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Fungal endophyte β-diversity associated with Myrtaceae species in an Andean Patagonian forest (Argentina) and an Atlantic forest (Brazil)

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ABSTRACT

The biogeography of microorganisms is poorly understood and how microbial diversity is structured is still an open debate. We investigated the processes underlying the fungal endophyte assemblages of phylogenetically related Myrtae host tree species at different spatial scales: regional, 101–5 000 km; local, 0–100 km; and microscale, 0–1 km. A total of 939 isolates was obtained and assigned to 51 distinct MOTUs based on the sequencing of the nrITS region. At regional scales, geographic distance was responsible for explaining the fungal community similarity, while, at a local scale, it was the environmental distance. Moreover, fungal endophytes exhibit preference in the colonization of *Luma apiculata* but not for *Myrceugenia ovata* var. *nanophylla*. Our results suggest that fungal endophytes are not randomly distributed and are influenced by both geographic and environment distances depending on the spatial scale analysed.

Introduction

Biogeography is the study of the distribution of biodiversity over space and time (Martiny et al., 2006), and can clarify the processes that generate and maintain diversity, such as dispersion, speciation, extinction, and species interactions (Brown and Lomolino, 1998). The community similarity between two groups often decreases as the geographic distance between them increases, a pattern observed in communities from all domains of life and known as distance decay (Nekola and White, 1999). Two primary explanations for this pattern have been proposed. Niche theory predicts that

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community similarity decreases with environmental distance, irrespective of geographic proximity, as a result of species differences along environmental gradients (Tilman, 1982). Neutral theory, in contrast, predicts that the decay of community similarity is caused by spatially limited dispersal, independent of environmental differences between sites (Hubbell, 2001). Although these two theories have been considered as contradictory, they are not mutually exclusive. Deterministic and stochastic processes are probably jointly responsible for structuring ecological communities (Chave, 2004; Dumbrell et al., 2009).

Microorganisms have long been regarded as cosmopolitan because they exhibit short generation times and large population sizes and disperse over long distances (Fenchel and Finlay, 2004), which prompted Baas Becking's hypothesis that "*everything is everywhere*, but, *the environment selects*" (the EisE hypothesis) (Baas Becking, 1934; Green and Bohannan, 2006). The past several years have witnessed an increase in debates about whether microorganisms exhibit similar biogeographic patterns to macroorganisms (Martiny et al., 2006). Furthermore, many works have shown that the microorganisms can exhibit biogeographic distribution patterns (Martiny et al., 2006).

The distance decay relationship is used to demonstrate how the processes of selection, drift, dispersal, and mutation shape biogeographic patterns (Hanson et al., 2012). Regarding the EisE hypothesis, the distance decay curve would be due to a gradient of selective factors, which are spatially autocorrelated (Martiny et al., 2011; Hanson et al., 2012). Therefore, organisms with different niche preferences are selected from the available pool of taxa as the environment changes with distance (Martiny et al., 2011). Distance decay patterns, and therefore β-diversity, can also be influenced by dispersal limitation. This effect should reflect the influence of historical processes on the current biogeographic patterns (Martiny et al., 2011).

Microbial biogeography studies were challenged following the advent of the molecular taxonomy of microorganisms, which allowed species to be distinguished more accurately (Fierer, 2008). The internal transcribed spacer (ITS1 and ITS2) and 5.8S regions of the nuclear ribosomal repeat unit are the most widely used molecular markers in fungal endophyte diversity studies (Promputtha et al., 2007; U'Ren et al., 2010) and have been used as the primary fungal barcode marker (Schoch et al., 2012).

This region is easily amplified and sequenced in all fungal lineages using universal primers (Nilsson et al., 2008); moreover, there are representative ITS sequence databases, such as INSC and Unite (Karsch-Mizrachi et al., 2012) for comparison. Fungal diversity remains relatively poorly explored, as evidenced by the small proportion of fungi that have been identified to date. Recent estimates have suggested that there are as many as 5.1 million fungal species, making fungi among the most diverse groups of organisms (Blackwell, 2011).

