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Forest fragment size and nutrient availability: complex responses of mycorrhizal fungi in native–exotic hosts

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Abstract In the past few decades, it has been widely accepted that forest loss due to human actions alter the interactions between organisms. We studied the relationship between forest fragment size and arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSE) colonization, and the AMF spore communities in the rhizosphere of two congeneric *Euphorbia* species (native and exotic/invasive). We hypothesized that these fungal variables will differ with fragment size and species status, and predicted that (a) AMF and DSE colonization together with AMF spore abundance and diversity would be positively related to forest fragment size; (b) these relationships will differ between the exotic and the native species; and (c) there will be a negative relationship between forest fragment size and the availability of soil nutrients (NH_4^+, NO_3^-, and phosphorus). This study was performed in the eight randomly selected forest fragments (0.86–1000 ha), immersed in an agricultural matrix from the Chaquean region in central Argentina. AMF root colonization in the native and exotic species was similar, and was positively related with forest fragment size. Likewise, AMF spore diversity and spore abundance were higher in the larger fragments. While DSE root colonization in the native host was positively related with forest fragment size, DSE colonization in the exotic host showed no relationship. Soil nutrients contents were negatively related with forest fragment size. In addition, NH_4^+ and NO_3^- were negatively correlated with AMF spores abundance and root colonization and with DSE colonization in the native species. The results observed in this study show how habitat fragmentation might affect the interaction between key soil components, such as rhizospheric plant-fungal symbiosis and nutrient availability. These environmental changes may have important consequences on plant community composition and nutrient dynamics in this fragmented landscape.

Keywords Habitat fragmentation · Arbuscular mycorrhizal fungi · Dark septate endophytes · Spores communities · Nutrients

Introduction

Forest loss and habitat fragmentation due to human actions are the most important causes of declining biodiversity (Saunders et al. 1991). Landscape modification and arrangement of remnant forest fragments have been shown to have major effects on population dynamics (Barbosa and Marquet 2002). Habitat

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fragmentation is a complex concept usually defined as a landscape-scale process that includes, besides fragmentation per se (i.e., a simple subdivision of habitat without habitat loss), a patchiness of the original matrix, habitat loss and patch isolation (Fahrig 2003). However, several studies commonly use forest fragment area as a good indicator of the relationship between habitat fragmentation and fungal organisms (Benitez-Malvido et al. 1999; Berglund and Jonsson 2001; Mangan et al. 2004; Brown et al. 2006; Peay et al. 2007; Jones et al. 2008). In addition, there are dozens of articles showing the ubiquitous species–area curve, representing the collective responses by population of different species to shifts in habitat area (Collinge 2009 and references therein). Nevertheless, the relationship between organisms and forest fragment size are species-specific and could be positive, negative, or neutral (Ewers and Didham 2006; Galetto et al. 2007).

Despite that belowground organisms have a major role in maintaining the ecosystem functioning and ecological processes (Fitter et al. 2004; Wardle et al. 2004), most of the ecological knowledge developed in fragmented landscapes has principally centered in aboveground organisms (mainly plants and animals) and their interactions. Few studies have examined the relationship between fragmented environments and belowground organisms, such as microarthropods, nematodes, and fungi (e.g., Gonzalez and Chaneton 2002; Mangan et al. 2004), and therefore the consequences of forest fragmentation on belowground biota still remains largely unknown. In particular, little is known about the relationship between the most widely distributed plant root symbionts, arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSE), and forest fragment size (Smith and Read 2008; Jumpponen and Trappe 1998; Mandyam and Jumpponen 2008). AMF are an important soil microbial component conferring benefits to different plant species, such as uptake of inorganic N and P (Smith and Read 2008), while DSE have been suggested to increase plant performance through organic N mineralization (Newsham 2011). Both fungal interactions with plant species might be affected by alterations in the abiotic environment such as soil nutrient availability (Smith and Jones 2004; Jumpponen 2001). In addition, these fungal symbionts may be directly affected by habitat fragmentation (Mangan et al. 2004). Some evidence suggests an inverse relationship between forest fragment size, nitrogen mineralization, and nutrition in plants due to a greater root biomass with higher N concentration in smaller fragments. Such amount of root decomposition into soil organic matter eventually changes biomass quality, quantity, and resulting N cycling parameters (Billings and Gaydess 2008). In addition, higher nutrient availability negatively affects the abundance and diversity of fungi (Treseder 2004 and references there in; Mandyam and Jumpponen 2008).

Another consequence of landscape fragmentation could be the emergence of favorable conditions for the establishment and invasion by exotic plant species (Hobbs and Huenneke 1992; Lindenmayer and Fischer 2006). Exotic plants might alter AMF communities by showing a different physiology, phenology, and life history than the native plants (Hawkes et al. 2006). They could favour certain AMF species and promote changes in AMF community composition present in their roots by supplying higher amounts of C (Wolfe and Klironomos 2005). In turn, AMF feedbacks on plants may have an important role on exotic establishment and growth (Klironomos 2002). On the other hand, DSE show a wide distribution without apparent host specificity (Mandyam and Jumpponen 2008), but the effect of plant composition on these fungi remain unknown.

