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Invasion of a xeric forest by an exotic tree species in Argentina: Impacts on the diversity of arbuscular mycorrhizal fungi and pre-existing mutualistic relationships

Camila Abarca[1*], Marcelo Daniel Barrera[2], Marta Cabello[1], Fabricio Valdés[1] and María Silvana Velázquez[1]

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ABSTRACT

This study aimed to analyse the effects of invasion by the exotic *Ligustrum lucidum* on mycorrhizal associations in forests of *Celtis tala,* and to determine the role that these fungi play in the invasion process. We analyzed colonization and response to mycorrhization of both plant species under greenhouse conditions after inoculation with soil obtained from patches of non-invaded native forests and invaded forests. The spores present in pots at the end of the experiment were counted and identified. Colonization of *L. lucidum* was greater in plants inoculated with invaded forest soil, whereas colonization of *C. tala* was greater in plants inoculated with non-invaded native forest soil. Principal component analysis of fungal species abundance showed differences in AMF composition according to host plant and forest type. The results show that *L. lucidum* establishes symbiosis with native AMF, which triggers changes in community structure. These changes favored the mycorrhization of *L. lucidum* and interfered with the mycorrhization of *C. tala*. AMF may have either a direct or indirect role in the invasion process, not only benefiting the invasive species but also preventing the regeneration of native plants.

Keywords: biological invasion, Glomeromycota, mycorrhizal colonization, xeric forest, *Celtis tala*, *Ligustrum lucidum*

Introduction

Biological invasions are one of the most important ecological disturbances. Invasive exotic plants can trigger multiple impacts on the ecosystems in which they are established, modifying the structure and dynamics of the native communities, the nutrient cycles, and the relationships between ecosystem components (Wang *et al.* 2016). Although these effects have been extensively documented, the operating mechanisms are still poorly understood.

The impact generated by an exotic plant on the microbial community of the rhizosphere may act as a factor for its invasion success (Xiao *et al.* 2014; Piper *et al.* 2015). The arbuscular mycorrhizal fungi (AMF, phylum Glomeromycota) are obligate biotrophs found in the rhizosphere of all terrestrial ecosystems, and they colonize the roots of 75 % of plant species (Brundrett 2009). The association of plants with AMF may be a key factor in the composition and other structural components of plant communities due to the wide variety of positive, neutral, or negative responses of different plant species to symbiosis (Klironomos *et al.* 2011).

1 Instituto de Botánica Spegazzini, Universidad Nacional de la Plata, 1900, La Plata, BA, Argentina
2 Laboratorio de Investigación de Sistemas Ecológicos y Ambientales, Universidad Nacional de la Plata, 1900, La Plata, BA, Argentina

* Corresponding author: camila.abrc@gmail.com

For this reason, the role of AMF in the establishment of exotic plants has been widely documented.

The mechanisms that drive AMF and invasive plant interactions are not completely clear yet. It has been observed that invasive plant species tend to associate with cosmopolitan AMF species or to form novel associations in their exotic ranges (Núñez & Dickie 2014). Zhang *et al.* (2010) found that the invasive forb *Solidago canadensis* alters soil AMF community composition because of a fungal-host preference, increasing some AMF species while reducing others. Checinska *et al.* (2019) performed a greenhouse experiment with native and exotic grasses and forbs and found that invasive species showed greater colonization percentages than native species. In this study, AM colonization was not positively correlated with performance in the field for exotic species, while native species showed a positive correlation between colonization and biomass and dominance. In this way, although exotic species may not depend on AMF to establish, they still can greatly affect native species by modifying or degrading AMF communities.

In this study, we evaluated the colonization rates and response to mycorrhization during the early stages of development of *Celtis tala*, commonly known as "tala", and *Ligustrum lucidum*. The tala tree is native to America, and it grows naturally in northwestern Buenos Aires province, Argentina, forming xeric forests named "talares". These talares are currently degraded by the presence and rapid expansion of *L. lucidum,* a tree of Chinese origin that has invaded many regions worldwide, including various forests in Argentina (Dascanio & Ricci 1988; Dascanio *et al*. 1994; Zamora Nasca *et al.* 2014).

