

# Ectomycorrhizal fungal communities associated to *Nothofagus* species in Northern Patagonia

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**Abstract** Ectomycorrhizal fungi constitute an important component of soil biota in *Nothofagus* forests in Patagonia. However, ectomycorrhizal fungal community is poorly known in this region. Here, we assess biodiversity and community compositions of ectomycorrhizal fungal species associated with *Nothofagus dombeyi*, *N. obliqua* and *N. alpina*. We selected three monospecific *Nothofagus* forest sites for each species within the boundaries of the Lanin National Park in Northern Patagonia. Ectomycorrhizal fungal species were identified based on morphotyping and rDNA (ITS and 28S rDNA) sequence analysis using both universal and taxon-specific primers. Contrary to previous studies on congeneric host trees, our results showed no significant differences among *Nothofagus* forest types in terms of fungal biodiversity and community composition. However, altitude had a strong effect on the structure of the ectomycorrhizal fungal community associated with *Nothofagus* spp.

**Keywords** ECM fungi · Richness · Community composition · *Nothofagus*

## Introduction

Ectomycorrhizal fungi (ECMF) form an important component of the forest ecosystems, mainly because of their capabilities in nutrient mobilization and soil aggregation (Smith and Read 2008). Despite that many species of ECMF are able to

associate with several unrelated host species (Horton and Bruns 1998; Kennedy et al. 2003), some notable exceptions have been observed, particularly in host species belonging to *Pinus* and *Alnus* (Molina et al. 1992). Host identity may strongly influence ECMF community composition, mainly when phylogenetically unrelated hosts are considered (Ishida et al. 2007; Tedersoo et al. 2008; Smith et al. 2009). Moreover, shifts in the ECMF communities can occur even in co-occurring host species belonging to the same genus (Aponte et al. 2010; Morris et al. 2008, 2009; Cavender-Bares et al. 2009; Kohout et al. 2011). Increasing evidence suggests that many dominant ECMF display some degree of host preference (Tedersoo et al. 2008) and such preferences may be dependent on host genetic variation (van der Heijden and Kuyper 2001) as well as influenced by environmental factors. Most recently, Morris et al. (2008) showed that ECMF assemblages vary within oak species from different sections and the same pattern was observed on oaks under diverse water availability regimes (Cavender-Bares et al. 2009).

In Patagonian temperate forests ecosystems, richness and biomass of hypogeous fruit-bodies of ECMF showed differences between forests dominated by either *Nothofagus dombeyi* or *N. pumilio*, and across gradients of altitude and precipitation (Nouhra et al. 2012a). At the regional and global scales, both temperature and precipitation strongly affect ECMF richness (Bahram et al. 2012; Tedersoo et al. 2012).

*Nothofagus* species are widely distributed in the Andean forests of southern Argentina and Chile below 33°S (Moreira-Muñoz 2004). In Lanin National Park in Northern Patagonia, five *Nothofagus* species form extensive monospecific forests stands, albeit some mixed forests also occur. In this region, the evergreen *N. dombeyi* (subgenus *Nothofagus*) alternates with forests stands of two other deciduous hosts: *N. obliqua* and *N. alpina*, (subgenus *Lophozonia*) and their distribution responds to different environmental conditions (Sabatier et al. 2011).

The ECMF taxa in the region including the Chilean side of the Andes, has been sparsely studied based on fruit-body

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collections (Moser and Horak 1975); Horak 1979; Garrido 1988; Garnica et al. 2003) and root tips with limited molecular identification (Palfner et al. 2008). Our main objective is to provide information about the ECMF communities in pristine *Nothofagus* forests of Southern Argentina and based on previous data we hypothesize that ECMF community composition differs among *N. dombeyi*, *N. obliqua*, and *N. alpina* forests types.

## Materials and methods

### Study area

The study sites are located in the Lanin National Park, Neuquén province, Northern Patagonia, Argentina (Table 1). The area is dominated by *Nothofagus* forests along the eastern foothills of the Andes. The climate is characterized by mean annual precipitation ranging from 1,800 to 2,250 mm (increasing to the west), and average monthly temperature ranging from 3 °C (July) to 15 °C in January (Vivanco and Austin 2008). Most of the rainfall occurs in March–September, falling as rain and snow. Summers are generally dry (Satti et al. 2003). The soils are Andosols (IUSS Grupo de Trabajo WRB (2007)) that are derived from postglacial volcanic ashes. The understory vegetation is mostly dominated by dense clumps of *Chusquea culeou* E. Desv. and a few other plants such as *Gaultheria mucronata* (L. f.) Hook. & Am., *Blechnum penna-marina* (Poir.) Kuhn, *Osmorrhiza chilensis* Hook. & Am., and *Polistichum mohrioides* (Bory) C. Presl. The dominant ectomycorrhizal host species in the study area include *N. dombeyi* (Mirb.) Oerst., *N. obliqua* (Mirb.) Oerst and *N. alpina* (Poepp. & Endl.) Oerst (Cabrera 1976). *N. dombeyi* is a widespread evergreen tree that usually grows on relatively acidic lowland soils (650–1,000 masl) that receive abundant rainfall; *N. obliqua* (650–1,150 masl) and *N. alpina* (800–1,350 masl) are relatively drought tolerant deciduous tree species that occur at mid- to low elevations in the region (Sabatier et al. 2011). The forests constituted a mosaic of

mono-dominant patches; a pattern that has also been observed in New Zealand *Nothofagus* forests (Wardle 1984). It is known that only *Nothofagus* spp. establish ECMF associations within the native vegetation of this area (Fontenla et al. 1998; Diehl et al. 2003, 2008).

