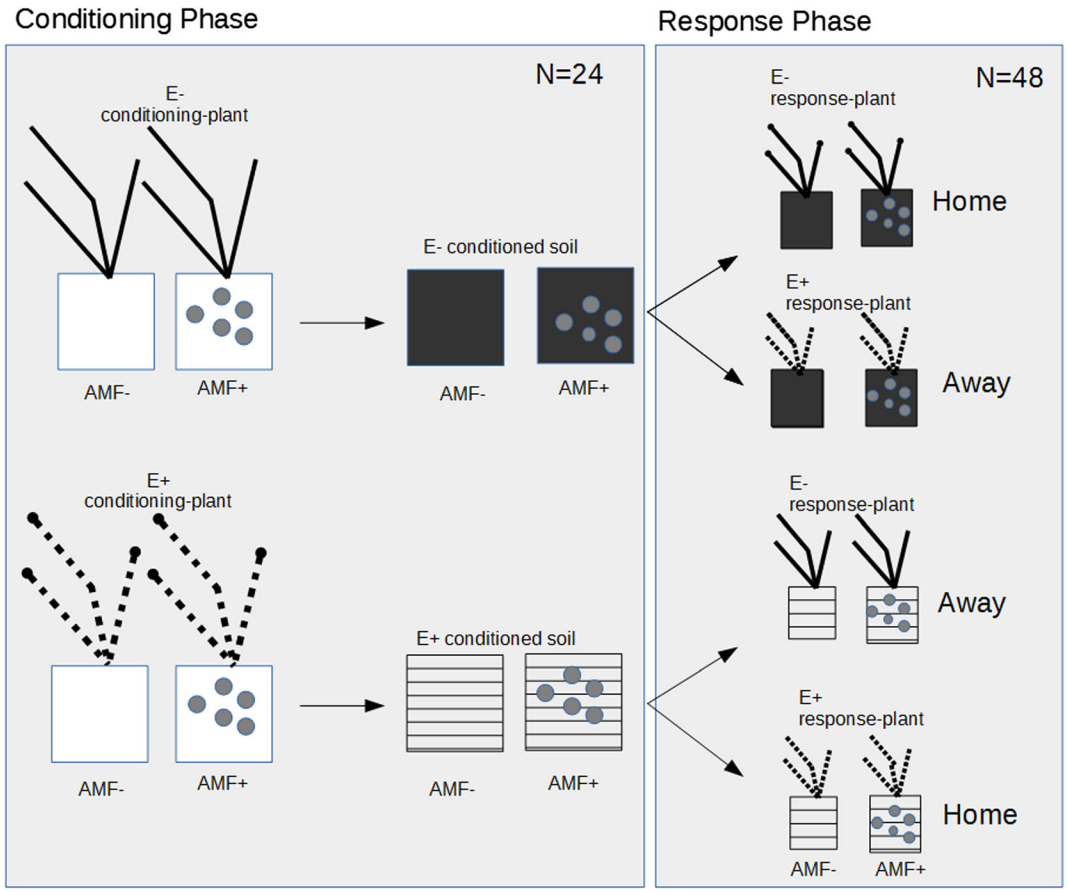

Fig. 1 Schematic of the experimental design divided into two phases. Conditioning phase: Endophyte-free (E-: continues lines) or endophyte-associated (E+, dashed lines) plants conditioned the soil inoculated or not with arbuscular mycorrhizal fungi (AMF+ or AMF -, with or without circles, respectively). Thus, the result of conditioning phase was E- and E+ conditioned soils (grey and patterned boxes) either with or without AMF presence. Response phase: E- and E+ response plants grew in each type of the conditioned soil, resulting in E- and E+ plants growing in home or away soils (i.e. conditioned by plants with the same or different endophytic status, respectively), in with or without AMF inoculation. N refers to the total number of experimental units in each phase

L. multiflorum Lam. were grown in different pots, either with or without the endophyte *Epichloë occultans* (C.D. Moon, B. Scott & M.J. Chr.) Schardl (E+ or E- plants, respectively), and inoculated or not with a combination of three AMF species (AMF+ or AMF-, respectively). In the response phase, E+ or E- *L. multiflorum* plants were grown in each one of the 4 types of conditioned soils (Fig. 1).

The experimental pots of the conditioning phase were filled with a mixture of sterile soil and sand (1:1, total C: 11.8 $mg.g^{-1}$, total N: 0.93 $mg.g^{-1}$). Soil came from the top (upper 10 cm) of Mollisol, whose plant community was a successional plot dominated by exotic dicots. In such community *L. multiflorum* presence was very low and thus, we avoided soils with microbial community selected by the grass. Moreover, soil was sterilized before the conditioning phase to reduce the amount AMF propagules. Sterilization was carried out by autoclaving the soil at 1 atm pressure, 100 °C, for 1 h, three times with 24 h interval.

To obtain endophyte-free and endophyte-associated *L. multiflorum* seeds, one year before the conditioning phase, we collected seeds from an old-field Pampean grassland (Carlos Casares, Argentina 34°06′S, 60°25′ W) dominated by a *L. multiflorum* population with ≈ 95% individuals associated to endophytes (Omacini et al. 2006). Half of seeds collected were treated with the fungicide triadimenol (0.5 g pa.100 g^{-1} seeds) to eliminate the endophyte. Fungicide-treated and untreated seeds were cultivated in adjacent 1 m^2 plots and the seeds produced by those plants were harvested and used in the conditioning phase as E- and E+ seeds, respectively. Microscopic observation of 30 seeds collected from each plot, stained with bengale rose (Bacon and

White 1994), confirmed that F1 of untreated seeds showed 95% of symbiotic individuals, and F1 of treated seeds, 0%.

