Differential hypogeous sporocarp production from *Nothofagus dombeyi* and *N. pumilio* forests in southern Argentina

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1

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Abstract: Mycorrhizal fungi that form hypogeous sporocarps are an important component of the temperate forest soil community. In many regions, such as the *Nothofagus* forest in the Patagonian Andes, this group of fungi has been poorly studied. Here we examined the spring and autumn community composition of ''sequestrate fungi'', based on sporocarp production in pure forests of *Nothofagus dombeyi* (evergreen) and *N. pumilio* (deciduous). We investigated the possible relationships between these communities and environmental factors over 2 y. The rarefaction curves and the minimal richness estimates converged at nearly the same level for each forest type, and the asymptotes suggested that the sampling effort was sufficient to capture most of the hypogeous sporocarp richness in these forest stands. In total 27 species were recovered. Basidiomycota, Ascomycota and Glomeromycota respectively accounted for nine, two and one genera. Species richness of hypogeous sporocarps varied in relation to forest type but not to season (fall and spring), whereas sporocarp biomass varied according to an interaction between season and forest type. Species richness and sporocarp biomass were positively correlated with rainfall and negatively correlated with altitude. In addition sporocarp species richness was positively related to number of trees per transect. We found that two different forest stands, each dominated by different species of *Nothofagus*, exhibited different hypogeous sporocarp communities.

Key words: biomass, fruit bodies, hypogeous richness, *Nothofagus* forest, Patagonia

INTRODUCTION

Ectomycorrhizal fungi (EMF) are an important component of the forest soil biota that are involved in processes such as transport, storage, and exchange of nutrients, improving soil conditions and maintaining the equilibrium of temperate forests (Smith and Read 2008). The diversity and abundance of EMF may have a major impact on forest development, productivity and the structure of the forest community (Amaranthus and Perry 1994). Climate (Claridge et al. 2000a, b; Kernaghan and Harper 2001), quality and quantity of leaf litter and woody debris (Amaranthus et al. 1994, Claridge et al. 2000b, Dickie et al. 2002, Smith et al. 2002) and forest nutrient dynamics (Johnson 1994, Lilleskov and Bruns 2001) affect the spatial and temporal distribution of EMF communities. Recent studies, mainly centered in phylogenetically unrelated ectomycorrhizal (ECM) hosts, have shown that host identity also influences EMF species composition (Ishida et al. 2007, Tedersoo et al. 2008, Smith et al. 2009). Fewer studies however have examined EMF species composition in congeneric host species (Morris et al. 2008, Tedersoo et al. 2009).

Many EMF lineages produce belowground reproductive structures called hypogeous sporocarps. These fungi are particularly important components of temperate forest ecosystems as a source of food for small mammals that consume the sporocarps and thereby disperse the spores (Maser et al. 1978, Amaranthus et al. 1994, Claridge 2002). In addition to their symbiotic capabilities and role in animal nutrition these fungi are important in several ecosystem processes such as effective nutrient mobilization and soil aggregation (Smith and Read 2008).

Here we studied the community composition of hypogeous sporocarps in forests of *Nothofagus dombeyi* (evergreen) and *N. pumilio* (deciduous) in spring and autumn over 2 y. We also examined the possible relationships of fungal community structure with certain stand variables such as number of tree individuals and mean stem diameter. *Nothofagus* species are among the most widely distributed trees in the Andean forests of southern South America. The two host species differ substantially in leaf physiology (Diehl et al. 2003, 2008; Vivanco and Austin 2008) and litter quality (Vivanco and Austin 2008). In addition it has been shown that photosynthesis can affect sporocarp production and affects EMF community composition (Andrew and Lilleskov

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TABLE I.   Location and climatic characteristics of the study sites

Forest type	Sites	Latitude (S)	Longitude (W)	Altitude (m)	Precipitation (mm)
Nothofagus dombeyi	1. La Angostura	40°49′02.6″	71°34′07.8″	898	1700
	2. Los Notros	41°04′59.7″	71°33′02.6″	821	1800
	3. Lago Mascardi	41°20′58.8″	71°35′07.5″	828	1750
	4. Los Moscos	41°20′52.2″	71°36′08.8″	830	1750
Nothofagus pumilio	1. Cerro Otto	41°08′45.7″	71°22′18.5″	1317	1500
	2. C° Challhuaco	41°14′56.2″	71°16′52.3″	1235	1500
	3. C° Challhuaco	41°15′24.9″	71°16′49.6″	1314	1500
	4. C° Tronador	41°11′56.6″	71°49′42.2″	1070	1750

2009). Due to important differences in habitat and tree physiology, we hypothesized that the biomass and community composition of hypogeous sporocarps differs between *N. dombeyi*- and *N. pumilio*-dominated forests in the region we studied. We predicted that both species richness and hypogeous sporocarp biomass would be greater in the *N. dombeyi* forest type because it is warmer, wetter and evergreen.

