Plant Ecol (2014) 215:1513–1525
DOI 10.1007/s11258-014-0410-3

Mycorrhizal fungi affect plant growth: experimental evidence comparing native and invasive hosts in the context of forest fragmentation

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Received: 18 March 2014 / Accepted: 3 September 2014 / Published online: 10 September 2014
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Abstract Forest fragmentation and biological invasions modify plant–mycorrhizal fungal interactions, but how these variations affect native and invasive plant vegetative and reproductive growth in a fragmented forest remain unknown. To test the effects of soil fungi from different forest fragment sizes on native and invasive plants, we conducted a greenhouse factorial experiment combining soil source (i.e., small and large forest fragments) and fungicide application (with and without fungicide) on two ruderal congeneric *Euphorbia* (*E. acerensis* and *E. dentata*, native and invasive, respectively). Soil fungi from small forest fragments promoted lower rates of mycorrhizal colonization than soil from large forest fragments in both plant species. In general, the source of soil fungi had no effect on vegetative and reproductive growth of both plant species. Fungicide application positively affected plant height and dry mass of the native host, while the fungicide application negatively affected height and neutrally affected growth of the invasive plant species. Reproductive traits were in general positively affected by fungicide application, although in some cases, they were dependent on soil source. Forest fragmentation might promote changes in soil conditions that negatively affect mycorrhizal colonization at levels without functional consequences for plant growth. However, landscape modifications that contribute to a more severe reduction in Arbuscular Mycorrhizal (AM) fungal colonization might certainly have important consequences on native and invasive plant growth.

Keywords Root symbionts · Ruderal plants · Reproductive traits · Greenhouse · Fungicide

Introduction

Arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSE) are the most widespread root symbionts. Several studies have shown that through their effects on plant nutrition, these fungal root symbionts may affect plant growth and reproduction (Treseder 2004; Mandyam and Jumpponen 2005; Smith and Read 2008; Newsham 2011). Nevertheless, the DSE functions on plant hosts are still being unraveled (Mandyam and Jumpponen 2014). Mycorrhizal fungi might differentially enhance nectar,

Communicated by G. Kernaghan.

Electronic supplementary material The online version of this article (doi:10.1007/s11258-014-0410-3) contains supplementary material, which is available to authorized users.

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pollen, flower, fruit, and seed production (Koide 2010; Varga 2010 and references therein) depending on the plant host identity (Gange and Smith 2005). In addition, it is well known that different combinations of AMF species promote different levels of mycorrhizal colonization (Jansa et al. 2008) and also affect the outcome of the plant–fungal interaction (van der Heijden et al. 1998; Lekberg and Koide 2005; Lehmann et al. 2012). Most of the studies evaluating plant reproduction use individual AMF cultures. However, it has been shown in a recent metanalysis that the effects of natural soil communities or mixed AMF cultures increase the plant performance more than individual cultures (Hoeksema et al. 2010).

The knowledge regarding how variation in soil fungal composition affects the performance of plant traits is of primary interest to understand plant population dynamics and community structure (Moora et al. 2004; Cahill et al. 2008). However, few studies have attempted to link soil fungi, plant growth, and reproduction in fragmented forests (Grilli et al. 2013). The process of forest fragmentation is an important driver of population dynamics and community structure (Didham et al. 2012). Many plants and animals are known to be affected by changes in the landscapes due to human activities. Continuous forest might moderate the impact of environmental conditions on plant populations (Laurance 2002). In contrast, it has been suggested that smaller forest fragments might be affecting plant growth rates due to the presence of warmer and drier air and soil and/or higher branch and treefalls that directly impact on forbs growth (Gagnon et al. 2011). In addition, evidence exists that smaller fragments might show increased wind turbulence, light availability on the forest floor, and reduced relative humidity (Chen et al. 1995, Laurance and Curran 2008). Despite this, little is known about what happens to belowground organisms (Mangan et al. 2004; Ewers and Didham 2006; Collinge 2009 and references therein).

It has been recently shown that AMF spore communities (spore diversity and total abundance) and root colonization in the rhizosphere of two congeneric annual forbs were negatively related to forest fragment size along a Chaquean forest fragmentation gradient in Central Argentina (Grilli et al. 2012). In addition, it has been suggested that soil biota might negatively affect growth of ruderal seedlings in forest fragments (Pizano et al. 2014). Thus, we expected that changes in the community composition of these widespread fungal root symbionts associated with the forest fragmentation process might have an impact on plant growth and reproduction in these plants.