The AMF inoculum consisted of a mixture of internal and external hyphae and spores (32 ± 3.4 spores.g^{-1}) of three fungi species known to colonize grasses and clovers: *Funneliformis mosseae* (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler (LPS SB1), *Simiglomus hoi* (S.M. Berch & Trappe) G.A. Silva, Oehl & Sieverd (BEG 104) and *Rhizophagus intraradices* (N.C. Schenck & G.S. Sm.) C. Walker & A. Schüßler (BAFC 3108). The inoculum was obtained from the multiplication of pure cultures of each fungus in plants of *Plantago lanceolata* L., *Lotus tenuis* L., and *Bromus unioloides* HBK. These plants were grown in pots with sterile perlite and vermiculite, watered with distilled water during the first week and with a modified (0.02 *mM* P) Hoagland's solution afterwards (methodology adapted from Grimoldi et al. (2005). When plants showed >60% of root length colonized by AMF, we stopped the watering. Thus, the inoculum consisted of the substrate, the plants roots and the spores contained in the pots. Additional pots were sown with the same plant species that grew under the same conditions but without AMF in order to obtain the control substrate applied to experimental pots of non-mycorrhizal treatments (AMF-).

Experimental setup

Soil conditioning phase

The conditioning phase was carried out in a greenhouse between June and December 2012. *L. multiflorum* plants were grown in 1.5 l pots (four plants per pot). Twelve pots were sown with E- seeds while other 12 were sown with E+ seeds. Furthermore, 25 g of AMF inoculum was added to half the pots with each endophytic status. The control substrate without AMF was incorporated to the remaining pots. Thus, we obtained four types of experimental units with six replicates. Pots were kept in a greenhouse, watered as needed, until mid-December. On 20th December, plants were senescing and watering was interrupted. We clipped shoot tissues and sieved the soil to be used in the second phase of the experiment. As a result, we obtained four types of conditioned soil: soils conditioned by endophyte-free or endophyte-associated plants with or without AMF (AMF+ and AMF-, respectively). After conditioning phase, AMF- soils showed no viable spores while AMF+ soils showed 33 ± 3 and 42 ± 4 spores.g^{-1} soil when conditioned by endophyte-associated and endophyte-free plants, respectively (García-Parisi et al. 2017). During the conditioning phase, endophyte-associated *L. multiflorum* plants without AMF produced 70 ± 7.3 g.pot-1 of belowground biomass while endophyte-free plants and endophyte-associated plants with AMF produced on average 28 ± 7.2 g.pot-1 of belowground biomass ($F_{1,20}$ = 14.4, P = 0.001).

Response phase

In the second phase, *L. multiflorum* plants were grown in the four types of conditioned soils (Fig. 1). From each of the 24 conditioning pots (experimental units), we obtained two sub-pots (180 ml, 240 g each), where we sowed one seed in each, either endophyte-free or endophyte-associated. As a result, we obtained a hierarchical factorial experiment. When the response plants were growing in soils conditioned by plants with their same endophytic status (i.e. E+ plants growing in soils conditioned by E+ plants, and E- plants in soils conditioned by E-), they were considered home soils. Alternatively, when the response plants were growing in soils conditioned by plants with different endophyte symbiotic status from their own (i.e. E+ plants growing in soils conditioned by E- plants, and E- plants in soils conditioned by E+ plants), they were considered away soils.

This phase was carried out for over three months in growth cabinets (Nuaire TM, Plymouth, USA) set at 20 °C, with a 16:8 h light/dark photoperiod at a photon flux density of 280umol.m^{-2}.s^{-1}. Plants were all located in the same cabinet but each sub-pot was located inside an individual container in order to avoid contamination among plants through leaching or splashing when watering. Pots were watered to field capacity, when necessary, by adding distilled water on the individual container.

Harvest and determinations

After three months, we harvested the E- and E+ response-plants. Shoots were clipped at soil surface. Roots were washed, and a subsample was cleared and Tryphan Blue-stained were examined under optical microscope at ×200 magnification to observe structures of AMF (Phillips and Hayman 1970). All shoots and roots were dried at 70 °C for 48hs, and their dry weight

recorded. The feedback effect was calculated as the ln (total biomass in home soil/total biomass in away soils). Phosphorus concentration (%) was determined in samples of roots and shoot (100 mg) which were ashed in a muffle furnace (4 h at 500 °C). The resulting ash was digested in HCl, and quantities of P were measured by phosphovanado-molybdate colorimetry (Hanson 1950). Reference material of ground grass leaves was included with every 10 samples to check digestion and analytical procedures.

Statistical analyses

Analyses were performed with linear mixed effect models (*lme*) with the package nlme using statistical software R (Pinheiro et al. 2015; R Core Team 2015). Feedback effect was calculated as the ratio between biomass of plant growing in home soil and biomass of plants growing in away soil (van der Putten et al. 1993; Brinkman et al. 2010), and was analysed with a model including AMF inoculation and endophytic status of response plant as fixed effects. Shoot and root biomass, P concentration and P content were analysed with models that included matching between previous and present endophytic status (home vs. away), AMF inoculation and endophytic status of response-plants as fixed effects, and the hierarchical organization (pot/sub-pot) as random effect. Total mycorrhizal colonization, arbuscular and vesicular colonization were analysed only for AMF+ treatments, including matching between previous and present endophytic status (home vs. away) and endophytic status of response-plants as fixed effects, and the hierarchical organization (pot/sub-pot) as random effect. The significance of the fixed factors in *lme* models was tested using Likelihood Ratio Test (LRT, Fox and Weisberg 2011).

