Diversity and growth-effects of ectomycorrhizal fungi of a *Nothofagus pumilio* forest in the Andes of Southern Chile

(Diversidad y efectos de crecimiento de hongos ectomicorrízicos en un bosque de Nothofagus pumilio en los andes del sur de Chile)

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ABSTRACT

Chilean temperate rainforests have unique climatic, edaphic and biotic conditions, constituting pre-industrial blueprint ecosystems. Mycorrhizal associations play a central role in the biogeochemical processes of these ecosystems´ functioning. Baseline forest ecology studies are necessary in order to better understand diversity patterns, specifically regarding mycorrhizal symbiosis. Therefore, here we describe the vegetation characteristics and the mycorrhizal relationships of vascular plants in a *Nothofagus pumilio* forest. We also describe, via morphological methods, the ectomycorrhizal diversity present in this forest. Additionally, we determined whether ectomycorrhizal inoculation confers positive growth effects on *N. pumilio* seedlings. We found that from 46 vascular plant species identified in this study, 42 (91%) were mycorrhizal and of these 33 (72%) were associated with arbuscular mycorrhizae (AM), two (the dominant trees *N. pumilio* and *N. dombeyi*) were forming ectomycorrhizae (EM), five were associated with ericoid mycorrhizae, two with orchid mycorrhizae, and four were non-mycorrhizal. Additionally, 26 EM species were detected of which 15 belong to *Cortinarius*. Finally, there were clear differences in the growth of *N. pumilio* seedlings inoculated with the ectomycorrhizal fungus *Laccaria laccata* compared to non-inoculated plants. We suggest that mycorrhizal fungi play a key role in seedling colonization of harsh environments such as the Andean treeline.

RESUMEN

Los bosques templados lluviosos de Chile tienen condiciones climáticas, edáficas y bióticas únicas, constituyendo ecosistemas preindustriales modelo. Las asociaciones micorrízicas juegan un rol central en los procesos biogeoquímicos del funcionamiento de estos ecosistemas. Por lo tanto, en este estudio describimos las características de la vegetación y las relaciones micorrízicas de las plantas vasculares de un bosque de *Nothofagus pumilio*. También describimos, vía métodos morfológicos, la diversidad ectomicorrízica presente en este bosque. Adicionalmente, determinamos si inoculaciones ectomicorrízicas confieren efectos de crecimiento positivos a semilleros de *N. pumilio*. Encontramos que, de 46 especies de plantas vasculares identificadas en este estudio, 42 (91%) son micorrízicas, y de estas, 33 (72%) están formando micorrizas arbusculares (AM), dos (los árboles dominantes *N. pumilio* y *N. dombeyi*) están asociados con ectomicorrizas (EM), cinco están asociadas con micorrizas ericoides, dos con micorrizas orquioides, y cuatro fueron nomicorrizadas. Adicionalmente, 26 especies de EM fueron detectadas, de las cuales 15 pertenecen a *Cortinarius*. Finalmente, hubo claras diferencias en el crecimiento de los semilleros de *N. pumilio* inoculados con el hongo ectomicorrízico *Laccaria laccata*, comparados a plantas no inoculadas. Sugerimos que los hongos micorrízicos juegan un rol clave en la colonización de ambientes severos por juveniles, como en el límite altitudinal andino.

INTRODUCTION

Southern Chile *Nothofagus* spp. forests, which extend from central Chile to Tierra del Fuego, present solely ectomycorrhizal (EM) associations as fungal partners. While Nothofagaceae forests exclusively form EM associations, being the only native ectomycorrhizal trees in Andean Patagonian forests[1,2,3,4,5,6,7] and have a high diversity of agaricoides, Valdivian and native coniferous forests are largely dominated by arbuscular mycorrhizae (AM)[8,9,10,11,12,13,14,15]. In EM or AM dominated forests, the reciprocal mycorrhizal types (either EM or AM, respectively) are not excluded, as they are often associated with understory plants. The dominant genus of EM fungi in Southern South American *Nothofagus* forests is *Cortinarius* with at least 200 species described to date[16,17]. Boletales and Russulales, which contribute to an important share of the fungal diversity in northern hemisphere ectotrophic forests, are scarce in Southern South America[18,19]. *Nothofagus pumilio* forests are usually located at the vegetation limit of the mountain region with a latitudinal extension that covers more than 2,000 km of distribution of this particular forest type at the treeline of the Andes Mountains[20].