AMF might benefit their hosts by providing access to soil nutrients among other profits (Smith and Read 2008). However, there is increasing evidence that several plants can also be negatively affected by AMF, notably plants of early successional stages with short life-cycles (Kardol e al. 2006; Rinaudo et al. 2010; Veiga et al. 2011; Urcelay et al. 2011). We have found that AMF root colonization was negatively correlated with plant growth in a native and an exotic ruderal *Euphorbia* in the field along a forest fragmentation gradient (Grilli et al. 2013). However, whether this relationship has functional consequences or not remains unknown. Moreover, it is possible that AMF–plant interaction outcomes differ between plant species and this could be particularly relevant if these fungi negatively affect native but not invasive hosts. In this way, soil fungal communities might be indirectly promoting invasive plant establishment and expansion as observed elsewhere (Philip et al. 2001; Callaway et al. 2001; Pringle et al. 2009; Inderjit and van der Putten 2010). This issue could be assessed by examining their effects on characteristics that are associated with the success of invasive plants such as vegetative (e.g., growth and specific leaf area) and reproductive (e.g., flower, seed, and pollen production) traits (Lake and Leishman 2004; Lloret et al. 2005).

According to the theoretical framework and field evidence, we expected that (a) the variation in soil fungal composition among fragment sizes would have consequences on plant growth and reproduction, (b) the negative correlation between mycorrhizal colonization and plant growth corresponds to negative effects of these fungi on plants, and (c) those negative effects on plant growth would be more marked in the native than in the invasive congeneric ruderal.

To test these predictions, we experimentally addressed the effects of soil fungi from large versus small forest fragments on vegetative and reproductive traits of a native and an invasive ruderal *Euphorbia* in the greenhouse.

Materials and methods

The plant species

Euphorbia acerensis Boiss. is a native forb to South America, and *Euphorbia dentata* Michx. is an exotic species to North and Central America. These are annual ruderal forbs occurring in Chaco forest fragments. Both plant species reach 45–50 cm tall and present similar reproductive biology. These annual herbs have a terminal or axillary cluster of flowers, which is called a 'cyathium' (inflorescence), with several cyathia densely clustered into a cyme with a cup-shaped involucre that contains one female flower surrounded by many male flowers (Subils 1977).

Study site and experimental design

The study was conducted in summer from January to March of 2010 (70 days) under greenhouse conditions at the Instituto Multidisciplinario de Biología Vegetal in Córdoba, Argentina. The greenhouse conditions were 25 °C, and controlled photoperiod of 17 h of light (summer light), and the plants were watered twice daily with tap water. Soil with mycorrhizal communities was collected in Chaco forest fragments of different sizes, from three small (<1 Ha) and three large forest fragments (>18 Ha) that were considered as replicates in the greenhouse experiment. The forest fragments are in the Río Ceballos locality of Córdoba Province at Central Argentina, and lie approximately between 31°11′19″S; 64°16′02″W; and 31°13′05″S; and 64°15′55″W (Fig. 1). The vegetation of the semiarid shrub-forest in fragments is characterized by *Acacia* spp., *Aspidosperma quebracho-blanco* Schltdl., *Zanthoxylum coco* Engl., *Prosopis* spp., Celtis ehrenbergiana Liebm., native and exotic herbs, and grasses, vines, and epiphytic plants. These forest fragments, which are rocky outcrops, are immersed in an agricultural matrix and remain in the landscape due to their unsuitability for agriculture. Forest fragments present an average isolation age of approximately 70 years. Because vegetative barriers are used to avoid fungicide drift in experimental studies (Perryman et al. 2009), soil was collected in the core of forest fragments. Moreover, the terrestrial application of fungicide on crops for control of pathogenic fungi used in this landscape allows minimal fungicide drift. Further description of the forest fragments is in Grilli et al. (2012).

Greenhouse experiment

Soils and seeds were collected randomly within forest fragments. Seeds from the six forest fragments of each category (i.e., small and large) were pooled to avoid any bias from plant population source. Seeds were germinated in autoclaved soil in a chamber under

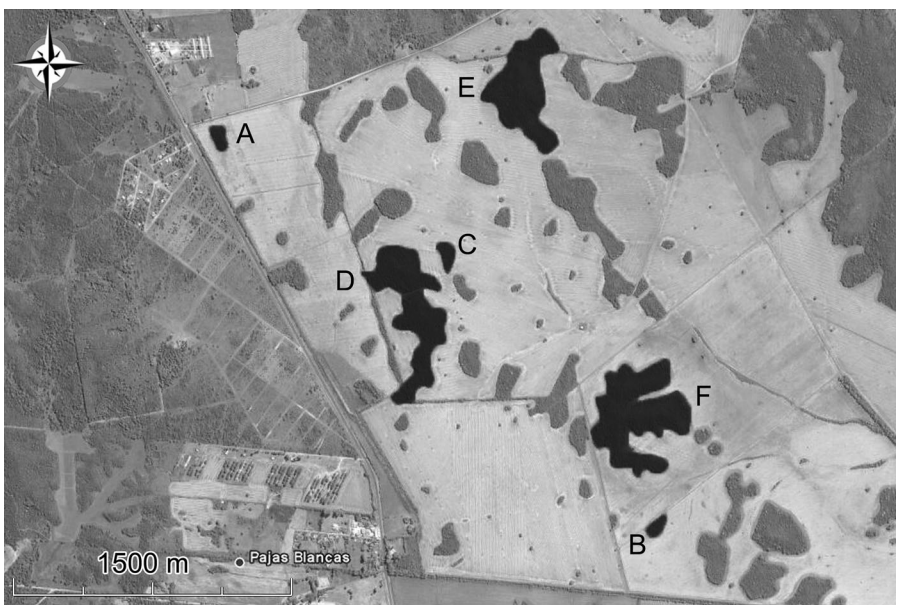

Fig. 1 Chaco forest fragments in an agricultural landscape in Central Argentina. Forest fragments used to collect soil for the greenhouse experiment are in *black*. Small (*A*, *B*, *C*) and large (*D*, *E*, *F*) forest fragments ($n = 6$)

