Forest Ecology and Management 310 (2013) 442–449

Contents lists available at ScienceDirect

Forest Ecology and Management

journal homepage: www.elsevier.com/locate/foreco

Linking mycorrhizal fungi and soil nutrients to vegetative and reproductive ruderal plant development in a fragmented forest at central Argentina

G. Grilli *, C. Urcelay, L. Galetto

Instituto Multidisciplinario de Biología Vegetal (CONICET-UNC), Vélez Sarsfield 1611, CC 495, 5000 Córdoba, Argentina
FCEFyN, Universidad Nacional de Córdoba, Córdoba, Argentina

ARTICLE INFO

Article history:
Received 21 May 2013
Received in revised form 21 August 2013
Accepted 23 August 2013

Keywords:
Arbuscular mycorrhizal fungi
Dark septate endophyte
Forest fragmentation
Nutrient availability
Plant sexual reproduction
Plant invasions

ABSTRACT

We studied the actual links between mycorrhizal fungi, nutrient availability and plant development in several sites of fragmented Chaco forests. Specifically, we evaluated whether arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSE) colonization and availability of soil nutrients are related to growth (biomass and plant height) and reproductive traits (pollen size and pollen and fruit production) of two ruderal, annual, congeneric *Euphorbia* species along a gradient of forest fragmentation. We assessed relationships between forest fragment size and isolation, nutrient availability, mycorrhizal fungal variables and plant development at eleven Chaquean forest fragments (0.5–1000 ha) immersed in an agricultural landscape in central Argentina. Mycorrhizal colonization was not related to forest fragment isolation, positively related to forest fragment size and negatively related to soil nutrient availability in both hosts along the fragmentation gradient. Plant reproductive and vegetative traits were related to mycorrhizal colonization. In general, plant height, biomass, and fruit production were negatively related to AMF, and pollen production was positively related to DSE, in both ruderal hosts (native and exotic). We found remarkable relationships along a forest fragmentation gradient that reliably link forest fragment size to lower nutrient availability and increased mycorrhizal colonization in native/exotic ruderal hosts, while negatively linking AMF colonization to plant development and reproductive success.

1. Introduction

Forests have been increasingly fragmented around the globe in the last decades, principally due to the advances of agricultural and urban frontier (Didham, 2010). Fragmented landscapes have been largely related to ecological patterns and processes of organisms occurring in the aboveground compartment of the ecosystem (Fahrig, 2003; Lindenmayer and Fischer, 2006; Didham et al., 2012). Thus, few studies have focused on relationships between belowground organisms (i.e. mycorrhizal fungi) and the forest fragmentation process (Mangan et al., 2004; Peay et al., 2007; Grilli et al., 2012). In particular, arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSE) are the most common and widespread fungal root symbionts. AMF colonize roots of approximately 80% of vascular plant species, and DSE were recorded in more than 600 plant species (Smith and Read, 2008; Newsham, 2011). There is substantial evidence showing that plant identity, nutrient availability and other environmental factors affect AMF colonization (Smith and Read, 2008). Forest fragmentation may be related to the factors affecting AMF colonization, such as exotic plant establishment and soil nutrient content (Lindenmayer and Fischer, 2007; Billings and Gaydess, 2008). We found a negative relationship of nutrient availability to AMF root colonization, spore abundance and diversity in the rhizosphere of two congeneric *Euphorbia* with increasing forest fragment size (Grilli et al., 2012). These complex relationships between forest fragmentation, nutrient availability, and AMF in the plant rhizosphere might be linked to aboveground plant growth and reproduction. However, most studies involving functional effects of forest fragmentation on plants have focused on aboveground biotic interactions, such as pollination and/or fruit dispersal, particularly in Chaco forests (Aguilar et al., 2006; Galetto et al., 2007; Grilli and Galetto, 2009; Ponce et al., 2012).

It is well known that nutrient acquisition, either directly or mediated by mycorrhizal fungi, varies among plants according to their resource acquisition strategies (Aerts and Chapin, 2000). For instance, functional measurements are difficult in long-lived perennial plant species with slow growth rates, in particular reproductive ones. Therefore, short-life cycle annual plants are a good study case for the understanding of forest dynamics in fragmented landscapes (Tscharntke et al., 2012). Success of ruderal plants in

---

* Corresponding author. Tel.: +54 3517594502.
E-mail address: ggrilli@imbiv.unc.edu.ar (G. Grilli).

0378-1127/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.foreco.2013.08.052

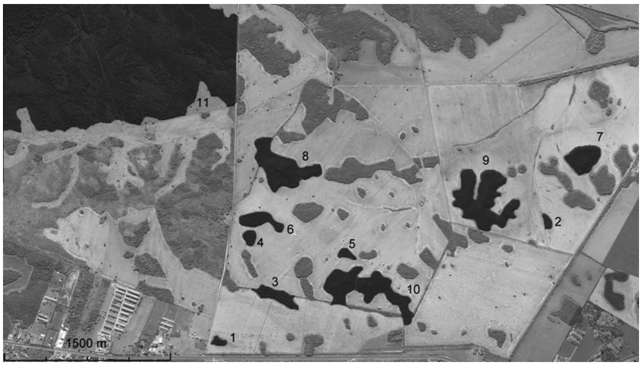

Fig. 1. Study area with the eleven forest fragments studied in black.

Table 1
GLMM outputs of mycorrhizal colonization in two ruderal *Euphorbia* plant species (native and exotic) along a fragmentation gradient ($n = 11$ forest fragments) in Córdoba, Argentina.

Term	d.f.	Deviance (χ^2)	*P*
(a) Total mycorrhizal colonization			
Species	1	41.31	**<0.0001**
LogArea	1	8.97	**0.002**
Isolation	1	1.04	0.31
LogArea * Species * Isolation	4	7.65	0.1
(b) Vesicular colonization			
Species	1	117.43	**<0.0001**
LogArea	1	1.17	0.19
Isolation	1	0.55	0.45
LogArea * Species * Isolation	4	49.25	**<0.0001**
(c) Native vesicular colonization			
LogArea	1	0.01	0.9
Isolation	1	1.18	0.27
(d) Exotic vesicular colonization			
LogArea	1	2.13	0.14
Isolation	1	0.32	0.56
(e) DSE colonization			
Species	1	7.22	**0.007**
LogArea	1	348.16	**<0.0001**
Isolation	1	0.44	0.5
LogArea * Species * Isolation	4	0.47	0.82

Bold values are significant at $P < 0.05$.

