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ORIGINAL PAPER

The enhancement of invasion ability of an annual grass by its fungal endophyte depends on recipient community structure

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Abstract Most terrestrial plants establish symbiotic associations with microorganisms that enable them to overcome abiotic or biotic filters in ecosystems. Here we investigated how aerial mutualisms involving invasive species may affect the recipient community's structure. We hypothesized that the endophyte *Epichloë occultans* enhances the ability of *Lolium multiflorum* to establish and colonize, but that success would depend on the structure and invasion resistance of the recipient grassland community. Seeds of *L. multiflorum* with high (E+) and low (E−) endophyte incidence were sown in plots located in grasslands with or without recent grazing history. Relative cover of *L. multiflorum* and floristic groups was determined during the growing season. Whereas we did not detect any endophyte effect in sites with grazing history, *L. multiflorum* cover was 63 % in E+ and 27 % in E− plots in sites without grazing history. As cover of *L. multiflorum* increased in these sites, the cover of warm- and cool-season grasses decreased in spring, with that of warm-season grasses continuing to decrease in summer. These decreases corresponded to 1.9, 3.7 and 1.6 %, for every % increase of *L. multiflorum* cover. Path analysis and posterior modelling predicted a greater impact of the endophyte on *L. multiflorum* cover than of seed addition when resident *L. multiflorum* cover was ≤20 %. This effect decreased asymptotically as *L. multiflorum* cover increased beyond 20 %. Our results suggest that the endophyte may boost the invasion ability of *L. multiflorum* particularly in natural grassland without grazing history with potential longer-term consequences for community structure and dynamics.

Keywords *Lolium multiflorum* · *Epichloë occultans* · Grass-endophyte symbiosis · Grassland functional groups

Introduction

Symbiotic associations with microorganisms enable plants to overcome some abiotic or biotic filters in ecosystems (Allen et al. 1989; Richardson et al. 2000; Suding et al. 2013). Thus, symbiotic microorganisms

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such as ectomycorrhizal or arbuscular mycorrhizal fungi which associate with a diverse array of plant species, may contribute critically to the invasion (Vellinga et al. 2009; Bunn et al. 2015). For instance, establishment of alien seedlings may be facilitated if these plants become rapidly colonised by the existing mycorrhizal fungal network of the recipient vegetation overcoming any particular competitive advantage of native species (Allen and Allen 1986; Marler et al. 1999; Mooney and Hobbs 2000; Klironomos 2002; Callaway et al. 2004). While the role of generalist soil symbiotic microorganisms has been widely studied (Richardson et al. 2000; Pringle et al. 2009 and references cited therein), the role of specific aerial endophytes in affecting invasions has been less frequently addressed (see, Rudgers et al. 2005; Uchitel et al. 2011; Saikkonen et al. 2013). Also, little is known about how this particular symbiosis—which arrives together with the introduced species—may affect the native species (van der Heijden et al. 1998; Callaway et al. 2008).

Recent evidence suggests that seed-born fungal endophytes of the genus *Epichloë* (Clavicipitaceae Schardl et al. 2004) may assist the worldwide expansion of certain host grasses (Clay and Holah 1999; Rudgers et al. 2004; Rudgers and Clay 2007; Rudgers and Orr 2009; Rudgers et al. 2010; Saikkonen et al. 2013). These obligate symbionts grow systemically throughout shoot tissues, including leaves, of many cool-season grasses and are vertically transmitted through seeds (Clay and Schardl 2002; Cheplick and Faeth 2009). Asexual *Epichloë* endophytes confer a suite of phenotypic traits that enhance the performance of host plants under stressful conditions while they obtain resources and suitable conditions for multiplication and dispersion (Malinowski and Belesky 2000; Clay and Schardl 2002; Cheplick and Faeth 2009). Host plants may benefit from fungal bioactive compounds which protect them from herbivores and, hence, increase their fitness (Clay 1988; Clay and Schardl 2002). Previous studies evaluating endophyte effects on both the hosts' ability to invade and to overcome the recipient communities' resistance to invasion, have focused on two widely distributed agronomic perennial grasses: *Schedonorus arundinaceus* (tall fescue; formerly *Festuca arundinacea*, *Lolium arundinaceum*; Catalán et al. 2007) and *Festuca pratensis* (meadow fescue; Rudgers et al. 2004; Saikkonen et al. 2013). Established plots of these grasses with high endophyte incidence have been more resistant to invasion (Clay and Holah 1999; Saikkonen et al. 2013) and at the same time, host plants have shown greater ability to invade experimentally assembled plant communities (Rudgers et al. 2005) suggesting that the endophyte may play an important role in community dynamics. However, the role of the endophyte in the initial stages of the invasion process and its impact on recipient communities under natural conditions has remained virtually unexplored.

Lolium multiflorum (Italian ryegrass) is a cool-season grass native to the European Mediterranean region and currently distributed worldwide [GBIF, http://www.gbif.org/species/4129708 (accessed February 2016)]. This species has an annual growth cycle in Mediterranean and cool-temperate humid climates. In temperate zones of South America, it was probably introduced accidentally as a weedy contaminant of wheat seed lots and subsequently disseminated with agriculture and cattle husbandry during the late seventeenth century (Parodi 1930). Current farming practices such as the use of *L. multiflorum* as annual winter forage likely boost its regional expansion throughout the Pampean region in Argentina (Rodríguez et al. 1998; Perelman et al. 2007; Tognetti et al. 2010). A survey carried out in the Flooding Pampa grasslands showed that naturalized populations of *L. multiflorum* have high (>90 %) incidence of endophyte *E. occultans* regardless of grazing history (Gundel et al. 2009b). Interestingly, *Epichloë* endophytes have not been documented in native grasses in this region (Iannone et al. 2011; 2012). Therefore, the European species *L. multiflorum* and *S. arundinaceus* are the only host grasses in the current flora that associate with foliar fungal endophytes (Gundel et al. 2009b; Iannone et al. 2011). While the *S. arundinaceus*-endophyte symbiosis with *E. coenophialum* (Leuchtmann et al. 2014) is highly toxic to livestock, the *L. multiflorum*-endophyte symbiosis with *E. occultans* seems to be harmless (De Battista 2005). Considering that *E. occultans* is an effective deterrent of insects and improves seedling recruitment and plant growth (Omacini et al. 2001; Uchitel et al. 2011; Gundel et al. 2012), this endophyte might also affect the invasion process of *L. multiflorum* in the Flooding Pampa grasslands.

In this paper, we investigate the effect of the fungal endophyte *E. occultans* on the invasion capacity of *L. multiflorum* in natural grasslands with different grazing histories in the Flooding Pampa (Argentina). In a field experiment, we simulated the arrival of *L. multiflorum* seeds with contrasting incidence of *E. occultans* to determine if the grass-endophyte symbiosis promotes the establishment of this annual grass and, if so, what changes of plant communities it would induce in communities shaped by their grazing history.