Results

Feedback effect shifted from negative to positive due to AMF inoculation (Fig. 2) for E- and E+ response-plants. (AMF: $F_{1,15} = 7.1$, $P = 0.02$; E: $F_{1,15} = 1.4$, $P = 0.25$; AMF x E: $F_{1,15} = 0.01$, $P = 0.95$). Total plant biomass was interactively affected by both the matching between endophytic status of previous and present plants and the inoculation with AMF (Table 1). Shoot biomass of plants growing in away soils (i.e. soils conditioned by plants with different symbiotic status) was higher than

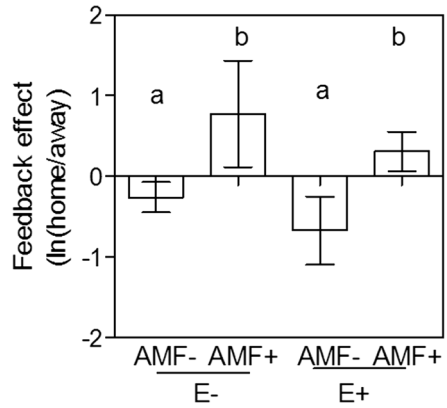

Fig. 2 Feedback effect (Ln (total biomass of plant growing in home soil/total biomass of plant growing in away soil) of endophyte-free (E-) and endophyte-associated (E+) *Lolium multiflorum* response-plants with or without arbuscular mycorrhizal fungi inoculation (AMF+ and AMF-, respectively)

when growing in AMF- home soils but not different from AMF+ home soils (Fig. 3). Indeed, AMF increased shoot biomass of plants growing in home soils by 100%. Root plant biomass was increased by about 65% as a result of AMF inoculation when plants were growing in home soils, but it was decreased when growing in away soils (Fig. 3).

Symbionts also influenced P acquisition. While P concentration (%) was reduced by about 15% in E+ response-plants, it was not affected either by the matching with previous endophytic status or by AMF inoculation (Table 1, Fig. 4, left panel). Instead, P content (mg. plant^{-1}) interactively depended on matching between previous and present endophytic status and AMF inoculation, similarly to plant biomass (Table 1, Fig. 4, left panel). Indeed, AMF inoculation doubled P content in home soils but did not affect it in away soils.

On plants growing in AMF+ soils, P content was interactively affected by matching between endophytic status of previous and present plants and arbuscular colonization, when including the latter as a predictive variable ($P < 0.01$, Fig. 4, right panel). We found no significant effect on P% or when including total colonization ($P > 0.1$, Fig. 4, right panel). This means that the response of P content to arbuscular colonization was different according to the matching with previous endophytic status of the soil (i.e. between home and away soils). Indeed, correlation between P content and arbuscular colonization was negative in away soils and positive in home soils (Spearman's rho Home: 0.52, Away: −0.83). Total AMF colonization and vesicular

Table 1 Chi square (χ^2) values from statistical analyses of plant response variables: Shoot and root biomass, Root length colonization (total, arbuscules and vesicules) and P content, as affected by the matching between previous and present endophytic status (home vs. aways soils), arbuscular mycorrhizal fungi inoculation (AMF) and endophyte association in response plant, and interactions among the three factors. Root length colonization was only analysed in AMF+ treatments

	Df	Biomass		Phosporous		Root Lenght Colonization		
		Shoot g.plant^{-1}	Root g.plant^{-1}	concentration %	content g.plant^{-1}	Total %	Arbuscules %	Vesicules %
Home-away soils (H-A)	1	**4.41***	0.02	0.95	0.01	2.04	**8.31****	1.82
AMF	1	**3.89***	0.22	1.16	**6.57***			
Endophyte (E)	1	0.80	0.59	**5.85***	2.08	0.01	**4.02***	0.29
H-A x AMF	1	**6.11***	**8.73****	0.98	**3.96***			
H-A x E	1	0.04	0.09	0.86	0.31	2.11	1.05	0.40
AMF x E	1	0.01	0.03	1.64	0.93			
H-A x AMF x E	1	0.56	0.53	2.09	2.69			

df degree of freedom of chi square (χ^2) test

* and ** indicate significance level (*P* values <0.05 and <0.01, respectively)

colonization were not affected by endophytic status of previous or present plant (Table 1). However, arbuscular colonization was higher in plants growing in their home soil rather than in away soils and in endophyte-associated plants rather than endophyte-free plants (Fig. 5). Colonization did not correlate with the number of AMF spores in the soil (Spearman's correlation, $P > 0.05$). Response-plants from AMF- treatments showed no mycorrhizal colonization.