This study aimed to analyze the effect that the expansion of *L. lucidum* has on the mycorrhizal associations already established in *C. tala* forests and to determine the role that AMF play in the invasion process. We hypothesized that *L. lucidum* would have the ability to establish symbiosis with some of the native AMF species and that its expansion would lead to changes in the fungal community, modifying the pre-existent mutualisms between native plant species and fungi.

Materials and methods

Study site

Samples were taken at El Destino (35°1' S, 57°17' W), a natural area belonging to "Parque Costero del Sur" Biosphere Reserve, located between the towns of Magdalena and Punta Indio, Buenos Aires province, Argentina. The climate is warm-temperate with a mean annual temperature of 15 °C, the highest maximum mean exceeding 22 °C in January and the lowest minimum mean being below 10 °C in August. The annual rainfall averages 97 cm, with two minima (ca. 6 cm) at the end of winter (August) and in midsummer (January) (Vervoorst 1967). The talares are restricted to the shore-banks deposited by the Platense marine transgression, consisting of a subsoil formed by shells of marine invertebrates and shallow sandy-humic soil. It is classified as a 'Typic Rendoll' or 'rendzina' (Vargas Gil *et al.* 1972; Sánchez *et al.* 1976).

Soil used as inoculum of AMF and seeds were collected from cords that have a tree structure with a spatial gradient in the proportion of *L. lucidum* W.T. Aiton (Oleaceae). Along this gradient, we established the sampling units: forest patches with native vegetation of *Celtis tala* Gill. ex Planch (Celtidaceae), *Scutia buxifolia,* and *Jodinha rhombifolia* (Hook. & Arn.) Reissek 1861 (NAT= non-invaded native forest soil) and from patches completely invaded by *L. lucidum* without native trees (INV=invaded forest soil), located 50 meters from the patch boundary. Three 100 m transects were established parallel to each other and approximately 500 m apart, at the ends of which both contrasting situations were located. Forty-five soil samples (15 subsamples from each site) were taken randomly from three representative sites of each situation (NAT and INV) and combined to form composite inocula. The soil samples were taken from forest soil and not only from *L. lucidum* and *C. tala* rhizosphere to obtain representative samples of the AMF community present in both contrasting situations. Seeds were collected randomly from litter at the sampling spots.

Greenhouse experiment

The experiment was performed from May to October 2018, under greenhouse conditions at "Instituto de Botánica Spegazzini" UNLP, La Plata, Argentina. The seeds of *C. tala* and *L. lucidum* were germinated in a mixture of perlite: vermiculite: terrafertil® (1:1:3) previously tyndalized (steamed for 30 minutes on three successive days). Three weeks after germination, seedlings of uniform size were selected, randomly transplanted to 3 L pots containing 2.5 L of the same substrate described above, and inoculated with 100 g/dry soil from the NAT and INV forest patches. Each pot contained a single plant. Plants grew under controlled temperature (20-25 °C), photoperiod (8/16 h day/night), and humidity (40-60 %) and were watered once a week with filtered water.

To suppress AMF colonization in control treatments, we used a commercial fungicide with Benomyl (50 mg of Benomyl in 100 ml of filtered water per 1 kg of substrate) (Koorem *et al.* 2012). This fungicide suppresses different types of rhizospheric fungi (*e.g.* pathogens, dark septate endophytes) and has been successfully used in numerous studies (Grilli *et al.* 2014). No signs of pathogenic fungi were found in non-treated plants. Therefore, we consider that the absence of AMF was the main effect of the fungicide, as frequently observed in this type of analysis (Gross *et al.* 2010; McCain *et al.* 2011; Deguchi *et al.* 2012). The fungicide was applied in AMF-pots at the beginning of the experiment and every three weeks until the end of the experiment (Gross *et al.* 2010).

The experiment consisted of five replicates x two species (*C. tala*, *L. lucidum*) x two treatments with fungicide (AMF+, AMF-) x two inoculum sources (NAT, INV). The 40 plants used in the experiment grew under greenhouse-controlled conditions and were rotated periodically to avoid spatial and environmental differences. After six months, we removed the plants from the pots and washed the roots carefully to avoid damage.

Mycorrhizal colonization

We evaluated the mycorrhizal colonization of 40 plants by staining fragments of fine roots in good conditions, following the methodology described by Phillips & Hayman (1970). The colonization percentages were calculated according to the segments method described by Cabello *et al.* (2013). We analyzed the presence of external mycelium (EM) (hyphae attached to the external surface of the roots), internal mycelium (IM), entry points (EP), arbuscules (Ar), vesicles (Ve), and coils (Co).