Altogether, the evidence suggests that fungal root symbiosis could be affected by forest fragmentation through complex pathways that include fragmentation *per se*, changes in nutrient dynamics and host identity in plant communities.

In this study, we analyzed the AMF and DSE colonization and the AMF spore communities in the rhizosphere of two congeneric species, *Euphorbia acerensis* (native) and *Euphorbia dentata* (invasive exotic), and nutrient availability along a fragmentation gradient in the Chaco forest in central Argentina. We hypothesized that fungal colonization and spore diversity would increase with forest fragment size, while nutrient availability would decrease with fragment size. Furthermore, these relationships may be stronger for the native than for the exotic congeneric species because native hosts should be co-adapted to the local microbial community (Hallett 2006; Johnson 2010).

Methods

Study site

This study was conducted in a fragmented landscape of the Chaco forest district in Córdoba, central Argentina (Cabrera 1976). The study area coordinates range between 31°11′19″S; 64°16′02″W; and 31°13′05″S; 64°15′55″W. The rainy season occurs between October and May with an 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. These forest fragments, which are rocky outcrops immersed in an agricultural matrix, remain in the landscape because of their futility for agriculture and have an average isolation age of approximately 60 years. In areas surrounding the fragments, soybean is sown in summer and spring, maize in summer, and wheat in winter. Eight fragments from 0.86 to 1000 ha were selected randomly in the landscape; these fragments represent a size gradient of the Chaco forest (Fig. 1). Despite the low size of smaller fragments, the plants were collected several meters from the border, avoiding major edge effects and selecting sites lacking visible signs of soil disturbance.

We considered for this study two abundant congeneric herbs in Chaco forest fragments: *E. acerensis* Boiss and *E. dentata* Michx. Both species present AMF and DSE fungal symbionts. Although this is only one pair of species, among herbs species there are similar values of root mycorrhizal colonization at Chaco forest (Urcelay and Battistella 2007). Both species have a similar reproductive biology, are annual herbs and can reach 45–50 cm of height (Subils 1977). Annual herbs were chosen because fragmentation effects might be better expressed on plants with short life cycles. *E. acerensis* is a native herb from the region, whereas *E. dentata* is an invasive exotic weed from North America. Individuals were collected between April and May of 2008, at the end of the rainy season. Ten individuals of each plant species per fragment were selected at the end of the reproductive stage; a total of 160 individuals, 80 per species. The congeneric species co-occur in similar microhabitats and were collected beneath understorey cover in the forest fragments. Plants were gathered together with 15 cm of soil depth surrounding the entire rhizosphere (most of the root system recovered) and brought to the laboratory.

AMF and DSE colonization

Roots were washed and separated carefully; then those with a diameter less than 2 mm were selected. All dead and damaged roots were discarded. Potassium hydroxide (KOH) was utilized to clarify 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 in semi permanent slides in polyvinyl-lactic acid-glycerol; we prepared one slide per individual because of 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 colonizations were determined following the magnified intersection method of McGonigle et al. (1990) using a compound microscope (Nikon optical, Model E200), 200× magnification. Percentage of root colonization (total, vesicle, arbuscule, and dark septate) was assessed as the proportion of total root intersections that were colonized.

Quantification of AMF spores

Spore density and diversity were determined from six samples of rhizospheric soil of randomly selected *Euphorbia* individuals per forest fragment: three from the native and three from the exotic. Spores were extracted from 50 g of soil using a wet sieving and centrifugal flotation technique (Daniels and Skipper 1982). Spores were counted using a light microscope Wild M5A, 500× magnification. Only viable spores (based in general appearance) were counted and then identified (when it was possible) to species level using morpho-taxonomic criteria (http://invam.caf.wvu.edu/HH). Spore density was expressed as number of spores in 100 g soil dry weight. We used spores to estimate Shannon's diversity index and

Fig. 1 Study site in the Chaco forest of central Argentina. In *black*, the studied fragments

evenness index of each AMF community. Evenness index (E) was calculated following Pielou (1969): $E = H'/\ln(S)$ where H' is the Shannon diversity index, and S is the species richness.

Soil nutrient analysis

Samples of soil were collected to assess nutrient availability and to characterize the soil of each forest fragment. Soil samples were stored at 4° C. Three subsamples of 100 g of soil per fragment were separated from the rhizospheric soil of randomly selected *Euphorbia* individuals and sent to the Edaphologic Laboratory of the Faculty of Agronomic Sciences (Universidad Nacional de Córdoba) where nutrients were measured. The methods adopted to measure nutrients were the Bray and Kurtz No. 1 for phosphorus; Kjeldahl for total nitrogen; direct potentiometry for nitrate and ammonia; Walkley and Black for organic matter; steam distillation method after treatment of the samples with ammonium acetate for cations; and 10 g air–dry soil mixed with 10-ml deionized water for pH (Sparks 1996). Additional data are given in Supplementary material 1.