controlled conditions (humidity and temperature). Two weeks post germination, seedlings of both species were transplanted in pots (800 g of mixture soil and autoclaved sand 1:3) with soil belonging to each of the six forest fragments (i.e., 10 seedling with and 10 without fungicide for each species and for each forest fragment). Mycorrhizal fungal colonization was reduced using a commercial fungicide (50 mg of benomyl in 100 ml of tap water for 1 kg of soil) with benomyl (Benlate); tap water was added in control pots to ensure that pots receive the same amount of liquid (Koorem et al. 2012). Fungicide was used to experimentally control the effects of the mycorrhizal and DSE fungi on plant traits. Fungicide suppresses different types of rhizospheric fungi (i.e., pathogens, DSE, and AMF). However, in non-fungicide treatment (i.e., control), we found no signs of pathogenic fungi in roots and negligible values of DSE colonization but similar levels of AMF colonization to those described in the field reported in Grilli et al. (2012) (see results). Therefore, we attribute the main effects of fungicide to absence of AMF as it is frequently found in the literature for this kind of study (e.g., Smith et al. 2000; Hartnett & Wilson 2002; Cahill et al. 2008; Gross et al. 2010; McCain et al. 2011; Becklin et al. 2011; Deguchi et al. 2012). Fungicide was applied at the beginning of the experiment and every 2 weeks until the end of the experiment (Gross et al. 2010). Thus, the full factorial design used was 10 plants × 2 species (native, invasive) × 2 fungicide treatment (control, fungicide) × 6 forest fragment sizes (3 large and 3 small). The 240 plants used in the experiment were followed during their growth until fruit production. Greenhouse irrigation and temperature conditions were fully controlled. Plants within the greenhouse were moved periodically to avoid spatial differences. Plants were harvested after 70 days when they had completed fruit ripening. Plants were removed from pots, and roots were carefully washed to avoid any accidental damage.

Plant vegetative and reproductive traits were assessed after collecting plants in the greenhouse. Plants were cut on the upper part of the radical system and divided into two parts (i.e., root and shoot). Then, plant height was recorded in the laboratory ($n = 10$ plants per fragment) using a meter from shoot's base to the apical meristem. Shoot and roots were dried at 60 °C during 3 days and weighed to obtain the variable root and shoot dry mass and root:shoot ratio. Specific leaf area (SLA) was calculated using four leaves per plant ($n = 960$) according to Cornelissen et al. (2003) and was expressed as $mm^2\ mg^{-1}$. Then, leaves were dried, weighed, and added to obtain the final quantity of the variable "shoot dry mass." One male flower per inflorescence per plant was collected from four plants of both species at each forest fragment when first inflorescences appeared and were preserved in 70 % ethanol. The number of pollen grains per flower was counted using a microscope (Nikon optical, Model E200), 200× magnification. Pollen size ($n = 4$ per plant) was measured with a metric ocular at 1,000× magnification. Inflorescence and fruit production were recorded at the lab immediately after plant collection at the end of the experiment, counting all developed infructecences (all the infructecences present in a plant) and fruits per plant ($n = 10$ plants per forest fragment).

Mycorrhizal colonization in plant roots

Mycorrhizal colonization was assessed in the 240 plant roots, staining all active roots with a diameter of less than 2 mm. All dead and damaged roots were discarded. Potassium hydroxide (KOH) was used to clear roots for a period of approximately 22–24 h. Then, the roots were washed with water and acidified in 10 % hydrochloric acid (HCL) for 30 min, to be dyed with 0.05 % aniline blue for 24–36 h (Grace and Stribley 1991; Brundrett et al. 1996). The roots were mounted on semi-permanent slides in polyvinyl-lactic acid-glycerol; we prepared one slide per individual due to the reduced rhizospheric system of most adult plants and, consequently, the low availability of active roots. We measured a minimum of 25 cm of roots per individual. AMF and DSE colonization rates were determined following the magnified intersection method of McGonigle et al. (1990) using a compound microscope (Nikon optical, Model E200), 200× magnification. One hundred intersections per sample were counted to assess wellstained irregular hyphae, vesicles, arbuscules, and regular brown septate hyphae. The quantity of intersections with mycorrhizal fungal structures was used to calculate root colonization percentages of total mycorrhizal, vesicles, arbuscules, and DSE.