Growth parameters

At the end of the experiment, we measured the vegetative growth parameters of the plants. In each plant, the stem (SL) and root lengths (RL) were measured from the base to the apical meristems using a meter. The aerial and radical structures were oven-dried at 60 °C until constant weight and weighed to obtain the dry biomass (B).

The "mycorrhizal dependence index" (MD) was calculated using the B values, following the formula: $MD = ([B_{\text{inoculated plants}} - B_{\text{non-inoculated plants}}] / B_{\text{inoculated plants}}) \times 100$.

Counting and identification of AMF spores

The isolation of the AMF spores present in the rhizospheric soil of each pot was performed by wet sieving and decanting of 100 g of soil using sieves of different mesh sizes (450, 105, 75, 30 μm) (Gerdemann & Nicolson 1973), followed by sucrose gradient centrifugation (Walker *et al.* 1982). Spore identification followed the descriptions of the International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (INVAM, USA, http://invam.cafwvu.edu) and Błaszkowski (2012). The assignment of AMF morphotypes to families and genera followed the consensus classification of AMF phylogeny (www.amf-phylogeny.com). In each sample, we determined the spore density (spore number in 100 g of dry soil) and relative abundance (ni/N) of each species. For each pot species, richness (S) and diversity indices were calculated.

Data analysis

The non-parametric Kruskal-Wallis test was used to analyze the colonization percentages between treatments. The growth parameters were compared with a bifactorial ANOVA, considering the use of fungicide (AMF+, AMF-) and source of inoculum (NAT, INV) as factors. The normality and homoscedasticity of variances were verified with Shapiro-Wilk´s and Levene´s tests. A Tukey test was used to identify differences between means.

We carried out a principal component analysis (PCA) with the AMF species relative abundance to explore differences between treatments. Data were transformed to log(x+1) and Bray-Curtis was used as a distance measure. The values of spore density, specific richness (S), and diversity indices were subjected to a bifactorial ANOVA considering the plant species and source of inoculum as factors. The normality and homoscedasticity of variances were verified with Shapiro-Wilk´s and Levene´s tests. A Tukey test was used to identify differences between means. Since we did not find spores in good condition in the AMF pots, we did not include them in the analyses.

All statistical analyses were carried out using InfoStat version 2016 (Di Rienzo *et al.* 2016) and PC-ORD 6.0 (McCune & Mefford 2006), with $\alpha = 0.05$.

Results

Colonization

Both species showed colonization by AMF and all the characteristic structures of the mycorrhizal activity, except for coils in the roots of *C. tala* (Fig. 1). The use of fungicide considerably reduced the mycorrhizal colonization of the roots of both species. Plants without fungicide showed total colonization ranging between 25 % and 95 %, whereas those treated with fungicide had less than 10 % colonization.

The total colonization of the invasive *L. lucidum* was greater in plants inoculated with invaded forest soil ($h = 13.36$, $p = 0.0037$). In *C. tala*, the total colonization percentages were higher in plants inoculated with non-invaded native forest soil, although the differences were not significant ($h = 7.70$, $p = 0.0502$). In *L. lucidum*, all fungal structures showed differences between treatments, whereas in *C. tala* only the percentages of EP and Ar differed ($p < 0.05$). Interaction between factors was not significant in all cases, except for IM in *L. lucidum*

Growth parameters and mycorrhizal dependence

The root length of *C. tala* was the only growth parameter that showed significant differences between treatments, being higher in the pots inoculated with INV soil ($f = 12.37$, $p = 0.0066$). The effect of the fungicide was not significant ($p > 0.05$) (Tab. 1). With regard to the MD index, the only negative value was observed in plants of *C. tala* inoculated with INV soil. In the other treatments, the index yielded positive values. Positive values indicate that mycorrhizal colonization is associated with higher biomass in the host plant, while negative values indicate that mycorrhization affects growth negatively.