We aimed at (i) determine the vegetation pattern and the mycotrophic status of the vascular plants, and (ii) determine the EM fungal species present in a *Nothofagus pumilio* forest located at the treeline of Puyehue National Park, Chile (40°S). Also, we hypothesized that a native ectomycorrhizal species fosters the growth of *Nothofagus pumilio* (a dominant and test species). To test this, we (iii) measured the effects EM fungal inoculations on *N. pumilio* seedlings.

MATERIALS AND METHODS

Study plot.
We selected one 30 m x 30 m plot in the Andes Mountains of Southern Chile in Puyehue National Park (40° 47' S – 72° 12' W), Antillanca sector (near Raihuen Crater). The plot was located within the altitudinal limit (1,150 – 1,200 m.a.s.l.) of a pristine, deciduous temperate forest of *Nothofagus pumilio*. The plot receives more than 7,000 mm of annual precipitation, mostly during winter (June to September), and the annual mean temperature is 4.5°C [21].

Vegetation patterns and mycorrhizal status.
A previous study has shown that the crown cover of this forest is 95%, and trees of this forest have a medium height of 15 m, a maximum height of 20 m, and an approximate age of 155 years[22]. Dasometric records indicate that the density of trees in the forest is 756 trees ha^{-1} with an average DBH of 13.3 cm and a basal area of 66.6 m^2 ha^{-1} [22]. The floristic composition of the vascular plants in the plot was determined according to Marticorena & Quezada (1985)[23], and life forms were determined according to Ellenberg & Mueller-Dombois (1966)[24]. In order to determine the types of mycorrhizal associations, fine root samples of at least five individuals of each species were extracted from soil close to the respective plant species, verifying the root attachment, transported to the laboratory, fixed in 70% alcohol, stained and observed under a microscope for fungal colonization[25]. For determining ectomycorrhizal associations, root samples were observed and described according to Agerer (1995)[26] and Palfner (2001)[5].

Determination of ectomycorrhizal fungal species.
The EM fungal species determination was carried out in the whole plot during periodic visits (every two months) for a period of three consecutive years. Fruiting bodies of EM fungi were collected according to Agerer (1991)[27] and fresh state morpho-anatomic identification was conducted[26,27,28]. Following identification, the fruiting bodies were dried and added to a reference collection. Taxonomic references of Garrido (1988)[1], Valenzuela et al. (1999)[4], and Gamundi & Horak (1993)[29] were consulted for species determination of gasteroid and agaricoid basidiomycetes, as well as ascomycetes.

Inoculation experiment with *Nothofagus pumilio* seedlings and ectomycorrhizal fungi.
Nothofagus pumilio seeds were collected in the plot and selected for subsequent stratification at 4°C. NaCl (0.1%) was applied for 30 s to aid germination, and then a treatment with gibberellic acid was applied for 24 h, in order to break the endogenic latency. To ensure that the inoculation species would be compatible with the seeds collected, the EM fungal species *Laccaria laccata* (Scop.) Berk. was collected in the plot, transported to the laboratory, the fungus was isolated from sporocarps, the mycelium grown in several petri dishes with malt Agar (2%) at 25°C. Following this, the fungi were cultured at 25°C in 1 L sterile recipients containing peat:vermiculite (2:1) substrate and modified Melin-Norkrans (MNM) medium (pH 5.0). Cultures were maintained until visible development of the mycelium's vegetative growth[30]. There were three treatments: a control with natural soil and no inoculum, natural soil (from the forest plot) with inoculum, and sterile soil (from the forest plot, sterilized with an autoclave at 121°C, 1 atm, for 30 minutes) with inoculum. Sterile and pre-germinated seedlings (one seedling per pot, 20 pots per treatment, in culture chambers) were selected for uniform height and a 3 cm radicle; selected seedlings were then planted in 1 L pots with natural and sterile soil.

A 50 milliliters volume of *L. laccata* inoculum solid substrate was added beneath the radical system of each plant. In total, 20 plants were used for each of the three treatments (60 plants in total); this 60 pots were randomly located in the greenhouse. The plants were left to grow in a greenhouse for 17 weeks where the soil humidity was maintained similar to what would be found in the field; water was supplied each week; no fertilization was implemented. At the end of the experimental period, the following morphological and biomass traits were measured for all plants: root collar diameter, shoot length, root length, fresh shoot weight, fresh root weight, shoot dry weight and root dry weight. The formation of *L. laccata*

mycorrhizae was confirmed in each of the inoculated plants using a stereomicroscope. Additionally, based on the above-mentioned morphological and biomass traits, the plant Quality Index (QI)[31] was calculated for the seedlings. The QI integrates several highly-correlated and non-correlated morphological growth traits, thus, giving a strong and objective proxy of young plant quality[31].