MATERIALS AND METHODS

Study area.—Sites were located within the Nahuel Huapi National Park (705 000 ha), in Neuquén and Rio Negro provinces, Argentina (TABLE I). The area is dominated by *Nothofagus* forest along the Andes. Mean annual precipitation is 500–2300 mm (increasing from E to W) and mean annual temperature is 5–10 C. Precipitation is concentrated in autumn and winter (Mar–Sep); whereas summers are generally dry (Satti et al. 2003). The soils are poorly developed Andisols, characterized by a high capacity to stabilize organic matter and store water (Mazzarino et al. 1998). The dominant ectomycorrhizal host species in the area are *Nothofagus antarctica* (G. Forster) Oerst, *N. dombeyi* (Mirb.) Oerst and *N. pumilio* (Poepp. & Endl.) Krasser. (Cabrera 1976). *N. dombeyi* usually associates with acidic lowlands that receive abundant rainfall. By contrast, *N. pumilio* forests are more tolerant of drier and colder conditions and grow at up to 1800 m. However both species coexist in regions with intermediate conditions forming mixed forests (Cabrera 1976).

The understory vegetation for both type of forest is dominated by small trees, *Lomatia hirsuta* (Lam.) Diels ex J.F. Macbr. and *Schinus patagonicus* (Phil.) I.M. Johnst. ex Cabrera, shrubs, *Azara microphylla* Hook. F., *Aristotelia chilensis* (Molina) Stuntz, *Berberis darwinnii* Hook., *B. buxifolia* Lam., *Ribes magellanicum* Poir., *Gaultheria mucronata* (L. f.) Hook. & Arn., *G. phillyreifolia* (Pers.) Sleumer, *G. caespitosa* Poepp. & Endl., and the bamboo *Chusquea culeou* E. Desv. Small perennial plants are *Alstroemeria aurea* Graham, *Blechnum penna-marina* (Poir.) Kuhn, *Codonorchis lessonii* (Brongn.) Lindl., *Leuceria thermarum* (Phil.) Phil., *Loasa argentina* Urb. & Gilg., *Osmorrhiza chilensis* Hook. & Arn. and *Polistichum mohrioides* (Bory) C. Presl. No differences have been recorded for the understory species composition in relation to forest type; however in southern Chile a different bamboo species (*Chusquea tenuiflora* Phil.)

is more abundant under *N. dombeyi* while the other species are more abundant under *N. pumilio* (Veblen et al. 1979). It also is known that only *Nothofagus* spp. establish ectomycorrhizal associations within this area (Fontela et al. 1998; Diehl et al. 2003, 2008).

Sampling design.—Study locations were established at eight 1 ha sites within the Nahuel Huapi National Park. Hypogeous sporocarps were sample at four sites in *N. dombeyi* and four in *N. pumilio* monospecific stands in spring (Nov) and autumn (Apr) 2005 and again in 2006. Five circular 4 m² plots were evenly distributed along three 40 m transects at each site. At each sampling time new transects and plots were established within the same sites, thus avoiding previously disturbed plots (Luoma et al. 2004). Across the eight sites a total of 24 transects and 120 plots (480 m²) were sampled during each of the four sampling occasions. The transect was the sampling unit in this study. A garden cultivator was used to carefully remove litter and soil inside the circular plots, raking to the depth of 10–15 cm to expose fungal sporocarps. An individual species collection was defined as a group of conspecific sporocarps occurring within a 50 cm radius. In addition we recorded the number of trees in each transect (including all trees located up to 2 m from each side of the transect line) and tree diameters to coarsely estimate the stand's structure.