Materials and methods

Study system

The study was conducted in a Flooding Pampa grassland dominated by humid mesophytic meadows, exclusively used for extensive beef cattle breeding during the last century (Pila, Buenos Aires province, Argentina, 36°30′S, 58°30′W; Perelman et al. 2001). The average carrying capacity of that grassland ranges between 0.6 and 0.8 animal units ha^{-1} yr^{-1} (Vecchio et al. 2008; Cid et al. 2011). Mean temperatures range from 8.2 °C in July to 21.4 °C in January. Mean annual rainfall is approximately 900 mm, relatively uniformly distributed throughout the year but with small peaks in autumn and spring. Soils are Typic Natraquolls [i.e. acidic, non-saline A horizon and saline, highly alkaline Bn horizon (Berasategui and Barberis 1992)]. Cattle grazing shaped communities and determined differences in their soil properties (Table S1, Fig. S1) (see, Lavado and Taboada 1987; Taboada and Lavado 1993; Chaneton and Lavado 1996), the relative cover of floristic groups (Table S2 and Fig. S2–S4) and the phylogenetic patterns (Fig. S7–S8). Accordingly, previous studies in the same grassland have described changes in productivity and spatially distinct floristic gradients, involving the replacement of native perennial grasses by annual and perennial exotic species, caused by grazing (Sala et al. 1986; Chaneton et al. 1988; Oesterheld and Sala 1990; Jacobo et al. 2006; Semmartin et al. 2007). Indeed, removal of cattle by long term exclosure led to increased biomass production by native grasses, and decreased the cover and richness of exotics through gap-colonization dynamics in this grassland (Perelman et al. 2001; Chaneton et al. 2002; Longo et al. 2013).

We established a total of 19 experimental sites in early autumn of 2009 (mid-March). All sites were 2 m wide and 2 m long and were surrounded by a fence that protected them from grazing during the experiment. Sites were randomly distributed in a grassland with (n = 12) or without recent (n = 7) grazing history in a total area covering ~200 ha (Fig. 1). Sites without recent grazing history (without grazing, hereinafter) were placed inside large fenced exclosures of 1000 m^2 that had been installed five years before the experiment began (2004) to prevent livestock grazing (Longo et al. 2013). Distances between sites ranged from 160 to 1160 m in sites with grazing history, and from 80 to 260 m in sites without grazing history. Our design assumed that sites were independent from each other and, actually, a posteriori spatial error distribution supported site independence (Fig. S5–S6).

Plant material

Lolium multiflorum seed with high endophyte incidence (>90 %, determined by microscopic examination of 50 stained seeds) had been previously collected from a post-agricultural grassland without grazing (Inland Pampa, 35°55°S, 61°09°W. See, Gundel et al. 2009a; Omacini et al. 2009). Half of the seeds were treated with the fungicide Triadimenol (5 mg g^{-1} seed) to obtain seed with low endophyte incidence level. Fungicide-treated and un-treated seeds were propagated in 1 m^2 plots under common environmental conditions at the experimental facilities of University of Buenos Aires (34°35′S, 58°35′W). Plants were allowed to cross-pollinate freely, which contributed to homogenization of the plant genetic background. Seed produced by these plants was harvested in late spring (December 2008). Endophyte incidence level was 3 ± 3 % and 93 ± 25 % (mean ± standard error) in fungicide-treated and un-treated seed pools, respectively. Germination tests according to ISTA rules were carried out under laboratory conditions (15/25 °C, 8 h light, 5 days). Germination rates were >95 % and did not differ between seed pools with high or low endophyte incidence ($p = 0.178$).

Experimental design and measurements

The experiment started in April 2009, when seeds were added, and was terminated in February 2010 (Fig. 1). Before sowing, we characterized plant community composition and soil properties in both sites (Table S1) and mowed aboveground biomass near

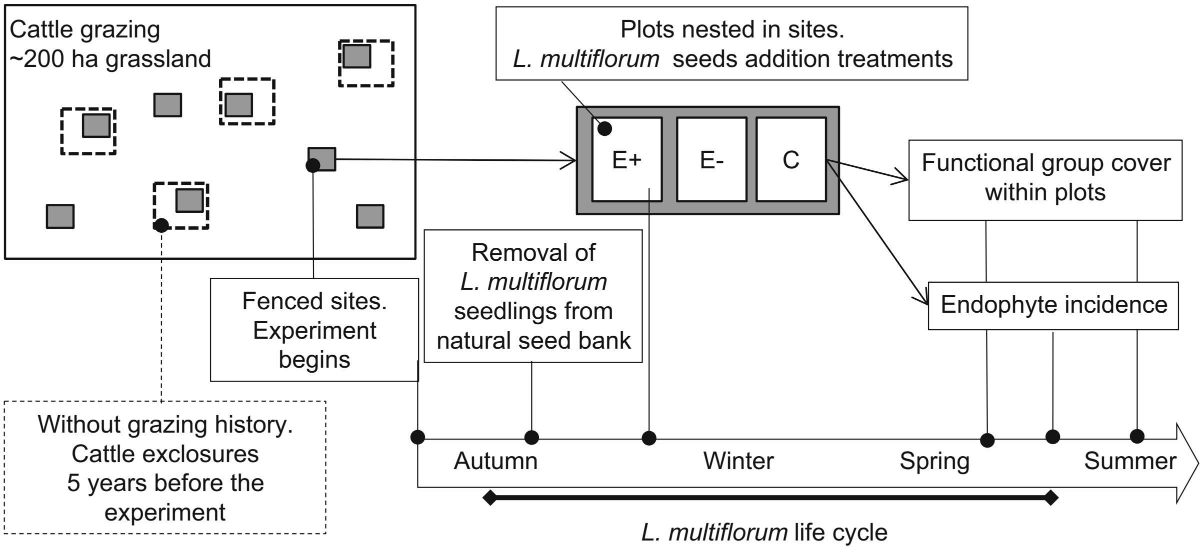

Fig. 1 Timeline showing the experimental setting in Pampean grassland. A total of 19 sites (2 m × 2 m) were fenced on sites with (n = 12; dominated by exotic species) or without (n = 7; dominated by native species) grazing history to protect them from cattle grazing at the beginning of the experiment (*dark grey rectangles*). Seven cattle-free exclosures of 1000 m^2 (termed 'sites without grazing history') were set up 5 years before the experiment began. Following fencing and mowing at near surface-level, *L. multiflorum* seedlings emerging from the soil seed bank were removed twice. Thereafter, seed addition treatments (E+, E− and control) were randomly assigned to one of three plots placed within each fenced site. During spring and summer, 7 and 10 months following seed addition, basal cover of functional groups (warm-season grasses, other than *L. multiflorum* cool-season grasses and forbs) was assessed in all plots. At these times reproductive tillers of *L. multiflorum* had started to flower and terminated their life cycle, respectively. The *bottom black line* shows the typical life cycle of *L. multiflorum* from autumn to spring. In late spring endophyte incidence was determined in seeds produced by *L. multiflorum* plants in each plot

ground level (mid-March). This disturbance was considered ecologically relevant, as it mimicked the effect of a strong overgrazing or low cutting height, factors that are known to facilitate invasion e.g. by promotion of germination and establishment of annuals (Rodríguez et al. 1998; Kulmatiski 2006). From end of March to mid-April 2009, *L. multiflorum* seedlings emerging from the soil seed bank were identified and carefully removed in order to reduce their natural cover. Then, within each site, we randomly placed three plots (0.6 × 0.9 m each), one each for sowing seed of *L. multiflorum* with high (93 %) or low (3 %) endophyte incidence, and one control. Sowings occurred on two occasions. At the end of April 2009, we added 3 g of seed per corresponding plot. Due to poor seedling emergence on both plots, we added another 5 g of seeds per corresponding plot in mid-June. These sowings corresponded to a total of approx. 13,000 viable seeds m^{-2}. Importantly, control plots (without seed addition) served to quantify resident *L. multiflorum* plants (that is *L. multiflorum* which germinated from the natural soil seed bank).

