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What happens to the mycorrhizal communities of native and exotic seedlings when *Pseudotsuga menziesii* invades Nothofagaceae forests in Patagonia, Argentina?

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ABSTRACT

Pseudotsuga menziesii is one of the most widely planted conifers in the Patagonian Andes of Argentina, having invading characteristics that are broadly reported. We studied the mycorrhizal status of seedlings along six Nothofagaceae + *P. menziesii* invasion matrices to investigate their role in the invasive process, according to these hypothesis: a) The abundance and richness of EM will be higher in seedlings grown in their own soil; b) In the presence of native EM inoculum, the invasive plant will be associated with generalist mycorrhizae (EM and/or AM), c) AM associations will be more abundant in *P. menziesii* seedlings grown in Interface or native forest soils, d) Mycorrhizal community differences between treatments will alter host fitness (growth and nutritional parameters). Seedlings from *Nothofagus dombeyi*, *N. antarctica*, *Lophozonia alpina*, *L. obliqua* and *Pseudotsuga menziesii* were set up in a soil-bioassay that included soils from non-invaded Nothofagaceae forests, pure *P. menziesii* plantations, and the interface between both. *Pseudotsuga menziesii* seedlings showed a decreasing, although never null, ectomycorrhizal (EM) colonization pattern from plantations to non-invaded forests, mainly with exotic EM species. *Hebeloma mesophaeum* and *Wilcoxina* sp. 1, two EM species with cosmopolitan distribution, were found to be shared by both tree species. *Hebeloma hiemale* and *Wilcoxina* sp. 1, common mycorrhizal partners of *P. menziesii* in Patagonia although not registered from Nothofagaceae forest, were found to be associated with *N. antarctica*, being the first report for both fungal species. *Pseudotsuga menziesii* seedlings showed the ability to form different arbuscular mycorrhiza (AM) colonization types (*Paris-*, *Arum-*, Both- and Intermediate-types) depending on the treatments, with significantly higher presence of Intermediate-type in the Interface treatment, where colonization was low. The shared EM species and the presence of different AM colonization types imply enhanced possibilities for invasive *P. menziesii* seedlings establishment and development. Seedling features and EM colonization rates evidenced that *P. menziesii* invasion could produce maladaptation (defined as a relative decline in host fitness due to altered mycorrhizal communities from native settings) of mycorrhizal communities, seriously injuring native ecosystem.

1. Introduction

One of the most perplexing questions of ecology is how some plants, when moved or introduced to new areas for productive purposes, can surpass the development of native species and be more abundant than in their natural range (Blossey and Nötzold, 1995; Elton, 1958; Hierro et al., 2005). Given the facts that the majority of plants depend at least on one fungal mutualism (Brundrett, 2009; Núñez and Dickie, 2014), and mutualistic interactions can prevent or facilitate biological invasions (Richardson et al., 2008; Núñez et al., 2009; Spence et al., 2011), a better understanding of invasion on plant communities requires a consideration of the role of fungal partner(s) in the symbiosis (Schnoor et al., 2011).

During the last decade several authors have warned about the invasive capability of *Pseudotsuga menziesii* (Mirb.) Blanco in native Nothofagaceae forests in Patagonia, Argentina (Núñez et al., 2009;

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Table 1
Sampling sites, with indication of matrix type, maximum effective recruitment distance (ERD), transect length, main understory species and sites features.