Hypogeous sporocarps were sampled in both spring (Nov) and autumn (Apr) for 2 y (2005, 2006) to capture the seasonal dichotomy in fruiting patterns and to increase the number of species detected (Fogel 1981, Luoma 1991, Smith et al. 2002). Sporocarp sampling coincided with peaks of seasonal fruiting as determined by previous visits to the region (L. Domínguez pers comm). Fresh sporocarps were macromorphologically described, photographed and dried within 12 h of collection. Microscopic characters were determined from hand-sectioned mounts in 5% KOH, phloxine and Melzer's reagent examined under a light microscope at 400× or 1000× magnification. Spore measurements included the largest and the smallest and at least 10 randomly selected spores from mature specimens; spore dimensions include ornamentation. Sporocarps were dried at 45 C for 12 h and weighed. Biomass of sporocarps was calculated as a sum of dry weights of all collections of each species during the four sample periods for a forest type. Biomass was expressed in grams of dry weight per hectare, standing crop per season (TABLE II).

TABLE II. Mean total hypogeous sporocarp standing crop (g/ha) 2005–2006 and number of sporocarp collections (in parentheses), grouped by forest type and collecting month

Fungal species	Nothofagus dombeyi		Nothofagus pumilio	
	Nov	Apr	Nov	Apr
Descomyces sp.	0.3 (3)	—	—	—
Hallingea purpurea	—	4.4 (4)	—	—
Gautieria inapire	—	7.4 (1)	—	—
Genea sp. 1	3.4 (1)	—	—	—
Genea sp. 2	2.6 (1)	—	—	—
Glomus sp.	—	1.6 (1)	—	—
Gymnohydnotrya sp.	—	0.2 (1)	—	—
Gymnopaxillus crubensis	—	5.8 (1)	—	—
Hysterangium crassipariete	8.5 (3)	454.6 (48)	1.6 (1)	—
Radiigera cf.*taylorii*	2.3 (1)	78.2 (1)	—	—
Radiigera sp.	26.5 (4)	10.7 (1)	97.5 (2)	26.9 (1)
Scleroderma sp.	0.4 (1)	—	—	—
Setchelliogaster sp.	5.6 (1)	—	—	—
Thaxterogaster albocanus	—	154.9 (20)	—	—
Thaxterogaster carneoroseus	—	32.2 (3)	253.7 (16)	—
Thaxterogaster dibaphoides	—	—	112.7 (8)	48.2 (6)
Thaxterogaster magellanicus	—	—	—	9.2 (2)
Thaxterogaster squamatus	0.1 (1)	2.7 (5)	—	2.3 (4)
Thaxterogaster violaceus	—	8.1 (1)	—	—
Thaxterogaster sp.1	4.3 (1)	—	2.9 (1)	11.1 (1)
Thaxterogaster sp.2	3.4 (1)	1.7 (1)	—	—
Thaxterogaster sp.3	10.7 (5)	—	—	—
Thaxterogaster sp.4	—	—	0.5 (1)	—
Thaxterogaster sp. 5	45.83 (9)	28 (1)	—	—
Thaxterogaster sp. 6	5.2 (1)	—	5.3 (1)	—
Thaxterogaster sp. 7	—	—	—	19.2 (1)
Thaxterogaster sp. 8	—	11.9 (1)	—	—
Totals	119.1	802.4	474.2	116.9
Number of species	14	15	7	6
Cumulative number of species	23		10	
Sum of mean spring and autumn biomass	921.5		591	

Specimens were identified to species (TABLE II) according to relevant literature (Castellano et al. 1989, Gamundi and Horak 1993, Montecchi and Lazzari 1993, Pegler et al. 1993, Singer and Smith 1960, Trappe and Castellano 1991). Unidentified collections were inferred as new taxa but are not described here.

Statistical analysis.—To study the effects of forest types on species richness of hypogeous fungi the number of collections of each species per transect were used to calculate rarefaction curves with the program EstimateS 8.2 (Colwell, 2006). To estimate the completeness of sampling at each forest type, minimal species richness estimates Chao2 and ACE were calculated with 100 permutations, sampling units (transects) were selected randomly without replacement.

The effects of forest type, season and year on species richness and sporocarp biomass, along with their corresponding interaction terms, were assessed with analysis of variance (ANOVA). The data were rank-transformed because they did not meet the ANOVA assumptions of normality and homogeneity of variance based on Shapiro-Wilks test. We also performed Spearman correlations between coarse forest structure variables (number and mean diameter of trees per transect) and species richness and biomass. The analyses were conducted with software Info Stat 1.0 (Di Rienzo et al. 2008).