Statistical analysis.
In order to test differences between the treatments of the inoculation experiment, a Tukey test was performed using the morphological traits measured (p value <0.05), using the R function TukeyHSD in R[32].

RESULTS

Vegetation pattern and mycorrhizal status.
Forty-six vascular plant species were identified in the *N. pumilio* plot (Table 1). The understory vegetation was mainly composed of *Chusquea montana* Phil., *Drimys andina* (Reiche) R.A. Rodr. et Quez., *Maytenus disticha* (Hook) Urban, *Berberis buxifolia* Lam., *Berberis serrate* Lechler, *Pernettya myrtilloides* (Mirb) Blume, *Pernettya pumila* (L.f.) Hook and *Ovidia andina* (P.et E.) Meins. The herbaceous included *Valeriana lapathifolia* Vahl. and *Viola reichei* Skottsb.

Four different types of mycorrhizal associations were found for the 42 vascular plants species studied (91% of total vascular plant species). The AM associations were present in several life forms in a total of 33 vascular plant species (72 % of total vascular plant species). Two tree species (4 % of total vascular plant species) were associated with EM fungi; these included *N. pumilio*, which was the dominant tree in the plot with 85% of canopy cover and *N. dombeyi*, which was less frequent with 5% of canopy cover. Ericoid mycorrhizae were present in five plant species (11%),

orchid mycorrhizae were present in two species (4%), and four plant species (9%) were non-mycorrhizal (Aspleniaceae, Proteaceae, Polypodiaceae, and Cyperaceae). The ectomycorrhizal and ericoid plant species were phanerophytes, while the orchid type was associated with typical herbs (cryptophytes).

Ectomycorrhizal fungi.
Twenty-six EM fungi species were identified in the forest plot (Table 2); most of EM species were identified by sporocarps, except two putative morphotypes which belong to the family Corticiaceae. *Cortinarius* spp. were confirmed to be associated with *N. pumilio* via root-colonization analysis. Brown spored EM fungi genera (*Cortinarius* and *Descolea*) accounted for 62% of the total EM species richness, a characteristic pattern in Andean-Patagonian *Nothofagus* forests. Another EM genus that was found to associate with several plants in the plot was *Laccaria*, which was a genus composed of two species. Other EM fungal genera were represented by only one species (including *Austropaxillus, Cenococcum, Gautieria, Inocybe, Russula,* and *Tricholoma*).

Inoculation experiment.
We found that the inoculation of *N. pumilio* with the EM fungi *Laccaria laccata* showed a positive effect on seedling growth traits (Table 3). *N. pumilio* seedlings in natural soil inoculated with the fungus had clearly higher growth than the *N. pumilio* control seedlings in natural soil without inoculum (Table 3). Both aboveground and belowground traits were significantly affected by the fungal inoculum treatments. Plants grown in natural soils with the EM fungi inoculation (Table 3) also had significantly increased growth (Table 3). The quality index was significantly higher in the inoculated treatments when compared to the control (Table 3).

Table 1. Vascular plant species present in the *Nothofagus pumilio* plot, with their respective family, life form and mycotrophic status.