To evaluate the response of resident species to *L. multiflorum* seed addition, we measured basal aerial cover using line interception methods (Canfield 1941) during spring when *L. multiflorum* plants had started to flower (end-November, 2009) and during summer at the end of the growing cycle (end-February, 2010). Basal cover was used as the variable characterizing the performance of each species in the community. We avoided destructive measures like biomass removal, as we wanted to keep the system undisturbed following seed additions. Basal cover of each species was measured along permanent 60 cm-long transects placed in each plot. Basal cover records distinguished between three different functional groups: cool-season grasses other than *L. multiflorum*, warm-season grasses, and forbs. Values were expressed as relative cover to allow comparisons among plots. The incidence level of the endophyte in each plot was re-evaluated in seeds produced by established *L.*

multiflorum plants in late spring (mid-December, 2009). Thirty seeds were collected from each plot, stained and examined under the microscope.

Statistical analyses

We used generalized linear mixed-effects models which account for plots nested in sites and specified the binomial family (link-"logit") (glmer function in lme4, Bates et al. 2014; Bolker et al. 2009). Models for endophyte incidence level and for *L. multiflorum* relative cover included grazing history (sites with and without recent cattle), seed addition (C: control, E− and E+ plots) and the interaction between both factors as predictors. The relative cover of *L. multiflorum* in spring represented its performance and estimated its colonization ability and invasion success as a function of endophyte status. Models to analyse relative cover of cool-season grasses other than *L. multiflorum*, warm-season grasses, and forbs included grazing history, seed addition, *L. multiflorum* cover and their double interactions as predictors. Given that *L. multiflorum* was manipulated experimentally, its cover was not included in relative cover analyses of cool-season grasses. The relative cover of the floristic groups in spring and summer were analysed individually due to changes in floristic composition between seasons. As an alternative, we replaced seed addition by endophyte incidence level in *L. multiflorum* as model predictor. This produced equivalent results. We diagnosed multicollinearity by means of the variance inflation factor (VIF; vif.mer function in R) and excluded terms with VIF values above 10. The model dispersion parameter (phi) was calculated according to the overdisp_fun (Uesugi and Kessler 2016) in order to evaluate model adequacy. Residual distribution was plotted with the binnedplot function (arm; Gelman and Su 2015) which generates a binned residual plot. Spatial correlation was evaluated by plotting the residuals semi-variograms.

We reduced the model estimated by maximum likelihood (ML) by removing non-significant terms in a stepwise fashion (single term deletion strategy), and selected the optimal models following Zuur et al. (2009). When overdispersion was detected, standard errors were adjusted by the squared root of the overdispersion (Gelman and Hill 2007) and corresponding adjusted statistics and *p* values were calculated. When interaction were detected, multiple comparisons tests were performed using the glht function (multcomp package; Hothorn et al. 2008) and *p* values were adjusted by the Benjamini and Yekutieli (2001) method. For logistic regression optimal models, the odds ratio was used to interpret the actual effects of estimated parameters on relative cover (i.e.: the proportion) of floristic groups (Rabe-Hesketh and Skrondal 2012). For each treatment, the odds ratio was obtained as the exponential of the sum of the parameters involved. Then, we calculated the percent change in the proportion (relative cover) by subtracting 1 from the odds ratio and multiplying by 100. For each model, estimated parameters, parameters back-transformed to the corresponding percentage, the statistics and *p* values for model selection procedure as well as multiple comparisons performed are presented in Supplementary material tables S3 to S10. There, we also show the AIC and the AIC difference with respect to the null model.

Finally, we used the path analysis technique to evaluate direct and indirect effects of seed addition on floristic groups as mediated by *L. multiflorum* cover. Three path analyses were performed for sites without grazing history (see Results) and each included two models: a model of *L. multiflorum* cover combined with warm-season grasses in spring and summer, and a model of cool-season grasses in spring. We used a general approach to path analysis, based on multiple univariate models as proposed by Shipley (2009; see also Clough 2012; Shipley 2013), to allow the incorporation of generalized linear models and mixed models. The sem.fit function (piecewiseSEM; Lefcheck 2015) was used to specify and test for the adequacy of the models included in the path analysis. The models involved in each path analysis represented the chain of effects from seed addition to relative covers of floristic groups. Specifically, this displayed the seed addition effect (i.e. that is the realized invasion) along a gradient from 5 to 85 % of *L. multiflorum* cover in the recipient community. Such a range of cover of *L. multiflorum* is common in the studied grassland (Perelman et al. 2001; Semmartin et al. 2010). We used this gradient to address the effect of high and low endophyte incidence in *L. multiflorum* seed additions along potential natural scenarios. All analyses were performed with the R-cran environment; version 3.2.3 (R Development Core Team 2007) and RStudio; version 0.99.489 (RStudio Team 2015).

Results

The experimental manipulation was successful in creating plots that differed significantly in endophyte incidence of *L. multiflorum* plants ($p < 0.001$; Table S3). Incidence levels were: 92 ± 3 % in E+ (mean ± standard error), 49 ± 7 % in E− and 72 ± 9 % in Control plots. Thus, the endophyte effect was represented by about a 40 % difference between the incidence in E+ and E− plots.

Both E+ and E− seed addition increased the relative cover of *L. multiflorum* in comparison with the control (Fig. 2). Remarkably, addition of E+ seeds caused a much greater enhancement of *L. multiflorum* relative cover than that of E− seeds in sites without grazing history, but not in sites with grazing history, as was indicated by a significant grazing history x seed addition interaction (without grazing history x E+: $Z = 4.99, p < 0.001$). In sites without grazing history, the estimated odds ratio of the relative cover of *L. multiflorum* was 22 and 4 times greater in E+ and E− plots than in control plots, respectively. These differences were also evident in the relative cover of *L. multiflorum* that was more than double in E+ relative to E− plots (i.e.: 63 vs. 27 %, respectively) in these sites.