Table 2
GLMM outputs of mycorrhizal colonization and nutrient availability along a fragmentation gradient ($n = 11$ forest fragments) in Córdoba, Argentina.

Term	d.f.	Deviance (χ^2)	*P*
(a) Total mycorrhizal colonization			
Nitrate	1	4.1	**0.04**
Ammonia	1	0.12	0.73
Phosphorus	1	10.53	**0.001**
Total nitrogen	1	1.28	0.25
Organic matter	1	0.35	0.55
C:N	1	4.77	**0.02**
Organic carbon	1	0.44	0.51
(b) Vesicular colonization			
Nitrate	1	6.48	**0.01**
Ammonia	1	0.8	0.37
Phosphorus	1	6.24	**0.01**
Total nitrogen	1	14.54	**0.0001**
Organic matter	1	2.48	0.12
C:N	1	0.2	0.65
Organic carbon	1	1.83	0.18
(c) DSE colonization			
Nitrate	1	10.43	**0.001**
Ammonia	1	1.62	0.2
Phosphorus	1	10.82	**0.001**
Total nitrogen	1	0.17	0.68
Organic matter	1	0.42	0.52
C:N	1	11.29	**0.0007**
Organic carbon	1	0.46	0.5

Bold values are significant at $P < 0.05$.

disturbed habitats has been attributed to rapid growth rates and to a comparably higher efficiency than other plants in acquiring available nutrients (Grime, 2001). Mycorrhizal fungi have been widely shown to be beneficial to their hosts, mainly due to enhancement in nutrient uptake (Smith and Read, 2008). Conversely, some ruderal plants appear to be less dependent or negatively affected by fungal symbionts (Brundrett, 1991; Hoeksema et al., 2010). In fact, vegetative development in ruderal plants could be negatively affected by AMF (Pérez and Urcelay, 2009; Rinaudo et al., 2010; Veiga et al., 2011; Urcelay et al., 2011). Accordingly, since reproductive traits also depend on resource availability, it might be expected that ruderal plant reproductive traits are also negatively affected by root symbionts (Koide, 2010). Flower, pollen, fruit and seed production might be increased, decreased or not affected by mycorrhizal fungi (Poulton et al., 2001; Poulton et al., 2002; Varga and Kytöviita, 2010; Varga, 2010 and references there in). However, this evidence does not support generalities about relationships between mycorrhizal fungi and hosts with different nutrient acquisition strategy. In this study we worked with two annual ruderal *Euphorbia plants* (exotic and native) that might be affected by forest fragmentation to evaluate the plant development process and its relationship with mycorrhizal fungal traits and nutrient availability. In addition, several studies have shown that the outcome of mycorrhizal associations might differ between native and invasive plant species (reviewed by Pringle et al., 2009). However, how mycorrhizal fungi affect ruderal plants according to their identity in fragmented forests remains unclear.

The aim of this study was to examine whether plant growth and reproductive traits of two ruderal, annual *Euphorbia* species (*E. acerensis* and *E. dentata*, native and exotic respectively) are related to mycorrhizal colonization and nutrient availability in a forest fragmentation gradient. We predicted that vegetative and reproductive ruderal plant development would be positively related to nutrient availability and negatively related to fungal variables along this forest fragmentation gradient. In this way we expected to provide novel evidence on the links of plant development and reproduction in a forest fragmentation gradient with patterns of mycorrhizal fungi and nutrient availability in a fragmented forest.

2. Methods

2.1. Study site

Chaco forests in central Argentina present high rates of landscape fragmentation (Zak et al., 2004), due to vast landscape modification with most original vegetation having disappeared (Fisher and Lindenmayer, 2007) and thus can be characterized as relictual forests. The study area ranges between 31°11’19”S; 64°16’02”W and 31°13’05”S; 64°15’55”W. The altitude varies from 600 to 640 m above sea level. The rainy season occurs between October and May with a mean annual precipitation of 750 mm (Luti et al., 1979; Moglia and Giménez 1998). Mean maximum and minimum temperatures are 26 and 10 °C, respectively. The vegetation of the semiarid shrub-forest in fragments is characterized by *Aspidosperma quebracho-blanco* Schltdl., *Acacia* spp., *Zanthoxylum coco* Engl., *Prosopis* spp., *Celtis ehrenbergiana* Liebm., native and exotic herbs and grasses, vines and epiphytic plants.

Table 3
GLMM outputs of vegetative and reproductive traits of two ruderal *Euphorbia* (native and exotic) plant species along a fragmentation and mycorrhizal colonization gradient ($n = 11$ forest fragments) in Córdoba, Argentina.

Term	*E. acerensis* (native)			*E. dentata* (exotic)	
	d.f.	Deviance (χ^2)	*P*	Deviance (χ^2)	*P*
(a) Plant height					
LogArea	1	5.22	**0.022**	2.45	0.12
Mycorrhizal colonization	1	5.28	**0.021**	7.59	**0.006**
Vesicular colonization	1	0.70	0.4	6.41	**0.01**
DSE colonization	1	1.51	0.22	14.64	**0.0001**
(b) Plant dry mass					
LogArea	1	1.28	0.25	3.96	**0.046**
Mycorrhizal colonization	1	0.37	0.54	0.58	0.46
Vesicular colonization	1	1.15	0.28	0.72	0.4
DSE colonization	1	0.15	0.69	0.42	0.52
(c) Fruit number					
LogArea	1	0.52	0.47	3.76	0.05
Mycorrhizal colonization	1	43.9	**<0.0001**	12.09	**0.0005**
Vesicular colonization	1	6.12	**0.013**	1.94	0.16
DSE colonization	1	1.21	0.27	14.28	**0.0002**
(d) Pollen number					
LogArea	1	0.11	0.74	6.45	**0.01**
Mycorrhizal colonization	1	0.48	0.49	1.23	0.27
Vesicular colonization	1	1.01	0.31	0.48	0.49
DSE colonization	1	13.32	**0.0002**	30.43	**<0.0001**
(e) Pollen size					
LogArea	1	0.45	0.5	0.04	0.85
Mycorrhizal colonization	1	0.15	0.7	0.12	0.74
Vesicular colonization	1	0.06	0.8	0.3	0.59
DSE colonization	1	1.74	0.18	0.02	0.90

Bold values are significant at $P < 0.05$.