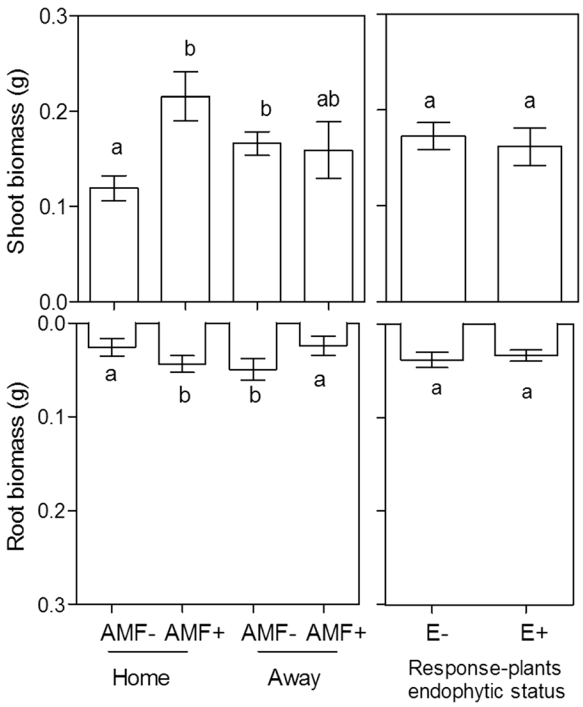

Fig. 3 Shoot and root biomass (g.plant^{-1}) of endophyte-free or endophyte-associated *Lolium multiflorum* plants (E- or E+ plants, respectively) growing in soils conditioned by plant with the same or different endophytic status (home or away soils) with or without arbuscular mycorrhizal fungi inoculation (AMF+ and AMF-, respectively). Different letters indicate significant differences among treatments for each plot ($P < 0.05$)

Discussion

Our findings highlight that AMF have a key role in intraspecific plant-soil feedback of grass-endophyte symbiosis. We detected that impact of AMF addition on *L. multiflorum* performance was independent of response plant endophytic status because it is a result of the matching between previous and present endophytic status (i.e. AMF increased shoot and root biomass both in endophyte-free and endophyte-associated plants only when growing in their home soil). Furthermore, we found that arbuscular colonization and P content increment in plants growing in home soil with AMF were higher than in away soils, irrespective of endophytic status. Indeed, we found that correlation between arbuscular colonization and P content in plants is positive when growing in home soil, and negative when growing in away soil. Thus, we reject the hypothesis that the legacy of endophyte-associated plants reduces the benefits conferred by mycorrhiza to endophyte-free plants but not to endophyte-associated plants.

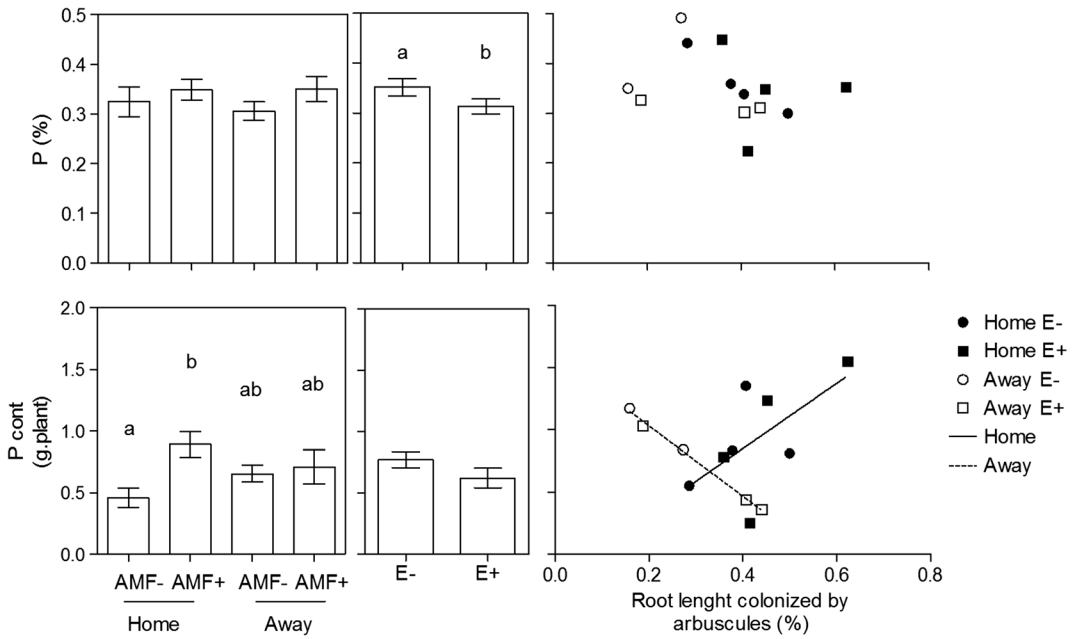

Fig. 4 *Left panel*: Phosphorous concentration (%) and content (g. plant $^{-1}$) of endophyte-free or endophyte-associated *Lolium multiflorum* plants (E- or E+ plants, respectively) growing in soils conditioned by plant with the same or different endophytic status (home or away soils) with or without arbuscular mycorrhizal fungi inoculation (AMF+ and AMF-, respectively). Different letters indicate significant differences among treatments for each plot ($P < 0.05$). Right panel: Relationship between phosphorous concentration (%, upper panel) or content (g. plant $^{-1}$, lower panel) and related to root length colonized by arbuscules (%) of endophyte-free or endophyte-associated *Lolium multiflorum* plants (E- or E+ plants, circles or squares, respectively) growing in soils conditioned by plant with the same or different endophytic status (home or away soils, filled or clear symbols) inoculated with arbuscular mycorrhizal fungi (AMF+). When interaction between the matching between endophytic status of previous and present plants and arbuscular colonization and arbuscular colonization is significant ($P < 0.05$), the regression line is drawn

Therefore, AMF availability reverses the direction of the feedback effect from negative to positive not only in the case of endophyte-free plants, but also of endophyte-associated plants.