We established three study sites (approx. 1 ha each) for each of the three monospecific *Nothofagus* forest types, thus totalizing nine sites randomly distributed in a 40×25-km area orientated west to east along the Andes and surrounding the lake Lacar basin (Sabatier et al. 2011), which is the natural distribution of *N. obliqua* and *N. alpina*. The distance between sites ranged from 4.9 to 31 km and their specific geographic location, host, and climatic characteristics are given in Table 1. At each site, eight soil samples (one sample taken from around one individual tree) were randomly collected with a spade, 20 cm deep and 25 cm in diameter, approximately 50–90 cm from the base of a tree. Soil sampling coincided with the peak of seasonal fruiting of ECMF, as determined by previous studies (Nouhra et al. 2012a). Samples were placed in plastic bags, labeled and stored at 4 °C until processing.

### Ectomycorrhiza morphotyping and DNA analysis

Roots from each sample were removed from the soil and gently rinsed in water. For each sample, all roots were placed under a stereomicroscope for examination at 10–40× magnification. The ectomycorrhizal root tips were separated carefully from the roots of non ectomycorrhizal plants and sorted into morphotypes according to their morphological and anatomical features. Criteria for sorting included diameter, branching pattern, mantle color, morphology of emanating hyphae and rhizomorphs as described in Agerer (1991). Clusters of ectomycorrhizal root tips belonging mostly to one individual morphotype from each root fragment and soil core were inserted into 1.5-ml Eppendorf tubes containing 500 µl 2 % CTAB DNA extraction buffer (2 % cetyltrimethylammonium bromide, 100 mM Tris–HCl (pH 8.0), 1.4 M NaCl, and 20 mM EDTA) and stored at –20 °C.

**Table 1** Location, host and climatic characteristics of the study sites

Sites	Host	Latitude	Longitude	Altitude (m)	Precip. (mm)	MAT (°C)
P5	<i>N. obliqua</i>	–40.154833	–71.506333	850	2,000	8.3
P7	<i>N. obliqua</i>	–40.125472	–71.657861	715	2,200	9.4
P6	<i>N. obliqua</i>	–40.147972	–71.489444	916	1,790	7.8
C6	<i>N. dombeyi</i>	–40.12375	–71.657861	690	2,000	9.4
C8	<i>N. dombeyi</i>	–40.141583	–71.470056	907	1,790	7.8
C10	<i>N. dombeyi</i>	–40.157466	–71.643902	714	2,000	9.4
R6	<i>N. alpina</i>	–40.130861	–71.657861	769	2,000	8.3
R8	<i>N. alpina</i>	–40.133028	–71.701	859	2,200	8.3
R4	<i>N. alpina</i>	–40.131556	–71.458972	974	1,790	7.2

MAT mean annual temperature

One to five root tips from each morphotype per soil core were subjected to DNA extraction using a Qiagen DNeasy 96 Plant Kit (Qiagen, Crawley, UK). PCR was performed using the primer ITSOF-T (5'-acttggtcatttagaggaagt-3') combined with LB-W (5'-cttttcattttccctcacgg-3') for Basidiomycota or ITS4 (5'-tctcgcgttattgatatgc-3') and ITS2 (5'-gctgcgtttctcatgatgc-3') that target all fungi. DNA resulting in low-quality sequences was re-amplified with taxon-specific primers as described in Tedersoo et al. (2011). For most phylotypes, 28S rDNA was amplified using primers LR0R (5'-accgcgtgaacttaagc-3') in combination with LB-Z (5'-aaaaatggcccactagaaact-3') or LR5 (5'-tcttgagggaacttcg-3'). The PCR products were checked on 1 % agarose gels under UV-light and purified using Exo-Sap enzymes (Sigma, St. Louis, MO, USA). Sequencing was performed using primer ITS5 (5'-ggaagtaaaagtcgtaacaagg-3'), LF340 (5'-tactgtgctgcgtatcg-3'), and/or ITS4 for the ITS region and CTB6 (5'-gcataatcaataagcggagg-3') for the 28S gene. Sequences were assembled into contigs and checked for quality using Sequencher 4.9 software (GeneCodes Corp., Ann Arbor, MI, USA). Sequences were assigned to species based on 97 % ITS sequence similarity threshold (excluding flanking rDNA genes) and to ECMF lineages according to Tedersoo et al. (2010). Identification was provided by running MegaBLAST and BLASTn searches of sequences against the International Sequence Database and UNITE separately or using a mass blaster function implemented in the PlutoF work bench (Abarenkov et al. 2010). ECMF taxon names were based upon the taxonomic level supported from the BLAST results (Table 2).

### Statistical analysis

In order to estimate the completeness of sampling at each forest type, the number of taxa identified from the root tips of each soil sample were used to calculate the rarefied species accumulation curves as implemented in EstimateS ver. 8.2 (Colwell 2006). To study the variation of the accumulating species richness of the ECMF communities among forest types, the minimal species richness estimate Chao2 was calculated with 100 permutations, samples being selected randomly without replacement with the above-mentioned program.

To address the effect of host on species richness for each *Nothofagus* species separately, we carried out a two-way analysis of variance (ANOVA). The data were rank-transformed when they did not meet the assumptions of ANOVA based on Shapiro–Wilks normality test. The analysis was conducted using the software Info Stat v. 1.0 (Di Rienzo et al. 2001).