Statistical analysis

We first examined mycorrhizal fungi colonization (total and vesicle AMF and DSE) along a forest fragmentation gradient in two plant species. Each mycorrhizal variable was analyzed with generalized linear mixed models (GLMM) in R v.2.12.0 (R Core Development Team 2010) with plant species (native and exotic) and LogArea as fixed effects, including the interaction term. Generalized mixed-effects models use the concept of random effects to emulate the randomness inherent in the data (Millar and Anderson 2004). "Individuals" term was nested within "forest fragments" and specified as random effect to avoid spatial pseudo-replication (e.g., Underwood 1997; Jauker et al. 2009; Douglas et al. 2010). AMF spore diversity and nutrients relationship with forest fragment size was analyzed with GLMM in the same way but excluding species effect. Models were fitted with a Gamma error structure and log-link. The significance of model terms was assessed by examining changes in the deviance using chi-square test.

The LogArea × Species interaction was marginally significant ($\chi^2 = 1.31$, $P = 0.07$) for DSE root colonization. Therefore, we analyzed DSE colonization of each plant species separately (native and exotic) (Table 1d–e).

The Spearman correlation coefficient was adopted to examine the relationships between mycorrhizal colonization and nutrient availability. We used the statistical package SPSS (15.0) for the correlation analyses.

Table 1 GLMM outputs of mycorrhizal colonization in two plant species (native and exotic) along a fragmentation gradient ($n = 8$) in Córdoba, Argentina

	Term	df	Deviance (χ^2)	P
(a) Total mycorrhizal colonization	Species	1	0.00	0.88
	LogArea	1	4.57	<0.0001
	LogArea × Species	1	0.00	0.99
(b) Vesicular colonization	Species	1	3.92	0.16
	LogArea	1	95.80	<0.0001
	LogArea × Species	1	0.33	0.68
(c) DSE colonization	Species	1	0.22	0.44
	LogArea	1	0.60	0.21
	LogArea × Species	1	1.31	0.07
(d) Native DSE colonization	LogArea	1	1.81	0.01
(e) Exotic DSE colonization	LogArea	1	0.095	0.65

Results

AMF and DSE colonization

The values of root colonization ranged between 30 and 95% AMF and 5–60% DSE. Colonization by vesicles in the roots of both species showed values between 0 and 25%. Colonization by arbuscules was very low ranging between 0 and 3%. AMF and DSE colonizations were observed in all the individuals of both species.

The patterns observed in total AMF colonization were not significantly different between species ($\chi^2 = 0.00$, $P = 0.88$). Total AMF colonization increased with forest fragment area size ($\chi^2 = 4.57$, $P \leq 0.0001$) (Table 1a; Fig. 2a). AMF colonization in both plant species reached the highest percentages in the continuous forest. In addition, AMF colonization by vesicles also increased with fragment area size ($\chi^2 = 95.8$, $P \leq 0.0001$) (Table 1b; Fig. 2b).

DSE root colonization showed no relationship with fragment area ($\chi^2 = 0.6$, $P = 0.21$). However, the interaction term LogArea × Species was marginally significant ($\chi^2 = 1.31$, $P = 0.07$). Therefore, we analyzed each species separately and found that DSE root colonization increased with fragment area on the native *E. acerensis* ($\chi^2 = 1.81$, $P = 0.01$) but not on the exotic *E. dentata* ($\chi^2 = 0.095$, $P = 0.65$) (Table 1d–e).

AMF spores abundance and diversity in the rhizosphere of plant species

Shannon's diversity index ($\chi^2 = 1.03$, $P = 0.004$) and abundance based on AMF spores occurrence increased significantly, except for abundance that was marginally significant ($\chi^2 = 2.46$, $P = 0.056$), with forest fragment size. Fragments larger than 6 ha tended to have higher spore abundance than the smallest fragments. While evenness showed no relationship with forest fragment size ($\chi^2 = 0.02$, $P = 0.69$) (Table 2a–c; Fig. 3a–c). When AMF spore morphospecies were examined individually, they displayed different responses to forest fragment size. We identified 12 morphospecies (Supplementary material 2) and among them, only three, belonging to Glomeraceae, increased with forest fragment size (Table 2d–e). Spore community composition in the rhizosphere of both *Euphorbia* were similar between the native and the exotic species (data not shown).

Nutrient availability and their relationship with mycorrhizal colonization

Soil chemical analyses revealed a significant negative relationship between fragment area and P, NO_3^-, and NH_4^+ availability (Table 3a–c; Fig. 4a–c).