These Chaco forest fragments, which are rocky outcrops immersed in an agricultural matrix, remain in the landscape due to their unsuitability for agriculture. Forest fragments have an average isolation age of approximately sixty years, due to conversion of original matrix surrounding forest fragments into crops (C. Torres, unpublished data). In areas surrounding the fragments, maize and soybean are sown during spring and summer, and wheat during autumn and winter. Eleven fragments from 0.86 to 1000 ha were selected randomly in the landscape, representing a size gradient (Fig. 1) avoiding fragments with visible signs of high disturbance. Isolation for each forest fragment was measured as the near neighbor distance (Fahrig 2003) as an additional indicator for the forest fragmentation process. Plant individuals were sampled again in six of the same forest fragments used in Grilli et al. (2012), and for this study five novel forest fragments were added to enlarge the gradient. Despite the low size of smaller fragments, the plants were collected several meters from the border, avoiding major edge effects.

We worked with two abundant, congeneric, ruderal herbs in Chaco forest fragments: *Euphorbia acerensis* Boiss and *E. dentata* Michx. Both species present AMF and DSE fungal colonization (Grilli et al., 2012). Annual herbs were chosen because fragmentation effects might be better expressed on plants with short life cycles. Both plant species have a similar reproductive biology, are annual herbs and can reach 45–50 cm height (Subils, 1977). *E. acerensis* is a native herb from the region, whereas *E. dentata* is an invasive exotic weed from North and Central America.

Individuals were collected between April and May of 2009, at the end of the rainy season. Ten sexually mature individuals of each plant species per forest fragment were randomly selected; a total of 220 individuals, 110 per species. These congeneric species co-occur in similar microhabitats and were collected in the understory of the forest fragments at the end of the reproductive stage. Plants were gathered together with soil surrounding the entire rhizosphere (most of the root system recovered) down to 15-cm depth and brought to the laboratory and stored at 8 °C.

2.2. AMF and DSE colonization

Roots with a diameter less than 2 mm were selected. All dead and damaged roots were discarded. Roots were stained as in Grilli et al. (2012) and mounted in semi-permanent slides in polyvinyllactic acid-glycerol; we prepared one slide per individual including all active roots. We measured a minimum of 25 cm of roots per individual. AMF and DSE colonization were determined following the intersection method of McGonigle et al. (1990) using a compound microscope (Nikon optical, Model E200) at 200× magnification. Percentage of root colonization (total, vesicular, arbuscular and dark septate) was assessed as the proportion of total root intersections that were colonized.

2.3. Vegetative and reproductive traits in plant hosts

One male flower per inflorescence of each plant was collected in four plants of both species at each forest fragment and preserved in 70% ethanol ($n = 44$ flowers of each species). The total number of pollen grains per flower was counted using a microscope (Nikon optical, Model E200), 200x magnification. Pollen size was measured with a metric ocular at 1000× magnification. Fruit production was recorded at the lab immediately after collection, counting all developed fruits per plant ($n = 10$ plants per fragment). These plants were then dried at 60 °C during three days and weighed to obtain "plant dry mass". Plant height was measured in the laboratory ($n = 10$ plants per fragment) from shoot base to the longest apical meristem using a caliper.