Soil nutrient analysis

Soil samples were collected and stored at 4 °C to assess nutrient availability. Three samples per each of the six forest fragments were sent to the Edaphologic Laboratory of the Faculty of Agronomic Sciences (Universidad Nacional de Córdoba) for soil nutrient analyses. Each sample of 10 g of dry soil was shaken in 50 ml of 1.0 M NaHCO3 for 30 min and was then filtered through Whatman # 42 filter paper. Phosphorus was determined colorimetrically using the ascorbic acid method, Bray and Kurtz n° 1 (Kuo 1996). The Kjeldahl method was used for the calculation of total nitrogen (Bremner 1996). Nitrate and ammonia were estimated using direct potentiometry (ORION Ionalizer 901; Mulvaney 1996).

Statistical analysis

Generalized linear mixed effect models (GLMMs) in R v.2.13.2 (R Development Core Team 2010) were used to analyze soil nutrients and mycorrhizal fungi effects on plant growth and reproduction using the function glmer or lmer (lme4 package, Bates and Sakar 2007). Fungal variables measured were "total mycorrhizal colonization (%)," "DSE colonization (%)," and "arbuscular colonization (%)." Vegetative and reproductive variables measured were "Plant height (cm)," "Plant dry mass (g)," "inflorescence number," "Fruit number," "Pollen size," and "Pollen grains per flower." Soil nutrient variables measured were "nitrate (ppm)," "ammonia (ppm)," "phosphorus (ppm)," "total nitrogen (%)," and "organic carbon (%)." The residuals of the models fitted were tested for normal distribution and homogeneity of variance with the functions shapiro.test and bartlett.test of the package stats. Because errors were not normally distributed or did not show homogeneity of variances, the models where fitted with different non-gaussian error distributions, such as poisson, binomial, or gamma distributions (Zuur et al. 2009). "Forest size" (small or large) and "Fungicide" (with or without benomyl) were used as fixed factors. Plant individuals and soil samples were nested within forest fragments and used as random term of the model in order to avoid the spatial pseudo-replication (Douglas et al. 2010). Interaction terms (Forest fragment size × fungicide) were tested and included when they significantly improved the model. Akaike information criterion was used to select goodness of fit of the models. The statistical significance of individual fixed effects was tested with z statistics for GLMMs.

Results

Soil nutrients and mycorrhizal colonization in plant roots

Soil nutrients (nitrate, ammonia, and phosphorus) were similar between soil samples of small and large forest fragments (Fig. S1). In addition, total nitrogen and the carbon: nitrogen relationship did not show significant differences between samples of different forest fragment sizes (Table S1). Both plant species showed higher total mycorrhizal colonization in larger forest fragments than in smaller ones (Table 1a; Fig. 2a, b). However, there were no differences in vesicular and DSE colonization between forest

Table 1 GLMM outputs of mycorrhizal colonization in two ruderal *Euphorbia* plant species (native and invasive) with factor forest fragment size (small and large; $n = 6$)

	n	Term	Coefficient	SE	z value	P
(a) Total mycorrhizal colonization						
Native plants	60	Fragment size	0.751	0.107	7.04	**<0.0001**
Invasive plants	60	Fragment size	0.334	0.162	2.05	**0.04**
(b) Vesicular colonization						
Native plants	60	Fragment size	0.529	0.462	1.15	0.25
Invasive plants	60	Fragment size	0.161	0.910	0.17	0.86
(c) DSE colonization						
Native plants	60	Fragment size	−0.337	0.377	−0.89	0.37
Invasive plants	60	Fragment size	0.143	0.534	0.27	0.79