Fungal endophytes inhabit healthy plant tissues during at least one stage of their life cycles without causing any apparent symptoms of disease or negative effects on the host (Petrini et al., 1992). Endophytes have been isolated from all studied plant groups, including bryophytes (U'Ren et al., 2010), pteridophytes (Petrini et al., 1992), gymnosperms (Soca-Chafre et al., 2011), and both monocotyledonous (Pinruan et al., 2010) and dicotyledonous angiosperms (Vaz et al., 2009; Vieira et al., 2012). Many studies have documented remarkable endophyte richness in tropical plants (Saikkonen et al., 1998; Vaz et al., 2009, 2012; Vieira et al., 2012). Similar trends have been observed in temperate environments, where a host tree may harbour dozens of fungal endophytes (Saikkonen et al., 1998; Stone et al., 2000).

The fungal endophyte can exhibit some host tree and/or tissue preference varying from high (McKenzie et al., 2000; Su et al., 2010; Peršoh, 2013) to low tissue specificity (Cannon and Simmons, 2002; Koukol et al., 2012). Many host-associated microorganisms exhibit patterns of genetic, morphological and functional differentiation that are related to the distribution of their hosts (Papke and Ward, 2004). To evaluate if fungal endophyte assemblages are correlated with their hosts, we selected Myrtaceae members because they can be found in diverse habitats, and there is a robust phylogeny for this group, making them a good candidate for comparisons of endophyte community diversity. Phylogenetic analyses of morphological and molecular data have suggested a Gondwanan origin of Myrtaceae, with the Myrtae tribe originating and diversifying in Australasia between 77 and 56 mya, when Australia was still connected to South America *via* warm-temperate Antarctic land bridges (Lucas et al., 2007). Myrtae is a monophyletic group of plants exhibiting probably a recent and rapid speciation, with long-distance dispersals more likely than vicariance to explain at least some of the intercontinental movements (Lucas et al., 2007).

We studied phylogenetically related Myrtae host species from a single clade, *Luma apiculata* and *Myrceugenia ovata* var. *nanophylla*. We included, as an outgroup, a Myrtae species *Eugenia neomyrtifolia* that is from a different clade. The main objective of our study was to determine whether environmental and/or geographical distances explain the fungal community assemblages diversity over different scales (regional, local or micro-scales) and host tree species.

Materials and methods

Study areas

Three different sites in Patagonia, Argentina were studied in Mar. 2009. These sites were located in the Andean Patagonian region, near the city of San Carlos de Bariloche, which is situated within Nahuel Huapi National Park. This region is characterised by native forests, which are dominated by *Nothofagus* spp. or native conifers, such as *Austrocedrus chilensis*, *Araucaria araucana*, *Fitzroya cupressoides* and *Pilgerodendrum uviferum* (Donoso, 2006). In Argentina, *L. apiculata* was collected from two different sites, the Arrayanes Forest and Puerto Blest; *M. ovata* var. *nanophylla* was collected from one site, Espejo Lake. In Brazil, the study was conducted at the Centro de Pesquisas e Conservação da Natureza Pró-Mata of Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), in São Francisco de Paula, Rio Grande do Sul state in Nov. 2009. This area is a confluence of three phytoecological regions, including *Araucaria* forest, Atlantic rainforest and a herbaceous-bushy formation known regionally as "hill-top

fields". *M. ovata* var. *nanophylla* and *E. neomyrtifolia* were collected from this Atlantic rainforest site (Fig 1). All the sampled sites were characterised by low anthropic impact and minimal atmospheric pollution.

Fungal endophyte isolation

Five visually healthy leaves were collected from each of the 20 trees of all Myrtaceae species. The trees were spaced approximately 5 m apart. All the leaves were stored in sterile plastic bags, and fungal isolation was performed on the same day as the collection. The leaves were surface-sterilised *via* successive dipping in 70 % ethanol (1 min) and 2 % sodium hypochlorite (3 min), followed by washing with sterile distilled water (2 min). After the leaf surface sterilisation, six fragments (approximately 4 mm^2) were cut from each leaf: one from the base, two from the middle vein, one from the left margin, one from the right margin and one from the tip (30 segments/individual; 600 segments/site; 3 000 segments overall). All the leaf fragments were plated onto potato dextrose agar (PDA, Difco, USA) supplemented with 100-μg ml^{-1} chloramphenicol (Collado et al., 1996). The plates were incubated at 15 °C for up to 60 d. To test the effectiveness of the surface sterilisation, 100 μl of the water used during the final rinse was plated on PDA to test for epiphytic microbial contaminants. Individual colonies were purified on PDA, and their morphologies were documented and photographed. The long-term preservation of mycelial samples was performed in sterile distilled water at room temperature. All the fungal isolates were deposited in the Culture Collection of Microorganisms and Cells of the Universidade Federal of Minas Gerais (UFMGCB).