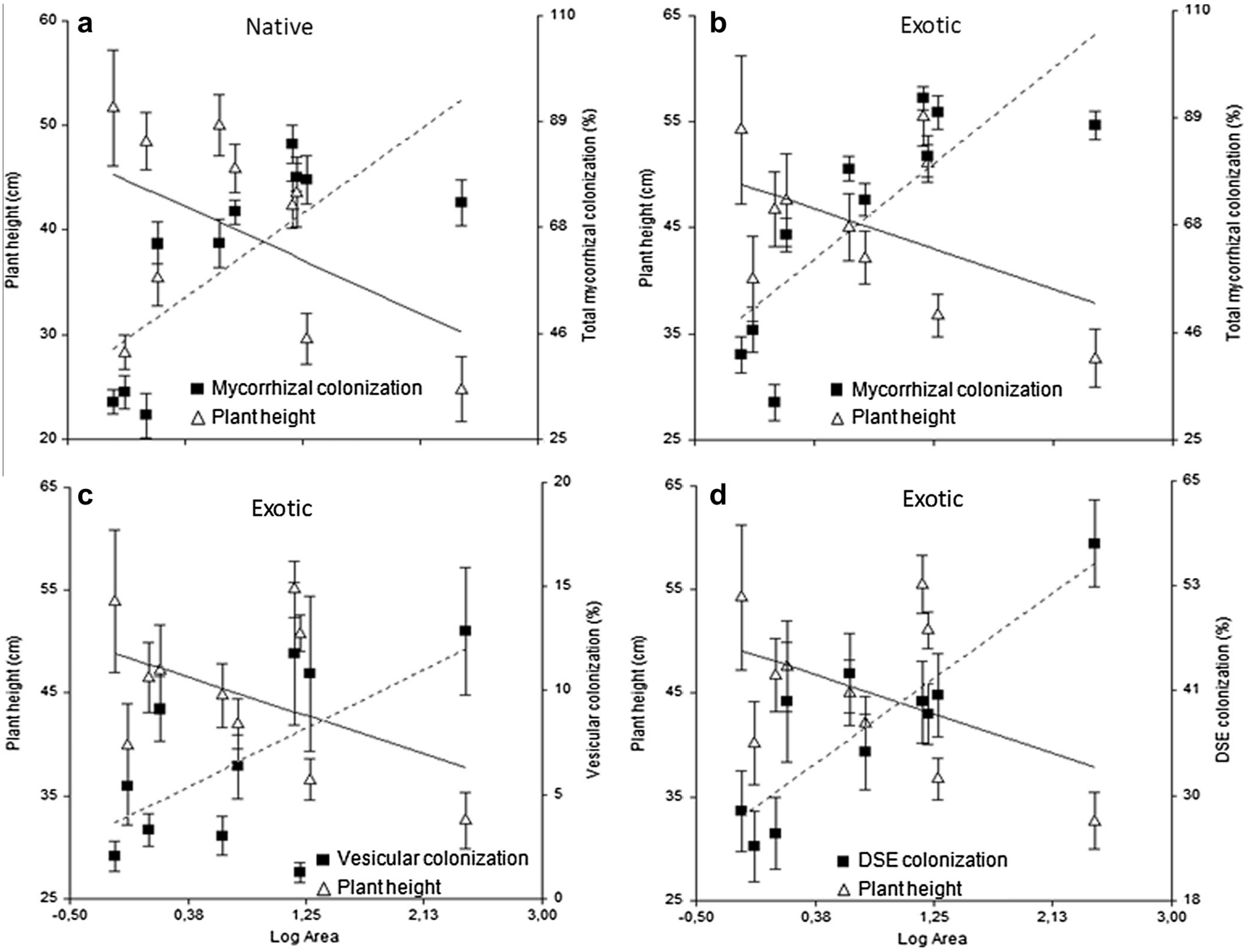

Fig. 2. Relationships between plant height in (a) the ruderal native *Euphorbia acerensis* and total mycorrhizal root colonization, (b) the exotic *Euphorbia dentata* and total mycorrhizal root colonization, (c) in both species vesicular colonization, and (d) both species and DSE colonization within Chaco forest fragment areas ($n = 11$) from Córdoba. Plant height is represented by solid lines (*open triangles*) and root colonization percentage is represented by dashed lines (*closed squares*). Data are the mean ± s.e.

2.4. Soil nutrient analyses

Soil samples were collected to assess nutrient availability and stored at 4 °C. Four samples (100 g from the rhizospheric soil of randomly selected *Euphorbia* individuals) per fragment were separated from one another and sent to the Edaphologic Laboratory of the Faculty of Agronomic Sciences (Universidad Nacional de Córdoba) for soil nutrient analyses. 10 g of dry soil was shaken in 50 ml of 1.0 M $NaHCO_3$ for 30 min and was then filtered through Whatman no. 42 filter paper, and phosphorus was determined colorimetrically using the ascorbic acid method Bray and Kurtz N°1 (Kuo, 1996), the Kjeldahl method was used for total nitrogen (Bremner, 1996), nitrate and ammonia were estimated using direct potentiometry (ORION Ionalizer 901) (Mulvaney, 1996), the Walkley and Black method was used for organic matter (Nelson and Sommers, 1996), the steam distillation method was used after treatment of the samples with ammonium acetate for cations, and 10 g air-dry soil mixed with 10 ml deionized water was used to determine pH (Sumner and Miller, 1996).

2.5. Statistical analyses

Generalized linear mixed effects models (GLMM) in R v.2.13.2 (R Development Core Team, 2010) were used to analyze relationships between mycorrhizal fungal colonization, soil nutrient availability, plant development and forest fragmentation (lme4 package, Bates and Sarkar, 2007). We used forest fragment size (Log Area), forest fragment isolation and species (native and exotic) as fixed factors. Individuals and flowers were nested within forest fragments and used as random terms to control spatial pseudo-replication. Models were fitted with a Poisson error structure and a log-link function. Model term significance was assessed based on model deviance changes with chi-squared (Douglas et al., 2010).

3. Results

3.1. Mycorrhizal fungi and nutrient availability at forest fragments

Root colonization rates ranged from 30% to 95% in AMF and 5–20% in DSE. Total AMF mycorrhizal colonization was positively related to forest fragment size but not to isolation (Table 1a), and was 5% higher in exotic hosts than in native plants (Table 1a). In addition, DSE colonization in exotic plants was positively related only to forest fragment size (Table 1d–e). Vesicular colonization showed a significant interaction term among area, isolation and species (Table 1b). However, no clear patterns emerge for vesicular colonization percentages from the interaction between forest area, forest isolation and plant species (results no showed). In