Reversing feedbacks due to AMF has been previously demonstrated without considering the interaction with other plant symbionts (Klironomos 2002; Bever 2002). We observed a negative feedback effect between plants with the same endophytic status without AMF, which disappears with the presence of an AMF community. While there are previous studies that have evaluated endophyte impact on intra and interspecific feedbacks, no focus has been placed on the influence AMF may have on the grass-endophyte symbiosis intraspecific feedback. For example, Matthews and Clay (2001) found a negative intraspecific feedback of endophyte-associated tall fescue plants, while Cripps et al. (2013) found that endophyte-free perennial ryegrass was affected by previous presence of endophyte-associated plants. Meanwhile, Rudgers and Orr (2009) demonstrated that microbial community mediates the negative effect of grass-endophyte symbiosis on tree establishment through plant-soil feedbacks. Recently, a positive effect of endophyte legacy was found on *T. repens* growth but without any AMF influence (García-Parisi et al. 2017). The findings presented here demonstrate that plant-soil feedback occurs at endophytic-status level, in addition to earlier findings that plant-soil feedbacks vary at intraspecific population level (i.e. different genotypes of the same plant species) and among plant species (Bever et al. 1997; Bever 2003; Kardol et al. 2007; Wagg et al. 2015).

We found that the symbiosis with AMF can be positive for both endophyte-free and endophyte-associated plants depending on the matching with the endophytic status of both grass generations. Previous studies detected that the simultaneous association with endophytes and AMF can produce antagonistic, additive and synergistic effects on host performance depending on AMF or endophyte species, and/or nutrient availability (Kong et al. 2004; Mack and Rudgers 2008; Liu et al. 2011; Vignale et al. 2016; Zhou et al. 2016). Surprisingly, we detected that *E. occultans* in *L. multiflorum* plants may

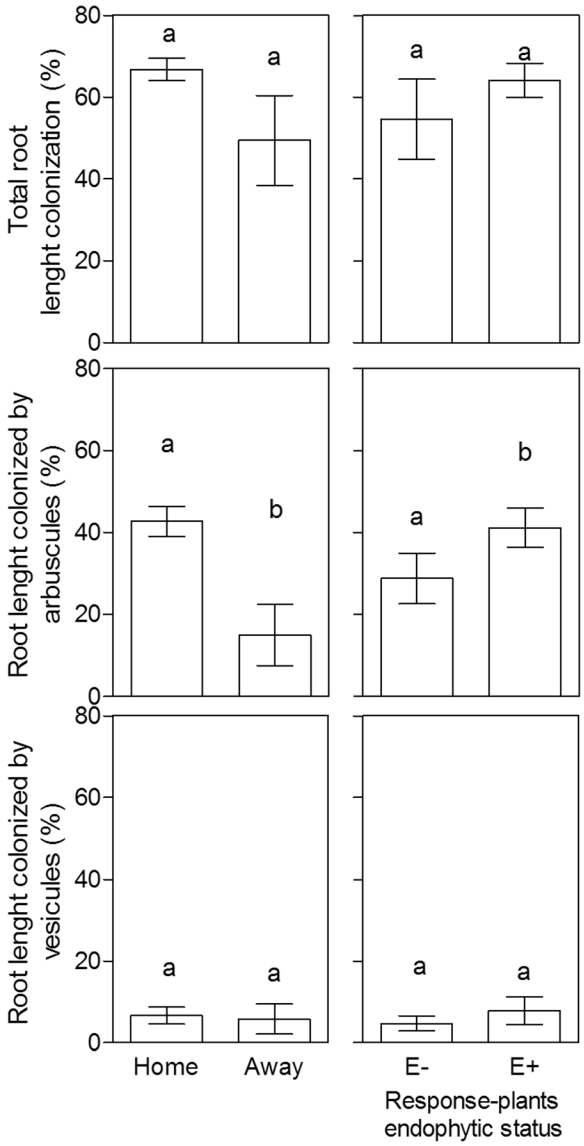

Fig. 5 Total, arbuscular and vesicular root length colonization (%)of endophyte-free or endophyte-associated *Lolium multiflorum* plants (E- or E+ plants, respectively) growing in soils conditioned by plants with the same or different endophytic status (home or away soils) inoculated with arbuscular mycorrhizal fungi. Different letters indicate significant differences between treatments for each plot ($P < 0.05$)

increase arbuscular colonization in host roots (see Omacini et al. 2006). This is the first time that this positive effect is detected in an exotic grass when both symbionts were sharing the host grass. Previously, an increase in host AMF colonization due to endophyte occurrence on shoot tissues was only detected in non-agronomic grasses such as *Poa bonariensis, Elymus histrix, Bromus setifolium, and Bromus auleticus* (Novas et al. 2009; Larimer et al. 2012; Arrieta et al. 2015; Vignale et al. 2016). Further research is needed to study the underlying mechanisms of the effects observed here.

Changes in plant response to AMF, together with this symbiosis establishment and functioning can be due to the AMF species involved (e.g. Larimer et al. 2012). Legacy of endophytic plants differed in the number of spores, which was relatively high and did not correlate with the AMF colonization. Thus, AMF identity can be the driving force in the feedback effect observed. Plants with different endophytic status would have contrasting AMF specificity among the three AMF species included or the ecotypes within them, as has been recently suggested for other grass-endophyte associations (Liu et al. 2011; Larimer et al. 2012; Zhou et al. 2016). Then, the positive AMF effect on plants growth and P acquisition in their home soil is probably explained by the fact that the most beneficial AMF genotype (ecotype or species) can be different for endophyte-free and endophyte-associated plants. Although we could identify the AMF species associated to endophyte-free and endophyte-associated plants, we found that the arbuscular colonization was positively correlated with P content in plants growing in home soil and negatively in plants growing in away soils. Hence, P exchange may be one of the currencies of this mutualism, at least under our simplified experimental conditions. Further research is needed in order to state if this effects are also observed when including the interactions with the native microbial soil community.