Name assignment to MOTUs (molecular operational taxonomic unit)

Pure cultures of the fungal isolates were grouped based on their morphological characteristics, including aerial mycelium formation, colony colour, surface texture and margin characters. At least 50 % of the fungal isolates of each morphospecies were identified by directly extracting their total genomic DNA and sequencing the ITS region of the rRNA gene. The extraction of DNA from filamentous fungi was performed according to Rosa et al. (2009). The complete ITS region of the rRNA gene was amplified using the universal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′), as described by White et al. (1990). The amplification of nrITS and the sequencing were performed as described by Vaz et al. (2009). The ITS sequences obtained were analysed in GenBank with BLASTn to search for similarity with the sequences deposited. Molecular operational taxonomic units (MOTUs) were defined using a 97 % ITS region identity threshold (O'Brien et al., 2005; Edgar, 2010; Sun et al., 2012).

Analysis of ecological data

The diversity was estimated using the Shannon (H') index ($H = -\Sigma ni/n \ln(ni/n)$, where ni is the number of individuals in the taxon i and n is the total number of individuals). Values of the Shannon index usually are between 1.5 and 3.5, with 1.5 representing the lowest diversity and 3.5 the highest (Gazis and Chaverri, 2010). Species accumulation curves were used to determine whether a sufficient number of samples had been obtained from each study site and were generated for each host tree species in each site. For these two analyses the

Fig 1 – Map of the geographical distribution of the host tree species.

Vegan package (Oksanen, 2009) was used. For calculations and statistical analyses, each individual fragment was considered a sample unit and a total of 3 000 sample units were evaluated.

The geographic distance among the collection sites were arranged by different scales: regional: 101–5 000 km, local; 0–100 km and microscale; 0–1 km. Nonmetric Multidimensional Scaling (NMDS) analyses were conducted to visualise the trends and groupings of the fungal endophytes at individual host tree level, so that the presence values obtained at the lower levels of sampling (leaf fragment and leaf) were summed for each individual host tree. The Jaccard distance was used in these analyses due to its simplicity, widespread use and more conservative measurement of community similarity than distances based on species abundance data, which are more sensitive to disturbances and local environmental differences (Nekola and White, 1999). The data were square-root transformed prior to the NMDS analysis to reduce the influence of the most abundant species (Clarke and Warwich, 2001; Joshee et al., 2009). The NDMS analysis was performed with a random starting configuration, using the package (Oksanen, 2009). The rate of distance decay of the fungal endophyte communities was calculated according to Nekola and White (1999), with the assumption that community similarities decrease with increasing geographical distance. The distance decay relationship was calculated as the slope of a least-squares linear regression on the (ln-transformed) geographic distance and the fungal endophyte community similarity measured by the Jaccard index. The slope of the distance–similarity relationship is one of the most common measures of β-diversity in ecological studies (Nekola and White, 1999). We chose to transform the geographic distance due to of our sampling scheme, which purposely sampled over many orders of magnitude; otherwise, the data points would have been highly skewed (Martiny et al., 2011). In addition, we tested whether the slope of the distance decay curve of each collection site was significantly different from zero using a randomisation procedure with 1 000 iterations.

The geographic distance was considered as how far each individual host tree was from each other at the same collection site and among collection sites, and we recorded the locations using a handheld GPS unit. The environmental variables measured at each site collection were elevation, precipitation and temperature. A principal components analysis (PCA) was performed and the dissimilarities were computed for the first component. To investigate the relationships among the fungal endophyte community similarity, geographic distance and the first component of the environmental characteristics across all the spatial scales, we used ranked partial Mantel tests (which assumes a monotonic, but not linear, relationship) in ecodist package (Goslee and Urban, 2007; Martiny et al., 2011). Correlations were examined with the Spearman correction, and the *P* values were based on 10 000 permutations.