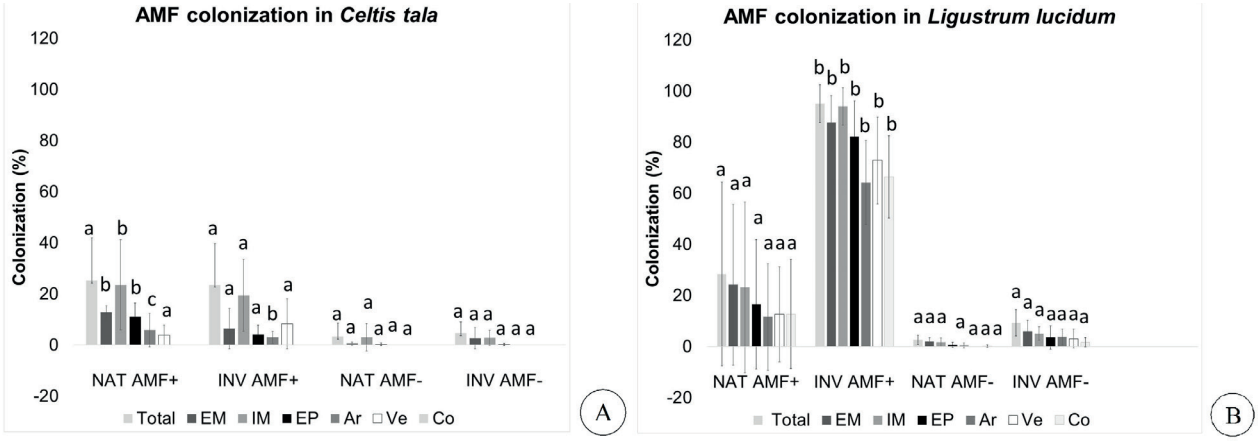

Figure 1. Percentages of external mycelium (EM), internal mycelium (IM), entry points (EP), arbuscules (Ar), vesicles (Ve), coils (Co) and total mycorrhizal colonization in roots of *Celtis tala* (**A**) and *Ligustrum lucidum* (**B**) inoculated with non-invaded native forest soil (untreated: NAT AMF+; treated with fungicide: NAT AMF-) and with invaded forest soil (untreated: INV AMF+; treated with fungicide: INV AMF-). Different letters indicate statistical difference among percentages (Tukey´s test, $p < 0.05$).

Table 1. Mean and standard deviation of growth parameters and mycorrhizal dependence (MD) of *Celtis tala* and *Ligustrum lucidum* inoculated with non-invaded native forest soil (untreated: NAT AMF+; treated with fungicide: NAT AMF-) and with invaded forest soil (untreated: INV AMF+; treated with fungicide: INV AMF-). Means followed by a different letter are significantly different (Tukey´s test, $p < 0.05$). Two-way ANOVA results: ns= non significant, * = $p < 0.05$, ** = $p < 0.01$,*** = $p < 0.001$.

Celtis tala		Stem length (cm)	Root length (cm)	Biomass (g)	MD
NAT	AMF+	13.49±3.30	8.56±3.26	20.36±5.91	0.19
	AMF-	11.67±1.86	4.8±0.72	16.46±2.57	
INV	AMF+	10.97±5.81	5.19±2.21	16.15±7.90	-0.41
	AMF-	14.77±5.02	6.64±1.85	22.81±6.86	
ANOVA results					
inoculum source		ns	ns	ns	
fungicide		ns	ns	ns	
inoculum source*fungicide		ns	*	ns	
Ligsutum lucidum		Stem length (cm)	Root length (cm)	Biomass (g)	MD
NAT	AMF+	84.90±11.36	33.20±3.70	27.02±5.33	0.03
	AMF-	102.00±8.60	38.20±9.01	26.24±1.98	
INV	AMF+	81.88±8.78	38.25±4.86	28.6±4.03	0.17
	AMF-	87.20±18.02	34.60±3.58	23.4±5.00	
ANOVA results					
inoculum source		ns	ns	ns	
fungicide		ns	ns	ns	
inoculum source*fungicide		ns	ns	ns	

Counting and identification of AMF spores

In the NAT inoculum, we identified spores of *Diversispora spurca*, *Glomus* sp., *Pacispora* sp.1, *Sclerocystis sinuosa*, *Entrophospora infrequens*, *Acaulospora delicata*, and *Scutellospora cerradensis*, whereas in INV, we found *Diversispora spurca*, *Glomus* sp., *Claroideoglomus etunicatum*, *Glomus aggregatum*, *Rhizophagus intraradices*, and *Sclerocystis sinuosa*.