The findings presented here may contribute to our understanding of the factors that determine endophyte incidence levels on grass populations. In Pampean grasslands, the level of endophyte incidence on *L. multiflorum* naturalized populations is very high, often about 95% (De Battista 2005; Gundel et al. 2009; Casas et al. 2016b). It has been suggested that this is a result of negative interactions such as competence or grazing, or management grassland history (Gundel et al. 2008; Gundel et al. 2011; García Parisi et al. 2012; Casas et al. 2016a). In this study we show that, acting through plant-soil feedback mechanisms, positive interactions such as mutualism with AMF can also shape symbiosis dynamics in naturalized populations. Plant-soil feedbacks have been proposed as ecological mechanisms that explain species coexistence and species invasion ability (Bever et al. 1997; Klironomos 2002; Bever 2002; van der Putten et al. 2013; van der Putten et al. 2016). We suggest that the feedback effects

observed in both endophyte-free and endophyte-associated plants can help to understand the dominance of one or the coexistence of both endophytic forms in Pampean grasslands. Thus, further research is needed to assess whether factors that impair AMF such as herbicide applications, grazing or even endophytes occurrence (García-Parisi et al. 2017; Chu-Chou et al. 1992; Druille et al. 2015; Druille et al. 2016) can modify this feedback effect under field conditions.

In conclusion, we demonstrated that AMF shifts the intraspecific feedback effect of endophyte-free and endophyte-associated grass from negative to positive. This reversion is supported by an increased arbuscular colonization and P content of AMF+ plants in plants growing in home soils. Indeed, the inverse relationship between arbuscular colonization and P acquisition of plants growing in home and away soils suggests that both the establishment and functioning of AMF symbiosis can determine plant response to home and away soils. Furthermore, we found that the outcome of simultaneous occurrence of foliar endophyte and AMF on grass performance depends on the matching with the endophytic status of plants previously grown in that soil. Thus, as far as we know, this is the first time that (1) the role of endophyte in interspecific plant-soil feedback of an annual grass is studied, and (2) it is demonstrated that AMF completely reverses the direction of the feedback effect not only of endophyte-free plants, but also of endophyte-associated grass plants. Our findings suggest that feedback effects between exotic plants and soil communities are relevant for interspecific plant interactions and also for plant-foliar symbionts dynamics.

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References

Antunes PM, Miller J, Carvalho LM et al (2008) Even after death the endophytic fungus of *Schedonorus phoenix* reduces the arbuscular mycorrhizas of other plants. Funct Ecol 22:912–918. doi:10.1111/j.1365-2435.2008.01432.x

Arrieta AM, Iannone LJ, Scervino JM et al (2015) A foliar endophyte increases the diversity of phosphorus-solubilizing rhizospheric fungi and mycorrhizal colonization in the wild grass *Bromus auleticus*. Fungal Ecol 17:146–154. doi:10.1016/j.funeco.2015.07.001

Bacon CW, White JFJ (1994) Biotechnology of endophytic fungi of grasses. CRC Press, Boca Raton

Bever JD (2002) Negative feedback within a mutualism: host-specific growth of mycorrhizal fungi reduces plant benefit. Proc R Soc B 269:2595–2601. doi:10.1098/rspb.2002.2162

Bever JD (2003) Soil community feedback and the coexistence of competitors: conceptual frameworks and empirical tests. New Phytol 157:465–473. doi:10.1046/j.1469-8137.2003.00714.x

Bever JD, Westover KM, Antonovics J (1997) Incorporating the soil community into plant population dynamics: the utility of the feedback approach. J Ecol 85:561–573. doi:10.2307/2960528

Bowatte S, Barrett B, Luscombe C et al (2011) Effect of grass species and fungal endophyte on soil nitrification potential. New Zeal J Agric Res 54:275–284. doi:10.1080/00288233.2011.606325

Breen JP (1994) Acremonium endophyte interactions with enhanced plant resistance to insects. Annu Rev Entomol 39:401–423. doi:10.1146/annurev.en.39.010194.002153

Brinkman EP, Van der Putten WH, Bakker E-J, Verhoeven KJF (2010) Plant-soil feedback: experimental approaches, statistical analyses and ecological interpretations. J Ecol 98:1063–1073

Buyer JS, Zuberer D, Nichols K, Franzluebbers AJ (2011) Soil microbial community function, structure, and glomalin in response to tall fescue endophyte infection. Plant Soil 339:401–412. doi:10.1007/s11104-010-0592-y

Casas C, Omacini M, Montecchia MS, Correa OS (2011) Soil microbial community responses to the fungal endophyte Neotyphodium in Italian ryegrass. Plant Soil 340:347–355. doi:10.1007/s11104-010-0607-8

Casas C, Gundel PE, Semmartin M et al (2016a) The enhancement of invasion ability of an annual grass by its fungal endophyte depends on recipient community structure. Biol Invasions 18:1853–1865. doi:10.1007/s10530-016-1129-y

Casas C, Torretta JP, Exeler N, Omacini M (2016b) What happens next? Legacy effects induced by grazing and grass-endophyte symbiosis on thistle plants and their floral visitors. Plant Soil 405:211–229. doi:10.1007/s11104-015-2644-9