To tease apart the relative importance of the environmental variables on fungal endophyte community similarity, we used multiple regression on matrices (*MRM* function) (Goslee and Urban, 2007). To reduce the effects of spurious relationships between variables, we performed the MRM test, removed the non-significant variables, and then repeated the test (Harrel, 2001; Martiny et al., 2011). Temperature and precipitation did not vary between the two collection sites in Brazil, thus it was not possible to evaluate the influence of these variables on the fungal endophyte dissimilarity between these two sites. To further investigate the relative importance of each predictor variable at the two spatial scales (regional: 101–5 000 km, local: 0–100 km), we investigated scale-specific MRM models. We tested the significance of each model by performing 10 000 permutations. All analyses were performed using the R program (R Development Core Team, 2005).

Results

Diversity of fungal endophytes

A total of 939 fungal endophyte isolates were obtained from 3 000 leaf fragments. Fifty-one distinct Molecular Operational Taxonomic Units (MOTUs) were identified based on the sequencing of the ITS region of rRNA. Twenty-two taxa were identified at the species level. Twenty-nine MOTUs exhibited a high divergence in the ITS region, with nucleotide differences from the other fungal sequences deposited in GenBank ranging from 4 to 11 % (Supplementary Table 1). All the taxa belonged to Ascomycota, except *Trametes* (Basidiomycota). The ascomycetous fungi were identified as members of the Sordariomycetes, Dothideomycetes, Leotiomycetes, Euromycetes and Pezizomycetes. *Pseudocercospora basintrucata* and *Xylaria* sp.1 were the most frequent taxa isolated from the *L. apiculata* in Arrayanes Forest and Puerto Blest collection sites (Andean Patagonian forest), respectively. *Mycosphaerella* sp. and *Xylaria enteroleuca* were most frequently isolated from *M. ovata* var. *nanophylla* from Espejo Lake (Andean Patagonian forest, Argentina) and the Atlantic rainforest (Brazil), respectively. The most frequent fungal species isolated from *E. neomyrtifolia* (Atlantic rainforest, Brazil) was *Colletotrichum* sp.1. Only species of the genus *Xylaria* were isolated simultaneously from all the host plants studied.

The accumulation curve did not reach an asymptote indicating that the sampling effort had not been sufficient to capture the total species richness of the culturable fungal endophytes (Fig 2). The diversity indexes are shown in Supplementary Table 2. The host trees of the Atlantic rainforest ecosystem in Brazil, *M. ovata* and *E. neomyrtifolia*, displayed the highest Shannon values. In contrast, both *L. apiculata* host trees exhibited low Shannon index values. *M. ovata* from the Atlantic rainforest (Brazil) presented greater diversity than the same species collected in the Andean Patagonian forest (Argentina), furthermore, they did not share any fungal endophyte species. Conversely, *L. apiculata* collected in two different Argentinean sampling sites shared six fungal endophyte species and *M. ovata* in the Atlantic forest shared four fungal endophyte species with *E. neomyrtifolia*.

The NMDS plots revealed a separation between the groups found at the three Andean Patagonian forest sites (Argentina) and those at the Atlantic rainforest sites (Brazil) (Fig 3A). When considering the fungal endophyte obtained from the same host tree species, the NMDS analysis of *L. apiculata* and *M. ovata* showed an overlap in the fungal endophyte assemblage in the former and a separation in the latter (Fig 3B, C).

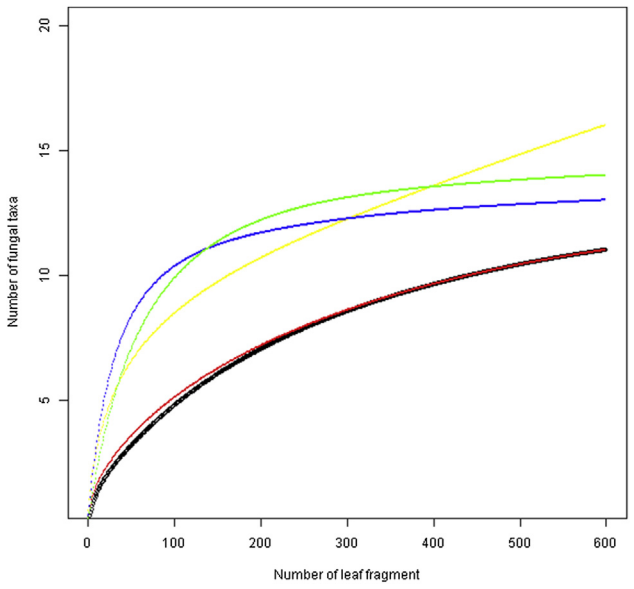


Fig 2 – Accumulation curves for the fungal endophytes of *Luma apiculata* (Arrayanes forest) (black), *Luma apiculata* (Puerto Blest) (red), *Myrceugenia ovata* var. *nanophylla* (Argentina) (yellow), *Myrceugenia ovata* var. *nanophylla* (Brazil) (blue), and *Eugenia neomyrtifolia* (Brazil) (green).