Species name	Family	Life-form/Mycorrhizal status
Acaena antarctica R. et P.	Rosaceae	Hem/AM
Acaena ovalifolia R. et P.	Rosaceae	Hem/AM
Adenocaulon chilensis Less	Asteraceae	Hem/AM
Asplenium dareoides A.N. Desv.	Aspleniaceae	Phan/NM
Berberis buxifolia Lam.	Berberidaceae	Phan/AM
Berberis serrato-dentata Lechler	Berberidaceae	Phan/AM
Blechnum chilense (Kaulf.) Mett.	Blechnaceae	Hem/AM
Blechnum magellanicum (A.N. Desv.) Mett.	Blechnaceae	Hem/AM
Blechnum penna-marina (Poir.) Kuhn.	Blechnaceae	Hem/AM
Chusquea montana Phil	Poaceae	Phan/AM
Codonorchis lessonii Lindl.	Orchidaceae	Cryp/OR
Drimys andina (Reiche) R.A. Rodr. et G. Forster	Winteraceae	Phan/AM
Embothrium coccineum J.R. et. G. Forster	Proteaceae	Phan/NM
Empetrum rubrum Vahl ex Willd.	Ericaceae	Phan/ER
Escallonia alpina Poepp. ex. DC.	Escalloniaceae	Phan/AM
Gaultheria phillyreifolia (Pers.) Sleumer	Ericaceae	Phan/ER
Gavilea lutea (Pers.) Correa	Orchidaceae	Cryp/OR
Grammitis magellanica A.N. Desv.	Polypodiaceae	Phan/NM
Greigia landbekii (Lechler ex Phil) Phil ex F. Phil	Bromeliaceae	Hem/AM
Gunnera magellanica Lam	Gunneraceae	Hem/AM
Hymenophyllum secundum Hook et Grev.	Hymenophyllaceae	Phan/AM
Lagenifera hirsuta (Poepp. ex Less) Dudley	Asteraceae	Hem/AM
Lagenifera hariotti (Franchet) Dudley	Asteraceae	Hem/AM
Lycopodium magellanicum (P. Beauv) Sw.	Lycopodiaceae	Cham/AM
Maytenus disticha (Hock. f.) Urban	Celastraceae	Phan/AM
Myoschilos oblonga R. et. P.	Santalaceae	Hem/AM
Myrceugenia chrysocarpa (Berg) Kausel	Myrtaceae	Phan/AM
Nothofagus dombeyi (Mirb.) Oerst	Nothofagaceae	Phan/EM
Nothofagus pumilio (P. et E.) Krasser	Nothofagaceae	Phan/EM
Osmorhiza chilensis H. et A.	Apiaceae	Hem/AM
Ovidia andina (P. et E.) Meisn.	Thymelaeaceae	Phan/AM
Perezia pedicularifolia Less	Asteraceae	Hem/AM
Perezia prenanthioides Less	Asteraceae	Hem/AM
Pernettya myrtilloides Zucc. ex Steud	Ericaceae	Phan/ER
Pernettya poeppigii (D.C.) Klotzsch	Ericaceae	Phan/ER
Pernettya pumila (L.f) Hook	Ericaceae	Phan/ER
Ranunculus chilensis D.C.	Ranunculaceae	Hem/AM
Ranunculus peduncularis J.E. Sm.	Ranunculaceae	Hem/AM
Ribes cucullatum Hook. et Arn.	Grossulariaceae	Phan/AM

Ribes magellanicum Poir.	Grossulariaceae	Phan/AM
Rubus geoides J.E. Sm.	Rosaceae	Hem/AM
Senecio acanthifolius Hombr. et Jacq.	Asteraceae	Hem/AM
Senecio trifurcatus (G. Forster) Less.	Asteraceae	Hem/AM
Uncinia brevicayulis (Thomars) Kunth.	Cyperaceae	Hem/NM
Valeriana lapathifolia Vahl.	Caprifoliaceae	Hem/AM
Viola reichi Skottsb.	Violaceae	Hem/AM

Life forms: Phan=phanerophytes, Cham=chamaephytes, Cryp=cryptophytes, Hem=hemicryptophytes. Mycorrhizal status: EM=ectomycorrhiza, AM=arbuscular mycorhiza, ER=ericoid, OR=orchid, NM=non mycorrhizal.

Table 2. Ectomycorrhizal (EM) fungi species identified in the *Nothofagus pumilio* plot.

Species name
Austropaxillus boletinoides (Singer) Bresinsky & Jarosch (b)
Cenococcum geophilum Fr. (b)
Cortinarius albocanus (E. Horak & M.M. Moser) Peintner & M.M. Moser (b)
Cortinarius austroacutus M.M. Moser (a)
Cortinarius austrolimonius M.M. Moser & E. Horak (a)
Cortinarius austrosalor M.M. Moser (a)
Cortinarius choloides E. Horak (a)
Cortinarius clorophanus M.M. Moser (a)
Cortinarius egenus E. Horak (a)
Cortinarius flammuloides E. Horak & M.M. Moser (a)
Cortinarius fulvoconicus M.M. Moser (a)
Cortinarius magellanicus Speg. (b)
Cortinarius pachynemeus M.M. Moser (a)
Cortinarius permagnificus E. Horak (a)
Cortinarius pugionipes M.M. Moser (a)
Cortinarius rubrobasalis M.M. Moser & E. Horak (a)
Cortinarius squamiger M.M. Moser (a)
Descolea antarctica Singer (b)
Gautieria inapire Palfner & E. Horak (b)
Inocybe bridgesiana Singer (a)
Laccaria laccata (Scop.) Cooke (b)
Laccaria ohiensis (Mont.) Singer (a)
Russula fuegiana Singer (b)
Tricholoma fusipes E. Horak (a)
"Nothofagirhiza reticulosa" *Morphotype a aff. Corticiaceae (b)
"Nothofagirhiza vinicolor" *Morphotype b aff. Corticiaceae (b)