Chu-Chou M, Guo B, An Z-Q et al (1992) Suppression of mycorrhizal fungi in fescue by the Acremonium coenophialum endophyte. Soil Biol Biochem 24:633–637. doi:10.1016/0038-0717(92)90041-U

Cripps MG, Edwards GR, McKenzie SL (2013) Grass species and their fungal symbionts affect subsequent forage growth. Basic Appl Ecol 14:225–234. doi:10.1016/j.baae.2013.01.008

De Battista JP (2005) Neotyphodium research and application in South America. In: Roberts C, West C, Spiers D (eds) Neotyphodium in cool season grasses. Blackwell Publishing, Ames, pp 63–69

Druille M, Cabello MN, García Parisi PA et al (2015) Glyphosate vulnerability explains changes in root-symbionts propagules viability in pampean grasslands. Agric Ecosyst Environ 202:48–55. doi:10.1016/j.agee.2014.12.017

Druille M, García-Parisi PA, Golluscio RA et al (2016) Repeated annual glyphosate applications may impair beneficial soil microorganisms in temperate grassland. Agric Ecosyst Environ 230:184–190. doi:10.1016/j.agee.2016.06.011

Fox J, Weisberg S (2011) An R companion to applied regression, 2nd edn. Sage, Thousand Oaks

Franzluebbers AJ (2006) Short-term responses of soil C and N fractions to tall fescue endophyte infection. Plant Soil 282: 153–164. doi:10.1007/s11104-005-5447-6

García Parisi PA, Casas C, Gundel PE, Omacini M (2012) Consequences of grazing on the vertical transmission of a fungal Neotyphodium symbiont in an annual grass population. Austral Ecol 37:620–628. doi:10.1111/j.1442-9993.2011.02325.x

García Parisi PA, Lattanzi FA, Grimoldi AA, Omacini M (2015) Multi-symbiotic systems: functional implications of the coexistence of grass-endophyte and legume-rhizobia symbioses. Oikos 124:553–560. doi:10.1111/oik.01540

García-Parisi PA, Lattanzi FA, Grimoldi AA et al (2017) Three symbionts involved in interspecific plant-soil feedback: epichloid endophytes and mycorrhizal fungi affect the performance of rhizobia-legume symbiosis. Plant Soil 412:151–162. doi:10.1007/s11104-016-3054-3

Grimoldi AA, Kavanová M, Lattanzi FA, Schnyder H (2005) Phosphorus nutrition-mediated effects of arbuscular mycorrhiza on leaf morphology and carbon allocation in perennial ryegrass. New Phytol 168:435–444. doi:10.1111/j.1469-8137.2005.01500.x

Gundel PE, Batista WB, Texeira M et al (2008) Neotyphodium endophyte infection frequency in annual grass populations: relative importance of mutualism and transmission efficiency. Proc R Soc B 275:897–905. doi:10.1098/rspb.2007.1494

Gundel PE, Garibaldi LA, Tognetti PM et al (2009) Imperfect vertical transmission of the endophyte Neotyphodium in exotic grasses in grasslands of the flooding pampa. Microb Ecol 57:740–748. doi:10.1007/s00248-008-9447-y

Gundel PE, Garibaldi LA, Martinez-Ghersa MA, Ghersa CM (2011) Neotyphodium endophyte transmission to *Lolium multiflorum* seeds depends on the host plant fitness. Environ Exp Bot 71:359–366. doi:10.1016/j.envexpbot.2011.02.002

Guo BZ, Hendrix JW, An Z-Q, Ferriss RS (1992) Role of Acremonium endophyte of fescue on inhibition of colonization and reproduction of mycorrhizal fungi. Mycologia 84: 882–885. doi:10.2307/3760286

Hanson WC (1950) The photometric determination of phosphorus in fertilizers using the phosphovanado-molybdate complex. J Sci Food Agric 1:172–173. doi:10.1002/jsfa.2740010604

Jenkins MB, Franzluebbers AJ, Humayoun SB (2006) Assessing short-term responses of prokaryotic communities in bulk and rhizosphere soils to tall fescue endophyte infection. Plant Soil 289:309–320. doi:10.1007/s11104-006-9141-0

Kardol P, Cornips NJ, van Kempen MM et al (2007) Microbe-mediated plant-soil feedback causes historical contingency effects in plant community assembly. Ecol Monogr 77:147–162. doi:10.1890/06-0502

Klironomos JN (2002) Feedback with soil biota contributes to plant rarity and invasiveness in communities. Nature 417: 67–70. doi:10.1038/417067a

Kong CH, Xu X, Zhou B et al (2004) Two compounds from allelopathic rice accession and their inhibitory activity on weeds and fungal pathogens. Phytochemistry 65:1123–1128. doi:10.1016/j.phytochem.2004.02.017

Larimer AL, Bever JD, Clay K (2012) Consequences of simultaneous interactions of fungal endophytes and arbuscular mycorrhizal fungi with a shared host grass. Oikos 121:2090–2096. doi:10.1111/j.1600-0706.2012.20153.x

Liu Q, Parsons AJ, Xue H et al (2011) Competition between foliar Neotyphodium lolii endophytes and mycorrhizal Glomus Spp. Fungi in *Lolium perenne* depends on resource supply and host carbohydrate content. Funct Ecol 25:910–920. doi:10.1111/j.1365-2435.2011.01853.x