To address the effect of host tree species on fungal community composition, we ran Adonis function in the Vegan package of R (Oksanen et al. 2010) using the

following options: Hellinger-transformed species frequency, Bray–Curtis distance measure and 999 permutations. Altitude was used as a covariate in this analysis. In parallel, Global Nonmetric Multidimensional Scaling ordination was performed using the same options.

### Results

In total, 81, 76, and 84 % of morphotype samples were successfully identified for *N. dombeyi*, *N. obliqua*, and *N. alpina*, respectively. The remaining root tip samples failed to amplify due to the moribund state of roots or co-colonization by diverse array of saprobes, particularly *Mortierella* spp. (Zygomycota) that were readily amplified with the supposedly basidiomycete-specific LB-W primer. Besides *Mortierella*, several root tip sequences corresponded to groups related to *Tulasnella* and *Rickenella*. Because these taxa were nested within non-ECMF groups and had no consistent ectomycorrhizal morphology, they were conservatively considered non-mycorrhizal and omitted from further analyses. Clustering of ITS sequences at 97 % similarity revealed 72 taxa of ECMF, including 38, 34, and 39 species associated with *N. dombeyi*, *N. obliqua*, and *N. alpina*, respectively, across the nine sites.

The ECMF community was dominated by Basidiomycota, but Ascomycota were highly diverse in these *Nothofagus* forests. Within the Basidiomycota, numerous species of the */cortinarius* (14 spp) and */inocybe* (12 spp.) lineages were found, albeit a few of them occurred with all three *Nothofagus* species (Fig. 1). Of Agaricales, five species of the */tricholoma* lineage, two species of the */descolea* lineage and a single species of the */hebeloma-alnicola* lineage were also recorded. Of other basidiomycetes, the */tomentella-thelephora* (5 spp.), */clavulina* (3 spp.), */tulasnella* (2 spp.), and */sebacina* (3 spp.) lineages were present in the fungal community (Table 2). Within the Ascomycota, *Cenococcum geophilum* was found on root tips of all three *Nothofagus* spp. Eight species of the Pezizaceae (belonging to the */pachyphloeus-amylascus* and */terfezia-peziza depressa* lineages) and eight species of Pyrenomataceae (including the */pulvinula*, */otidea*, */tarzetta*, */genea-humaria*, and */wilcoxina* lineages), occurred in various sites (Table 2). Two species of the */tuber-helvella* lineage were present in both *N. alpina* and *N. obliqua* samples. Several species groups of the Pezizales order could not be matched with any of the known ectomycorrhizal or non-mycorrhizal taxa (cf. Tedersoo et al. 2010, 2013). Also, a single species in the Helotiales was recorded for the *N. alpina* community (Table 2).

The ECMF community was dominated with a few frequent species and numerous rare species with a single occurrence (32 singletons). The most frequent species included *Cortinarius* sp. 7 (11samples), Pyrenomataceae sp. 8 (9),

**Table 2** List of taxa obtained from the DNA sequence analysis results of the ITS1/5.8S/ITS2 region from ECM morphotypes based on Gen Bank blast searches. Best BLASTn matches of the entire internal transcribed spacer (ITS) region to database sequences are shown for the ECMF root tips for the three *Nothofagus* spp