In the native *E. acerensis*, total AMF colonization, colonizations by vesicles and by DSE were negatively correlated with NO_3^- and NH_4^+

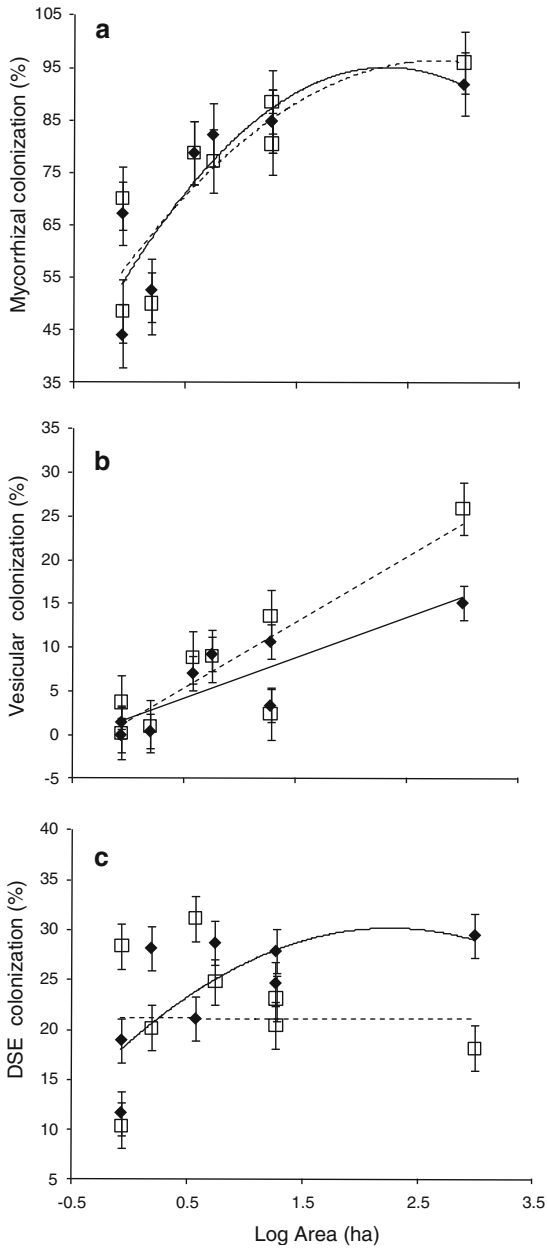

Fig. 2 Relationship between fragment size and fungal colonization: **a** total mycorrhizal colonization of (*filled diamond*) *E. acerensis* and (*open square*) *E. dentata*, **b** vesicular colonizations of (*filled diamond*) *E. acerensis* and (*open square*) *E. dentata*, and **c** DSE colonizations of (*filled diamond*, *solid line*) *E. acerensis* and (*open square*, dashed line) *E. dentata* with Chaco forest fragments areas ($n = 8$) from Córdoba, Argentina. Data are the mean ± SE

contents and electric conductivity of soil. In the exotic *E. dentata*, total AMF colonization was negatively correlated with NO_3^- and NH_4^+ contents. In addition, colonization by arbuscules in *E. dentata* showed a strong negative correlation with total nitrogen in soil (Table 4).

Discussion

AMF–DSE colonization and AMF spore diversity

As far as we know, this is the first evidence showing that AMF spore diversity and mycorrhizal colonization in the rhizosphere of native and exotic plants is negatively related to forest fragment size. These results contrast with the only study published to date, which evaluated AMF spore diversity in a fragmented landscape in Panama (Mangan et al. 2004). These authors found no differences in species richness and Shannon diversity in forests with different sizes.

It is worth mentioning that soil AMF spore composition may not necessarily reflect root-colonizing AMF diversity (Clapp et al. 1995; Sykorova et al. 2007). In addition, differences might exist between classical morphotaxonomy and sequence-based taxonomy. Because both methods present limitations (Hibbett et al. 2009), it seems that they are rather complementary than mutually exclusive. Certainly, further research using molecular tools would contribute to have a better picture (Öpik et al. 2010) regarding the relationships between forest fragmentation and AMF communities in the study region.

Nutrient availability and its relationship with mycorrhizal fungi in forest fragments

The observed negative relationships between nutrient availability and forest fragment size are consistent with a recent study in a fragmented landscape in the northern hemisphere despite differences in geographical location, plant communities, and soil characteristics (Billings and Gaydess 2008). Reduction of nitrogen and phosphorus availability with increasing fragment size could be one of the possible explanations for the enhanced AMF colonization in both plant species and AMF spore abundance and diversity. This conjecture is supported by many field studies that show a negative influence of increasing nutrient availability on mycorrhizal fungi (Treseder

Table 2 GLMM outputs of AMF communities in the rhizosphere of two plant species (native and exotic) along a fragmentation gradient ($n = 8$) in Córdoba, Argentina

	Term	df	Deviance (χ^2)	*P*
(a) Shannon index	Species	1	0.012	0.75
	LogArea	1	1.029	0.004
	LogArea × Species	1	0.012	0.33
(b) Evenness	Species	1	0.022	0.19
	LogArea	1	0.021	0.69
	LogArea × Species	1	0.053	0.53
(c) Total spores abundance	Species	1	0.111	0.69
	LogArea	1	2.462	0.056
	LogArea × Species	1	1.214	0.18
(d) *Glomus* sp2	LogArea	1	14.81	0.043
(e) *Glomus* sp7	LogArea	1	16.714	<0.0001
(f) *Glomus* (*Sclerocistys*)	LogArea	1	21.474	0.029
(g) *Glomus* sp9	LogArea	1	51.81	0.076

Only morphospecies with significant relationship with forest fragment area are shown

2004 and references therein). The strong negative correlation between AMF colonization and nitrogen (ammonia and nitrate), but not phosphorous, availability found here, suggests that mycorrhizal colonization is more sensitive to nitrogen than to phosphorous in this ecosystem.