A plot of the fungal endophyte community similarity *versus* the geographic distance for each pairwise set of samples at regional scale revealed a significant negative distance decay curve for the fungal endophyte community (slope = −0.01, $P < 0.0001$) (Fig 4). At local scale the distance decays were also significant for Argentina (slope = −0.02, $P < 0.0001$, Fig 4) and Brazil (slope = −0.05, $P < 0.0001$, Fig 4). Furthermore, a microscale analysis of the slope of this curve revealed significant variation between the host trees transects. There were significantly negative distance decay slopes for *M. ovata* (slope = −0.015, $P = 0.03$) from Puerto Blest in the Andean Patagonian forest, *M. ovata* (slope = −0.11, $P < 0.0001$) and *E. neomyrtifolia* (slope = −0.03, $P = 0.002$) from the Atlantic rainforest in Brazil. However, the distance decays slope for *L. apiculata* in the Arrayanes Forest and Puerto Blest did not differ significantly from zero ($P = 0.139$ and $P = 0.766$, respectively).

The ranked partial Mantel tests revealed that the dissimilarity in the fungal community was significantly correlated with geographic distance ($\rho = 0.15$, $P < 0.05$), but not with environmental distance ($P = 0.9$) at regional scale (Table 1). MRM were used to investigate the relative importance of the factors contributing to these correlations. Over a regional scale, the MRM model explained a low proportion of the variability in the fungal endophyte community similarity ($R^2 = 5$ %, $P = 0.05$; Table 2). The geographical distance ($\beta = 0.78$, $P = 0.0001$), elevation ($\beta = -0.20$, $P = 0.05$) and air temperature ($\beta = -0.58$, $P = 0.001$) significantly contributed to the partial regression coefficients. Precipitation did not play a significant role ($P = 0.957$) in describing the model.

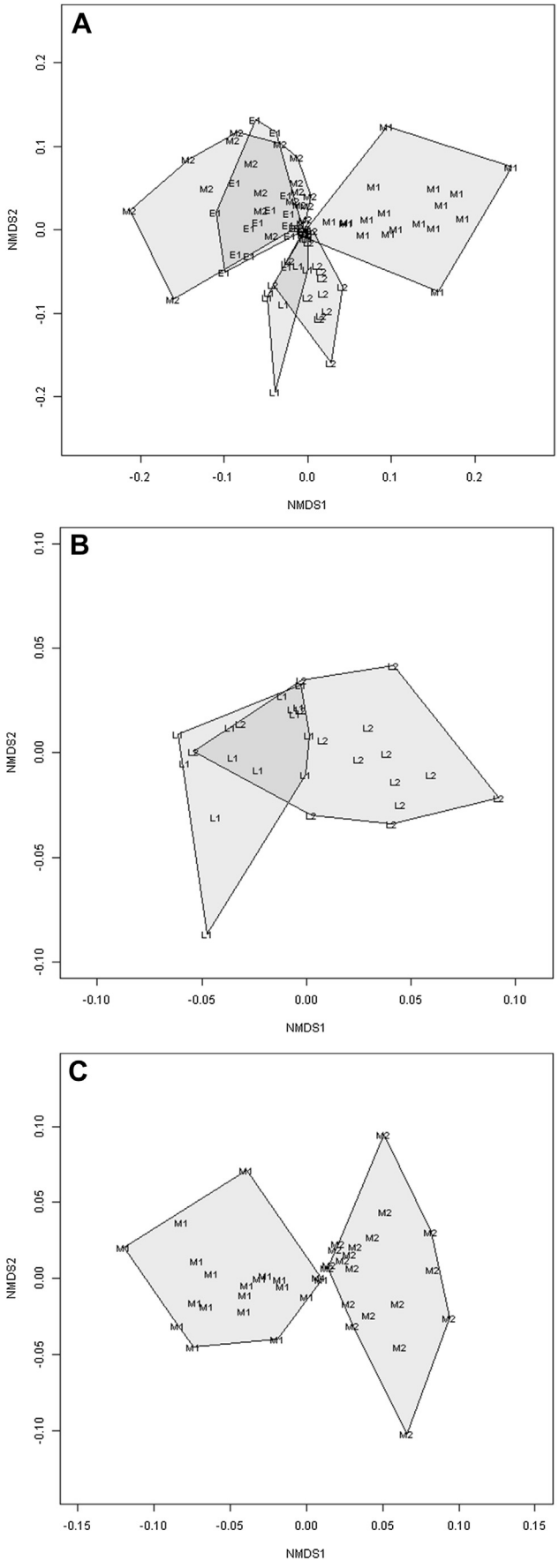
The fungal endophyte dissimilarity was significantly correlated to environmental distance at local scale (Table 1). The unexplained variance in the MRM model was different for Argentina and Brazil, 14 % and 68 %, respectively. The geographic distance did not influence the fungal endophyte community similarity; however, the environmental variables were significant in explaining the model in each site (Table 2). When considered the same host tree species, it was observed that only geographic distance was statistically significant for *M. ovata* (Table 1). The multiple regressions on matrices (MRM) for *L. apiculata* and *M. ovata* were not done because there is no variation on environmental variables inside each collection site.