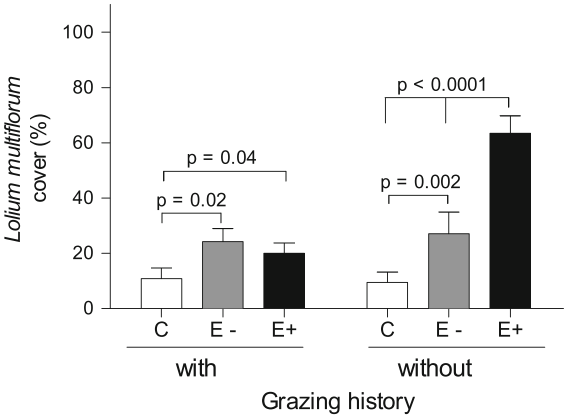

Fig. 2 *Lolium multiflorum* relative cover (%) in grasslands with and without grazing history and, subjected to three seed addition treatments: control plots without seed addition (C), plots with the addition of *L. multiflorum* seeds with low or high *E. occultans* incidence (E− and E+ plots, respectively). The resident community of grassland with grazing history was dominated by exotic species, whereas that without grazing history was dominated by native species. *Bars* show mean values (±standard error). The *p* values show significant differences of pairwise comparisons (Table S4)

The relative cover of warm-season grasses was independent of grazing history and seed addition (E+ and E−). However, this functional group was affected by the relative cover of *L. multiflorum* in both spring and summer. Thus, the model indicated that the cover of warm-season grasses decreased as the cover of *L. multiflorum* increased in both seasons (spring: $Z_{adj} = -3.70$, $p < 0.001$; summer; $Z_{adj} = -2.27$, $p = 0.023$; Fig. 3a, b). These effects were associated with a decrease of the cover of warm-season grasses of 1.9 and 1.6 % for each percent increase in the relative cover of *L. multiflorum* in spring and summer, respectively. Again, these effects were independent of grazing history and seed addition, and resulted from transformation of the odds ratio for warm-season grasses cover in spring (0.980) and summer (0.984), respectively.

Similar to the warm-season grasses, addition of E+ and E− seeds did not influence the relative cover of cool-season grasses (other than *L. multiflorum*) either in spring or summer. But, results were different in each season. The cover of cool-season grasses decreased as the cover of *L. multiflorum* increased in spring. This effect tended to be stronger in sites without grazing history than in sites with grazing history, as was indicated by a marginal interaction between grazing history and *L. multiflorum* cover ($Z_{adj} = -1.86$, $p = 0.062$; Fig. 3c). According to this interaction, the cover of cool-season grasses decreased by 0.66 % in sites with, and by 3.75 % in sites without grazing history for each percent increase in *L. multiflorum* cover. Again, these effects were independent of seed addition and resulted from the transformation of the odds ratio of cool-season grasses cover with (0.99) or without (0.96) grazing history, respectively. On the other hand, the cover of cool-season grasses (other than *L. multiflorum*) did not depend on grazing history, seed addition or the cover of *L. multiflorum* in summer (Fig. 3 d). Likewise, these predictor variables had not a significant effect on the cover of forbs in spring and summer (Fig. 3e, f).

For sites without grazing history, three path analyses—for cover of warm-season grasses in spring and in summer and cool-season grasses in spring—fit data adequately (warm-season grasses in spring: Fisher $C = 0$, $p = 1$; warm-season grasses in summer: Fisher $C = 0.02$, $p = 1$; cool-season grasses in spring: Fisher $C = 0.32$, $p = 1$). Overall, posterior model representation predicted that positive effects of

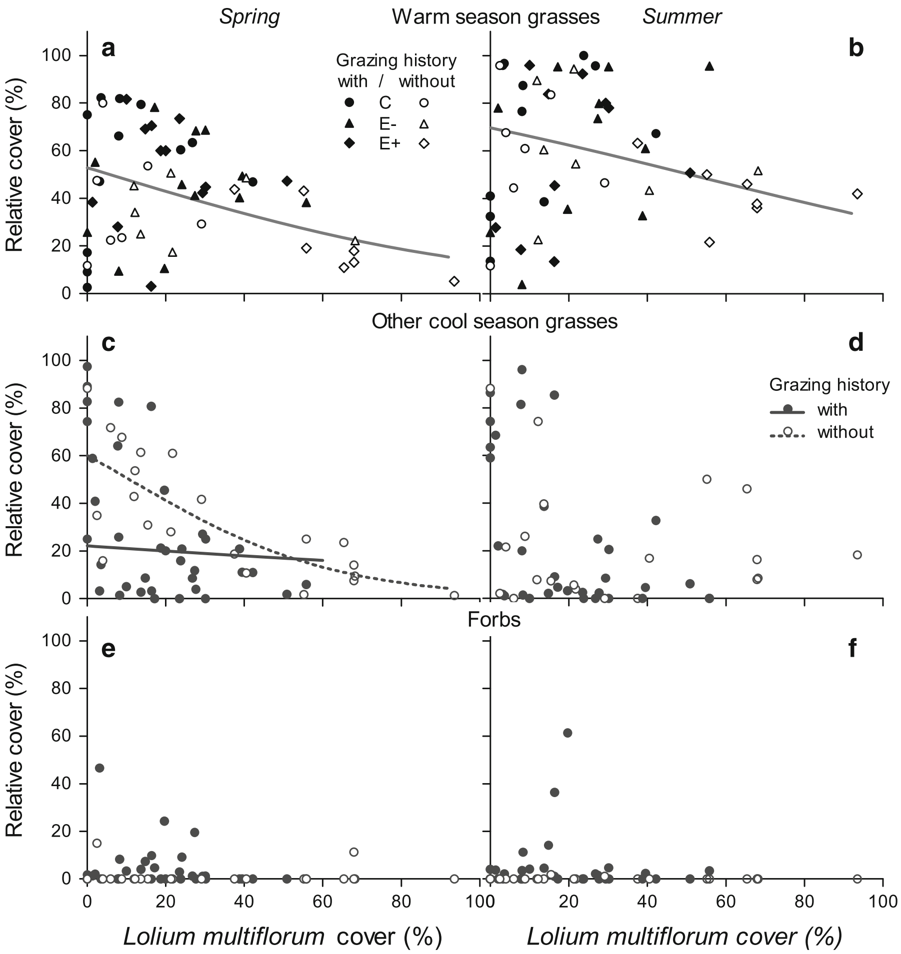

Fig. 3 Cover of warm-season grasses (**a**, **b**), cool-season grasses other than *L. multiflorum* (**c**, **d**) and forbs (**e**, **f**) in relation to the cover of *L. multiflorum* (all expressed as %). *Plots* show sites with (*black symbols*) and without (*white symbols*) grazing history (which were dominated by exotic and native species, respectively) in spring (*left*) and summer (*right*). Treatments are designated thus: control, *circles*; E−, *triangles* and E+, *diamonds*. *Lines* show the best model fit curve. In the case of the relative cover of warm-season grasses, cover of *L. multiflorum*: $Z_{adj} = -3.70$, $p < 0.001$ in spring (**a**) and, $Z_{adj} = -2.27$, $p = 0.023$ in summer (**b**). In the case of the relative cover of cool-season grasses other than *L. multiflorum*, grazing history × cover of *L. multiflorum*: $Z_{adj} = -1.86$, $p = 0.062$ in spring (**c**)

seed addition (for both E+ and E− treatments) on the final cover of *L. multiflorum* decreased as its cover in the recipient community increased until it reached a plateau (Fig. 4a). Specifically, the models predicted that the addition of E+ seeds had the greatest impact on the final cover of *L. multiflorum* when its initial cover in the recipient community was about 20 %. While, the addition of E− seeds had the greatest impact on the final cover of *L. multiflorum* when its initial cover in the recipient community was about 30 %. Moreover, the relative effect of the endophyte (E+/E− minus 1; having subtracted recipient community cover) on final cover of *L. multiflorum* was >2 for low initial cover of *L. multiflorum* in the recipient community. This meant that the endophyte effect on the cover of *L. multiflorum* was more important than the seed addition effect when the initial cover of *L. multiflorum* in the recipient community was low (<10 %). This relative endophyte effect was 1 when *L. multiflorum* cover was about 20 %, meaning that the effects of endophyte presence and seed addition on the final cover of *L. multiflorum* were similar. At 80 % initial cover of *L. multiflorum* in the recipient community, the relative endophyte effect was 0.3, indicating a greater importance of seed addition than endophyte presence on the cover of *L. multiflorum*. These relationships implied that the endophyte effect accounted for a 15 % reduction of the cover of warm-season grasses in both spring and summer, and for a 33 % reduction of the cover of cool-season grasses (other than *L. multiflorum*) in spring when the initial cover of *L. multiflorum* in the recipient community was 10 to 15 %. The size of the endophyte effect decreased asymptotically to almost zero when initial cover of *L. multiflorum* was very high (Fig. 4b, c). Note that similar analyses were not performed for sites