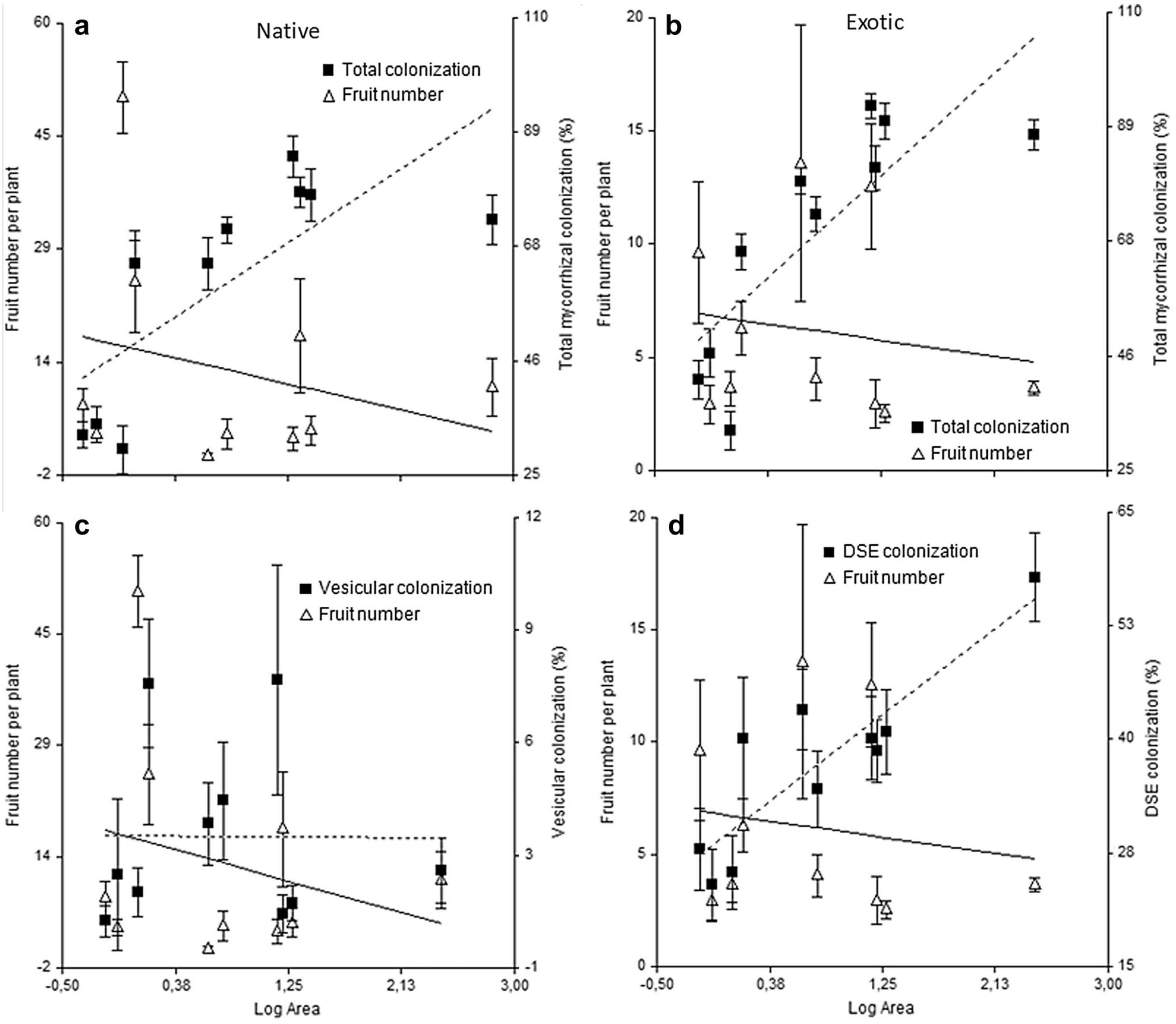

Fig. 3. Relationships of fruit production in the ruderal native *Euphorbia acerensis* with (a) total mycorrhizal colonization and (c) vesicular colonization; and fruit production in the ruderal exotic *Euphorbia dentata* with (b) total mycorrhizal colonization and (c) DSE colonization in Chaco forest fragment areas ($n = 11$). Fruit production is represented by solid lines (*open triangles*) and root colonization percentage is represented by dashed line (*closed squares*). Data are the mean ± s.e.

consequence, the interaction term was explored by analyzing separately vesicular colonization in the roots of native and invasive species and no significant relationships were evidenced for forest fragment size or isolation (Table 1c–d). DSE colonization in the native *E. acerensis* was not related to forest fragment size or isolation.

Soil nutrient availability (nitrate, ammonia and phosphorus) showed no relationship with increasing forest fragment size. Nonetheless, colonization by AMF (total) and DSE were negatively related to nitrate, phosphorus and C:N ratio, while vesicular colonization was negatively related to nitrate, phosphorus and total nitrogen (Table 2).

3.2. Relationships between plant species development and forest fragment size, mycorrhizal fungi, and nutrient availability

Plant height was negatively related to forest fragment size in the native *E. acerensis* (Tables 3a and 4a–b), total AMF colonization in both *Euphorbia* species (Table 3a; Fig. 2a and b), and vesicular and DSE colonization in the exotic *E. dentata* (Table 3a; Fig. 2c and d). Plant dry mass was negatively related to forest fragment size only in the exotic *E. dentata* (Table 3b).

In addition, plant reproductive traits showed varied relationships with forest fragment size and mycorrhizal colonization (Table 3 c, d). Fruit production was negatively related to total mycorrhizal colonization in both species, but not related to forest fragment size (Table 3 c; Fig. 3a and b). It was also negatively related to vesicular colonization in the native and DSE colonization in the exotic species (Fig. 3c and d). Pollen production was positively related to forest fragment size in the exotic and DSE colonization in both species (Table 3d; Fig. 4a and b). Pollen size was not related to forest fragment size or mycorrhizal fungal colonization for either plant species (Table 3e).

Plant height was positively related to nitrate in the native *E. acerensis* (Table 4a). Plant dry mass showed no relationship to soil nutrients in both plant species (Table 4b). Fruit production was positively related to nitrate, ammonia and C:N ratio in the native but not in the exotic species (Table 4c). Pollen grain number was

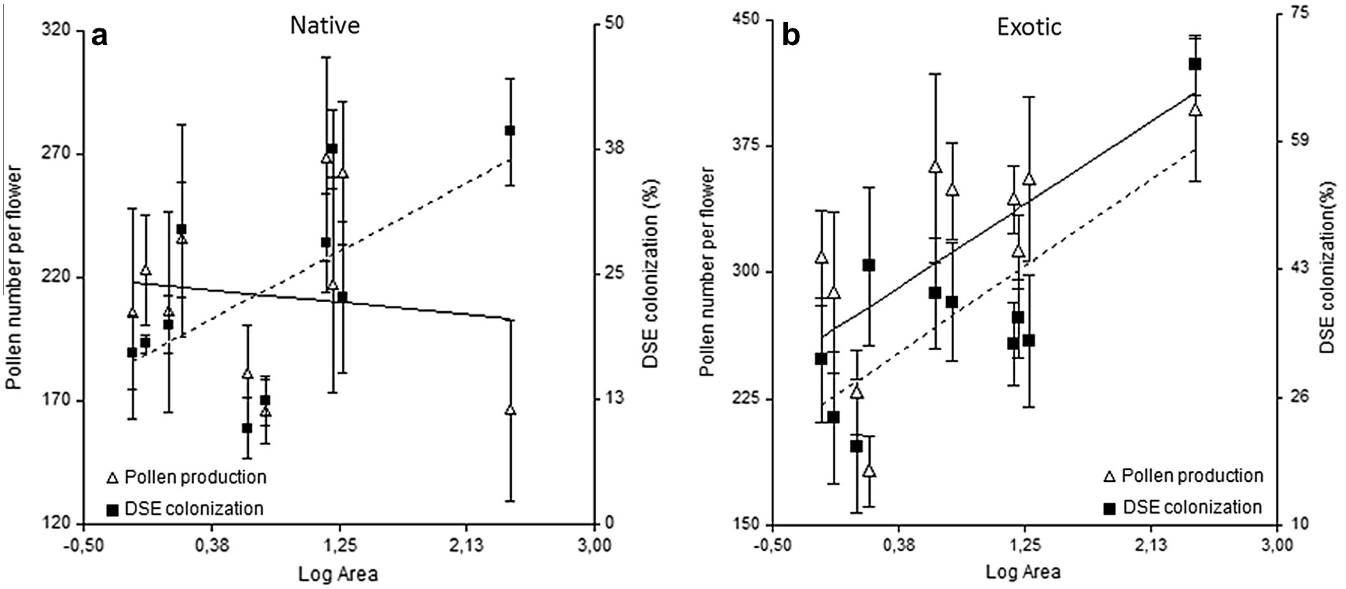

Fig. 4. Relationships between pollen number and DSE colonization in (a) the native Euphorbia acerensis and (b) the exotic *Euphorbia dentata* in Chaco forest fragment areas (*n* = 11). Pollen number is represented by solid lines (*open triangles*) and DSE colonization percentage is represented by dashed lines (*closed squares*). Data are the mean ± s.e.

positively related only to nitrate in the exotic *E. dentata* and pollen size of both species was not related to nutrient availability (Table 4d).