Discussion

We used a culture-based approach and the sequencing of ITS region of the rRNA gene to assess the diversity and taxon composition of fungal endophytes associated with phylogenetically related *Myrtaceae* species. The ITS region was chosen because this region has the highest probability of allowing the successful identification of a broad range of fungi (Schoch et al., 2012). Diversity patterns of fungal endophyte community from host trees in an Atlantic forest in Brazil (Supplementary Table 2) were slightly lower than those in the tropical angiosperm hosts *Hevea brasiliensis* (*Euphorbiaceae*) in Peru (Gazis and Chaverri, 2010); *Guarea guidonia* (*Meliaceae*) from El verde site in Puerto Rico (Gamboa and Bayman, 2001); *Cereus jamacaru* (*Cactaceae*) from Brazil (Bezerra et al., 2013) and *Solanum cernuum* in Brazil (*Solanaceae*, Vieira et al., 2012). However, the Shannon index was similar to the following host trees: *Kunzea ericoides* (*Myrtaceae*) in New Zealand (Joshee et al., 2009) and many other woody perennials in Western Ghats in India (Suryanarayanan et al., 2011).

The Shannon diversity of host trees from Andean Patagonian forest (Argentina) from the present work were lower than those observed from hosts in some temperate ecosystems: *Dryas integrifolia* (*Rosaceae*) in Canada (Higgins et al., 2007); *Ulmus macrocarpa* (*Ulmaceae*) (Sun et al., 2012); *Nothofagus* sp. (*Fagaceae*) (Johnston et al., 2012); *Quercus* sp. (*Fagaceae*) and *Rhododendrum* sp. (*Ericaceae*) (Li et al., 2012); *Tinospora cordifolia* (*Menispermaceae*, Mishra et al., 2012). Moreover, similar indexes were obtained from *Betula platyphylla* (*Betulaceae*) and *Quercus liaotungensis* (*Fagaceae*) (Sun et al., 2012).

Both the number and size of the sampled fragments have important effects on the number of species isolated: when the size of the leaf fragments is reduced while their number is increased, the number of isolated fungal species increases (Gamboa et al., 2002). The number of leaf fragments sampled in the present work was higher than those of other studies of fungal endophytes associated with tropical (Gazis and Chaverri, 2010; Chen et al., 2011) and temperate (Mishra et al., 2012; Langenfeld et al., 2013; Matsumura and Fukuda, 2013) ecosystems. However, the sampling of 600 leaf fragments per host tree was not sufficient to adequately capture the richness of the culturable endophytes, which was confirmed by the species accumulation curve analysis (Fig 2). The curves of all host trees did not reached a plateau, a pattern frequently found in samples from tropical environments (Gazis and Chaverri, 2010; Joshee et al., 2009), indicating that more samples are needed to estimate the real diversity of fungal endophytes.