Identification by: (a)=(4); (b)=this study. "*Nothofagirhiza reticulosa*" described by Palfner (2001)[5], "*Nothofagirhiza vinicolor*" described by Palfner & Godoy (1996)[2].

Table 3. Inoculation experiment, showing the growth effects of the ectomycorrhizal (EM) fungus *Laccaria laccata* in different morphological and biomass traits of *Nothofagus pumilio* seedlings.

Treatment	Root collar diameter (mm)	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	Q.I.
Control	0.20	8.1	11.4	0.50	0.24	0.12	0.11	0.47
Natural soil + inoculum	0.33*	10.1*	13.1*	0.71*	1.30*	0.22*	0.30*	0.53*
Sterile soil + inoculum	0.34*	13.1*	13.2*	1.33*	1.71*	0.47*	0.41*	1.04*

Q.I.: Quality Index, resulting by combining the measured morphological traits, according to Ritchie (1984)[31]. *denotes statically significant differences between the control and the treatments with fungal inoculum (Tukey test, p value < 0.05).

DISCUSSION

The vascular flora found in the forest plot (Table 1) is typical of Chilean temperate rainforests located at the vegetation limit of the Andean treeline and was best classified as *Drimys andina* – Nothofagetum[33]. Other studies have documented that this forest differs in age and growth characteristics compared to lowland temperate rainforests, likely due to the effects of frequent natural disturbances[34,35]; lowland *N. pumilio* forests tend to be older and taller than the forest described here. The vascular flora described here has varied mycorrhizal associations: *Nothofagus* species were found to be exclusively associated with EM fungi, while most other plant species (72%) were dominated by AM fungi. Furthermore, ericoid mycorrhizae are typically associated with the families Empetraceae and Ericaceae. Additionally, orchid mycorrhizae were found in two Orchidaceae species. One species of the genus *Gunnera*, which is known to take part in a facultative tripartite association (AM fungi and the N-fixing cyanobacteria *Nostoc punctiforme*[36]), was also found in the plot (Table 1). Non-mycorrhizal plants included a representative of Cyperaceae, two species of epiphytic ferns (Aspleniaceae and Polypodiaceae), and one species of

Proteacaeae (non-mycorrhizal and which has preoteoid roots, a nutrient-uptake adaptation). In the region, all these families are typically considered non-mycorrhizal[9,10,11].

Although the study of fungal diversity has been traditionally dependent on fruiting bodies, as in this study, there is an increasing necessity of comparing these results with those from molecular and metagenomic studies[37,38]. The Puyehue National Park, where this study took place, has been recently studied in terms of its AM fungal diversity[14,15], and in its whole soil fungal diversity[7], including all fungal functional groups (EM, AM, saprophytic fungi, lichens, etc.). Although these mentioned metagenomic studies were not conducted on the exact plot as this study, we hope to give future insights for fungal diversity comparisons using traditional and modern taxonomic methods.

Ectomycorrhizal fungi species and their ecosystem roles in *N. pumilio* forests.
The diversity of agaricoid fungi recorded in Patagonian forests is comparable to similar temperate forest ecosystems in the Northern hemisphere[6] (Table 2). This result is in accordance with previous studies in the region[1, 2, 3, 4, 5, 6]. Excluding *Nothofa-*

gus spp., most of the vascular flora in the region is dominated by AM fungi[8,9,10,11,12,13,14,15]. There is not much certainty about species-specific associations between the ten Chilean Nothofagaceae species and their mycobionts; it is yet unknown even whether there are differences in the EM community structure of evergreen and deciduous forests. However, the results here provide some evidence that Southern Andean *N. pumilio* forests are distinct, not only based on EM species composition but also in terms of the morphology of the encountered fungal taxa. The dominance of *Cortinarius* spp. in association with *N. pumilio* was confirmed by this study. *Cortinarius* represented the 58% (15 species) of the total EM fungal richness. Most registered EM fungal species are common in *Nothofagus* forests in Chile and in limitrophous zones that span a wide latitudinal and altitudinal range in Argentina[1,5,39]. Examples of these common species are *Austropaxillus boletinoides, Cortinarius flammuloides, C. magellanicus, Descolea antarctica, Laccaria laccata, Russsula fuegiana* and *Tricholoma fusipes.*