It has been suggested that DSE might be nutritionally important for plants (Jumpponen 2001; Barrow and Osuna 2002). A recent meta-analysis shows that DSE promote mineralization of organic N to inorganic N forms available for plants (Newsham 2011). Moreover, DSE may increase plant biomass and N and P tissue contents (Newsham et al. 2009; Alberton et al. 2010; Newsham 2011). In this study, similar to that observed for AMF, DSE colonization in the native *Euphorbia* showed an inverse relationship with nitrogen availability along the fragmentation gradient. This evidence suggests the existence of a nutritional role in this plant-fungal symbiosis (Alberton et al. 2010; Newsham 2011). The lack of relationship between DSE colonization in the exotic *Euphorbia* and habitat fragmentation and/or nutrient availability suggests the functional role of the plant-DSE interaction is context dependent.

These results could be related to direct consequences of area size reduction (Ewers and Didham 2006). For example, smaller fragments present higher light intensity, temperature variability, and soil disturbance that in turn could negatively affect mycorrhizal fungi, either colonization, or spore communities (Hayman 1974; Heinemeyer et al. 2003; Staddon et al. 2003; Urcelay et al. 2009). In comparison to other AMF lineages (e.g., Gigasporaceae and Acaulosporaceae), Glomeraceae develops higher intraradical colonization rates (Maherali and Klironomos 2007). Therefore, reductions in AMF colonization with decreasing fragment size could be a direct consequence of the observed reductions of spore abundance in Glomeraceae.

Mycorrhizal fungi and their relationship with native and exotic hosts

Mycorrhizal fungi might affect directly (positive or negative) plant growth depending on the host identity (i.e., native or exotic) (Wolfe and Klironomos 2005). For example, AMF root colonization rates were positively related with growth and development of exotic annual herbs in France (Fumanal et al. 2006). However, the outcome of plant–fungus interaction might be the result of the co-adaptation time between hosts. It has been suggested that symbiosis in long-term partners may be tighter than in novel combinations of partners (Hallett 2006; Johnson 2010). The results observed here partially support this hypothesis. According to our results, this might apply to DSE but not AMF. Although the identity of the fungi colonizing plant roots remains unknown, the differential responses of AMF and DSE to the fragmentation gradient suggests that the tightness of the relationship or the sensitivity of the symbiotic

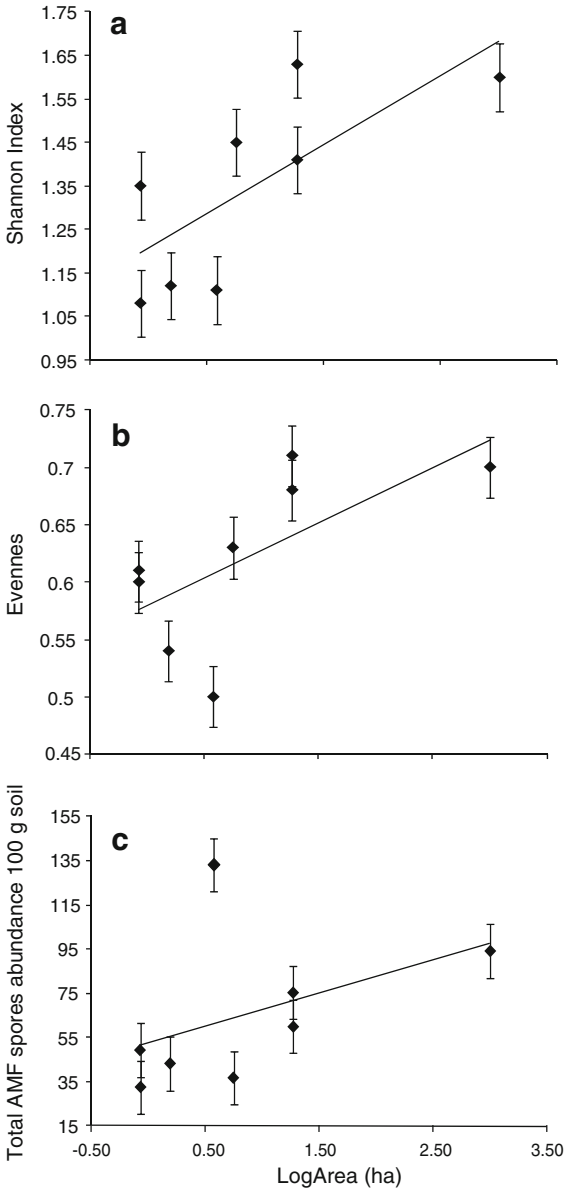

Fig. 3 Relationship between fragment size and AMF spores: **a** mean values of Shannon's diversity index, **b** evenness, and **c** total AMF spore abundance per 100 g of soil of AMF spore morphotypes with Chaco forest fragment areas ($n = 8$) from Córdoba, Argentina. Data are the mean ± SE

interaction with DSE depends on host identity. Molecular identification of the fungal lineages that are colonizing the roots in both plant species in the fragmentation gradient may provide further insights on the observed patterns. Certainly this issue deserves further attention in future studies.