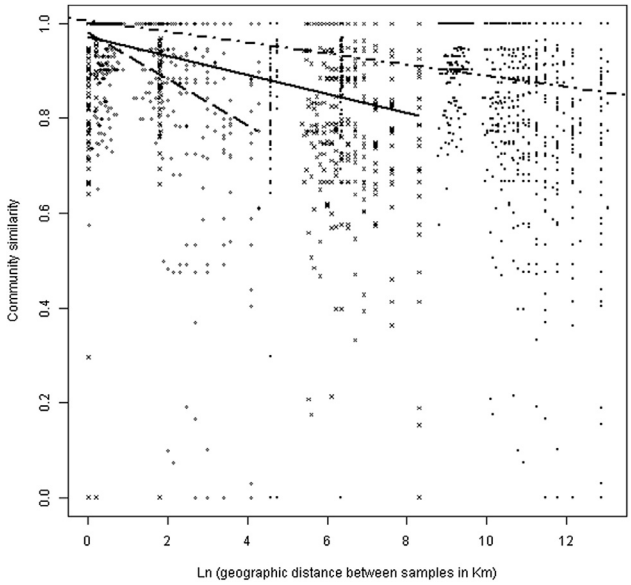


Fig 4 – Distance decay relationship for fungal endophyte communities. Pairwise community similarities were calculated using the Jaccard index and plotted against the natural logarithms of the distances among the study sites. The lines denote the linear regression from regional scale (– – –), local scale: Argentina (———) and Brazil (– – –). All slopes were significantly smaller than zero.

There is an ongoing debate as to whether microorganisms exhibit biogeographical patterns similar to those of macroorganisms or whether such patterns are obscured by the population sizes and dispersal capabilities of microorganisms (Martiny et al., 2011). The process of selection, drift, dispersal and mutation can influence the distance decay relationship. Usually, the selection and drift generate that relationship, dispersal counteracts it, and mutation modifies its variance (Hanson et al., 2012). Some works considered that the large size of population, and the small size and short generation time of microorganisms result in a cosmopolitan distribution, assuming that there is no dispersal limitation (Fenchel and Finlay, 2004). However, many works have shown the distance effect in the distribution of microorganisms, even in a global distributed, spore-forming bacterium *Myxococcus xanthus* (Vos and Velicer, 2008) and the archaeon *Sulfolobus* (Whitaker et al., 2003). In our work, at regional scale, the NMDS analysis suggested that the fungal endophyte assemblages were shaped at a local scale (Fig 3). The distance

Fig 3 – Nonmetric multidimensional scaling (NMDS) plots showing the differences in the fungal endophyte community compositions at (A) Regional scale and associated with (B) *Luma apiculata* (C) *Myrceugenia ovata* var. *nanophylla* species. Andean Patagonian forest (Argentina): L1 – *Luma apiculata* (Arrayanes Forest); L2 – *L. apiculata* (Puerto Blest) and M1 – *Myrceugenia ovata* var. *nanophylla* (Espejo Lake). Atlantic forest (Brazil): M2 – *Myrceugenia ovata* var. *nanophylla* and E1 – *Eugenia neomyrtifolia*.

Table 1 – Comparison of the fungal endophyte partial Mantel test results, where Spearman ρ is the correlation between the fungal endophyte community dissimilarity and geographic distance or environmental distance

Correlation between fungal endophyte	Controlling for:	Regional scales		Local scale				Host tree species			
				Argentina		Brazil		*L. apiculata*		*M. ovata*	
		ρ	P	ρ	P	ρ	P	ρ	P	ρ	P
Geographic distance	Environmental distance	0.15	<0.05	−0.03	0.65	−0.09	0.85	−0.02	0.55	0.27	<0.05
Environmental distance	Geographic distance	−0.09	0.94	0.11	0.06	0.11	0.06	0.05	0.22	0.0003	0.50

The environmental variables were first examined using a principal components analysis (PCA) that considered the elevation, precipitation and temperature of each collection site. The *P* values are one-tailed and based on 10 000 permutations.

(2 324 km) between these two countries reflects the large environmental differences between them, evidencing that geographic distance is the main factor responsible for the differences at regional scale due to the decrease in migration rates. Moreover, the distance effect was corroborated by the distance–decay relationship (Fig 4) and its strong effect described in the partial Mantel regression (Table 1). Therefore, we suggest that geographical isolation, and consequently dispersal limitation, are primarily responsible for the fungal endophyte assemblage at regional scale.

The dispersal of a microorganism can be defined as the movement and successful establishment of an individual from one location to another (Hanson et al., 2012). Then, initially, there is a dispersal of fungal endophytes and subsequently the environmental conditions select for those taxa that are relatively better adapted to those conditions. This can explain the environmental influence of the variables elevation and temperature (MRM analysis, Table 2) at regional scale.