Sites	GPS point	Matrix type	ERD (m)/ Transect length (m)	Understory species[a]	Treatment	AM SD[b]	AP[c]	MT[d]	SpH[e]	OM[f]	PC[g]	TN[h]	ST[i]	EC[j]	CaC[k]	MgC[l]	KC[m]	NaC[n]
Corcovado	Lat.: −43.63, Long.: −71.44	*P. menziesii - N. antarctica*	55/235	COSP, SCPA, LOHI, FAIM, MACH, CYSC, RUAC, ACPI, ACSP, POLI, POLA, HOSP, FRCH, OSCH, OBAN, RUAD.	Forest	16	810	9	5.81	18.5	21	0.43	Sandy loam	0.09	6.80	3.00	0.30	1.30
					Interface	18	810	9	6.49	13.6	33	0.66	Sandy loam	0.20	4.50	6.25	0.53	1.00
					Plantation	50.8	810	9	5.80	14.9	23	0.33	Loamy sand	0.08	9.30	1.00	0.40	1.00
Foyel	Lat.: −41.67, Long.: −71.45	*P. menziesii - N. antarctica*	86.4/266.4	SCPA, LOHI, MABO, MACH, CHCU, MUHA, VISP, FRCH, BEBU, MUDE, POLI, POLA, FEAR, BRAU, OSCH, ACPI.	Forest	19.6	1490	9.58	5.87	21.7	25	0.82	Sandy loam	0.14	14.30	6.80	0.80	0.90
					Interface	26	1490	9.58	6.49	12.2	34	0.47	Sandy loam	0.11	16.00	7.50	0.91	0.80
					Plantation	24.4	1490	9.58	5.94	19.6	20	0.75	Loamy	0.08	17.80	9.80	0.60	0.90
ENFORSA	Lat.: −41.23, Long.: −71.42	*P. menziesii - N. antarctica*	78.2/258.2	SCPA, MACH, LOHI, EMCO, BEBU, OSCH, POLI, POLA, FEAR, BRAU, FRCH, VISP., MUDE, PLLA, RUAD, MUHA, ACPI, TRRE.	Forest	18	1490	9.58	5.26	17.1	18	0.67	Sandy loam	0.14	11.80	7.80	1.00	1.10
					Interface	21.6	1490	9.58	5.13	17.4	31	1.02	Sandy loam	0.18	8.75	5.75	0.51	0.90
					Plantation	49.6	1490	9.58	5.52	10.6	13	0.30	Sandy loam	0.06	16.30	8.30	0.80	0.90
Isla Victoria	Lat.: −40.97, Long.: −71.53	*P. menziesii - N. dombeyi*	218.6/398.6	BEBU, RUAD, MACH, VISP, MUHA, SCPA, ARCH, LOHI, AUCH, LUAP, CHCU, MUDE, POLI, POLA, FEAR, BRAU, ROEG.	Forest	8.8	1544	8.68	6.32	9.6	22	0.32	Sandy loam	0.04	10.80	5.30	0.40	1.00
					Interface	4.4	1544	8.68	6.07	12.9	35	0.46	Sandy loam	0.09	14.75	6.00	0.76	0.90
					Plantation	16.4	1544	8.68	5.75	14.7	26	0.28	Sandy loam	0.14	12.50	4.80	0.50	1.30
Est. Quechuquina	Lat.: −40.15, Long.: −71.59	*P. menziesii - L. alpina - L. obliqua*	44.61/224.61	CHCU, MACH, ARCH, MUHA, TAOF, SCPA, POLI, POLA, FEAR, BRAU, TRRE, OSCH, FRCH, CATH, CIVU, ALAU, RUAD, BEBU, RUAC, AUCH, MOPE.	Forest	18	1834	12.2	5.17	16.1	22	0.36	Sandy loam	0.44	9.80	3.50	0.40	2.10
					Interface	15.2	1834	12.2	5.67	13.1	29	0.62	Sandy loam	0.19	8.00	6.75	0.43	0.60
					Plantation	25.6	1834	12.2	5.5	13.2	37	0.33	Sandy loam	0.26	12.00	7.00	0.50	1.80
Est. Newmeyer	Lat.: −40.12, Long.: −71.32	*P. menziesii - N. dombeyi*	19/199	CHCU, SOAC, SASP, PRCE, MACH, LOHI, MUHA, OSCH, RUAD, POLI, POLA, FEAR, BRAU.	Forest	21.6	2258	12.2	5.7	26.5	33	0.54	Sandy loam	0.32	22.30	11.30	0.70	1.90
					Interface	20	2258	12.2	5.57	8.25	35	0.31	Sandy loam	0.24	12.50	5.25	1.03	0.90
					Plantation	19.2	2258	12.2	6.79	9.8	12	0.28	Loamy sand	0.08	17.50	2.30	0.20	1.20

[a] *Understory species:* ACPI: *Acaena pinnatifida;* ACSP: *A. splendens;* ALUA: *Alstroemeria aurea;* ARCH: *Aristotelia chilensis;* AUCH: *Austrocedrus chilensis;* BEBU: *Berberis buxifolia;* BRAU: *Bromus auleticus;* CATH: *Carduus thoerneri;* CHCU: *Chusquea culeou;* CIVU: *Cirsium vulgare;* COSP: *Colletia spinossisima;* CYSC: *Cytisus scoparius;* EMCO: *Embothrium coccineum;* FAIM: *Fabiana imbricata;* FEAR: *Festuca argentina;* FRCH: *Fragaria chiloensis;* HOSP: *Hordeum* sp.; LOHI: *Lomatia hirsuta;* LUAP: *Luma apiculata;* MABO: *Maytenus boaria;* MACH: *M. chubutensis;* MOPE: *Montia perfoliata;* MUDE: *Muticia decurrens;* MUHA: *Muehlenbeckia hastulata;* OBAN: *Obidia andina;* OSCH: *Osmorrhiza chilensis;* POLA: *Poa lanuginosa;* POLI: *P. ligulata;* PLLA: *Plantago lanceolata;* PRCE: *Prunus cereseae;* ROEG: *Rosa eglanteria;* RUAC: *Rumex acetosella;* RUAD: *Ruhmora adiantiformis;* SASP: *Sambucus* sp.; SCPA: *Schinus patagonicus;* SOAC: *Sorbus acuaiparia;* TAOF: *Taraxacum officinale;* TRRE: *Trifolium repens;* VISP: *Vicia* sp.

[b] AM SD: AM spore density (spores/100 gr. Of dry soil).