Taxon	Best BLASTn identified ITS match			Host species
	UNITE accession	Specimen	% identity	
<i>Austropaxillus boletinoides</i>	UDB007002	<i>Austropaxillus boletinoides</i> HM135660 <sup>a</sup>	100.0	<i>N. dombeyi</i>
<i>Cenococcum</i> sp 1	UDB007042	<i>Cenococcum geophilum</i> AY394919	99.0	<i>N. dombeyi</i>
<i>Clavulinaceae</i> sp 1	UDB007001	<i>Clavulinaceae</i> sp. AJ534710	92.5	<i>N. dombeyi</i>
<i>Clavulinaceae</i> sp 4	UDB007059	<i>Clavulina</i> sp. FN 669178	84.0	<i>N. dombeyi</i>
<i>Cortinarius</i> sp 1	UDB007046	<i>Cortinarius venetus</i> FJ039601	95.0	<i>N. dombeyi</i>
<i>Cortinarius</i> sp 9	UDB007009	<i>Cortinarius subcastanellus</i> GU222270	94.0	<i>N. dombeyi</i>
<i>Cortinarius</i> sp 3	UDB007010	<i>Cortinarius idahoensis</i> FJ039595	94.0	<i>N. dombeyi</i>
<i>Cortinarius</i> sp 7	UDB007066	<i>Cortinarius cycneus</i> GU222297	95.0	<i>N. dombeyi</i>
<i>Cortinarius</i> sp 4	UDB007018	<i>Cortinarius balteatus</i> FJ039613	96.0	<i>N. dombeyi</i>
<i>Cortinarius</i> sp 12	UDB007038	<i>Cortinarius ionomataius</i> GU222303	89.0	<i>N. dombeyi</i>
<i>Cortinarius</i> sp 6	UDB007045	<i>Cortinarius cretux</i> AY669622	93.0	<i>N. dombeyi</i>
<i>Descomyces</i> sp 1	UDB007003	<i>Descomyces</i> sp. DQ328217	94.0	<i>N. dombeyi</i>
<i>Genea</i> sp 1	UDB007030	<i>Genea harknesii</i> DQ218282	91.0	<i>N. dombeyi</i>
<i>Inocybe</i> sp 1	UDB007011	<i>Inocybe jacobii</i> HQ604371	89.0	<i>N. dombeyi</i>
<i>Inocybe</i> sp 2	UDB007033	<i>Inocybe sambucina</i> AM882757	85.0	<i>N. dombeyi</i>
<i>Inocybe</i> sp 3	UDB007037	<i>Inocybe phaeoleuca</i> FN550907	89.0	<i>N. dombeyi</i>
<i>Inocybe</i> sp 5	UDB007028	<i>Inocybe</i> sp FN550934	93.0	<i>N. dombeyi</i>
<i>Inocybe</i> sp 7	UDB007067	<i>Inocybe glabrescens</i> HQ604216	86.0	<i>N. dombeyi</i>
<i>Inocybe</i> sp 9	UDB007068	<i>Inocybe hystrix</i> AM882810	86.0	<i>N. dombeyi</i>
<i>Pachyphloeus</i> sp 1	UDB007050	<i>Pachyphloeus melanoanthus</i> EU543194	83.0	<i>N. dombeyi</i>
<i>Pachyphloeus</i> sp 4	UDB007017	<i>Pachyphloeus marroninus</i> EU427549	88.0	<i>N. dombeyi</i>
<i>Pezizaceae</i> sp 5	UDB007065	<i>Peziza saccardoana</i> JF908541	82.0	<i>N. dombeyi</i>
<i>Pezizaceae</i> sp 3	UDB007056	<i>Peziza michelii</i> DQ200839	81.0	<i>N. dombeyi</i>
<i>Pyrenomataceae</i> sp 1	UDB007008	<i>Pulvinula convexella</i> UDB000987	87.9	<i>N. dombeyi</i>
<i>Pyrenomataceae</i> sp 2	UDB007007	<i>Otidea</i> sp FJ404767	89.0	<i>N. dombeyi</i>
<i>Pyrenomataceae</i> sp 4	UDB007051	<i>Pseudaleuria quinaultiana</i> EU669428	90.0	<i>N. dombeyi</i>
<i>Pyrenomataceae</i> sp 5	UDB007029	<i>Wilcoxina mikolae</i> AY880942	89.0	<i>N. dombeyi</i>
<i>Pyrenomataceae</i> sp 6	UDB007006	<i>Pezizomycetes</i> sp. GQ153151	85.0	<i>N. dombeyi</i>
<i>Sebacinaceae</i> sp 2	UDB007041	<i>Sebacina</i> sp DQ974768	93.0	<i>N. dombeyi</i>
<i>Thaxterogaster</i> sp 2	UDB007005	<i>Thaxterogaster levisporus</i> DQ328148	93.0	<i>N. dombeyi</i>
<i>Tomentella</i> sp 1	UDB007004	<i>Tomentella</i> sp. AJ534914	95.8	<i>N. dombeyi</i>
<i>Tomentella</i> sp 2	UDB007016	<i>Tomentella</i> sp DQ 974782	92.0	<i>N. dombeyi</i>
<i>Tomentella</i> sp 5	UDB007024	<i>Tomentella</i> sp AF430289	98.0	<i>N. dombeyi</i>
<i>Tricholomataceae</i> sp 1	UDB007039	<i>Tricholoma orirubens</i> DQ389734	92.0	<i>N. dombeyi</i>
<i>Tricholomataceae</i> sp 4	UDB007061	<i>Tricholoma sejunctum</i> FJ845448	88.0	<i>N. dombeyi</i>
<i>Tulasnellaceae</i> sp 1	UDB007040	<i>Tulasnella tomaculum</i> AY373296	80.0	<i>N. dombeyi</i>
<i>Tulasnellaceae</i> sp 2	UDB007019	<i>Epulorhiza</i> sp. AB369937	85.0	<i>N. dombeyi</i>
<i>Cenococcum</i> sp 1	UDB007118	<i>Cenococcum geophilum</i> AY394919	98.0	<i>N. obliqua</i>
<i>Clavulinaceae</i> sp 1	UDB007074	<i>Clavulinaceae</i> sp. AJ534710	87.0	<i>N. obliqua</i>
<i>Clavulinaceae</i> sp 3	UDB007107	<i>Clavulina</i> sp. FN 669173	88.0	<i>N. obliqua</i>
<i>Cortinarius</i> sp 1	UDB007116	<i>Cortinarius venetus</i> FJ039601	96.0	<i>N. obliqua</i>
<i>Cortinarius</i> sp 14	UDB007078	<i>Cortinarius vaginatus</i> AY669609	100.0	<i>N. obliqua</i>
<i>Cortinarius</i> sp 8	UDB007111	<i>Cortinarius variosimilis</i> FJ717596	96.0	<i>N. obliqua</i>
<i>Cortinarius</i> sp 9	UDB007119	<i>Cortinarius subcastanellus</i> AY669623	96.0	<i>N. obliqua</i>
<i>Cortinarius</i> sp 7	UDB007077	<i>Cortinarius cycneus</i> GU222297	97.0	<i>N. obliqua</i>