Previous studies have shown that fungal endophyte community assemblage is affected by climate, with temperature and humidity being the most important variables for explaining fungal diversity (Talley et al., 2002). Although distance decays were significant at local scale, when all variables were simultaneously compared in the Mantel and MRM analyses, the environment variables were the most important for explaining the fungal endophyte community. Cottenie (2005), comparing many studies about community structure, showed that in most of them a higher variation in microbial composition can be explained by environmental distance than by geographic distance; the environment effect was more important at small spatial scales. Moreover, Fierer and Jackson (2006) also found an environmental effect at local scales in soil bacterial community diversity.

Table 2 – Results of the multiple regressions on matrices (MRM) analysis of the fungal endophytes by spatial scale

	Regional scale $R^2 = 0.05^{**}$	Local scale	
		Argentina $R^2 = 0.86^{***}$	Brazil $R^2 = 0.32^{***}$
Ln (geographic distance)	0.78**		
Altitude	−0.20*	−11.79***	0.11*
Temperature	−0.58**	−15.32***	–
Precipitation	–	22.31***	–

The variation (R^2) of the community dissimilarity that is explained by the remaining variables and the partial regression coefficients (β) of the final model are shown. Where a partial regression is shown, its significance level (*via* one-way tests) is <0.005. $^{*}P \leq 0.01$, $^{**}P \leq 0.001$, $^{***}P \leq 0.0001$. The water precipitation, altitude and temperature were measured at each collection site. Temperature and water precipitation not varied between the two collection sites in Brazil, then it was not possible to evaluate the influence of these variables on the fungal endophyte dissimilarity.

The varying importance of geographic distance and environmental parameters at different spatial scales likely reflects differences in their underlying variability at those scales (Martiny et al., 2011). The variables considered in our study, probably, were not sufficient to explain most of the variability in fungal endophyte community similarity (Table 2), suggesting that abiotic and biotic factors may not be assessed. At local scale the unexplained variance was lower than at regional scale. The higher value obtained for Brazil could be explained by the fact that temperature and precipitation did not vary between the two collection sites, and then elevation was the only environmental variable used in the analysis.

Analysing the fungal endophyte similarity from the same host tree species, the only non-significant distance decay slope was observed for *L. apiculata*. The Mantel partial analysis showed that fungal endophyte dissimilarity was not significantly correlated to environmental variables nor to geographic distance. Moreover, the NDMS showed an overlap among fungal endophyte community associated to this host tree species in the two collections sites. These results suggest that some fungal endophyte taxa could probably show preference in the colonization of the *L. apiculata* as reported by Johnston et al. (2012), Sun et al. (2012) and Matsumura and Fukuda (2013). However, to confirm this statement, it is necessary to study the temporal and seasonal variation patterns.

The geographic distance was significant in explaining the fungal endophyte dissimilarity in *M. ovata* var. *nanophylla*, and there was a non-overlap in the NMDS analysis. The fungal endophyte community is more similar when the host substratum is more important than environmental variables (Taylor et al., 2000). However, this does not appear to be the case for *M. ovata* var. *nanophylla* and this same pattern was also found in palms from different ecosystems (Taylor et al., 2000). Our results suggest that in *M. ovata* var. *nanophylla* there is no host tree preference for species of fungal endophyte, and the establishment of the mycobiota is related to the geographic distance.

In woody plants, non-systemic endophytes are horizontally transmitted by spores and/or hyphal fragmentation

among plants (Faeth and Hammon, 1997; Saikkonen et al., 1998). This mode of transmission may explain the significant effect of distance decay in *M. ovata* var. *nanophylla* (Argentina and Brazil) and *E. neomyrtifolia* (Brazil), suggesting that the mycobiota associated with the surrounding forest trees are probably responsible for the fungal endophytes associated with these species.

Our work showed that fungal endophyte communities are not randomly distributed and are influenced by both environmental and geographic distances, depending on the spatial scale analysed. Fungal endophyte community similarity was influenced by geographical distance and, consequently, dispersal limitation, at regional scale; at local scale, the environment distance was the most important. Furthermore, fungal endophytes exhibited preference in the colonization of *L. apiculata* but not in *M. ovata* var. *nanophylla*. To confirm the host preference colonization we will study the influence of time on the fungal endophyte community composition in these tree species.

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Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.funeco.2013.12.008.

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