[c] *AP: annual precipitation (mm).*

[d] MT: mean temperature (°C).

[e] SpH: soil pH.

[f] OM: organic matter (%).

[g] PC: soil phosphorous content (mg/kg of soil).

[h] TN: total soil nitrogen content (%).

[i] ST: soil texture.

[j] EC: Electrical conductivity (ds/m).

[k] Calcium content (meq/100 g).

[l] Magnesium content (meq/100 g).

[m] Potassium content (meq/100 g).

[n] Sodium content (meq/100 g).

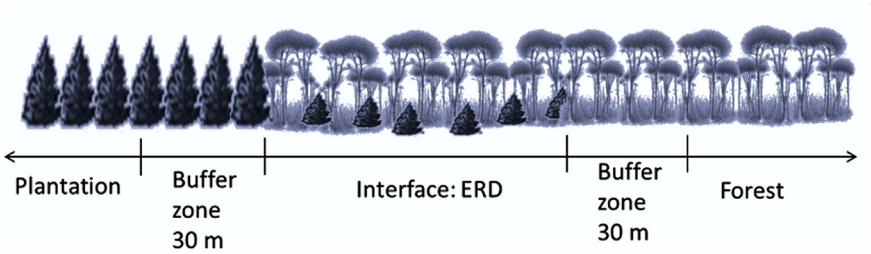

Fig. 1. Sampling design.

Orellana and Raffaele, 2010; Richardson et al., 2008; Sarasola et al., 2006; Simberloff et al., 2002, 2003). Numerous investigations have established that Nothofagaceae spp. and *Pseudotsuga menziesii* are ectomychorrizal (EM) (Barroetaveña and Rajchenberg, 2003; Barroetaveña et al. 2006, 2007; Fontenla et al., 1998; Godoy and Palfner, 1997; Horton et al., 1999; Peredo, 1987), and the presence of arbuscular mycorrhiza (AM) in *P. menziesii* seedlings has been reported (Cázares and Smith, 1996; Salgado Salomón et al., 2013b, 2014).

Arbuscular mycorrhizal fungi, present in all shrubs and grasses from Nothofagaceae forests (Fontenla et al., 1998) are globally distributed and are generally believed to exhibit low host specificity (Giovannetti and Sbrana, 1998; Bonfante and Genre, 2010). It can be expected then that *P. menziesii* could exploit this situation by increasing their AM association capability using available native AM inoculum. On the other hand, EM fungi have been claimed as host-specific (Smith and Read, 2008). Even though some EM fungal partners also lack specificity (Molina et al., 1992), the most of EM fungi are exclusive for some plants group, e.g. *Suillus* and *Rhizopogon* are specialized genera on members of the Pinaceae (Kretzer et al., 1996; Kretzer and Bruns, 1999; Mujic et al., 2014), with closely related plant species showing a similar EM fungi community associated (Waterman et al., 2011, 2012). Based on these considerations, the big taxonomic distances between *Pseudotsuga* and Nothofagaceae species, with only 27.6% shared fungi genera out of 58 reported for *P. menziesii* in its natural range (Singer, 1969; Garrido, 1986; Gamundí and Horak, 1993; Barroetaveña et al., 2007), foresees no crossover between EM fungal communities. Only reported shared EM taxa [*Hebeloma mesophaeum* (Pers.) Fr., *Cenococcum geophilum* Fr., *Lacaria laccata* (Scop.) Fr.] with cosmopolitan distribution are expected to be found associated with both forest types.

The essential role of mycorrhizal associations in *P. menziesii* establishment and growth (Trappe and Strand, 1969; Wright, 1971) has been long ago established. The strategy of tree invasion ability subsidized by the co-invasion with their mycorrhizal partners has been shown by Dickie et al. (2010) for co-invasive *Pinus contorta* Douglas ex Loudon in *Nothofagus* forests, Moeller et al. (2015) for *P. menziesii* in *Fuscospora* (*Nothofagus*) *cliffortioides* (Hook. f.) forests, Bogar et al. (2015) for *Alnus glutinosa* and *Salix fragilis* invasion all in New Zealand and Hayward et al. (2015) in mixed forests in Isla Victoria (Patagonia, Argentina).

Nonetheless, these previous studies did not analyze the mycorrhizal situation of the invaded Nothofagaceae forest, the impact on their growth and nutritional status nor the AM status of *P. menziesii* in invaded matrices. On the other hand, *P. menziesii* invasive range in Patagonia has been very restricted sampled to study the mycorrhizal role (Hayward et al., 2015; Núñez et al., 2009; Simberloff et al., 2002, 2003). The aim of this study is to understand the knock-on belowground effects on the mycorrhizal community available for all trees in a broader invading *P menziesii* context in Patagonia Argentina, by elucidating the EM, AM inoculum potential in Nothofagaceae + *P. menziesii* matrices for both tree species and the effects of belowground factors on seedling growth and nutritional status. Due the high specificity of EM associations, the more generalist behavior of AM and the big genomic distance between both forest species, we hypothesize:

a) The abundance and richness of EM will be higher in seedlings grown in their own soil.
b) In the presence of native EM inoculum, the invasive plant will be associated with generalist mycorrhizae (EM and/or AM).
c) AM associations will be more abundant in *P. menziesii* seedlings grown in Interface (defined as Nothofagaceae + *P. menziesii* matrix) or native forest soils.
d) Mycorrhizal community differences between treatments will alter host fitness (growth and nutritional parameters).