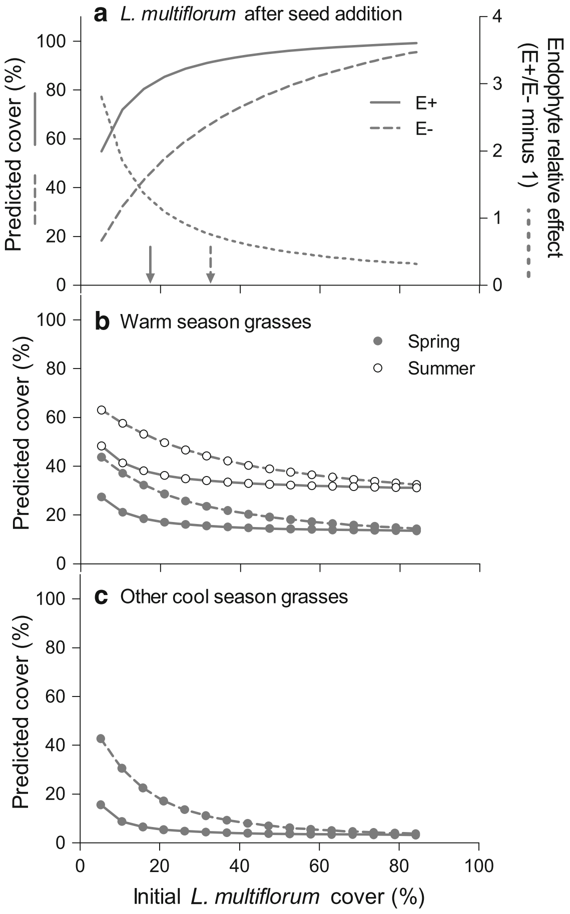

Fig. 4 Predicted effects of seed addition on the cover of *L. multiflorum* (**a**), warm-season grasses in spring and summer (**b**), and cool-season grasses in spring (**c**) along a gradient of initial cover of *L. multiflorum* in the recipient communities without grazing history. In panel (**a**), the *left axis* shows the cover of *L. multiflorum* predicted for E+ and E− seed additions (*full* and *dashed lines*, respectively); the *right axis* shows the endophyte relative effect (E+/E− minus 1). *Arrows* indicate the greatest impact estimated as its final minus its initial cover. The output of this model was used to simulate the consequent effect on the relative cover of cool- (other than *L. multiflorum*) and warm-season grasses (**b** and **c**). The central panel (**b**) shows the relative cover of warm-season grasses in spring and summer (*black* and *white* symbols, respectively). The lower panel (**c**) gives the relative cover of cool-season grasses (other than *L. multiflorum*). In all cases, the E+ and E− seed addition effects are indicated with *full and dashed lines*, respectively, and the area between both *lines* represents the endophyte effect

with grazing history, as there was no evidence of an endophyte effect on the cover of *L. multiflorum*. For the same reason, the analyses were not performed for cool-season grasses in summer or forbs.

Discussion

Our results show that the systemic fungal endophyte *E. occultans* increased the ability of an annual grass to become integrated into a community without recent grazing history and had effects particularly on warm- and cool-season grasses functional groups. The endophyte effect on *L. multiflorum* cover suggested an enhancement of host establishment and competitive ability; benefits that were evident where cover of other competitive exotic species was low (that is in sites without grazing history). In these sites, improved performance of *L. multiflorum* conferred by the endophyte fungus might be explained by a combination of multiple direct and indirect mechanisms such as deterrence of herbivores (e.g. insects) or seed predators or greater water use efficiency. In turn, *L. multiflorum* cover seemed to be the main control for reducing the cover of both warm- and cool-season grasses, a conclusion that was also supported by our global modelling. Importantly, we did not detect interactions between seed addition and *L. multiflorum* cover that would have suggested endophyte effects mediated by alternative mechanisms such as allelophathic effects.

E. occultans enhanced the invasion ability of *L. multiflorum* in grassland depending on community filters strength

Effects of fungal endophytes on invasion success and community structure have been tested in previous studies (e.g.: Rudgers et al. 2005, 2007; Saikkonen et al. 2013). However, its importance for annual grasses invasion with respect to recipient communities with potential contrasting resistance has rarely been considered. Our results emphasize the importance of the functional composition and diversity of the initial plant assemblage (Tilman 2001; Mitchell et al. 2006) in determining the effects of the endophytes on its host as well as on community structure. In this study, the endophytic fungus markedly enhanced the invasion ability of an annual grass in a community that was dominated by native grasses (in sites without recent grazing history). This result is in line with 'Darwin's naturalisation hypothesis' which predicts that exotic species more distantly related to native communities are more successful in early stage of species succession, as they share fewer natural enemies and compete less intensively for resources with the native species (Daehler 2001; Li et al. 2015). The mechanism may also be taken to suggest that the endophyte generates a sort of niche differentiation either by enabling a differential use of resources or a greater protection against natural enemies which would favours its hosts' invasion success, in line with the limiting similarity hypothesis (Abrams 1983). In addition, the clipping pre-treatment may have weakened the native species' competitive ability relative to the emerging *L. multiflorum* seedlings, thus providing beneficial conditions for the establishment of endophyte-enhanced *L. multiflorum* in the community without recent grazing history. On the contrary, communities in sites with grazing history were composed of highly competitive exotic species (which were phylogenetically more closely related with *L. multiflorum*, Fig. S7–S8). These species have replaced the native cool-season grasses, and may have reinforced the filter to invasion (Kulmatiski 2006) diluting (or erasing) the beneficial effects of the symbiosis.

In spite of the fact that our experiment did not test specific mechanisms, we speculate that less favourable environmental conditions (e.g. lower soil moisture and nutrient availability, see Fig. S1, Table S1) as well as a greater phylogenetic distance between *L. multiflorum* and the recipient community (Fig. S7–S8) may have permitted endophytic *L. multiflorum* plants to express competitive advantages in sites without grazing history. In these sites compared to sites with grazing history the endophyte might enable increased resource acquisition from the soil (e.g.: Ravel et al. 1997; Kane 2011; Vázquez-de-Aldana et al. 2013; García Parisi et al. 2014), protection against invertebrate herbivores, pathogens (Omacini et al. 2001, 2006; Sugawara et al. 2006; Ponce et al. 2009; Pérez et al. 2013) or granivores (Uchitel et al. 2011). Remarkably, protective endophyte effects at the seed and seedling stages (Omacini et al. 2009; Uchitel et al. 2011; Gundel et al. 2012) may be particularly important for grasses such as *L. multiflorum* which have no persistent soil seed bank and for which recruitment in newly invaded sites is seed-limited (Levine et al. 2004; Mitchell et al. 2006; Gundel et al. 2009b).