After the bioassay, we identified 12 morphospecies of Glomeromycota belonging to the families Acaulosporaceae, Claroideoglomeraceae, Diversisporaceae, Glomeraceae, Pacisporaceae, and Gigasporaceae. As shown in Table 2, *A. delicata* was recovered in all treatments. The density of spores showed no significant differences between treatments ($p > 0.05$). The specific richness (S) was affected by both the inoculum source and the host species, being higher in *C. tala* than in *L. lucidum* and in NAT than in INV soil. The evenness (E) and diversity indices (H´ and D) were higher in *C. tala* and showed no significant differences between NAT and INV soils (Tab. 3).

The first two axes of the PCA (Fig. 2) explained 49.99 % of the total variation of AMF species relative abundance and separated two sets of samples with no overlap corresponding to the pots of *C. tala* and *L. lucidum*. Considering Axis 3, the total variation explained by the first three axes was 64.38 %, and samples were grouped according to the source of inoculum (*i.e.*, non-invaded native and invaded forest soil). It is important to note that *C. tala* samples showed a greater variation, while those of *L. lucidum* were grouped together at a close distance, indicating a high similarity between them.

Table 2. Species of Glomeromycota recovered at the end of the experiment from *Celtis tala* and *Ligustrum lucidum* pots inoculated with non-indaved native (NAT) and invaded (INV) forest soil.

	Celtis tala		*Ligustrum lucidum*	
	NAT	INV	NAT	INV
Acaulospora delicata	x	x	x	x
Acaulospora sp.	x	0	0	0
Claroideoglomus claroideum	x	0	0	0
Claroideoglomus etunicatum	0	0	x	0
Diversispora spurca	x	0	0	0
Funneliformis coronatum	x	0	0	0
Glomus sp.	0	0	x	x
Pacispora sp.1	x	0	0	0
Pacispora sp. 2	0	x	0	0
Sclerocystis sinuosa	0	x	0	0
Scutellospora sp.	0	x	0	0
Septoglomus constrictum	0	x	0	0

Discussion

We verified that AMF colonized both *C. tala* and *L. lucidum*. Velázquez (2011) and Irrazabal (2007) previously registered both species as hosts of these fungi under field conditions. Both species had the highest colonization percentages when they received, as a source of inoculum, soil from the forest where they were dominant in structure. Our results may indicate that *L. lucidum* has the ability to establish symbiosis with the native AMF during the early stages of invasion, as observed in plants inoculated with NAT soil, triggering changes in the microbiota that maximize the colonization rates, as observed in plants inoculated with INV soil. Zubek *et al.* (2016) showed that when an exotic plant is able to benefit from the association with native AMF, it tends to modify the relative abundance of the resident fungal species. Consequently, it interferes with the pre-existing mutualism relationships and negatively affects native plants by decreasing the available resources and the diversity of the soil microbiota.

In this study, *C. tala* showed the highest colonization in the presence of inoculum from the non-invaded native forest soil. This difference was significant in the case of entry points and arbuscules, which are structures cited as indicators of functional symbiosis (Smith & Read 2008). The changes in the soil microbiota induced by the invasion of *L. lucidum* mentioned above could explain the low percentages of colonization observed in *C. tala* plants inoculated with