RESULTS

During this study 168 collections of hypogeous sporocarps were obtained. They were separated into 27 species within nine genera of Basidiomycota, two of Ascomycota and one of Glomeromycota (TABLE II). Sixteen of these taxa were not identified to species, and these are referred to as undescribed taxa that await further taxonomic assessment.
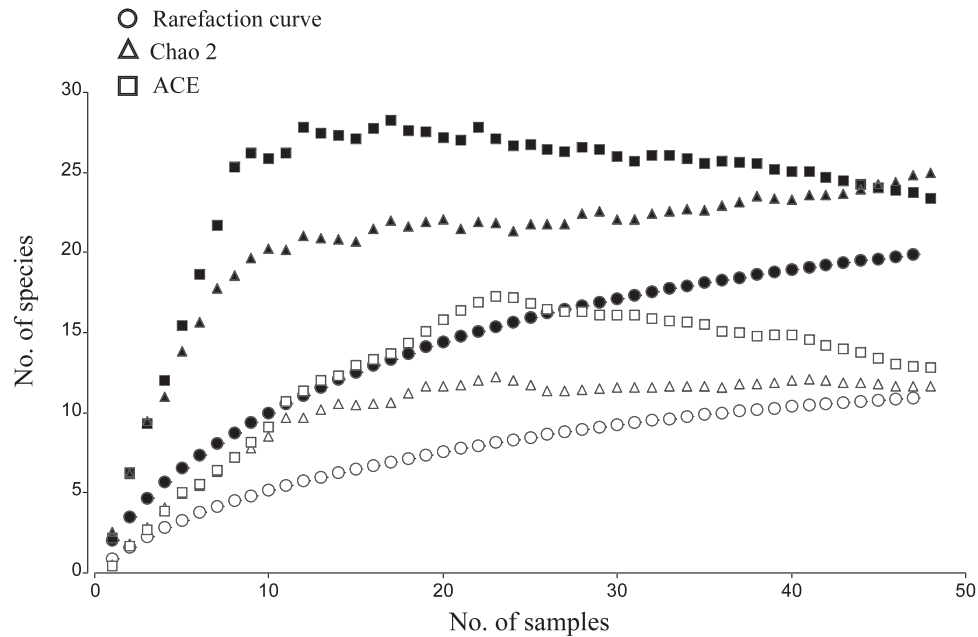
FIG. 1. Coleman rarefaction curve, ACE and Chao2 minimal species richness estimates based on hypogeous fungal sporocarp collections in *Nothofagus dombeyi* (black) and *N. pumilio* (white) forests.

The largest number of collections belonged to the genus *Thaxterogaster,* followed by *Hysterangium* (90 and 52 respectively). These two genera accounted for 86% of total collections. *Hysterangium crassipariete* (52 collections) dominated in April in terms of abundance and biomass of sporocarps. *Hysterangium crassipariete* Castellano & J.J. Muchovej and *Thaxterogaster albocanus* E. Horak & M.M. Moser were among the most frequent taxa in April, particularly under *N. dombeyi.* Other abundant species, such as *T. dibaphoides* E. Horak (8%) and *T. carneoroseus* E. Horak (11.5%) and *T. squamatus* Halling (6%), produced sporocarps in November and April over 2 y (TABLE II).

Species richness and sporocarp biomass.—Rarefaction curves nearly approached the same level for both forest types, suggesting that the sampling design was adequate to detect most of the hypogeous species. In the case of minimal species richness estimates, ACE and Chao2 also approached the asymptote with increasing sample size, indicating the presence of approximately two times the number of species for *N. dombeyi* and *N. pumilio* forest types respectively (FIG. 1). Species richness and sporocarp biomass showed significant differences between forest types. Stands of *N. dombeyi* had higher mean and total species richness and sporocarp biomass compared to *N. pumilio* (TABLE III; FIGS. 1, 2). Only six species within the genera *Hysterangium, Radiigera,* and *Thaxterogaster* were observed in both forest types.

There were significant differences between sample years. Both *Nothofagus* forest types showed higher mean and total species richness and sporocarp biomass in 2006 compared to 2005 (TABLE III, FIG. 2) A significant interaction term between forest type and season was evidenced by sporocarp biomass (TABLE III, FIG. 2). No other significant interaction term was observed between forest type, season and year. Both species richness and sporocarp biomass were positively correlated with rainfall and negatively with altitude (TABLE IV).