E. occultans shaped the recipient community structure mediated by *L. multiflorum* cover

Our results provide support for the idea that the endophyte effect shaped community structure and controlled filter strength of the community with effects that persisted even after the growth cycle of this annual grass. Certainly, it is necessary to evaluate if the lasting endophyte effect (i.e. the reduction in the cover of warm-season grasses during spring and summer) might facilitate the invasion of others species with longer-term consequences for community dynamics. For instance, Longo et al. (2013) observed that experimental removal of warm-season grasses dramatically increased exotic grass and forb biomass. In addition, empirical evidence from these grasslands showed a first transient peak of *L. multiflorum* after one year of grazing followed by a delayed increase in perennial rosette-like forbs only after five years of continuous grazing (Chaneton et al. 2002). Remarkably, our results suggest that the endophyte may be playing an important role on plant community structure which has not been considered before especially early after a disturbance. Besides, the production of endophytic *L. multiflorum* seeds and their arrival to the soil seed bank may increase during the following growing seasons. In particular, considering the low *L. multiflorum* seed dispersion (Williams and Bartholomew 2005), this mechanism may enhance the persistence of endophyte controls on the invaded plant community. In parallel, our global modelling suggested that initial *L. multiflorum* cover in the recipient community may have controlled the invasion of endophytic *L. multiflorum*. We are not aware of experiments that have addressed the effect of the invasion of this grass-endophyte symbiosis on recipient communities with a range of different initial *L. multiflorum* covers.

While the reduction in the cover of warm-season and cool-season grasses other than *L. multiflorum* was explained by the cover of *L. multiflorum*, we can not disregard simultaneous effects of alternative mechanisms. For example, *Trifolium pratense* growing next to endophytic *Festuca rubra* exhibited a greater reduction in plant growth (i.e. lesser shoot and root biomass production) than plants growing next to non-endophytic *F. rubra* (Vázquez-de-Aldana et al. 2012). That result was not explained by *F. rubra* biomass or phosphorus content of *T. pratense* suggesting that the effect may have resulted from the release into the soil of some chemical compound(s) with allelopathic effects. Studies with *S. arundinaceus* in symbiosis with *E. coenophialum* detected negative soil feedbacks on shrubs (Orr et al. 2005; Rudgers and Orr

2009) but not on forbs (Matthews et al. 2001). In the specific case of the *L. multiflorum–E. occultans* symbiosis, the endophyte was found to increase its hosts' nitrogen uptake (García Parisi et al. 2014) and shift the structure and function of the microbial community in the rhizosphere (Casas et al. 2011). This symbiosis also affected mycorrhizal colonization in non-host counterparts (Omacini et al. 2006) and the nodulation by nitrogen-fixing bacteria in legume species (García Parisi et al. 2014). Certainly, these mechanisms might also affect neighbouring species in the community.

Field conditions favoured high incidence levels of *E. occultans* in *L. multiflorum* population

Remarkably, the incidence of *E. occultans* in *L. multiflorum* seeds harvested from E− plots presented a strikingly higher incidence than that in the added seed pool (49 vs. 3 %, respectively). This result was independent of grazing history and highlights two main aspects. First, plots may have an important *L. multiflorum* soil seed bank (even though it is not considered persistent), replenished by large amounts of seed every growing season, from plants in situ and surroundings (seed dispersion). Despite our effort to remove seedlings of *L. multiflorum* just prior to the seed addition experiment, external seeds could have arrived in all three plots (control, E− and E+). However, this mechanism was probably weak as *L. multiflorum* seed production and dispersion normally occurs during December and January, several months before the experiment began. Second, the increase in *E. occultans* incidence in E− plots may have resulted from a superior competitive ability of the resident plants with high level of endophyte incidence relative to the added E− *L. multiflorum* seeds. Meanwhile, mechanisms such as competition, differential seed predation or herbivory by insects may also be involved. Indeed, this evidence is also consistent with the high incidence of this symbiosis in the control plot (72 %) and throughout the grasslands of the Pampa region (De Battista 2005; Gundel et al. 2009a) and other similar systems (Clay 1990; Clay et al. 2005). Final levels of endophyte incidence in E− plots suggest that this grass is under a strong selection for the symbiosis with *E. occultans* (Clay and Schardl 2002).

Summarizing, this work demonstrates that fungal endophytes can play an important role in assisting an annual host grass to invade natural temperate grasslands. Aerial endophytes in grasses which are vertically transmitted (i.e. seed-born) may present a critical advantage increasing the chances for this ruderal species to pass through the various ecological filters to invasion resistance (Uchitel et al. 2011). Ultimately, the reduction in the cover of other grasses could have consequences for community structure (Levine 2000). Understanding the impact of aerial fungal endophytes on communities assemblage and ecosystem processes over time will require explicit focus on their interactions with other trophic levels.

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References

Abrams P (1983) The theory of limiting similarity. Annu Rev Ecol Syst 14:359–376

Allen EB, Allen MF (1986) Water relations of xeric grasses in the field: interactions of mychorrhizas and competition. New Phytol 104:559–571

Allen MF, Richards JH, Busso CA (1989) Influence of clipping and water status on vesicular-arbuscular mycorrhizae of two semi-arid tussock grasses. Biol Fertil Soils 8:285–289

Bates D, Maechler M, Bolker B et al (2014) lme4: Linear mixed-effects models using Eigen and S4. R package version. 1.1-7

Benjamini Y, Yekutieli D (2001) The control of the false discovery rate in multiple testing under dependency. Ann Stat 29:1165–1188. doi:10.1214/aos/1013699998

Berasategui LA, Barberis LA (1992) Los suelos de las comunidades vegetales de la región Castelli-Pila, Depresión del Salado (Pcia. de Buenos Aires). Rev Fac Agron 3:13–25

Bolker BM, Brooks ME, Clark CJ et al (2009) Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol Evol 24:127–135. doi:10.1016/j.tree.2008.10.008

Bunn RA, Ramsey PW, Lekberg Y (2015) Do native and invasive plants differ in their interactions with, arbuscular mycorrhizal fungi? A meta-analysis. J Ecol. doi:10.1111/1365-2745.12456

Callaway RM, Thelen GC, Rodriguez A, Holben WE (2004) Soil biota and exotic plant invasion. Nature 427:731–733. doi:10.1038/nature02322

Callaway RM, Cipollini D, Barto K et al (2008) Novel weapons: invasive plant suppresses fungal mutualists in America but not in its native Europe. Ecology 89:1043–1055

Canfield RH (1941) Application of the line interception method in sampling range vegetation. J For 39:388–394

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

Catalán P, Torrecilla P, Stace CA (2007) A systematic approach to subtribe Loliinae (Poaceae: Pooideae) based on phylogenetic evidence. Aliso J Syst Evol Bot 23:380–405

Chaneton EJ, Lavado RS (1996) Soil nutrients and salinity after long-term grazing exclusion in a Flooding Pampa grassland. J Range Manag 49:182–187