Table 3 GLMM outputs of nutrients availability along a fragmentation gradient ($n = 8$) in Córdoba, Argentina

	Term	df	Deviance (χ^2)	P
(a) NO_3	LogArea	1	11.58	<0.0001
(b) NH_4	LogArea	1	1371.7	0.0015
(c) P	LogArea	1	5386.1	0.024

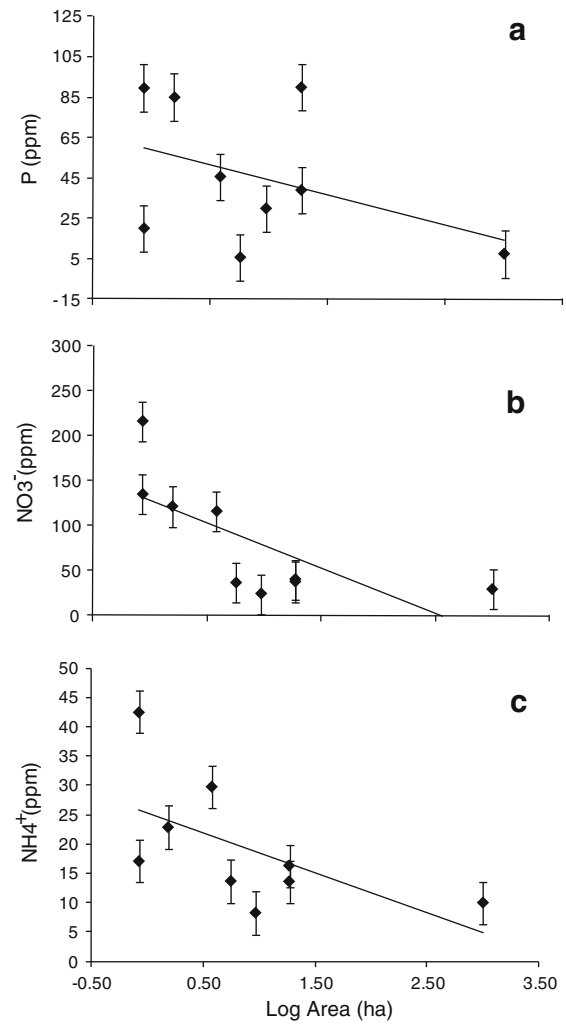

Fig. 4 Relationship between fragment size and nutrient availability: **a** P (ppm), **b** NO_3^- (ppm), and **c** NH_4^+ (ppm) concentrations with Chaco forest fragment areas ($n = 8$) from Córdoba, Argentina. Data are the mean ± SE

Conclusions

To our knowledge, this is the first report that shows a negative relationship of the interaction between plants

Table 4 Spearman correlation's coefficient between mycorrhizal colonization in two Euphorbia species (*E. acerensis* and *E. dentata*, native and invasive, respectively) and edaphic soil conditions of eight forest fragments of Chaco forests of Córdoba, Argentina

	Euphorbia acerensis				*Euphorbia dentata*			
	TMC	VC	AC	DSEC	TMC	VC	AC	DSEC
Total nitrogen	−0.54	−0.44	−0.14	−0.48	−0.49	−0.48	−0.74*	0.22
NO_3^-	−0.91**	−0.93**	−0.25	−0.86**	−0.83*	−0.86**	−0.13	−0.04
NH_4^+	−0.91**	−0.86**	−0.07	−0.76*	−0.81*	−0.83*	−0.43	0.02
P	−0.31	−0.36	0.18	−0.45	−0.21	−0.38	−0.18	−0.24
Carbon	−0.19	−0.07	−0.34	−0.10	−0.17	−0.14	−0.67	−0.14
C:N rate	0.62	0.69	−0.25	0.69	0.64	0.62	−0.06	0.19
Organic matter	−0.19	−0.07	−0.34	−0.09	−0.17	−0.14	−0.68	0.14
pH	−0.36	−0.45	−0.39	−0.24	−0.54	−0.31	−0.31	−0.69
Ca	−0.31	−0.41	−0.07	−0.62	−0.17	−0.45	−0.36	−0.26
Mg	−0.57	−0.58	−0.29	−0.33	−0.46	−0.58	−0.08	−0.57
K	−0.09	−0.23	−0.10	−0.23	−0.04	−0.30	−0.08	−0.53
Na	−0.23	−0.14	−0.09	−0.23	−0.26	−0.02	0.53	0.24
Electric conductivity	−0.88**	−0.86**	0.15	−0.81*	−0.81*	−0.79*	−0.21	−0.02
CIC	−0.60	−0.55	−0.07	−0.48	−0.45	−0.52	−0.04	−0.33

TMC total mycorrhizal colonization, *VC* vesicular colonization, *AC* arbuscular colonization, *DSEC* dark septate endophytes colonization

* $P < 0.05$; ** $P < 0.01$

and mycorrhizal fungi (i.e., AMF and DSE) with forest fragment size. The strong negative correlations between nitrate and ammonia with AMF colonization and spores communities suggest that nitrogen availability more than phosphorus, could be a key factor underlying the population's dynamics of rhizospheric fungi in forest fragments. In the case of DSE symbiosis, the relationship differs between the native and the exotic and was not related to nutrient availability. This suggests that the effects of forest fragmentation on DSE depend on host identity.