**Table 2** (continued)

Taxon	Best BLASTn identified ITS match			Host species
	UNITE accession	Specimen	% identity	
<i>Cortinarius</i> sp 15	UDB007083	<i>Cortinarius teraturgus</i> AF389151	95.0	<i>N. obliqua</i>
<i>Descolea</i> sp 1	UDB007127	<i>Descolea antarctica</i> AF325647	99.0	<i>N. obliqua</i>
<i>Descomyces</i> sp 1	UDB007069	<i>Descomyces</i> sp. DQ328217	94.0	<i>N. obliqua</i>
<i>Helvellaceae</i> sp 1	UDB007073	<i>Helvella beatoni</i> GQ981530	83.0	<i>N. obliqua</i>
<i>Inocybe</i> sp 11	UDB007135	<i>Inocybe calida</i> AM882760	88.0	<i>N. obliqua</i>
<i>Inocybe</i> sp 2	UDB007120	<i>Inocybe sambucina</i> AM882757	84.0	<i>N. obliqua</i>
<i>Inocybe</i> sp 5	UDB007091	<i>Inocybe</i> sp FN550934	91.0	<i>N. obliqua</i>
<i>Inocybe</i> sp 6	UDB007138	<i>Inocybe ambigua</i> AM882800	89.0	<i>N. obliqua</i>
<i>Inocybe</i> sp 9	UDB007079	<i>Inocybe hystrix</i> AM882810	87.0	<i>N. obliqua</i>
<i>Laccaria</i> sp 1	UDB007070	<i>Laccaria glabripes</i> HQ533019	97.0	<i>N. obliqua</i>
<i>Pachyphloeus</i> sp 1	UDB007098	<i>Pachyphloeus melanoxanthus</i> EU543194	83.0	<i>N. obliqua</i>
<i>Pachyphloeus</i> sp 3	UDB007082	<i>Pachyphloeus virescens</i> EU543198	89.0	<i>N. obliqua</i>
<i>Pezizaceae</i> sp 1	UDB007087	<i>Peziza badia</i> DQ384574	88.0	<i>N. obliqua</i>
<i>Pezizaceae</i> sp 2	UDB007072	<i>Peziza</i> sp FN669234	83.0	<i>N. obliqua</i>
<i>Pezizaceae</i> sp 3	UDB007103	<i>Peziza michelii</i> DQ200838	81.0	<i>N. obliqua</i>
<i>Pezizaceae</i> sp 4	UDB007097	<i>Peziza infossa</i> DQ974817	82.0	<i>N. obliqua</i>
<i>Pyrenomataceae</i> sp 2	UDB007095	<i>Otidea</i> sp FJ404767	89.0	<i>N. obliqua</i>
<i>Pyrenomataceae</i> sp 4	UDB007115	<i>Pseudaleuria quinaultiana</i> EU669428	90.0	<i>N. obliqua</i>
<i>Pyrenomataceae</i> sp 7	UDB007125	<i>Tarzetta</i> sp AJ969614	86.0	<i>N. obliqua</i>
<i>Pyrenomataceae</i> sp 8	UDB007089	<i>Pyrenomataceae</i> sp. DQ220465	95.0	<i>N. obliqua</i>
<i>Sebacinaeae</i> sp 1	UDB007080	<i>Sebacina</i> aff <i>epigaea</i> AF490393	95.0	<i>N. obliqua</i>
<i>Tomentella</i> sp 1	UDB007109	<i>Tomentella</i> sp. AJ534914	96.0	<i>N. obliqua</i>
<i>Tomentella</i> sp 5	UDB007114	<i>Tomentella</i> sp. AF430289	95.0	<i>N. obliqua</i>
<i>Tricholomataceae</i> sp 2	UDB007123	<i>Tricholoma saponaceum</i> FJ845442	87.0	<i>N. obliqua</i>
<i>Tricholomataceae</i> sp 3	UDB007100	<i>Tricholoma fulvum</i> DQ658855	95.0	<i>N. obliqua</i>
<i>Tricholomataceae</i> sp 5	UDB007096	<i>Tricholoma myomyces</i> AY082607	86.0	<i>N. obliqua</i>
<i>Tulasnellaceae</i> sp 2	UDB007088	<i>Epulorhiza</i> sp. AB369933	86.0	<i>N. obliqua</i>
<i>Athelia</i> sp 1	UDB007153	<i>Athelia singularis</i> GQ162813	96.0	<i>N. alpina</i>
<i>Cenococcum</i> sp 1	UDB007166	<i>Cenococcum geophilum</i> AY394919	98.0	<i>N. alpina</i>
<i>Clavulinaceae</i> sp 1	UDB007165	<i>Clavulinacea</i> sp. AJ534710	86.0	<i>N. alpina</i>
<i>Cortinarius</i> sp 8	UDB007213	<i>Cortinarius variosimilis</i> FJ717596	95.0	<i>N. alpina</i>
<i>Cortinarius</i> sp 4	UDB007161	<i>Cortinarius</i> cf. <i>balteatus</i> FJ157117	96.0	<i>N. alpina</i>
<i>Cortinarius</i> sp 5	UDB007181	<i>Cortinarius amoenus</i> AF389160	97.0	<i>N. alpina</i>
<i>Cortinarius</i> sp 7	UDB007204	<i>Cortinarius cyneus</i> GU222297	97.0	<i>N. alpina</i>
<i>Cortinarius</i> sp 6	UDB007192	<i>Cortinarius cretax</i> AY669622	91.0	<i>N. alpina</i>
<i>Cortinarius</i> sp 12	UDB007211	<i>Cortinarius ionomataius</i> GU222303	95.0	<i>N. alpina</i>
<i>Cortinarius</i> sp 19	UDB007193	<i>Cortinarius walkeri</i> AY669632	95.0	<i>N. alpina</i>
<i>Genea</i> sp 1	UDB007144	<i>Genea harknesii</i> DQ218282	95.0	<i>N. alpina</i>
<i>Hebeloma</i> sp 2	UDB007195	<i>Hebeloma theobrominum</i> FJ816621	92.0	<i>N. alpina</i>
<i>Helotiales</i> sp 1	UDB007171	<i>Helotiales</i> sp EF093148	88.0	<i>N. alpina</i>
<i>Inocybe</i> sp 2	UDB007162	<i>Inocybe sambucina</i> AM882757	86.0	<i>N. alpina</i>
<i>Inocybe</i> sp 5	UDB007150	<i>Inocybe</i> sp FN550934	85.0	<i>N. alpina</i>
<i>Inocybe</i> sp 12	UDB007177	<i>Inocybe auricomella</i> FN550900	89.0	<i>N. alpina</i>
<i>Inocybe</i> sp 13	UDB007182	<i>Inocybe tenebrosa</i> AM882967	86.0	<i>N. alpina</i>
<i>Inocybe</i> sp 14	UDB007183	<i>Inocybe godeyi</i> AJ889954	85.0	<i>N. alpina</i>
<i>Inocybe</i> sp 6	UDB007178	<i>Inocybe ambigua</i> AM882800	88.0	<i>N. alpina</i>
<i>Inocybe</i> sp 7	UDB007168	<i>Inocybe glabrescens</i> HQ604216	88.0	<i>N. alpina</i>