Chaneton EJ, Facelli JM, Leon JC (1988) Floristic changes induced by flooding on grazed and ungrazed lowland grasslands in Argentina. J Range Manag 41:495–499

Chaneton E, Perelman S, Omacini M, León RC (2002) Grazing, environmental heterogeneity, and alien plant invasions in temperate pampa grasslands. Biol Invasions 4:7–24. doi:10.1023/A:1020536728448

Cheplick GP, Faeth SH (2009) Ecology and evolution of the grass-endophyte symbiosis. Oxford University ress, New York

Cid MS, Fernández Grecco RC, Oesterheld M et al (2011) Grass-fed beef production systems of Argentina's flooding pampas: understanding ecosystem heterogeneity to improve livestock production. Outlook Agric 40:181–189. doi:10.5367/oa.2011.0040

Clay K (1988) Fungal endophytes of grasses: a defensive mutualism between plants and fungi. Ecology 69:10–16. doi:10.2307/1943155

Clay K (1990) Fungal endophytes of grasses. Annu Rev Ecol Syst 21:275–297. doi:10.1016/j.pbi.2012.03.007

Clay K, Holah J (1999) Fungal endophyte symbiosis and plant diversity in successional fields. Science (80-) 285:1742–1744

Clay K, Schardl CL (2002) Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. Am Nat 160:99–127

Clay K, Holah J, Rudgers JA (2005) Herbivores cause a rapid increase in hereditary symbiosis and alter plant community composition. PNAS 102:12465–12470. doi:10.1073/pnas.0503059102

Clough Y (2012) A generalized approach to modeling and estimating indirect effects in ecology. Ecology 93:1809–1815. doi:10.1890/11-1899.1

Daehler CC (2001) Darwin's naturalization hypothesis revisited. Am Nat 158:324–330. doi:10.1086/321316

De Battista J (2005) Neotyphodium research and aplication In South America. In: Roberts CA, West CP, Spiers DE (eds) Neotyphodium in cool-season grasses, 1st edn. Blackwell Publishing Ltd, Oxford, pp 65–71

García Parisi PA, Lattanzi FA, Grimoldi AA, Omacini M (2014) Multi-symbiotic systems: functional implications of the coexistence of grass-endophyte and legume-rhizobia symbioses. Oikos 124:553–560

Gelman A, Hill J (2007) Data analysis using regression and multilevel/hierarchical models. Cambridge University Press, Cambridge

Gelman A, Su YS (2015) arm: data analysis using regression and multilevel/hierarchical models. R package version 1.8-6. https://CRAN.R-project.org/package=arm

Gundel PE, Garibaldi LA, Tognetti PM et al (2009a) 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, Martínez-Ghersa MA, Garibaldi LA, Ghersa CM (2009b) Viability of Neotyphodium endophytic fungus and endophyte-infected and noninfected *Lolium multiflorum* seeds. Botany 87:88–96. doi:10.1139/B08-119

Gundel PE, Martínez-Ghersa MA, Omacini M et al (2012) Mutualism effectiveness and vertical transmission of symbiotic fungal endophytes in response to host genetic background. Evol Appl 5:838–849. doi:10.1111/j.1752-4571.2012.00261.x

Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. Biom J 50:346–363

Iannone L, White J Jr, Giussani L et al (2011) Diversity and distribution of *Neotyphodium*-infected grasses in Argentina. Mycol Prog 10:9–19. doi:10.1007/s11557-010-0669-2

Iannone LJ, Novas V, Young CA et al (2012) Endophytes of native grasses from South America: biodiversity and ecology. Fungal Ecol 5:357–363. doi:10.1016/j.funeco.2011.05.007

Jacobo EJ, Rodríguez A, Bartoloni N, Deregibus VA (2006) Rotational grazing effects on rangeland vegetation at a farm scale. Rangel Ecol Manag 59:249–257

Kane KH (2011) Effects of endophyte infection on drought stress tolerance of Lolium perenne accessions from the Mediterranean region. Environ Exp Bot 71:337–344. doi:10.1016/j.envexpbot.2011.01.002

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

Kulmatiski A (2006) Exotic plants establish persistent communities. Plant Ecol 187:261–275. doi:10.1007/s11258-006-9140-5

Lavado RS, Taboada MA (1987) Soil salinization as an effect of grazing in a native grassland soil in the Flooding Pampa of Argentina. Soil Use Manag 3:143–148. doi:10.1111/j.1475-2743.1987.tb00724.x

Lefcheck JS (2015) piecewiseSEM: piecewise structural equation modeling in R for ecology, evolution, and systematics. Methods Ecol Evol. doi:10.1111/2041-210X.12512

Leuchtmann A, Bacon CW, Schardl CL et al (2014) Nomenclatural realignment of *Neotyphodium* species with genus *Epichloë*. Mycologia 106:202–215. doi:10.3852/13-251

Levine JM (2000) Species diversity and biological invasions: relating local process to community pattern. Science 288:852–854

Levine JM, Adler PB, Yelenik SG (2004) A meta-analysis of biotic resistance to exotic plant invasions. Ecol Lett 7:975–989. doi:10.1111/j.1461-0248.2004.00657.x

Li SP, Cadotte MW, Meiners SJ, Hua ZS, Shu HY, Li JT, Shu WS (2015) The effects of phylogenetic relatedness on invasion success and impact: deconstructing Darwin's naturalisation conundrum. Ecol Lett 18:1285–1292. doi:10.1111/ele.12522

Longo G, Seidler TG, Garibaldi LA et al (2013) Functional group dominance and identity effects influence the magnitude of grassland invasion. J Ecol 101:1114–1123. doi:10.1111/1365-2745.12128

Malinowski DP, Belesky DP (2000) Adaptations of endophyte-infected cool-season grasses to environmental stresses: mechanisms of drought and mineral stress tolerance. Crop Sci 40:923–940

Marler MJ, Zabinski CA, Callaway RM (1999) Mycorrhizae indirectly enhance competitive effects of an invasive forb on a native bunchgrass. Ecology 80:1180–1186. doi:10.1890/0012-9658(1999)080[1180:MIECEO]2.0.CO;2

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

Mitchell CE, Agrawal AA, Bever JD et al (2006) Biotic interactions and plant invasions. Ecol Lett 9:726–740. doi:10.1111/j.1461-0248.2006.00908.x

Mooney HA, Hobbs RJ (2000) Global change and invasive species: where do we go from here. In: Hobbs RJ (ed) Invasive species in a changing world. Island Press, Washington, DC, pp 425–434

Oesterheld M, Sala OE (1990) Effects of grazing on seedling establishment: the role of seed and safe-site availability. J Veg Sci 1:353–358. doi:10.2307/3235711

Omacini M, Chaneton EJ, Ghersa CM, Müller CB (2001) Symbiotic fungal endophytes control insect host-parasite interaction webs. Nature 409:78–81. doi:10.1038/35051070

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, Chaneton EJ, Bush L, Ghersa CM (2009) A fungal endosymbiont affects host plant recruitment through seed- and litter-mediated mechanisms. Funct Ecol 23:1148–1156. doi:10.1111/j.1365-2435.2009.01582.x

Orr SP, Rudgers JA, Clay K (2005) Invasive plants can inhibit native tree seedlings: testing potential allelopathic mechanisms. Plant Ecol 181:153–165. doi:10.1007/s11258-005-5698-6