Previous studies from other ecosystems have shown negative relationships between fragmented landscapes and different fungal groups, including ectomycorrhizal fungi (Dickie and Reich 2005; Peay et al. 2007) and wood-decaying fungi (Berglund and Jonsson 2001; Edman et al. 2004). Despite the important phylogenetic and functional differences between these fungal groups, all the evidence together suggests a widespread sensitivity of fungi to forest fragmentation. It is worth mentioning that those studies, like this one, refer to data collected at one time period and to a unique fragmented landscape suggesting that these patterns should be further validated considering temporal and landscape variation of fungal communities.

Overall, the results observed in this study show how an important environmental threat, such as forest fragmentation, affects key soil interactions such as rhizospheric plant–fungal symbiosis. Differences in DSE colonization between native and exotic species along the fragmentation gradient may imply differential growth responses of these plant species to the fungal symbionts. Certainly, this issue deserves further studies because of the implications it has on important processes such as biological invasions at fragmented landscapes.

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References

Alberton O et al (2010) Dark septate root endophytic fungi increase growth of Scots pine seedlings under elevated CO_2 through enhanced nitrogen use efficiency. Plant Soil 328:459–470

Barbosa O, Marquet PA (2002) Effects of forest fragmentation on the beetle assemblage at the relict forest of Fray Jorge, Chile. Oecologia 132:296–306

Barrow JR, Osuna P (2002) Phosphorus solubilization and uptake by dark septate fungi in four wing saltbrush, *Atriplex canescens* (Pursh) Nutt. J Arid Environ 51: 449–459

Benitez-Malvido J et al (1999) Leaf-fungal incidence and herbivory on tree seedlings in tropical rainforest fragments: an experimental study. Biol Conserv 91:143–150

Berglund H, Jonsson BG (2001) Predictability of plant and fungal species richness of old-growth boreal forest islands. J Veg Sci 12:857–866

Billings SA, Gaydess EA (2008) Soil nitrogen and carbon dynamics in a fragmented landscape experiencing forest succession. Landsc Ecol 23:581–593

Brown N et al (2006) Macrofungal diversity in fragmented and disturbed forests of the Western Ghats of India. J Appl Ecol 43:11–17

Brundrett M et al (1996) Working with mycorrhizal in forestry and agriculture. ACIAR Monograph 32

Cabrera A (1976) Regiones fitogeográficas de Argentina. ACME, Buenos Aires

Clapp JP, Young JPW, Merryweather JW, Fitter AH (1995) Diversity of fungal symbionts in arbuscular mycorrhizas from a natural community. New Phytol 130:259–265

Collinge SK (2009) Ecology of fragmented landscapes. The Johns Hopkins University Press, Baltimore, MD

Daniels BAD, Skipper HD (1982) Methods for the recovery and quantitative estimation of propagules from soil. In: Schenck NC (ed) Methods and principles of mycorrhizal research. The American Phytopathological Society, St. Paul, MN

Dickie IA, Reich PB (2005) Ectomycorrhizal fungal communities at forest edges. J Ecol 93:244–255

Douglas et al (2010) Variation in arthropod abundance in barley under varying sowing regimes. Agric Ecosyst Environ 135:127–131

Edman M et al (2004) Abundance and viability of fungal spores along a forestry gradient—responses to habitat loss and isolation? Oikos 104:35–42

Ewers RM, Didham RK (2006) Confounding factors in the detection of species responses to habitat fragmentation. Biol Rev 81:117–142

Fahrig L (2003) Effects of habitat fragmentation on biodiversity. Annu Tev Ecol Evol Syst 34:487–515

Fitter AH et al (2004) Global environmental change and the biology of arbuscular mycorrhizas: gaps and challenges. Can J Bot 82:1133–1139

Fumanal B et al (2006) Which role can arbuscular mycorrhizal fungi play in the facilitation of *Ambrosia artemisiifolia* L. invasion in France? Mycorrhiza 17:25–35

Galetto L 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

Gonzalez A, Chaneton EJ (2002) Heterotroph species extinction, abundance and biomass dynamics in an experimentally fragmented microecosystem. J Anim Ecol 71: 594–602

Grace C, Stribley DP (1991) A safer procedure for routine staining of vesicular-arbuscular mycorrhizal fungi. Mycol Res 95:1160–1162

Hallett SG (2006) Dislocation from coevolved relationships: a unifying theory for plant invasion and naturalization? Weed Sci 54:282–290

Hawkes CV, Belnap J, D'Antonio C, Firestone. MK (2006) Arbuscular mycorrhizal assemblages in native plant roots change in the presence of invasive exotic grasses. Plant Soil 281:369–380