2. Materials and methods

2.1. Sampling sites

A soil bioassay with seedlings acting as mycorrhizal inoculum trap was set up to evaluate the mycorrhizal inoculum potential present at six sites with *P. menziesii* plantations adjacent to Nothofagaceae spp. forests. Study sites were located in NW Patagonia, Argentina, in habitats belonging to the Deciduous Forest District, Sub-Antarctic Province, Sub-Antarctic Domain (Cabrera and Willink, 1980) (Table 1).

Soil sampling at each site was carried out along a 200–400 m transect that included three situations considered as treatments: Nothofagaceae forest without *P. menziesii* invasion (FOREST), Nothofagaceae + *P. menziesii* matrix along the effective recruitment distance (ERD, Sarasola et al., 2006) (INTERFACE), and *P. menziesii* plantation lacking Nothofagaceae specimens (PLANTATION) (Table 1, Fig. 1). The transects had different length according of the ERD, varying between 200 and 400 m, details of each transect length can be seen in Table 1. A mixed soil sample of 3 kg was taken from each sampling unit (3 treatments per site, 6 sites, 18 composite soil samples in total) and kept in brand-new plastic bags.

A non-sampled "*buffer zone*" was kept between each treatment in order to avoid border effects on EM inocula (Fig. 1).

Details of sampling sites location, matrix type, maximum effective recruitment distances, main understory species and sites features are presented in Table 1.

2.2. Bioassay setup

Pseudotsuga menziesii, *Nothofagus antarctica* (G.Forst.) Oerst., *N. dombeyi* (Mirb.) Oerst., *Lophozonia alpina* (=*Nothofagus alpina*) (Poepp. & Endl.) Heenan & Smissen and L. *obliqua* (=*Nothofagus obliqua*) (Mirb.) Heenan & Smissen commercial quality seeds were cleaned and conditioned in accordance with Willan (1991) and stratified for 24 h in water and then stored at 4 C in a refrigerator for 60 days. Sterile seedlings were obtained in a growth chamber at 17/19 C, 48–55% relative air humidity, and 16 h photoperiod with 1400 lux radiation for 30 d. Clean, 250 cm^3 flower pots were filled with a 1:1 (v/v) mix of soil obtained from each sampling unit and sterilized pumicite (oven, 120 C for 48 h). As a control, 50 flower pots, 10 per tree species, were filled with sterilized soil (mixing soil from each treatment from all sites) autoclaved at 1.2 atm for 30 min, mixed (1:1, v/v) with sterilized pumicite (oven, 120 C for 48 h; van der Heijden et al. 1998) in order to check for inoculum contamination through watering or other sources. Seedlings grown in sterilized pumicite (oven, 120 C for 48 h) were

transplanted into flower pots comprising 10 replicates per species, and three treatments per site (FOREST, INTERFACE and PLANTATION); in total, 420 seedlings were planted. Seedlings were randomly arranged and grown for 12 months in a greenhouse devoted exclusively to this experiment and watered regularly as needed, with water obtained directly from a well through pipes, in order to minimize mycorrhizal inoculum contamination.

2.3. Mycorrhizal status evaluation

Arbuscular mycorrhizal and EM colonization percentages for each seedling were estimated following Brundrett et al. (1996), using the complete root system. Ectomycorrhizal colonization percentage for each seedling (EM%) was estimated as:

EM% = (number of EM tips/total tips of the root system)*100

And AM colonization percentage (AM%) was estimated as:

AM% = (number mycorrhizal intersects/total intersects)*100

EM morphotypes were characterized and classified according to Goodman et al. (1996), Barroetaveña et al. (2006, 2007), Agerer (1994, 1995, 2001) and Agerer and Rambold (2004–2016). Morphotype richness per seedling was calculated as the most probable number of different morphotypes found per unit sampling (seedling). EM morphotypes were used to estimate evenness index (E), which was calculated following Pielou (1969).

Completed the EM evaluation, root samples for all tree species were cut into 10 mm lengths portions (approx. 600 mg per seedling) to fit in Tissue-Tek plastic capsules (Fisher Scientific Co., Pittsburgh, PA), cleared in 10% KOH for 30 min at 100 °C under water bath and 15% H_2O_2 overnight at room temperature. Cleared samples were immersed 60 min at 4 C in a staining solution of 0.05% trypan-blue in lactoglycerol, rinsed with tap water and stored in lactoglycerol at 4 C until microscopic examination (Cázares and Smith, 1996).