4. Discussion

4.1. Linking plant development to mycorrhizal fungi and soil nutrients in a fragmentation gradient

To our knowledge, this is the first study to highlight complex relationships among soil properties, fungal variables and ruderal plant development in the context of fragmented landscape. Until now, few empirical studies identifying alterations of important interactions such as plant-mycorrhizal fungal have contributed to improve the theoretical framework regarding fragmented forests (Mangan et al., 2004; Peay et al., 2007; Grilli et al., 2012). The lack of relationship of plant-fungal interaction with forest fragment isolation might be due to environmental variables (e.g. nutrient availability) being more important than spatial structure limiting dispersal ability of mycorrhizal fungi (Lekberg et al., 2007). Theoretical links have been proposed between above and belowground biotic processes within the abiotic context (Wardle et al., 2004; van der Heijden et al., 2008). Both plant species showed negative relationships between vegetative developments (i.e. plant height in native species and plant dry mass in the exotic) and forest fragment size. In contrast, reproductive traits were not related to forest fragment size – except for pollen production in the exotic plant species –, but were negatively related to AMF colonization and, in some cases, positively to soil nutrients. This could suggest that if there is a truly reduction in fitness of *Euphorbia* with forest fragment size, it would be due to effects on initial establishment rather than on established plants per se. In turn, nutrient availability (nitrate and phosphorus) was negatively related to mycorrhizal colonization. These relationships are consistent with evidence suggesting that an increase in nutrient availability negatively affects mycorrhizal fungi (Treseder, 2004 and references there in). However, we cannot discard that higher irradiation together with lower humidity as forest fragment size decreases might also be playing a role in the plant-fungi interaction at this fragmented landscape. These relationships observed in the field might reflect the effect of nutrient availability and mycorrhizal fungi on flower and fruit production evidenced in the greenhouse by experimental studies (Obeso, 2002; Varga, 2010 and references there in).

The inverse relationship between plant development and AMF colonization is coherent with the negative effects of AMF fungi on growth of ruderal plants observed in recent studies (Pérez and Urcelay, 2009; Rinaudo et al., 2010; Veiga et al., 2011; Urcelay et al., 2011). In contrast, positive relationships between DSE and pollen production in both species could indicate that DSE is supplying some resources that enhance male reproductive success. This is consistent, albeit not conclusive, with the assumption that DSE promotes plant host development (mainly with N uptake) (Jumpponen, 2001; Newsham, 2011). Regarding plant invasion processes in fragmented forests, similarities between relationships shown by native and invasive *Euphorbia* could be due to a low degree of host plant species specificity in mycorrhizal fungi (i.e. AMF and DSE) (Davison et al., 2011) and not to their duration of naturalization in the area. Still, fine nuances between relationships observed in native and invasive plant species were also observed and cannot be discarded. These nuances could be suggesting functional differences between native and exotic hosts (Ordonez et al., 2010).

Overall, it seems that the relationships between plant development and forest fragment size might be linked to or mediated by mycorrhizal fungal colonization and soil nutrient availability. It is worth mentioning that despite these suggestive relationships, the nature of the functional relationships between AMF and DSE fungi, soil nutrients and *Euphorbia* plant growth and reproduction warrant experimental testing. Documenting interactions between above and belowground biota is extremely important for understanding the impacts of the fragmentation processes in the context of extended land use changes (Wardle et al., 2004), especially if we consider the importance of mycorrhizal fungi on plant diversity and productivity (van der Heijden et al., 2008). The results depicted here reveal the complex nature of those relationships and empirically highlight some of the theoretical links that underlie that complexity.

Table 4
GLMM outputs of vegetative and reproductive traits of two ruderal *Euphorbia* (native and exotic) plants along a forest fragmentation and mycorrhizal colonization gradient ($n = 11$ forest fragments) in Córdoba, Argentina.

Term	*E. acerensis* (native)			*E. dentata* (exotic)	
	d.f.	Deviance (χ^2)	*P*	Deviance (χ^2)	*P*
(a) Plant height					
Nitrate	1	4.77	**0.029**	1.22	0.27
Ammonia	1	0.17	0.68	0.03	0.87
Phosphorus	1	0.4	0.53	1.36	0.24
Total nitrogen	1	0.27	0.61	0.78	0.38
Organic matter	1	0.25	0.62	0.79	0.37
C:N	1	0.05	0.82	1.25	0.26
Organic carbon	1	0.25	0.62	0.79	0.37
(b) Plant dry mass					
Nitrate	1	0.27	0.6	1.15	0.28
Ammonia	1	0.25	0.62	2.87	0.09
Phosphorus	1	0.08	0.78	0.05	0.83
Total nitrogen	1	0.09	0.77	0.27	0.6
Organic matter	1	0.11	0.74	0.18	0.67
C:N	1	0.27	0.6	0.23	0.64
Organic carbon	1	0.11	0.74	0.25	0.62
(c) Fruit number					
Nitrate	1	64.34	**<0.0001**	0.08	0.78
Ammonia	1	82.24	**<0.0001**	1.74	0.19
Phosphorus	1	0.44	0.51	1.18	0.29
Total nitrogen	1	0.04	0.84	0.14	0.71
Organic matter	1	0.43	0.51	0.13	0.72
C:N	1	20.38	**<0.0001**	0.19	0.67
Organic carbon	1	0.31	0.58	0.13	0.72
(d) Pollen number					
Nitrate	1	1.33	0.25	6.7	**0.008**
Ammonia	1	1.78	0.18	1.8	0.18
Phosphorus	1	0	0.98	0.16	0.69
Total nitrogen	1	0.23	0.64	1.21	0.27
Organic matter	1	0.25	0.62	1.2	0.27
C:N	1	0.11	0.73	0.04	0.84
Organic carbon	1	0.25	0.68	1.2	0.27
(e) Pollen size					
Nitrate	1	0.13	0.71	0.42	0.52
Ammonia	1	0.21	0.65	0.01	0.93
Phosphorus	1	0.03	0.86	0.27	0.6
Total nitrogen	1	0.02	0.9	0	0.1
Organic matter	1	0.02	0.9	0	0.1
C:N	1	0.53	0.47	0	0.1
Organic carbon	1	0.02	0.9	0	0.1

Bold values are significant at $P < 0.05$.