Significant values are pointed in bold

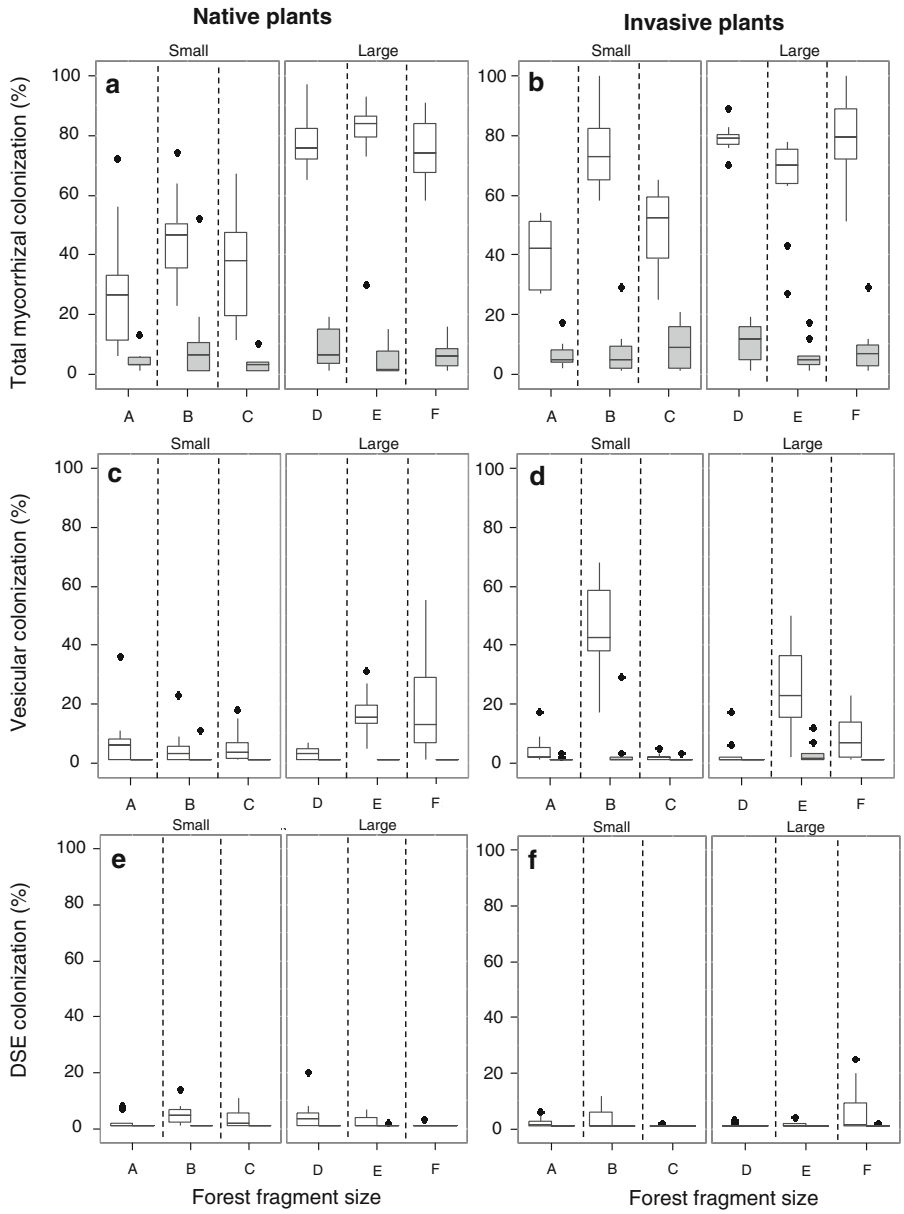

Fig. 2 Mycorrhizal colonization in two ruderal *Euphorbia* plant species (native and invasive) in two forest fragment sizes (small and large). Total mycorrhizal colonization (**a**, **b**), Vesicular colonization (**c**, **d**), and Dark Septate Endophyte (DSE) colonization (**e**, **f**) in plants without (*open square*) and with fungicide (*filled square*). Box-plot: median, first, and third quartile and 95 % confidence interval of median

fragments, and average values were consistently low (Table 1, Fig. 2c, f). Arbuscular colonization was present only in 4 of the 240 plant roots. Therefore, these data were not considered in the statistical analyses. Fungicide consistently reduced fungal colonization in roots. Native and invasive hosts treated with fungicide showed total colonization rates by AMF lower than 10 %. In contrast, plants without fungicide reached significantly higher total colonization rates (between 30 and 80 %) ($GLMM_{native}$ $z = 40.59$, $P < 0.001$; $GLMM_{invasive}$ $z = 43.10$, $P < 0.0001$). Fungicide also effectively reduced vesicular colonization ($GLMM_{native}$ $z = 16.91$, $P < 0.0001$; $GLMM_{invasive}$ $z = 20.63$, $P < 0.0001$) and DSE colonization ($GLMM_{native}$ $z = 8.19$, $P < 0.0001$; $GLMM_{invasive}$ $z = 6.70$, $P < 0.0001$) in native and invasive plants. It is worth mentioning that in the case of DSE, values always averaged below 6 %, suggesting a negligible biological significance of the statistical difference.

Effects of reduced AM fungal colonization on plant vegetative growth

Vegetative growth in both plant species showed no differences between small and large forest fragments (Table 2; Fig. 3a, d). However, reduced AM fungal colonization positively affected dry biomass (Table 2; Fig. 3a) and plant height in the native species (Table 2; Fig. 3c). In contrast, the invasive host showed no differences in plant dry mass, but a decrease in plant height when AM fungal colonization was reduced (Fig. 3d; Table 2). Root:shoot ratio and SLA showed no differences between fungicide treatments in both species.