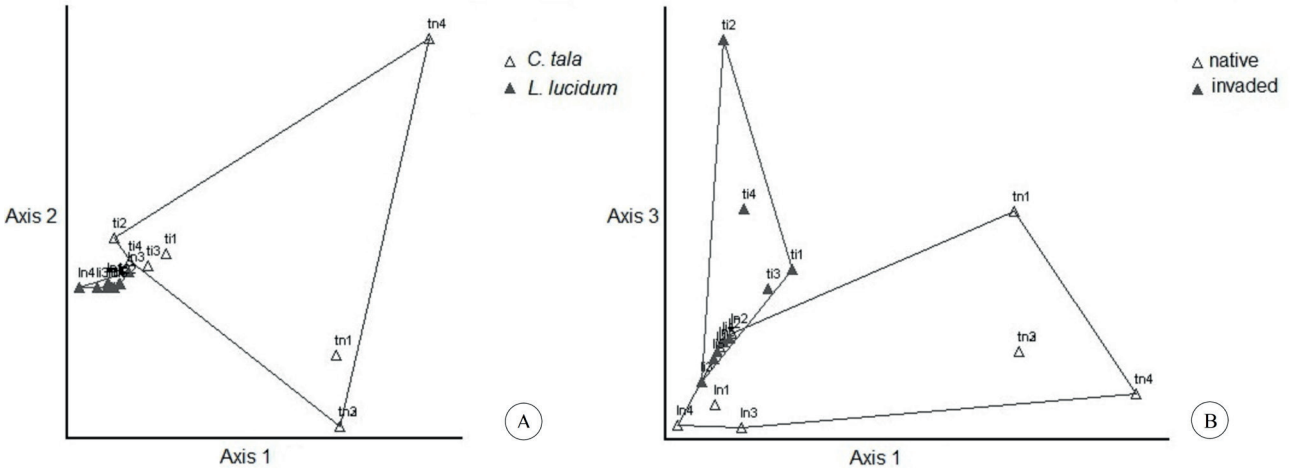

Figure 2. Principal Component Analysis (PCA) of the abundance of AMF species recovered from the pots. Axis 1 and 2, showing the distribution of the samples according to host species (*Celtis tala* and *Ligustrum lucidum*) (**A**). Axis 1 and 3, showing the distribution of the samples according to inoculum source (non-invaded native and invaded forest soil) (**B**).

Table 3. Mean and standard deviation of the number of Glomeromycota spores in 100 g of dry soil (spore density), specific richness (S), evenness (E), Shannon-Wiener diversity index (H´) and Simpson diversity index (D) from pots of *Celtis tala* and *Ligustrum lucidum* inoculated with the non-invaded native and invaded forest soil. Means followed by a different letter are significantly different (Tukey´s test, $p < 0.05$). Two-way ANOVA results: ns= non significant, * = $p < 0.05$, ** = $p < 0.01$,*** = $p < 0.001$.

		Spore density (spores/100g of soil)	S	E	H´	D
Celtis tala	NAT	28 ± 4.76 ^{a}	5.5 ± 0.57 ^{c}	0.86 ± 0.06 ^{b}	1.47 ± 0.19 ^{b}	0.73 ± 0.06^{b}
	INV	14.5 ± 3.10 ^{a}	2.75 ± 0.50 ^{bc}	0.89 ± 0.13 ^{b}	0.86 ± 0.19 ^{b}	0.53 ± 0.1^{b}
Ligustrum lucidum	NAT	65.4 ± 63.66 ^{a}	1.8 ± 1.30 ^{ab}	0.12 ± 0.19 ^{a}	0.11 ± 0.18 ^{a}	0.07 ± 0.12^{a}
	INV	26.4 ± 27.98 ^{a}	1 ± 0.70 ^{a}	0.14 ± 0.30 ^{b}	0.13 ± 0.30 ^{a}	0.06 ± 0.13^{a}
ANOVA results						
host species		ns	*	**	**	**
inoculum source		ns	*	*	ns	ns
host species*inoculum		ns	ns	*	ns	ns

INV soil. Thus, the establishment of exotic species might interfere in the linkages between native host species and AMF. Stinson *et al.* (2006) previously reported this strategy of invasion for *Alliaria petiolata*, a European invasive species of North American forests.

Soil changes caused by the invasive species can alter the relationship between the microbiota and the native host species, negatively affecting its growth and favoring the invasion (Hawkes *et al.* 2006). This mechanism may explain the negative value of mycorrhizal dependence observed in this study in *C. tala* plants inoculated with invaded forest soil. In this treatment, the negative effect on growth may be due to the symbiosis located at the parasitic extreme of the mutualism-parasitism-continuum, according to the model proposed by Johnson & Graham (2013). This could be a key factor for the establishment of *L. lucidum* in *C. tala* forests, which may hamper the regeneration of the native species in the invaded areas.