Relationship between fungal composition and stand structure.—Species richness and sporocarp

TABLE III. ANOVA values for richness and sporocarp biomass of hypogeous fungal species for 2005 and 2006. Sources of variation in the analysis were ''forest type'', ''season'' and ''year''

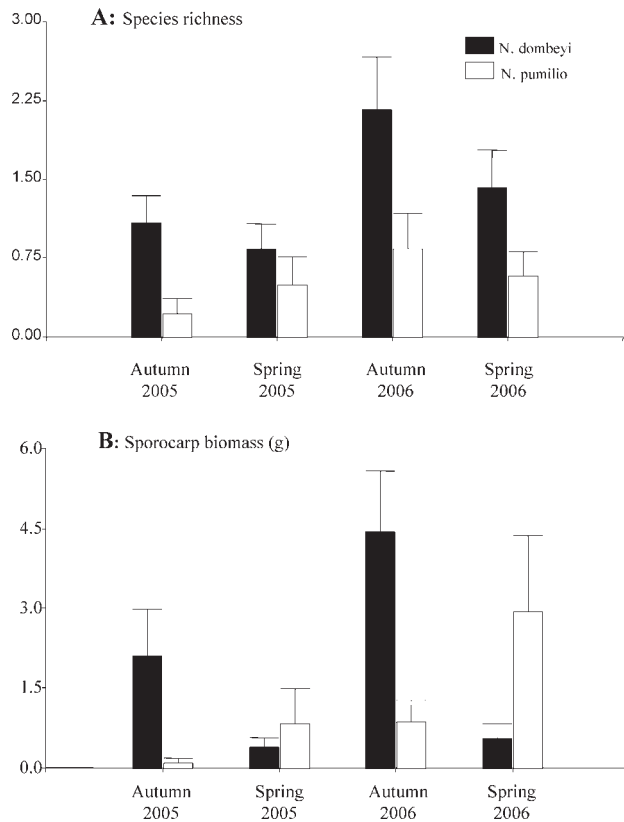|	Hypogeous fungal species			
	Richness		Sporocarp biomass	
Factors	F	P	F	P
Forest type (F)	18.22	< **0.0001**	13.93	**0.0003**
Season (S)	0.45	0.5052	1.69	0.1971
Year (Y)	5.80	**0.0181**	4.94	**0.0288**
FS	1.24	0.2685	6.17	**0.0149**
FY	0.13	0.721	0.19	0.6669
SY	0.64	0.4267	0.18	0.6762
FSY	0.17	0.6796	0.04	0.8479

FIG. 2. Hypogeous fungal species richness (A) and sporocarp biomass in g (B) over 2 y in *Nothofagus dombeyi* and *N. pumilio* forests. Error bars indicate $\pm$ 1 SE (n = 8).

biomass were significantly correlated with number of trees per transect but not with mean diameter (TABLE IV). These patterns were consistent within forest type (data not shown).

DISCUSSION

In this study we observed greater species richness and hypogeous sporocarp biomass in *N. dombeyi* as compared to *N. pumilio* forests. The rarefaction curves and the minimal richness estimates converged at about the same level for each forest type, suggesting that the sampling effort was almost sufficient to capture the hypogeous sporocarp richness in these forest stands at the regional scale.

Seasonal and precipitation influence.—At our study sites rain typically falls from mid-autumn (May) through spring (Oct–Nov), with snow during the winter and a water deficit in summer (Bava 1997). Species richness was not significantly affected by season when comparing November vs. April samples between the two forest types. However biomass in April was consistently greater in *N. dombeyi* forest stands while *N. pumilio* stands observed greater biomass in November. These contrasting patterns account for the significant interaction term between forest type and season. Despite the positive relationship between average precipitation and biomass, the contrasting patterns between forest types by season suggest that differences in precipitation between *N. dombeyi* and *N. pumilio* forest stands do not fully explain biomass production patterns.

Several studies from Australia have documented differences in diversity and biomass of sporocarps in relation to seasonal rainfall and temperature patterns (Claridge et al. 1993, 2000a; Johnson 1994). States and Gaud (1997) found a significant reduction in hypogeous sporocarp production associated with decreasing precipitation. O'Dell et al. (1999) suggested that EMF fungal species richness and sporocarp biomass have a unimodal or hump-shaped distribution in relation to precipitation values in a study of a moisture gradient transect established within the Olympic Peninsula in North America. They found that both variables increased linearly with average annual precipitation up to intermediate values (ca. 2500 mm/y) but were lower again in one extremely wet site. In tropical climates hypogeous sporocarp production shows different responses, with "hydrophilic" species increasing abundance and biomass at higher rainfall values and "hydrophobic" species showing the inverse trend (Abell 2008). All in all the evidence suggests that average precipitation generally correlates with sporocarp richness and biomass but this relationship is dependent on biomes and host trees.