**Table 2** (continued)

Taxon	Best BLASTn identified ITS match			Host species
	UNITE accession	Specimen	% identity	
<i>Inocybe</i> sp 8	UDB007187	<i>Inocybe aeruginascens</i> 949591	87.0	<i>N. alpina</i>
<i>Pachyphloeus</i> sp 1	UDB007175	<i>Pachyphloeus melanoxanthus</i> JF908511	86.0	<i>N. alpina</i>
<i>Pachyphloeus</i> sp 3	UDB007210	<i>Pachyphloeus virescens</i> EU543198	88.0	<i>N. alpina</i>
<i>Pezizaceae</i> sp 1	UDB007172	<i>Peziza badia</i> DQ384574	86.0	<i>N. alpina</i>
<i>Pezizaceae</i> sp 5	UDB007141	<i>Peziza saccardoana</i> JF908541	82.0	<i>N. alpina</i>
<i>Pezizaceae</i> sp 2	UDB007147	<i>Peziza</i> sp FN669234	83.0	<i>N. alpina</i>
<i>Pyrenomataceae</i> sp 4	UDB007184	<i>Pseudaleuria quinaultiana</i> EU669386	85.0	<i>N. alpina</i>
<i>Pyrenomataceae</i> sp 5	UDB007176	<i>Wilcoxina mikolae</i> AY880942	89.0	<i>N. alpina</i>
<i>Pyrenomataceae</i> sp 8	UDB007146	<i>Pyrenomataceae</i> sp. DQ220465	95.0	<i>N. alpina</i>
<i>Russulaceae</i> sp 1	UDB007154	<i>Russula</i> sp GU222292	96.0	<i>N. alpina</i>
<i>Russulaceae</i> sp 2	UDB007174	<i>Russula pallidospora</i> DQ422032	86.0	<i>N. alpina</i>
<i>Sebacinaceae</i> sp 1	UDB007179	<i>Sebacina</i> aff <i>epigea</i> AF490393	95.0	<i>N. alpina</i>
<i>Sebacinaceae</i> sp 3	UDB007170	<i>Tremellodendron pallidum</i> AF384862	95.0	<i>N. alpina</i>
<i>Thaxterogaster</i> sp 1	UDB007142	<i>Thaxterogaster albocanus</i> AF325599	97.0	<i>N. alpina</i>
<i>Tomentella</i> sp 1	UDB007148	<i>Tomentella</i> sp. AJ534914	95.0	<i>N. alpina</i>
<i>Tomentella</i> sp 3	UDB007152	<i>Tomentella</i> sp AJ534912	93.0	<i>N. alpina</i>
<i>Tomentella</i> sp 4	UDB007145	<i>Tomentella fuscocinerea</i> GU214812	93.0	<i>N. alpina</i>
<i>Tuberaceae</i> sp 1	UDB007212	<i>Tuber</i> sp AB553455	84.0	<i>N. alpina</i>
<i>Tulasnellaceae</i> sp 2	UDB007188	<i>Epulorhiza</i> sp. AB369938	82.0	<i>N. alpina</i>

<sup>a</sup> Identification based on rDNA nuclear large subunit (nLSU) sequence.

*Clavulina* sp.1 (9), *Inocybe* sp. 5 (9), *Inocybe* sp. 2 (8) and *Tomentella* sp.1 (8) (Fig. 1).

Rarefaction curves did not approach the asymptote suggesting that further sampling would increase the number of ECMF species at the regional scale (Fig. 2). According to Chao2 estimator, the numbers of fungal species do not substantially differ between *Nothofagus* species (Fig. 2).

The ANOVA analysis indicated that there were no differences between ECMF species richness and *Nothofagus* forests types ( $F=0.31$ ;  $p=0.7357$ ).

The multivariate analysis revealed that forest type had no significant effect on ECMF community composition ( $F_{2,5}=1.18$ ;  $P=0.216$ ). Despite the narrow altitudinal range (700 m) between the lowland and mid-elevation sites in the study region, altitude had a significant effect on community structure ( $F_{1,5}=1.71$ ;  $P=0.029$ ), explaining 18.9 % of variation. In the GNMDS ordination, the two primary axes explained 90.9 % of variation in the fungal community (Stress=9.1) and covered 81.0 % ( $P<0.001$ ) of the altitudinal effect (Fig. 3).