We recorded twelve species of Glomeromycota from the pots. Four out of the seven AMF species identified in the NAT inoculum were found in the pots: *D. spurca* and *Pacispora* sp. 1 in *C. tala* pots, *Glomus* sp. in *L. lucidum* pots, and *A. delicata* in both treatments. Similarly, three out of the six species present in the INV inoculum were recovered after the bioassay: *S. sinuosa* in *C. tala* pots, *Glomus* sp. in *L. lucidum* pots, and *A. delicata* in both treatments. On the other hand, spores of *Acaulospora* sp., *C. claroideum*, *C. etunicatum*, *F. coronatum*, *Pacispora* sp. 1, and *S. constrictum* were recovered from the pots although they had not been found in the inocula. These results indicate that *C. tala* and *L. lucidum* are favorable hosts and that they promote the sporulation of different AMF species.

Acaulospora delicata was the only species present in all treatments, whereas the rest of the species were specific to each treatment. In the case of *C. tala*, *Acaulospora* sp., *D. spurca*, *C. claroideum*, *F. coronatum*, *Pacispora* sp. 1 were found in the pots inoculated with NAT soil, whereas *Pacispora* sp. 2, *S. sinuosa,* and *Scutellospora* sp.1 were observed in those inoculated with the INV soil. With regard to *L. lucidum*, *C. etunicatum* was present exclusively in the pots with NAT soil, while *Glomus* sp. was found in both treatments. Pots of *C. tala* showed the presence of six families (Acaulosporaceae, Glomeraceae, Claroideoglomeraceae, Diversisporaceae, Pacisporaceae, and Gigasporaceae), while pots of *L. lucidum* had only Acaulosporaceae, Glomeraceae, and Claroideoglomeraceae. Therefore, it is important to note that *L. lucidum* showed a smaller number of families than *C. tala*. In addition, pots of *C. tala* inoculated with both sources of inoculum had a greater richness and diversity of Glomeromycota than *L. lucidum*. The richness found in *C. tala* pots could indicate that this native tree tends to promote the development of a greater variety of AMF species than the exotic one. However, this result does not allow us to establish a direct relationship between the diversity of spores and the percentages of colonization observed, because *L. lucidum* showed the highest colonization. Besides, we should consider that the AMF species found are not necessarily the same species colonizing the roots (Fernández Bidondo *et al.* 2018 and reference therein). Although we were not able to identify the mechanisms underlying the AMF-host interaction for the plant species studied, we can confirm that these species interact with AMF in a different way, which was reflected in both the values of mycorrhizal dependence and the colonization percentages.

It is interesting to mention that the AMF species recovered from *L. lucidum* pots belong to the families Acaulosporaceae, Claroideoglomeraceae, and Glomeraceae. These families are commonly found in disturbed environments, which suggests great adaptability, probably due to low host specificity and great tolerance to abiotic stresses (Chagnon 2013; Asmelash 2016). Furthermore, *Claroideoglomus etunicatum* is one of the most widely distributed species of AMF and has been registered in diverse ecosystems including Alaska and Namibia (http://invam.caf.wvu.edu). Although these results are insufficient to conclude that *L. lucidum* tends to relate to generalist and cosmopolitan AMF species, as suggested by Núñez & Dickie (2014), it would be interesting to conduct new studies to explore this hypothesis.

The PCA considering the relative abundance of AMF species grouped the samples by both plant species and source of inoculum, although the type of host was the factor that contributed the most to this separation. This indicates that there were differences in the diversity of fungal propagules between sites and in the AMF species with which each plant species establishes the symbiosis. Our results are in agreement with the laboratory tests of Bever *et al.* (1996) and Leal *et al.* (2009), who found that the AMF diversity was more related to the host species than to the source of inoculum.

In our study, the growth parameters of *C. tala* and *L. lucidum* showed no differences between treatments. However, we cannot rule out that mycorrhization influences plant fitness under field conditions at other developmental stages not evaluated herein (*i.e.*, germination, flowering, fruit production).

Our results suggest that the invasion of the exotic *L. lucidum* in *C. tala* forests may be acting as a factor that determines changes in the diversity of AMF and affects the mutualism relationships that occur between native plants and these fungi. Therefore, we confirm the initial hypothesis of this study, that *L. lucidum* establishes mutualisms with some AMF species from the talares and its spread tends to modify the fungal community and pre-existent interactions between native plants and fungi. Although future studies that deepen the understanding of these interactions are required, this work contributes to the understanding of the mechanisms that operate in the invasion phenomena, demonstrating that AMF can play a role in this process, not only benefiting the invasive species but also preventing the regeneration of native plants.

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