The upper soil layers in the study area consists mainly of deposits of broad pyroclastic particles produced by repeated past volcanic eruptions. This material promotes rapid drainage of rainwater and snowmelt and, due to its dark color, heats up considerably when exposed to direct sunlight. Resulting water stress, which adds to highly variable temperatures during diurnal and annual cycles, would likely favor basidiomycete dominance, as these fungi form secotioid or hypogeous fruiting bodies (examples include *Cortinarius albocanus* and *Gautieria inapire*). As indicated by Moser & Horak (1975)[16] and Horak & Moser (1965)[40], the success of secotioid ectomycorrhizae under the mentioned conditions is evident by the high diversity of sequestrate *Cortinarius* species in Andean Patagonian *Nothofagus* forests.

An alternative response to soil water stress would be a retreat of the mycelia towards deeper and moister soil layers, and this would be correlated with the formation of fruiting bodies with long, radicating stipes during the rainy season. This is the exact pattern of *Cortinarius pugionipes*, which has been repeatedly observed at the study region; so far, *C. pugionipes* has been only observed in *N. pumilio* forests[16] (according to Moser & Horak (1975) and the present study) and may be a specific mycobiont of this tree species. Likewise, the unidentified ectomycorrhizal morphotypes (mostly dense mycelial mats under the leaf litter) are putative members (taxa) of the Corticiaceae: "*Nothofagirhza vinicolor*" and "*Nothofagirhiza reticulata*". These taxa so far have been found associated exclusively with *N. pumilio*. "*N. vinicolor*" is easy to detect in the field by the dark red color of its periferical mat-like mycelium and has been observed in several Andean *N. pumilio* forests and in the coastal mountains of south-central Chile[5]. So far "*N. vinicolor*" has not been found in association with any other *Nothofagus* species. Morphological methods should be complemented with molecular methods; sequencing soil samples from Argentinian *Nothofagus* forests, Marín *et al,* (2017a)[7] and Nouhra *et al,* (2013)[41] found similar EM genera to those described in this study.

The only Ascomycete among the identified EM species is the cosmopolitan anamorphic species *Cenococcum geophilum,* member of the Gloniaceae family[42]. *C. geophilum* has been reported in a wide range of natural and managed forests and in greenhouse and nursery cultures[3,5]. It seems to be particularly common in soils periodically prone to water stress, which reflects the conditions of our study plot. The most interesting EM species found is probably the hypogeous basidiomycete *Gautieria inapire*. This species is so far only known from the type locality associated with *N. pumilio*[5,43], where it forms semihypogeous fruiting bodies in dense clusters. This is another striking example of functional adaptation to adverse environmental conditions, as the fruiting bodies emerge from

dense mycelial mats directly beneath the soil surface. This results in moisture retention, improving conditions for the survival of primordia during late summer and early autumn before the rainy season starts. Another important role of the mentioned mycelial mats may be the formation of a nursery bed for tree seedling establishment and survival, as has been suggested by Griffiths et al (1991)[44] for the closely related northern hemisphere species *Gautieria monticola*.

The feedback between soil, mycorrhizae, and plants is especially important for plant growth at forest altitudinal limits[45,46]. In conservation programs, if certain plants and fungi are present, there is a considerable potential for *N. pumilio* seedling production and health (Table 3). This would have strong implications for a country, such as Chile, that is highly dependent on the forestry industry, where sustainable local production remains to be achieved.

CONCLUSIONS

Most of the vascular flora (91%) in the pristine forest had mycorrhizal associations, demonstrating the importance and prevalence of root symbionts in the ecology of forest ecosystems. This study should be considered as a baseline for further studies of mycorrhizal diversity and ecology in this particular forest ecosystem. Based on identification of fruiting bodies, we listed a total of 26 EM fungi species in the *N. pumilio* forest at the treeline of Puyehue National Park, Chile. However, molecular methods are definitely needed to further identify soil biota and to provide a complementary view of the diversity and functionality of this important component of the ecosystem. The inoculation assays with EM fungi showed a potential for using inoculations as a tool in restoration programs, especially in commonly disturbed (earthquakes, fires, volcanic events) areas of the region.

ACKNOWLEDGEMENTS

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