Relationship between stand characteristics and hypogeous sporocarp production.—It has been widely shown that stand structure depends on species composition and age. Here we found that number of trees, but not

TABLE IV. Correlation analysis between fungal variables, precipitation, altitude and forest structure variables

Variables	Precipitation		Altitude		Number of trees		Mean diameter of trees	
	R	P	R	P	R	P	R	P
Species richness	0.5654	**< 0.0001**	−0.3112	**0.0028**	0.4285	**< 0.0001**	−0.0489	0.6337
Sporocarp biomass	0.5256	**< 0.0001**	−0.2722	**0.009**	0.4711	**< 0.0001**	−0.1361	0.1848

diameter (as surrogate of age), positively correlated with sporocarp richness and biomass. Amaranthus et al. (1994) observed that in relatively dry regions of Oregon hypogeous sporocarp production was greater in mature stands where it was associated with abundant decaying coarse woody debris. In contrast, in mesic stands in Oregon's central Cascade Range Smith et al. (2002) found that despite sporocarp biomass being higher in ''young'' and ''rotation age'' stands as compared to old growth forest; there was no difference in species richness among the different *Pseudotsuga menziesii* stand age classes. These differences among studies probably reflect the diverse physiographic settings and composition of the studied forest communities and variations in detection and annual production of sporocarps.

Altitude influence.—The monospecific stands of *N. dombeyi* and *N. pumilio* occur at different average altitudes. We observed that the lowest biomass values were obtained for the subalpine *N. pumilio* forests (1150–1320 m). These forests occur in belts an average of 500 m above the *N. dombeyi* forests (650–850 m). The negative correlation between altitude and fungal richness and biomass that we observed is in accordance with a study from the Canadian Rocky Mountains that showed decreasing EMF species richness with elevation. They concluded that the reduction in sporocarps was in response to a number of physical factors, such as changes in edaphic properties and microclimate conditions (Kernaghan and Harper 2001).

Stand type influence on species richness and biomass of hypogeous sporocarps.—The results of this study supported our hypothesis that fungal richness and hypogeous sporocarp biomass would be greater in the warmer, wetter and evergreen *N. dombeyi* forest type. In addition we observed that *Hysterangium crassipariete* and *Thaxterogaster albocanus*, two of the most prolific species in terms of sporocarp production, were more common in *N. dombeyi* forest stands than in *N. pumilio* stands, suggesting some type of habitat selection effect on these species (Molina et al. 1992, Nantel and Newman 1992).

Together with stand features discussed above host plant also might have a great influence on associated fungal communities. For example evergreen and deciduous species leaves are known to differ in chemical composition, with evergreen showing higher lignin contents than deciduous (Aerts, 1995, Diehl et al. 2008). It has been shown that *Nothofagus* species display differential leaf life spans and differ in the decomposition process within their monospecific forest stands. That is each host produces effects on the belowground properties that enhanced decom-

position of their own litter in comparison to litter from other congeneric species (Vivanco and Austin 2008). This could be explained by a decomposer community developing that is more efficient in the breakdown of the litter produced in situ (Gholz et al. 2000, Vivanco and Austin 2008). Accordingly, leaf litter qualities could influence EMF communities because these symbiotic fungi also have some saprotrophic capabilities (Conn and Dighton 2000, Dighton et al. 2000). Our results may indicate that *N. dombeyi* has a more diverse EMF community that also contributes to the decomposition of the litter. Morris et al. (2008) suggested that congeneric tree species with differences in their biological life strategies can create distinct ecological niches for EMF.

The relatively low number of hypogeous species observed under *N. dombeyi* and *N. pumilio* compared with other ECM temperate forests could be attributed partly to the biogeographic isolation of the *Nothofagus* stands in southern South America and the short term of sampling. However new species are expected to occur in the vast area and varied landscapes of the Andes dominated by *Nothofagus*. It is worth mentioning that within the native vegetation only *Nothofagus* species forms ECM symbiosis in the Patagonian forests, which might constrain the pool of potential ''host generalist'' symbiotic fungal partners. Although experimental evidence is lacking, higher host diversity could be related to higher EMF richness in a given area (Bruns 1995).

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Authors Queries

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