Each positive intersected root fragment was microscopically analyzed in order to check the presence of intercellular/intracellular hyphae, arbuscules (dichotomously branched, haustorium-like structures arising from intercellular hyphae), hyphal coils, arbuscular coils (hyphal coils with fine arbuscule-like branches on their surfaces), spores and vesicles. AM types were classified following Brundrett et al. (1996), Dickson (2004) and Smith and Smith (1997). Seedlings with *Arum*- and *Paris*-types structures in the same root system were called “Both”, and with intermediate structures between both types were called “Intermediate”.

2.4. AM spore soil extraction and counting

250 g of soil per treatment and site were analyzed. Arbuscular mycorrhizal spores were collected by wet-sieving and sucrose decanting method (adapted from Ianson and Allen, 1986), and counted. Spore density was expressed as the number of AM spores in 100 gr dry soil (Duponnois et al., 2001).

2.5. Seedling features

Seedling features included measurements of shoot height (H, from apex to collar), collar diameter (CD, measured at ground level), shoot dry weight (SDW, oven, 60 C for 72 h), and root specific length index [RSLI, RSLI = root system length (mm)/root system weight (g)] (Eissenstat, 1991; Tani et al., 2003). Foliar contents of N, P, K, Mg and Ca were evaluated following Sadzawka et al. (2004) in INTA Bariloche (San Carlos de Bariloche, Río Negro, Argentina).

2.6. Molecular identification of EM morphotypes

Molecular identification was performed for EM and ECM, looking for possible shared species by the different tree species.

Morphotypes obtained from the bioassays were morphological and anatomically described, washed twice with sterile distilled water and stored at −18 C in CTAB (100 mM Tris-HCl (pH 8.0), 1.4 M NaCl, 20 mM EDTA, 2% CTAB).

Representative fresh root-tips of each characterized morphotype from the bioassay were selected for rDNA extraction. Five to ten root-tips from each morphotype were washed with 100 µL of ultrapure water and cut into 4–5 pieces with a sterile scalpel, resuspended in 20 µL extraction solution (Kit AmpPlant Extract-N-1KT XNAP2, Sigma, St. Louis Missouri), incubated at 95 C for 10 min, then following the manufacturer's instructions with modifications described by Avis et al. (2003).

The internal transcribed spacer region (rDNA ITS1-5.8S-ITS2) was amplified using primers *ITS1F* and *ITS4* (White et al., 1990; Gardes and Bruns, 1993) and *LR5* and *LR53* 28S (Huhndorf et al., 2004). PCR was performed using the RED Extract-N-Amp Plant PCR Kit (Sigma, St. Louis Missouri). PCR reactions were performed in My Cycler™ thermocycler, (BioRad) with the protocol adapted from Barroetaveña et al. (2010) for ITS region: an initial denaturation at 95 C (10 min), followed by 41 cycles of denaturation at 94 C (45 s), primer annealing at 54 C (45 s) and elongation at 72 C (1 min), with a final elongation step at 72 C (10 min), while for 28S region was an initial denaturation at 94 C (90 s), followed by 25 cycles of denaturation at 95 C (45 s), primer annealing at 52 C (40 s) and elongation at 72 C (2 min), followed by 15 cycles of denaturation at 95 C (45 s), primer annealing at 52 C (40 s) and elongation at 72 C (2 min with additional extension time of 5 s per cycle), with a final elongation step at 72 C (10 min) (Reeb et al., 2004). PCR products were separated on 1% agarose (w/v) stained with Gel-Red ™ (Biotium, California, USA) and visualized under UV illumination.

The ITS and 28S PCR products that presented multiple bands were cloned. PCR purified products were cloned using a pGEM®-T easy vector system following the procedures outlined by the manufacturer (Promega, Madison, USA). The amplified fragments were purified and sequenced in at the DNA Synthesis and Sequencing Facility, Macrogen (Seoul, Korea).

Identification of detected fungi was conducted searching for similar sequences using BLAST (Altschul et al., 1997) at the NCBI and UNITE (Kõljalg et al., 2013) using default settings. When UNITE species hypothesis was not found, the GenBank number was reported.

The nomenclature and classification of the identified species follow Index Fungorum (www.indexfungorum.org), Deemy (http://www.deemy.de) and/or PubMed (www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi). Sequences generated in this study were deposited in the GenBank of the National Center for Biotechnology Information (NCBI);

2.7. Statistical analysis

To perform the statistical analysis, the AM%, EM%, EM richness and evenness data matrix was corrected, subtracting the average values of controls seedlings abundance and richness per species (values presented Annex 1, Tables 4 and 5). Arbuscular mycorrhiza colonization percentage, AM types' incidence, AM spore density, EM%, EM richness, evenness and seedling features between treatments did not meet the assumptions of normal distribution and equal variances using the Shapiro-Wilk and Levene tests, respectively (Everitt, 2005). Therefore, differences in EM%, EM richness and evenness between treatments were analyzed using a three-way generalized linear mixed model (3W-GLMM) with the restricted maximum likelihood estimation method. Sites were treated as blocks (incorporated as a random effect) and different soils (PLANTATION, INTERFACE and FOREST) and forest type (*P. menziesii* and Nothofagaceae spp.) as fixed-effects (Di Rienzo et al.