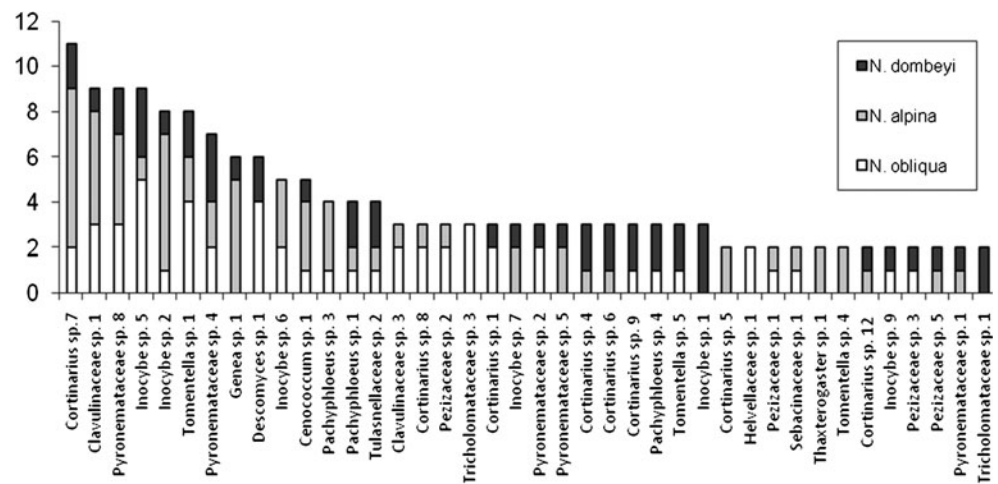
## Discussion

The community structure of ECMF in these *Nothofagus* forests of Patagonia is relatively diverse in terms of taxonomic

groups, mostly involving members of the /cortinarius, /inocybe, /tomentella-thelephora, /clavulina and /tricholoma lineages. The /russula-lactarius lineage was relatively poor in species, whereas the /suillus- rhizopogon, /boletus and /pisolithus-scleroderma lineages were not recovered in our sampling, which is a unique pattern at the global scale (Tedersoo et al. 2012). The paucity of these groups and the high proportion of /cortinarius and /inocybe are also consistent with fruit-body surveys in *Nothofagus* forests of southern South America (Moser and Horak 1975; Garnica et al. 2003).

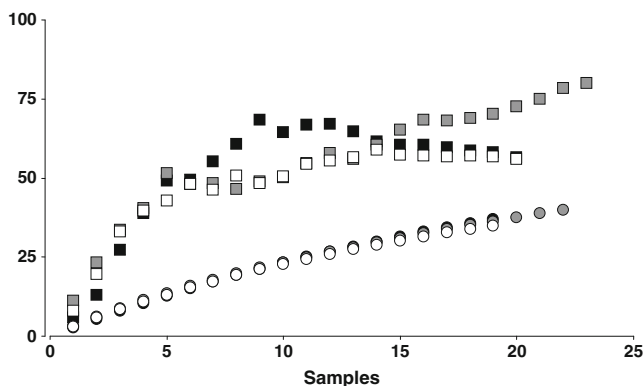
The overall ECMF community is relatively similar to that of other *Nothofagus*-dominated ECMF communities in New Zealand and Australia, where the /cortinarius; /descolea; /inocybe; and /tomentella-thelephora lineages are highly diverse (Dickie et al. 2009; Tedersoo et al. 2008; 2009). In particular, the presence of the /descolea and /austropaxillus lineages are characteristic of Southern temperate *Nothofagus* habitats (Pirozynski 1983; Tedersoo et al. 2010). Nonetheless, in the case of /russula-lactarius, several collections of hypogeous specimens of *Cystangium*, *Gymnomycetes*, *Macowanites*, and *Arcangeliella* have been recently collected from the same area and are being used in morphological studies (Nouhra et al., unpublished). Although fruit-bodies of *Scleroderma* and *Boletus* have been previously recorded in the region (Horak and Moser 1966; Horak 1977; Nouhra et al. 2012b; Palfner 2005), taxa in the /suillus-rhizopogon, /boletus and /pisolithus-

**Fig. 1** Relative frequency of ECMF taxa found in eight soil cores from each site of *Nothofagus dombeyi*, *N. alpina* and *N. obliqua* at Lanin National Park



scleroderma lineages were not detected below ground in this study. These fungal lineages were absent or poorly represented on root tips in Australian wet sclerophyll and temperate rain forests as well (Tedersoo et al. 2008; 2009) which is in contrast with their relative abundance in the Northern hemisphere (Taylor et al. 2000; Horton and Bruns 2001). However, the presence of a single *Laccaria* species in Northern Patagonia contrasts with a high contribution of this genus to ECMF biodiversity in Australia (Tedersoo et al. 2008; 2009) and New Zealand in areas subjected to timber harvesting and increased N availability (Dickie et al. 2009). It is worth mentioning that sampling sites in this study have little or no anthropic alterations and an average estimated age of the forests that ranges between 200–300 years.