5. Conclusions

Our results suggest that global change promoting conversion of natural forests into productive lands and isolated remnants of natural vegetation might affect plant-fungal interaction outcomes that eventually influence plant population dynamics of fragmented Chaco forests. In particular, both congeneric ruderal *Euphorbia*, might be more successful growing in small forest fragments due to a generally higher soil nutrient availability (Grime, 2001). Furthermore, these small forest fragments showed lower mycorrhizal colonization than large forest fragments, a factor that could also benefit the development of these ruderal plants if we consider that ruderals are more likely to be parasitized by mycorrhizal fungi in the context of the mutualism–parasitism continuum of plant-fungal interactions (Urcelay et al., 2011; Johnson and Graham, 2013). The differences between these native and exotic congeneric ruderals deserve experimental study to elucidate the functional consequences of variation in fungal colonization and soil nutrients along the gradient of fragmented forest.

All in all, the relationships shown in this study suggest that mycorrhizal fungi are actively involved in fragmented forest dynamic. Despite the unknown mechanisms underlying these relationships, the patterns observed here provide novel evidence on how the links between above- and belowground compartments in ecosystems vary with forest fragment size (Wardle et al., 2004; van der Heijden et al., 2008). More studies would provide clues regarding the direction, magnitude, and mechanisms of the effects that underlie these links.

Acknowledgements

We thank Secretaría de Ciencia y Técnica (Universidad Nacional de Córdoba) and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) for financial support. Two anonymous reviewers provided useful comments and suggestions that improved the final version of this manuscript. CU and LG are researchers and GG is a fellowship in the National Council (CONICET).

References

Aerts, R., Chapin, F.S.I.I.I., 2000. The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. Adv. Ecol. Res. 30, 1–67.

Aguilar, R., Ashworth, L., Galetto, L., Aizen, M.A., 2006. Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. Ecol. Lett. 9, 968–980.

Bates, D.M., Sarkar, D., 2007. Lme4: linear mixed-effects models using S4 classes. R package version 0.9975-12.

Billings, S.A., Gaydess, E.A., 2008. Soil nitrogen and carbon dynamics in a fragmented landscape experiencing forest succession. Landsc. Ecol. 23, 581–593.

Bremner, J.M., 1996. Nitrogen-total. In: Sparks, D.L. (Ed.), Methods of Soil Analysis, Part 3, Chemical Methods – SSSA Book Series no. 5, Madison, Wisconsin, pp. 1085–1121.

Brundrett, M.C., 1991. Mycorrhizas in ecosystems. Adv. Ecol. Res. 21, 171–313.

Davison, J., Öpik, M., Daniell, T.J., Moora, M., Zobel, M., 2011. Arbuscular mycorrhizal fungal communities in plant roots are not random assemblages. FEMS Microbiol. Ecol. 78, 103–115.

Didham, R.K., 2010. Ecological Consequences of Habitat Fragmentation. In: Encyclopedia of Life Sciences (ELS). John Wiley and Sons Ltd., Chichester.

Didham, R.K., Kapos, V., Ewers, R.M., 2012. Rethinking the conceptual foundations of habitat fragmentation research. Oikos 121, 161–170.

Douglas, D.J.T., Vickery, J.A., Benton, T.G., 2010. Variation in arthropod abundance in barley under varying sowing regimes. Agric. Ecosys. Environ. 135, 127–131.

Fahrig, L., 2003. Effects of habitat fragmentation on biodiversity. Annu. Rev. Ecol. Evol. Syst. 34, 487–515.

Fisher, J., Lindenmayer, D.B., 2007. Landscape modification and habitat fragmentation: a synthesis. Global Ecol. Biogeogr. 16, 265–280.

Galetto, L., Aguilar, R., Musicante, M., Astegiano, J., Ferreras, A., Jausoro, M., et al., 2007. Fragmentación de hábitat, riqueza de polinizadores, polinización y reproducción de plantas nativas en el Bosque Chaqueño de Córdoba, Argentina. Ecol. Austral. 17, 67–80.

Grilli, G., Galetto, G., 2009. Remoción de frutos de una especie invasora (*Lantana camara* L.) en el Bosque Chaqueño de Córdoba (Argentina). Ecol. Austral. 19, 149–156.

Grilli, G., Urcelay, C., Galetto, G., 2012. Forest fragment size and nutrient availability: complex responses of mycorrhizal fungi in native–exotic hosts. Plant Ecol. 213, 155–165.

Grime, J.P., 2001. Plant Strategies, Vegetation Processes, and Ecosystem Properties, 2nd ed. Wiley, Chichester.

Hoeksema, J.D., Chaudhary, V.B., Gehring, C.A., Johnson, N.C., Karst, J., Koide, R.T., Pringle, A., Zabinski, C., Bever, J.D., Moore, J.C., Wilson, G.W.T., Klironomos, J.N., Umbanhowar, J., 2010. A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. Ecol. Lett. 13, 394–407.

Johnson, N.C., Graham, J.H., 2013. The continuum concept remains a useful framework for studying mycorrhizal functioning. Plant Soil 363, 411–419.

Jumpponen, A., 2001. Dark septate endophytes – are they mycorrhizal? Mycorrhiza 11, 207–211.

Koide, R.T., 2010. Mycorrhizal symbiosis and plant reproduction. In: Koltai, H., Kapulnik, Y. (Eds.), Arbuscular Mycorrhizas: Physiology and Function, 2nd ed. Springer, pp. 297–320.

Kuo, S., 1996. Phosphorus. In: Sparks, D.L. (Ed.), Methods of Soil Analysis. Part 3. Chemical Methods. SSSA Book Series, vol. 5. Soil Science Society of America, Madison, WI, pp. 869–919.

Lekberg, Y., Koide, R.T., Rohr, J.R., Wolfe, L.A., Morton, J.B., 2007. Role of niche restrictions and dispersal in the composition of arbuscular mycorrhizal fungal communities. J. Ecol. 95, 95–105.

Lindenmayer, D.B., Fischer, J., 2006. Habitat fragmentation and landscape change: an ecological and conservation synthesis. Island Press, Washington, DC.

Lindenmayer, D.B., Fischer, J., 2007. Tackling the habitat fragmentation panchreston. Trends Ecol. Evol. 22, 127–132.

Luti, R., Beltrán de Solis, M.A., Galera, F.M., Ferreira, N.M., Berzal, M., Nores, M., Herrera, M.A., Cabrera, J.A.C., 1979. VI. Vegetación. In: Vázquez, J.B., Miatello, R.A., Roqué, M.E. (Eds.), Geografía física de la provincia de Córdoba. Boldt, Buenos Aires, pp. 297–368.