2010). To check the robustness of the observed patterns considering the presence of contaminants in Control seedlings, all data for the OTUs that were found contaminating the controls were removed and the GLMM performed again.

Differences in AM%, AM types' incidence, AM spores' density and seedling features between treatments were analyzed by generalized linear mixed models (GLMM) with a restricted maximum likelihood estimation method, and sites were treated as blocks and incorporated as a random effect. Subsequent comparisons with the DGC test (exclusive groups formation test) were performed (Di Rienzo et al. 2002) in R for R-DCOM (Di Rienzo et al. 2010).

Sites with less than 3 seedlings per treatment were not included in the statistical analysis. To further analyze the relationships between seedling features and mycorrhizal status, Pearson correlation (r) tests were conducted, including shoot height, collar diameter, shoot dry weight, root specific length index, total foliar content of N, P, K, Ca, Mg, EM richness and EM%.

All the analyses were performed at the 0.05 significance level, using the statistical package InfoStat for Windows version 2011 (Di Rienzo et al. 2011).

To examine the effect of treatment and host on EM fungal community structure (i.e. richness and composition) accounting for site, an analyses of similarity (ANOSIMs) using PRIMER v7 was performed (Clarke and Gorley, 2015).

3. Results

3.1. Ectomycorrhizal abundance

Twenty-three morphotypes were found with different proportions in each treatment and site, 11 from *P. menziesii* and 12 from Nothofagaceae seedlings.

Ectomycorrhizal colonization percentages ranged between 24.7 and 68.2% in *P. menziesii* and between 29.6 and 73.7% in Nothofagaceae seedlings (Table 2). The tree-way generalized linear mixed model showed interaction between treatment and species with significant differences in EM% ($P = 0.0042$, F = 7.41, 3W-GLMM) and seedlings grown in its own soil presented higher values (Nothofagaceae-FOREST and *P. menziesii*-PLANTATION). Lowest colonization values were found in *P. menziesii* seedlings grown in forest soil (*P. menziesii*-FOREST) (Fig. 2 A). This analysis was performed with corrected data (subtracting Control contaminations, values presented in Table 2d), the analysis excluding the contaminant OTUs showed a conserved pattern within tree genera level (Fig. 2 B). Although, a clear difference appears between tree genera, showing that *P. menziesii* seedling were more affected by contaminating OTUs. For all analysis, Nothofagaceae seedlings from Foyel and Est. Quechuquina sites were not included because high seedling mortality drastically reduced the ***n*** statistical number.

3.2. Ectomycorrhizal fungal richness and diversity

Mean EM richness per seedling in *P. menziesii* ranged between 1.8 and 5.0, with a maximum of 8 and a minimum of 1 morphotype per seedling (Table 2). For Nothofagaceae, mean EM richness ranged between 3.3 and 6.3 with a maximum of 7 and a minimum of 1 morphotype per seedling (Table 2). The identity of different OTUs found in this study are summarizing in Table 3. Three-way generalized linear mixed models showed that there was interaction between treatment and species, with significant differences in EM richness ($P < 0.0001$, F = 257.28, 3W-GLMM) and with higher values in *P. menziesii* seedlings, but without differences between treatment. On the other hand, Nothofagaceae seedlings showed a higher and significant EM richness in INTERFACE soil than FOREST and PLANTATION (Fig. 2). Ectomycorrhizal diversity showed significant differences between treatments ($P < 0.0001$, F = 71.36, 3W-GLMM), but these differences are trivial from a biological point of view. Anyway, both tree types displayed a greater EM species evenness in seedlings grown in soil of the other tree type than in its own soil and INTERFACE area (Fig. 2, Table 2).

P. menziesii control seedlings presented 21.1 EM%, with a mean seedling's EM richness of 2.2 (Table 2). Nothofagaceae control seedlings showed 27.6 EM%, with a mean seedling EM richness of 2.6 (Table 2 and information in Annex 1, Tables 4 and 5).

3.3. Shared EM fungi and root endophytes detected

Three EM species were found to be shared by both forest types: *Hebeloma mesophaeum* (Pers.) Quél, a *Wilcoxina* sp., and a Pyronemataceae sp. *Hebeloma mesophaeum* was abundant in PLANTATION and FOREST for *P. menziesii* and in FOREST for *N. dombeyi*, while *Wilcoxina* sp. was abundant in INTERFACE for both *P. menziesii* and *N. antarctica*. An uncultured Pyronemataceae fungus was found in *P. menziesii*, *N. dombeyi*, *N. antarctica*, *L. alpina* and *L. obliqua*, and was abundant in FOREST and INTERFACE for all Nothofagaceae spp. and slightly found in *P. menziesii* seedlings growing in FORESTS.