Effects of reduced AM fungal colonization on plant reproductive growth

Reproductive growth of both plant species showed no significant differences between small and large forest fragments (Fig. 4, Table 3, except for pollen grains per flower in the invasive species that was lower with reduced AM fungal colonization in large forest fragments). In contrast, reduced AM fungal colonization positively affected reproductive growth in the native and the invasive hosts. Both species showed higher inflorescence production with reduced AM fungal colonization, albeit inflorescence production in the invasive only showed differences between fungicide treatments at large forest fragments (Table 3, Fig. 4a, b). Fruit number was higher when AM fungal colonization was reduced in both species. However, significant interaction terms limited differences between treatments to smaller fragments in native hosts and to larger fragments in invasive plants (Table 3; Fig. 4c, d). In addition, reduced AM fungal colonization in native hosts positively affected pollen grain production per flower in both forest fragment sizes. In turn, reduction of fungal colonization in the invasive negatively affected pollen production in

Table 2 GLMM outputs of vegetative traits in two ruderal *Euphorbia* plant species (native and invasive) with two factors, fungicide (with and without; $n = 10$) and forest fragment size (small and large, $n = 6$)

	n	Term	Coefficient	SE	*z* value	*P*
(a) Plant dry mass						
Native plants	120	Fungicide	0.517	0.15	3.42	**0.0006**
		Fragment size	−0.074	0.22	−0.33	0.74
Invasive plants	120	Fungicide	−0.030	0.22	−0.14	0.89
		Fragment size	−0.137	0.29	−0.47	0.64
(b) Plant height						
Native plants	120	Fungicide	0.262	0.04	7.49	**<0.0001**
		Fragment size	−0.117	0.10	−1.15	0.25
Invasive plants	120	Fungicide	0.078	0.03	2.55	**0.01**
		Fragment size	−0.017	0.11	−0.15	0.87

Significant values are pointed in bold

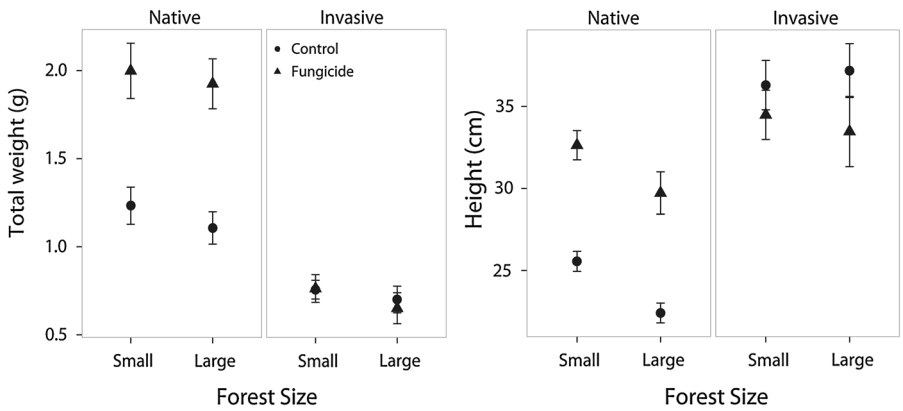

Fig. 3 Vegetative growth in two ruderal *Euphorbia* plant species (native and invasive) in two forest fragment sizes (small and large). Total plant dry mass (**a**, **b**) and Plant height (**c**, **d**) in plants without fungicide (*open square*) and with fungicide (*filled square*). Box-plot: median, first, and third quartile and 95 % confidence interval of median