Mack KML, Rudgers JA (2008) Balancing multiple mutualists: asymmetric interactions among plants, arbuscular mycorrhizal fungi, and fungal endophytes. Oikos 117:310–320. doi:10.1111/j.2007.0030-1299.15973.x

Malinowski DP, Alloush GA, Belesky DP (2000) Leaf endophyte Neotyphodium coenophialum modifies mineral uptake in tall fescue. Plant Soil 227:115–126

Malinowski DP, Belesky DP, Hill NS et al (2008) Influence of phosphorus on the growth and ergot alkaloid content of Neotyphodium coenophialum-infected tall fescue (*Festuca arundinacea* Schreb.) Plant Soil 198:53–61

Matthews JW, Clay K (2001) Influence of fungal endophyte infection on plant-soil feedback and community interactions. Ecology 82:500–509

Novas MV, Cabral D, Godeas AM (2005) Interaction between grass endophytes and mycorrhizas in *Bromus setifolius* from Patagonia, Argentina. Symbiosis 40:23–30

Novas MV, Iannone LJ, Godeas AM, Cabral D (2009) Positive association between mycorrhiza and foliar endophytes in *Poa bonariensis*, a native grass. Mycol Prog 8:75–81. doi:10.1007/s11557-008-0579-8

Novas MV, Iannone LJ, Godeas AM, Scervino JM (2011) Evidence for leaf endophyte regulation of root symbionts: effect of Neotyphodium endophytes on the pre-infective state of mycorrhizal fungi. Symbiosis 55:19–28. doi:10.1007/s13199-011-0140-4

Omacini M, Chaneton EJ, Ghersa CM (2005) A hierarchical framework for understanding the ecosystem consequences of endofhyte-grass symbioses. In: Roberts C, West CP, Spiers DE (eds) Neotyphodium in cool-season grasses current research applications. Blackwell Publishing, Boston, pp 141–161

Omacini M, Eggers T, Bonkowski M et al (2006) Leaf endophytes affect mycorrhizal status and growth of co-infected and neighbouring plants. Funct Ecol 20:226–232. doi:10.1111/j.1365-2435.2006.01099.x

Omacini M, Semmartin M, Perez LI, Gundel PE (2012) Grass-endophyte symbiosis: a neglected aboveground interaction with multiple belowground consequences. Appl Soil Ecol 61: 273–279. doi:10.1016/j.apsoil.2011.10.012

Pérez LI, Gundel PE, Omacini M (2016) Can the defensive mutualism between grasses and fungal endophytes protect non-symbiotic neighbours from soil pathogens? Plant Soil 405: 289–298. doi:10.1007/s11104-015-2568-4

Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Br Mycol Soc 55:158–163

Pinheiro JC, Bates D, DebRoy S et al (2015) {nlme}: Linear and Nonlinear Mixed Effects Models. R package version 3.1-121

Ponce M, Bompadre MJ, Scervino JM et al (2009) Flavonoids, benzoic acids and cinnamic acids isolated from shoots and roots of Italian rye grass (*Lolium multiflorum* lam.) with and without endophyte association and arbuscular mycorrhizal fungus. Biochem Syst Ecol 37:245–253. doi:10.1016/j.bse.2009.03.010

R Core Team (2015) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Rasmussen S, Parsons AJ, Newman JA (2009) Metabolomics analysis of the *Lolium perenne*–Neotyphodium lolii symbiosis: more than just alkaloids? Phytochem Rev 8:535–550. doi:10.1007/s11101-009-9136-6

Rudgers JA, Orr S (2009) Non-native grass alters growth of native tree species via leaf and soil microbes. J Ecol 97:247–255. doi:10.1111/j.1365-2745.2008.01478.x

Rudgers JA, Koslow JM, Clay K (2004) Endophytic fungi alter relationships between diversity and ecosystem properties. Ecol Lett 7:42–51. doi:10.1046/j.1461-0248.2003.00543.x

Van Der Putten WH, Van Dijk C, Peters B (1993) Plant-specific soil-borne diseases contribute to succession in foredune vegetation. Nature 362:53–56. doi:10.1038/362053a0

van der Putten WH, Bardgett RD, Bever JD et al (2013) Plant-soil feedbacks: the past, the present and future challenges. J Ecol 101:265–276. doi:10.1111/1365-2745.12054

van der Putten WH, Bradford MA, Pernilla Brinkman E et al (2016) Where, when and how plant–soil feedback matters in a changing world. Funct Ecol 30:1109–1121. doi:10.1111/1365-2435.12657

Van Hecke MM, Treonis AM, Kaufman JR (2005) How does the fungal endophyte Neotyphodium coenophialum affect tall fescue (*Festuca arundinacea*) rhizodeposition and soil microorganisms? Plant Soil 275:101–109. doi:10.1007/s11104-005-0380-2

Vignale MV, Iannone LJ, Pinget AD et al (2016) Effect of epichloid endophytes and soil fertilization on arbuscular mycorrhizal colonization of a wild grass. Plant Soil 405:279–287. doi:10.1007/s11104-015-2522-5

Wagg C, Boller B, Schneider S et al (2015) Intraspecific and intergenerational differences in plant–soil feedbacks. Oikos 124:994–1004. doi:10.1111/oik.01743

Zhou Y, Li X, Qin J et al (2016) Effects of simultaneous infections of endophytic fungi and arbuscular mycorrhizal fungi on the growth of their shared host grass Achnatherum Sibiricum under varying N and P supply. Fungal Ecol 20:56–65. doi:10.1016/j.funeco.2015.11.004