Mangan, S.A., Eom, A.-H., Adler, G.H., Yavitt, J.B., Herre, E.A., 2004. Diversity of arbuscular mycorrhizal fungi across a fragmented forest in Panama: insular spore communities differ from mainland communities. Oecologia 141, 687–700.

McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.L., Swan, J.A., 1990. A method which gives an objective measure of colonization of roots by vesicular–arbuscular mycorrhizal fungi. New Phytol. 115, 495–501.

Moglia, G., Giménez, A.M., 1998. Rasgos anatómicos característicos del hidrosistema de las principales especies arbóreas de la región chaqueña argentina.. Rev. Invest. Agraria Sist. Rec. Forest. 7, 53–71.

Mulvaney, R.L., 1996. Nitrogen—inorganic forms. In: Sparks, D.L., Page, A.K.L., Helmke, P.A., Soltanpour, P.N., Tabatabai, M.A., Johnston, C.T., Summer, M.E. (Eds.), Methods of Soil Analysis, Part 3: Chemical Methods. Soil Science Society of America, Madison, WI, pp. 1123–1184.

Nelson, D.W., Sommers, L.E., 1996. Total carbon, organic carbon, and organic matter. In: Sparks, D.L., (Ed.), Methods of Soil Analysis, Part 3, Chemical Methods – SSSA Book Series no. 5, Madison, Wisconsin, pp. 961–1010.

Newsham, K.K., 2011. A meta-analysis of plant responses to dark septate root endophytes. New Phytol. 190, 783–793.

Obeso, J.R., 2002. The cost of reproduction in plants. Tansley review no. 139. New Phytol. 155, 321–348.

Ordonez, A., Wright, I.J., Olff, H., 2010. Functional differences between native and alien species: a global-scale comparison. Funct. Ecol. 24, 1353–1361.

Peay, K.G., Bruns, T.D., Kennedy, P.G., Bergemann, S.E., Garbelotto, M., 2007. A strong species–area relationship for eukaryotic soil microbes: island size matters for ectomycorrhizal fungi. Ecol. Lett. 10, 470–480.

Pérez, M., Urcelay, C., 2009. Differential growth response to arbuscular mycorrhizal fungi and plant density in two wild plants belonging to contrasting functional types. Mycorrhiza 19, 517–523.

Ponce, A.M., Grilli, G., Galetto, L., 2012. Frugivory and ornitochorous fruits removal in Chaco forest fragments of Córdoba (Argentina). Bosque 33, 33–41.

Poulton, J.L., Koide, R.T., Stephenson, A.G., 2001. Effects of mycorrhizal infection, soil phosphorus availability and fruit production on the male function in two cultivars of *Lycopersicon esculentum*. Plant, Cell Environ. 24, 841–849.

Poulton, J.L., Bryla, D., Koide, R.T., Stephenson, A.G., 2002. Mycorrhizal infection and high soil phosphorus improve vegetative growth and the female and male functions in tomato. New Phytol. 154, 255–264.

Pringle, A., Bever, J.D., Gardes, M., Parrent, J.L., Rillig, M.C., Klironomos, J.N., 2009. Mycorrhizal symbioses and plant invasions. Annu. Rev. Ecol. Evol. Syst. 40, 699–715.

R Core Development Team, 2010. R: a language and environment for statistical computing. <http://www.R-project.org>.

Rinaudo, V., Bàrberi, P., Giovannetti, M., van der Heijden, M.G.A., 2010. Mycorrhizal fungi suppress aggressive agricultural weeds. Plant Soil 333, 7–20.

Smith, S.E., Read, D.J., 2008. Mycorrhizal symbiosis. Academic Press, New York, pp. 787.

Subils, R., 1977. Las especies de *Euphorbia* de la República Argentina. Kurtziana 10, 83–248.

Sumner, M.E., Miller, W.P., 1996. Cation exchange capacity and exchange coefficients. In: Sparks, D.L. (Ed.), Methods of Soil Analysis, Part 3, Chemical Methods – SSSA Book Series no. 5, Madison, Wisconsin, pp. 1085–1121.

Treseder, K.K., 2004. A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO_2 in field studies. New Phytol. 164, 347–355.

Tscharntke, T., Tylianakis, J.M., Rand, T.A., Didham, R.K., Fahrig, L., Batáry, P., Bengtsson, J., Clough, Y., Crist, T.O., Dormann, C.F., Ewers, R.M., Fründ, J., Holt, R.D., Holzschuh, A., Klein, A.M., Kleijn, D., Kremen, C.L., Landis, D.A., Laurance, W., Lindenmayer, D., Scherber, C., Sodhi, N., Steffan-Dewenter, I., Thies, C., van der Putten, W.H., Westphal, C., 2012. Landscape moderation of biodiversity patterns and processes – eight hypotheses. Biol. Rev. 87, 661–685.

Urcelay, C., Tecco, P.A., Pérez, M., Grilli, G., Longo, M.S., Battistella, R., 2011. Mycorrhizal status and responsiveness of early successional communities from chaquean region in central Argentina. In: Pagano, M. (Ed.), Mycorrhiza: Ocurrence in Natural and Restored Environments, 1st ed. Nova Science Publishers, New York, pp. 147–163.

van der Heijden, M.G.A., Bardgett, R.D., Van Straalen, N.M., 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol. Lett. 11, 296–310.

Varga, S., 2010. Review: effects of arbuscular mycorrhizas on reproductive traits in sexually dimorphic plants. Span. J. Agric. Res. 8, 11–24.

Varga, S., Kytöviita, M.-M., 2010. Gender dimorphism and mycorrhizal symbiosis affect floral visitors and reproductive output in *Geranium sylvaticum*. Funct. Ecol. 24, 750–758.

Veiga, R.S.L., Jansa, J., Frossard, E., van der Heijden, M.G.A., 2011. Can arbuscular mycorrhizal fungi reduce the growth of agricultural weeds? PlosOne 6, e27825.

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., van der Putten, W.H., Wall, D.H., 2004. Ecological linkages between aboveground and belowground biota. Science 304, 1629–1633.

Zak, M.R., Cabido, M., Hodgson, J.G., 2004. Do subtropical seasonal forests in the Gran Chaco, Argentina, have a future? Biol. Conserv. 120, 589–598.