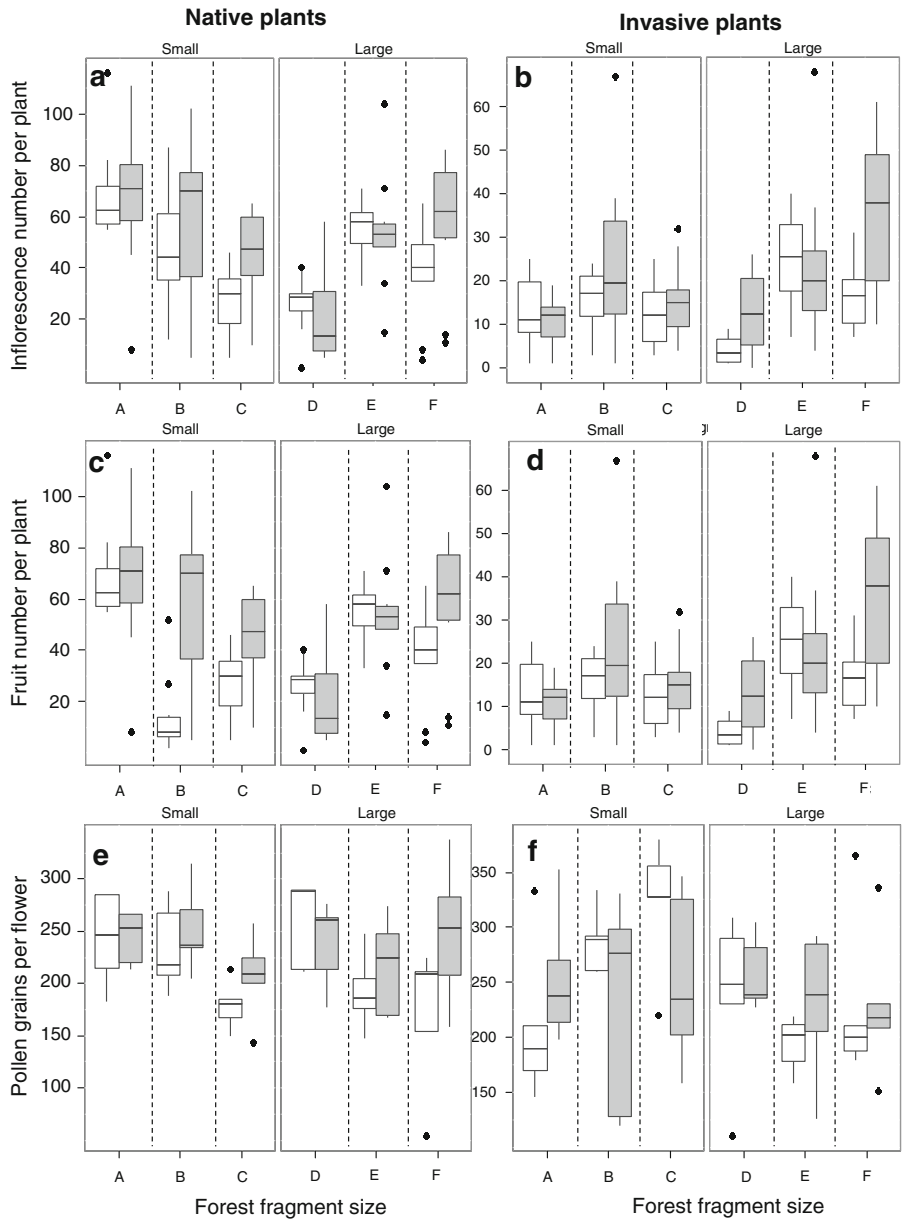

Fig. 4 Reproductive growth in two ruderal *Euphorbia* plant species (native and invasive) in two forest fragment sizes (small and large). Inflorescence number (**a**, **b**), fruit number (**c**, **d**), and pollen grain number (**e**, **f**) in plants without fungicide (*open square*) and with fungicide (*filled square*). Box-plot: median, first, and third quartile and 95 % confidence interval of median

small fragments while it positively affected pollen production in large forest fragments, explaining the significant interaction term (Table 3, Fig. 4f). Both plant species showed similar pollen grain sizes between AM fungal colonization treatments (Table 3).

Discussion

Fungal colonization in roots

Patterns of total AMF colonization in the native and in the invasive plant species under controlled greenhouse

Table 3 GLMM outputs of reproductive traits in two ruderal *Euphorbia* plant species (native and invasive) with two factors, fungicide (with and without; $n = 10$) and forest fragment size (small and large, $n = 6$)

	n	Term	Coefficient	SE	z value	P
(a) Inflorescence number						
Native plants	120	Fungicide	0.148	0.027	5.55	**<0.0001**
		Fragment size	−0.270	0.270	−1.00	0.32
Invasive plants	120	Fungicide	−0.229	0.066	−3.46	**<0.0001**
		Fragment size	0.262	0.318	0.82	0.41
		Fung × Frag size	−0.251	0.090	−2.80	**0.005**
(b) Fruit number						
Native plants	120	Fungicide	−0.443	0.038	−11.47	**<0.0001**
		Fragment size	−0.271	0.277	−0.978	0.33
		Fung × Frag size	0.343	0.055	6.17	**<0.0001**
Invasive plants	120	Fungicide	−0.231	0.066	−3.49	**<0.0001**
		Fragment size	0.262	0.318	0.82	0.41
		Fung × Frag size	−0.248	0.089	−2.76	**0.005**
(c) Pollen grains per flower						
Native plants	120	Fungicide	0.096	0.017	5.55	**<0.0001**
		Fragment size	−0.024	0.080	−0.30	0.76
Invasive plants	120	Fungicide	−0.104	0.022	−4.60	**<0.0001**
		Fragment size	−0.215	0.064	−3.33	**<0.0001**
		Fung × Frag size	0.186	0.033	5.62	**<0.0001**
(d) Pollen size						
Native plants	120	Fungicide	0.006	0.054	0.11	0.91
		Fragment size	0.003	0.054	0.05	0.96
Invasive plants	120	Fungicide	0.039	0.043	0.91	0.36
		Fragment size	0.037	0.043	0.86	0.39

Significant values are pointed in bold

conditions varied between small and large forest fragments. In contrast to our prediction, these differences in mycorrhizal colonization had no consequences on vegetative and reproductive growth in these annual ruderal hosts. Despite the fact that Grilli et al. (2012) found a negative relationship between nutrient availability and fragment size, there were no significant differences between large and small forest fragments here. This might explain in part the lack of differences in plant growth between fragment size treatments. It is worth mentioning that differences in AM colonization between forest fragments sizes do not necessarily reflect differences in AMF community composition in roots.

It has been suggested that ruderal plants might be related to ruderal AMF, mainly Glomeraceae (formerly Group A), because associations between AMF and plant hosts might be filtered according to shared functional traits among partners (Chagnon et al. 2013). This is consistent with recent findings on AMF composition in roots of these ruderal plants (Grilli et al. unpublished). Our results suggest that changes in AMF spore composition in the rhizosphere together with changes in percentages of mycorrhizal colonization (Grilli et al. 2012, 2013) might not be accompanied by changes in mycorrhizal functioning, suggesting some kind of functional resilience for the interactions between AMF and ruderal plants (Johnson et al. 2005). Nonetheless, below certain thresholds in the percentage of mycorrhizal colonization, such as those promoted by fungicide amendment, AMF functioning is certainly affected (see below).