Molecular techniques revealed that two known endophytes were found in *P. menziesii* roots, namely *Phialocephala fortinii* Wang & Wilcox (Helotiales) and a *Cordana* sp. Preuss (Sordariales) (Stoyke et al., 1992; Addy et al., 2005).

3.4. Arbuscular mycorrhizal abundance and type

Arbuscular mycorrhizal colonization percentages ranged between 12.2 and 62.1% in *P. menziesii* seedlings (Table 2). The generalized linear mixed model showed significant differences in AM% ($P < 0.0001$, F = 19.77, GLMM) between treatment with lowest colonization values for INTERFACE soil (Fig. 3). Although the 4 types of colonization were present in all treatments, the incidence was significantly different between treatments. *Paris*- and Both-types were significantly more abundant in PLANTATION treatment ($P < 0.0001$, F = 36.76; $P < 0.0001$, F = 20.65; GLMM, respectively), *Arum*-type incidence was significantly higher in FOREST treatment ($P < 0.0001$, F = 57.81, GLMM), while Intermediate-type was significantly higher in INTERFACE treatment ($P = 0.0002$, F = 14.75, GLMM; Fig. 3). Arbuscular mycorrhizal soil spores density ranged between 4.4 and 50.8 spores/100 gr of dry soil (Table 1) and was significantly higher in PLANTATION treatment ($P < 0.0001$, F = 55.77, GLMM; Fig. 3). Arbuscular mycorrhizal colonization in Nothofagaceae spp. was never detected.

3.5. Bioassay seedling features

Seedling measurements and nutritional parameters were variable between treatments and sites (Table 2). Nevertheless, Nothofagaceae seedlings growing in INTERFACE soil showed significant smaller values for shoot dry weight, shoot height and collar diameter ($P < 0.0001$, F = 29.95, GLMM; $P < 0.0001$, F = 69.83, GLMM; $P < 0.0001$, F = 202.89, GLMM, respectively, Fig. 4, Table 2). For *P. menziesii* seedlings only shoot height showed significant differences, being seedlings growing in PLANTATION significant taller than those grown in INTERFACE or FOREST soils ($P < 0.0001$, F = 508.5, GLMM). All tree species showed significant correlation between shoot dry weight and EM% (r = 0.81, $P < 0.0001$; r = 0.84, $P = 0.0007$, respectively). *Pseudotsuga menziesii* seedlings also showed significant correlations between shoot dry weight and foliar P content with EM richness (r = 0.77, $P = 0.0002$; r = 0.75, $P = 0.0005$, respectively), while Nothofagaceae seedlings did it between shoot height and EM% (r = 0.78, $P = 0.003$). For *P. menziesii* seedlings, AM% showed significant correlations with collar diameter and foliar P content (r = 0.71, $P = 0.00089$; r = 0.78, $P = 0.0002$, respectively).

3.6. Similarity analyses of richness

The community similarity assessment across the landscape did not

Table 2
Mycorrhizal colonization and Nothofagaceae and *P. menziesii* seedlings features per treatment and site.