Species of Ascomycota contributed 23 % to the richness of ECMF on roots of *Nothofagus* spp., suggesting their important role in these forest ecosystems, as shown for northern temperate forests (Morris et al. 2008; Smith et al. 2007; Tedersoo et al. 2006). Tedersoo et al. (2006) predicted the relatively high proportion of Pezizales in drought- and fire-stressed ecosystems, which is conflicting with their great abundance in these wet, pristine temperate rain forests of South America. Certain

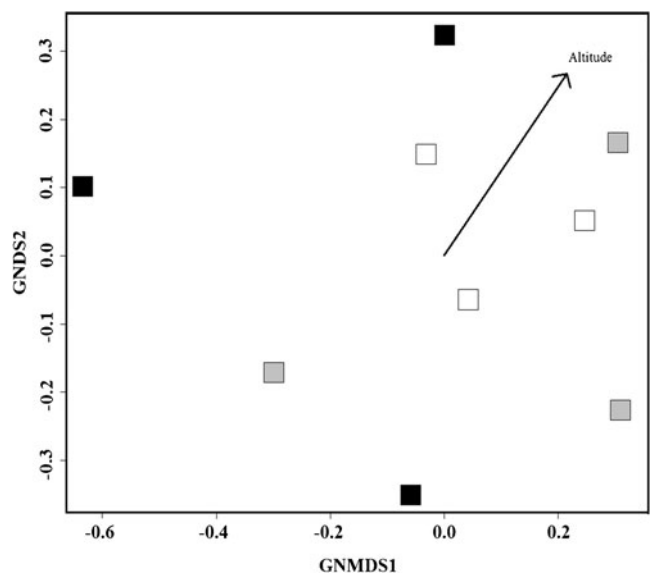


**Fig. 2** Coleman rarefaction curve (circles) and Chao2 (squares) minimal species richness estimates in *Nothofagus dombeyi* (black symbols), *N. obliqua* (open symbols) and *N. alpina* (gray symbols) forests

closely related species of Pyronemataceae could not be reliably placed to any pre-defined lineages and these may contribute to yet unrecognized shifts to ectomycorrhizal habit (Tedersoo et al. 2013).

In terms of species richness and composition of the fungal associates, we detected no significant differences among forest types. We anticipate that with our current sample size, small differences remain outside the detection limit. Similar to other studies (Horton and Bruns 1998; Kennedy et al. 2003; Richard et al. 2005; Nara 2006; Ishida et al. 2007; Morris et al. 2008; Tedersoo et al. 2008), the most common mycobionts, such as species of *Cenococcum*, *Clavulina*, *Inocybe*, and *Tomentella* had no host preference, associating with all three *Nothofagus* species.

Despite differences in leaf chemistry and physiology of evergreen and deciduous leaves (Aerts 1995; Vivanco and Austin 2008), the ECMF community was not structured by



**Fig. 3** Global Nonmetric Multidimensional Scaling graph showing the relative importance of altitude (vector) on ECMF community composition in *Nothofagus dombeyi* (black squares), *N. obliqua* (open squares) and *N. alpina* (gray squares) forests

host species. This contrasts with previous studies revealing substantial differences between co-occurring congeneric host species from different sections that exhibited different physiology (Morris et al. 2008). Our results thus suggest that ECMF species associating with *Nothofagus* in South America have not evolved high niche differentiation for living host roots or organic soil structured by congeneric trees. Further studies on contrasting soil types and across the soil profile are required to determine the overall effect of soils on the ECMF communities of *Nothofagus*.

Various studies have observed that within a local forest site, commonly fruiting species may have a limited occupancy on root tips (Dahlberg 2001; Gardes and Bruns 1996). This pattern was shown by *Thaxterogaster* and *Hysterangium* which were infrequent on root tips, but constituted the most prolific fruiters in the hypogeous sporocarps record from under nearby *N. dombeyi* sites (Nouhra et al. 2012a).

ECMF communities from other Southern hemisphere locations showed higher diversity of recovered species, with 123 taxa and estimated number of 210–247 spp. as the minimal species richness in a mix forest of *Acacia* sp., *Pomaderris* sp., *Eucalyptus* sp., and *Nothofagus* sp., in Tasmania (Tedersoo et al. 2008), and 140 distinct ECMF T-RFLP types from harvested and unharvested *Nothofagus* forests in New Zealand. Both studies showed relatively higher number of associated ECM taxa, however they were carried out in diverse settings, sampling designs, stand ages, and in one case included ectomycorrhizal hosts species from different plant families. In fact, Bruns (1995) suggested that higher host diversity as occurring in the mixed conifer and eucalyptus forests in North America and Australia, respectively (Claridge et al. 1993; Hunt and Trappe 1987; Johnson 1994; Smith et al. 2002), leads to increased litter heterogeneity, constituting a possible determinant of ECMF diversity.

Despite the narrow altitudinal range observed between the three different forests types in our study, altitude had a significant effect on the ECMF community structure. Until recently, the effects of altitude on ECMF diversity and composition have been poorly studied. Bahram et al. (2012) observed that both host genus and altitude play a substantial role in structuring the ECMF communities along a pronounced altitudinal gradient (100–2700 m) in northern Iran. In that study, altitude had a significant negative effect on ECMF richness. Similarly, Gomez-Hernandez et al. (2012) reported on decline in macrofungal richness and substantial community change along an elevation gradient (100–3,500 m) in Central Mexico. However, the change in macrofungal community was largely ascribed to dramatic shifts in vegetation, especially proportion of ectomycorrhizal trees. Within the Patagonian altitudinal gradient, precipitation mostly contributed to the shift in species richness and biomass of fruit-bodies of hypogeous ECMF taxa in *N. pumilio* and *N. dombeyi* forests (Nouhra et al. 2012a). All these studies suggest that various

environmental factors may strongly affect the diversity and composition patterns of ECMF at the local and regional biogeographic scales.

## Conclusions

Our study provides novel information about the diversity and community composition of ectomycorrhizal root symbionts in *Nothofagus* forests in Northern Patagonia. In contrast to certain previous field studies on congeneric trees (Morris et al. 2008), the analysis of ECMF biodiversity (i.e., identity, richness, and community) reveals high similarity among *Nothofagus* tree species from different subgenera and contrasting leaf traits. Despite these differences, the composition of the ECMF communities is influenced by altitude.

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