Hayman DS (1974) Plant growth responses to vesicular-arbuscular mycorrhiza. VI. Effect of light and temperature. New Phytol 73:71–80

Heinemeyer A et al (2003) Impact of soil warming and shading on colonization and community structure of arbuscular mycorrhizal fungi in roots of a native grassland community. Glob Change Biol 10:52–64

Hibbett DS et al (2009) Fungal ecology catches fire. New Phytol 184:279–282

Hobbs RJ, Huenneke LF (1992) Disturbance, diversity, and invasion: implications for conservation. Conserv Biol 6:324–337

Jauker F et al (2009) Pollinator dispersal in an agricultural matrix: opposing responses of wild bees and hoverflies to landscape structure and distance from main habitat. Landsc Ecol 24:547–555

Johnson NC (2010) Resource limitation is a driver of local adaptation in mycorrhizal symbioses. PNAS 107: 2093–2098

Jones MD et al (2008) Location relative to a retention patch affects the ECM fungal community more than patch size in the first season after timber harvesting on Vancouver Island, British Columbia. For Ecol Manage 255: 1342–1352

Jumpponen A (2001) Dark septate endophytes—Are they mycorrhizal? Mycorrhiza 11:207–211

Jumpponen A, Trappe JM (1998) Dark septate endophytes: a review of facultative biotrophic root-colonizing fungi. New Phytol 140:295–310

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

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

Luti R et al (1979) VI Vegetación. In: Vázquez JB, Miatello RA, Roqué ME (eds) Geografía física de la provincia de Córdoba. Boldt, Buenos Aires, pp 297–368

Maherali H, Klironomos JN (2007) Influence of phylogeny on fungal community assembly and ecosystem functioning. Science 316:1746–1748

Mandyam K, Jumpponen A (2008) Seasonal and temporal dynamics of arbuscular mycorrhizal and dark septate endophytic fungi in a tallgrass prairie ecosystem are

minimally affected by nitrogen enrichment. Mycorrhiza 18:145–155
Mangan SA et al (2004) Diversity of arbuscular mycorrhizal fungi across a fragmented forest in Panama: insular spore communities differ from mainland communities. Oecologia 141:687–700
McGonigle TP et al (1990) A method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytol 115:495–501
Millar RB, Anderson MJ (2004) Remedies for pseudoreplication. Fish Res 70:397–407
Moglia G, Giménez AM (1998) Rasgos anatómicos característicos del hidrosistema de las principales especies arbóreas de la región chaqueña argentina. Rev de Invest Agraria Sist y Rec Forest 7:53–71
Newsham KK (2011) A meta-analysis of plant responses to dark septate root endophytes. New Phytol 190:783–793
Newsham KK et al (2009) Mycorrhizas and dark septate root endophytes in polar regions. Fungal Ecol 2:10–20
Öpik M et al (2010) The online database Maarj*AM* reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). New Phytol 188: 223–241
Peay KG et al (2007) A strong species-area relationship for eukaryotic soil microbes: island size matters for ectomycorrhizal fungi. Ecol Lett 10:470–480
Pielou E (1969) An introduction to mathematical ecology. Wiley Interscience, New York, p 286
R Core Development Team (2010) R: a language and environment for statistical computing. http://www.R-project.org
Saunders DA et al (1991) Biological consequences of ecosystem fragmentation. Conserv Biol 5:18–32
Smith MD, Jones SD (2004) Exploring functional definitions of mycorrhizas: Are mycorrhizas always mutualisms? Can J Bot 82:1089–1109
Smith SE, Read DJ (2008) Mycorrhizal symbiosis. Academic, New York, NY
Sparks DL (1996) Methods of soil analysis. Part 3. Chemical methods. ASA, SSSA, CSSA, Madison, WI
Staddon PL et al (2003) Mycorrhizal fungal abundance is affected by long-term climatic manipulations in the field. Glob Change Biol 9:186–194
Subils R (1977) Las especies de *Euphorbia* de la República Argentina. Kurtziana 10:83–248
Sykorova Z, Ineichen K, Wiemken A, Redecker D (2007) The cultivation bias: different communities of arbuscular mycorrhizal fungi detected in roots from the field, from bait plants transplanted to the field, and from a greenhouse trap experiment. Mycorrhiza 18:1–14
Treseder KK (2004) A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO_2 in field studies. New Phytol 164:347–355
Underwood AJ (1997) Experiments in ecology. Cambridge University Press, Cambridge, p 504
Urcelay C, Battistella R (2007) Colonización micorrícica en distintos tipos funcionales de plantas herbáceas del centro de Argentina. Ecol Austral 17:179–188
Urcelay C et al (2009) Mycorrhizal community resilience in response to experimental plant functional type removals in a woody ecosystem. J Ecol 97:1291–1301
Wardle DA et al (2004) Ecological linkages between aboveground and belowground biota. Science 304:1629–1633
Wolfe BE, Klironomos JN (2005) Breaking new ground: soil communities and exotic plant invasion. Bioscience 55: 477–487