Effects of AMF suppression on plant vegetative growth

The response of plant vegetative growth to fungal suppression could be mostly attributed to AMF because general negligible amounts of DSE colonization were found in all the experimental treatments. The

increases in dry mass and plant height when AM fungal colonization was reduced in the native *Euphorbia* are consistent with recent findings on other ruderal plants (Pérez and Urcelay 2009; Rinaudo et al. 2010; Veiga et al. 2011). The negative effects of AMF on these native forbs could place the symbiosis in the parasitic side of the mutualism–parasitism continuum (Johnson and Graham 2013). In addition, we cannot discard that lack of arbuscules as a reason for the apparent parasitic relationship between fungi and host plants, since these structures represent the major exchange sites between fungi and plant hosts (Smith and Read 2008). Instead, AMF have no effects on the invasive plant growth, except for an increment on the height of the invasive forbs. Certainly we cannot discard that fungicide might be releasing the plants of pathogenic fungi affecting plant growth, in particular the native species that might be released from their natural enemies (Klironomos 2002). A recent study in forest fragments of tropical forest in Colombia showed that ruderal plant growth could be negatively affected by non-AMF soil biota from their home habitat (Pizano et al. 2014). Nevertheless, it is important to highlight that pathogenic fungi were not evidenced in plant roots of our study.

It has been suggested that decreases in root: shoot ratios and increases in SLA might be mediated by nutrient availability and positively related to plant growth (e.g., Meziane and Shipley 1999; Aerts and Chapin 2000). The lack of response of root: shoot ratio and SLA observed here might be related to the negative and neutral effects of AMF on biomass in these plants. Indeed, our findings for root: shoot ratios agree with the results of a recent meta-analysis on forbs and support the idea that when AMF negatively affect growth, these fungi are less likely to promote asymmetries in the root: shoot ratio of plant hosts (Veresoglou et al. 2012).

Effects of AMF suppression on plant reproductive growth

Reproductive traits in both species, native and invasive, showed a positive fitness response when fungal colonization was suppressed. These results are in accordance with those depicted for vegetative growth in the native but not for the invasive species. Differences in vegetative and reproductive response to AMF in the invasive plant could be a consequence of a plastic response to novel constraints faced in the non-native range (e.g., Claridge and Franklin 2002). Due to carbon constraints imposed by fungal root symbionts, the plastic response of the invasive plant might be the selective allocation to vegetative plant growth, but not to reproductive traits. In this way, the congeneric invader seems to "fare better" than the native in the invasive range (Hawkes 2007).

Reduced AM fungal colonization positively affected inflorescence and fruit production in both hosts. These findings are similar to those recently reported on other ruderal hosts (Rinaudo et al. 2010; Veiga et al. 2011), although they differ from others in which positive effects of AMF on ruderal plant reproductive traits have been observed (Gange and Smith 2005; Poulton et al. 2001, Aguilar-Chama and Guevara 2012). However, we should be cautious in our comparison, since our study is the only one that uses natural soil as inocula instead of pure cultures of AMF or a limited mix of them. The significant interaction term observed for the number of inflorescences per plant and fruit production in the invasive *Euphorbia* indicates that, despite the overall positive effect of fungal suppression on reproduction, this positive trend varies between soils from different forest fragment sizes. All in all, the negative effects of AMF on the reproductive growth of these species would certainly have consequences on their population dynamics.

Conclusions

To our knowledge, the links between soil fungi and plant vegetative and reproductive growths in the context of forest fragmentation were experimentally tested for the first time here. The differences in the percentage of mycorrhizal colonization in roots between plants growing in soils from a gradient of fragments sizes in the field (Grilli et al. 2012) and between small and large fragments in the greenhouse (the study herein) without clear effects on plant growth suggest functional resilience between fungal communities (Johnson et al. 2005) and ruderal plants. Growth and reproduction of the native species were negatively affected by AMF. However, the root symbionts in the congeneric invasive plant had positive, neutral, and negative effects on height, biomass, and reproductive traits, respectively. The negative effects of AMF on reproductive growth of these species may certainly have important

consequences on their population dynamics. Our findings from the greenhouse experiment are evidence that AMF might have direct implications on several key plant ecological processes related to plant population dynamics which, in turn, are linked to relevant community processes such as the expansion of invasive plants over their non-native range.

Acknowledgments We thank Julia Galetto for text editing and English advice, Secretaría de Ciencia y Técnica (Universidad Nacional de Córdoba) and Concejo Nacional de Investigaciones Científicas y Técnicas (CONICET) for financial support. CU and LG are researchers in CONICET, and MSL and GG are fellows of the same institution.

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