Parodi LR (1930) Ensayo fitosociológico sobre el partido de Pergamino. Rev Fac Agron Vet B Aires 7:65–271

Perelman SB, Leon RJC, Oesterheld M (2001) Cross-scale vegetation patterns of Flooding Pampa grasslands. J Ecol 89:562–577

Perelman SB, Chaneton EJ, Batista WB et al (2007) Habitat stress, species pool size and biotic resistance influence exotic plant richness in the Flooding Pampa grasslands. J Ecol 95:662–673. doi:10.1111/j.1365-2745.2007.01255.x

Pérez LI, Gundel PE, Ghersa CM, Omacini M (2013) Family issues: fungal endophyte protects host grass from the closely related pathogen Claviceps purpurea. Fungal Ecol 6:379–386. doi:10.1016/j.funeco.2013.06.006

Ponce MA, 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

Pringle A, Bever JD, Gardes M et al (2009) Mycorrhizal symbioses and plant invasions. Annu Rev Ecol Evol Syst 40:699–715. doi:10.1146/annurev.ecolsys.39.110707.173454

R Development Core Team (2007) R: a language and environment for statistical computing. R Development Core Team, Vienna

Rabe-Hesketh S, Skrondal A (2012) Dichotomous or binary responses. In: Multilevel and longitudinal modeling using stata, vol II, 3rd edn. Stata Press, pp 501–574

Ravel C, Courty C, Coudret A, Charmet G (1997) Beneficial effects of Neotyphodium lolii on the growth and the water status in perennial ryegrass cultivated under nitrogen deficiency or drought stress. Agronomie 17:173–181

Richardson DM, Allsopp N, D'Antonio CM et al (2000) Plant invasions-the role of mutualisms. Biol Rev Camb Philos Soc 75:65–93

Rodríguez A, Jacobo E, Deregibus VA (1998) Germination behaviour of Italian ryegrass in flooding pampa rangelands. Seed Sci Res 8:521–528

RStudio Team (2015) RStudio: integrated development for R. RStudio, Inc., Boston, MA. http://www.rstudio.com/

Rudgers JA, Clay K (2007) Endophyte symbiosis with tall fescue: how strong are the impacts on communities and ecosystems? Fungal Biol Rev 21:107–124. doi:10.1016/j.fbr.2007.05.002

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

Rudgers JA, Mattingly WB, Koslow JM (2005) Mutualistic fungus promotes plant invasion into diverse communities. Oecologia 144:463–471. doi:10.1007/s00442-005-0039-y

Rudgers JA, Holah J, Orr SP, Clay K (2007) Forest succession suppressed by an introduced plant–fungal symbiosis. Ecology 88:18–25

Rudgers JA, Fischer S, Clay K (2010) Managing plant symbiosis: fungal endophyte genotype alters plant community composition. J Appl Ecol 47:468–477. doi:10.1111/j.1365-2664.2010.01788.x

Saikkonen K, Ruokolainen K, Huitu O et al (2013) Fungal endophytes help prevent weed invasions. Agric Ecosyst Environ 165:1–5

Sala OE, Oesterheld M, León RJC, Soriano A (1986) Grazing effects upon plant community structure in subhumid grasslands of Argentina. Vegetatio 67:27–32. doi:10.1007/BF00040315

Schardl CL, Leuchtmann A, Spiering MJ (2004) Symbioses of grasses with seedborne fungal endophytes. Annu Rev Plant Biol 55:315–340. doi:10.1146/annurev.arplant.55.031903.141735

Semmartin M, Oyarzabal M, Loreti J, Oesterheld M (2007) Controls of primary productivity and nutrient cycling in a temperate grassland with year-round production. Austral Ecol 32:416–428. doi:10.1111/j.1442-9993.2007.01706.x

Semmartin M, Bella C, Salamone I et al (2010) Grazing-induced changes in plant species composition affect plant and soil properties of grassland mesocosms. Plant Soil 328:471–481. doi:10.1007/s11104-009-0126-7

Shipley B (2009) Confirmatory path analysis in a generalized multilevel context. Ecology 90:363–368. doi:10.1890/08-1034.1

Shipley B (2013) The AIC model selection method applied to path analytic models compared using a d-separation test. Ecology 94:560–564. doi:10.1890/12-0976.1

Suding KN, Harpole WS, Fukami T et al (2013) Consequences of plant—soil feedbacks in invasion. J Ecol 101:298–308. doi:10.1111/1365-2745.12057

Sugawara K, Inoue T, Yamashita M, Ohkubo H (2006) Distribution of the endophytic fungus, *Neotyphodium occultans* in naturalized Italian ryegrass in western Japan and its production of bioactive alkaloids known to repel insect pests. Grassl Sci 52:147–154. doi:10.1111/j.1744-697X.2006.00060.x

Taboada MA, Lavado RS (1993) Influence of cattle trampling on soil porosity under alternate dry and ponded conditions. Soil Use Manag 9:139–143. doi:10.1111/j.1475-2743.1993.tb00943.x

Tilman D (2001) Functional diversity. Encycl Biodivers 3:109–120

Tognetti PM, Chaneton EJ, Omacini M et al (2010) Exotic vs. native plant dominance over 20 years of old-field succession on set-aside farmland in Argentina. Biol Conserv 143:2494–2503. doi:10.1016/j.biocon.2010.06.016

Uchitel A, Omacini M, Chaneton E (2011) Inherited fungal symbionts enhance establishment of an invasive annual grass across successional habitats. Oecologia 165:465–475. doi:10.1007/s00442-010-1740-z

Uesugi A, Kessler A (2016) Herbivore release drives parallel patterns of evolutionary divergence in invasive plant phenotypes. J Ecol. doi:10.1111/1365-2745.12542

van der Heijden MGA, Boller T, Wiemken A, Sanders IR (1998) Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. Ecology 79:2082–2091. doi:10.1890/0012-9658(1998)079[2082:DAMFSA]2.0.CO;2

Vázquez-de-Aldana BR, Zabalgogeazcoa I, García-Ciudad A, García-Criado B (2012) An *Epichloë* endophyte affects the competitive ability of Festuca rubra against other grassland species. Plant Soil 362:201–213. doi:10.1007/s11104-012-1283-7

Vázquez-de-Aldana BR, García-Ciudad A, García-Criado B et al (2013) Fungal endophyte (*Epichloë festucae*) alters the nutrient content of *Festuca rubra* regardless of water availability. PLoS ONE 8:1–14. doi:10.1371/journal.pone.0084539

Vecchio MC, Golluscio RA, Cordero MI (2008) Cálculo de la receptividad ganadera a escala de potrero en pastizales de la Pampa Deprimida. Ecol Austral 18:213–222

Vellinga EC, Wolfe BE, Pringle A (2009) Global patterns of ectomycorrhizal introductions. New Phytol 181:960–973. doi:10.1038/ng.473

Williams R, Bartholomew P (2005) Factors affecting Italian ryegrass (*Lolium multiflorum* L.) seed distribution. In: Murphy JJ (ed) Utilization of grazed grass in temperate animal systems. Wageningen Academic Publishers, Wageningen, p 248

Zuur AF, Ieno EN, Walker NJ et al (2009) Mixed effects models and extensions in ecology with R. Springer, Berlin