Sites	Treatment	Tree species	No of surviving seedlings	%EM[a]	%EM corrected	Richness per seedling	AM%	H[b] (cm)	CD[c] (mm)	SDW[d] (gr)	RSLI[e] (mm/gr)	Total %N	Total %P	Total %K	Total %Ca	Total % Mg
Corcovado	Forest	*N. antarctica*	7	45.26	32.48	5.57	0.00	13.17	4.17	0.75	3540.9	1.05	0.09	1.08	0.24	0.78
		P. menziesii	7	27.92	5.54	2.14	23.08	12.3	2.5	0.52	1703.88	1.08	0.1	0.4	0.33	0.1
	Interface	*N. antarctica*	3	29.58	17.80	4.33	0.00	6.13	2.5	0.13	4216.3	NC	0.1	1.03	0.41	0.66
		P. menziesii	8	39.83	6.25	2.13	27.41	9.03	2.13	0.4	2932.08	0.83	0.07	0.34	0.28	0.09
	Plantation	*N. antarctica*	9	40.29	36.13	5.33	0.00	11.53	3.28	0.41	3834.2	1.11	0.09	1.11	0.27	0.48
		P. menziesii	7	44.04	12.39	2.86	27.71	13.56	2.71	0.69	1365.3	1.17	0.07	0.39	0.3	0.1
Foyel	Forest	*N. antarctica*	6	43.73	38.46	4.33	0.00	13.1	3.25	0.46	2984.98	1.3	0.09	0.84	0.23	0.59
		P. menziesii	5	31.6	6.44	1.8	21.23	14.12	2.9	0.81	1536.05	1.19	0.,16	0.38	0.38	0.1
	Interface	*N. antarctica*	1	73.66	25.79	6	0.00	13.5	3.5	0.82	3257.78	1.09	0.12	1.02	0.27	0.34
		P. menziesii	8	40.06	18.36	2.5	14.15	10.28	2.09	0.35	1710.34	0.9	0.07	0.31	0.3	0.12
	Plantation	*N. antarctica*	8	50.61	46.90	6.25	0.00	22.48	5.06	1.35	2810.68	1.01	0.11	1.29	0.27	0.56
		P. menziesii	3	44.49	20.13	3	28.05	14.67	2.75	0.73	1667.48	0.98	0.12	0.51	1.17	0.09
ENFORSA	Forest	*N. antarctica*	9	59.12	51.21	5.11	0.00	23.7	4.64	1.15	3210.09	1.58	0.09	0.98	0.19	0.68
		P. menziesii	10	24.67	3.33	2.9	21.91	10.09	2.12	0.43	2440.88	0.86	0.06	0.33	0.38	0.09
	Interface	*N. antarctica*	7	48.65	36.62	6.29	0.00	16.27	4.29	0.87	3127.07	1	0.09	1.27	0.26	0.82
		P. menziesii	5	40.8	11.40	2.4	12.78	11.48	2	0.4	2218.21	0.95	0.07	0.38	0.36	0.08
	Plantation	*N. antarctica*	10	45.96	38.42	6.2	0.00	19.58	4.35	1.05	3836.06	1.04	0.08	1.15	0.21	0.55
		P. menziesii	10	46.89	8.70	4.17	25.75	14.99	2.92	0.85	1490.03	0.66	0.04	0.24	0.4	0.08
Isla Victoria	Forest	*N. dombeyi*	9	63.00	28.60	4.33	0.00	19.73	3.8	0.93	2778.72	0.81	0.23	0.96	0.22	0.71
		P. menziesii	10	48.59	12.24	4.67	37.79	11.68	2.67	0.91	1118.57	0.93	0.26	0.77	0.32	0.10
	Interface	*N. dombeyi*	3	40.81	20.30	3.33	0.00	13.72	2.45	0.36	4603.70	0.89	0.08	0.90	0.19	0.49
		P. menziesii	10	47.77	12.93	4.27	42.09	12.05	2.68	0.98	1110.26	0.79	0.22	0.80	0.28	0.07
	Plantation	*N. dombeyi*	4	37.37	22.12	3.75	0.00	17.34	3.1	0.51	2797.99	1.01	0.18	0.87	0.29	0.31
		P. menziesii	10	66.4	17.94	5.00	43.03	21.06	3.28	1.21	892.98	0.7	0.23	0.68	0.97	0.09
Est. Quechuquina	Forest	*L. alpina-L. obliqua*	1	69.21	47.40	4.00	0.00	9.5	4.4	0.52	1447.32	NE	NE	NE	NE	NE
		P. menziesii	10	51.99	14.31	4.24	40.49	12.73	3.08	1.23	1005.61	0.79	0.17	0.54	0.32	0.09
	Interface	*L. alpina-L. obliqua*	7	60.09	42.63	4.43	0.00	10.93	3.43	0.56	1388.46	1.25	0.19	2.04	0.51	1.04
		P. menziesii	10	52.27	15.48	4.71	31.05	13.42	2.99	1.19	1205.89	0.78	0.15	0.61	0.38	0.09
	Plantation	*L. alpina-L. obliqua*	0	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
		P. menziesii	10	53.38	17.29	4.92	46.69	10.46	2.83	0.79	1438.92	0.71	0.32	0.6	0.28	0.15
Est. Newmeyer	Forest	*N. dombeyi*	8	63.68	56.45	4.75	0.00	21.76	3	0.77	4839.11	0.94	0.17	0.83	0.19	0.31
		P. menziesii	10	49.86	12.45	3.89	62.1	11.43	4.45	0.71	1241.49	1.03	0.23	0.49	0.81	0.18
	Interface	*N. dombeyi*	6	51.08	41.61	5.5	0.00	16	3.47	0.85	3292.86	0.83	0.1	1.11	0.22	0.69
		P. menziesii	10	60.52	17.89	4.94	31.36	13.57	3.39	1.3	1306.94	0.95	0.23	0.5	0.4	0.08
	Plantation	*N. dombeyi*	8	54.96	40.07	5.25	0.00	19.57	4.1	1.12	2733.59	0.9	0.12	1.96	0.4	0.62
		P. menziesii	10	68.17	16.09	4.45	23.18	12.73	3.46	1.54	1102.41	0.82	0.13	0.51	1.15	0.15
Control		*N. antarctica*	4	20.74	NE	2.00	0.00	8.94	2.75	0.37	3586.50	1.23	0.14	1.01	0.48	0.77
		N. dombeyi	8	34.52	NE	3.25	0.00	14.63	2.91	0.52	3038.73	0.9	0.22	1.09	0.28	0.64
		L. alpina	0	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
		L. obliqua	0	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
		P. menziesii	15	21.12	NE	2.2	13.39	12.47	2.67	0.94	1866.56	0.83	0.085	0.405	0.295	0.08

[a] Raw values.
[b] H: Seedlings shoot height (cm).
[c] CD: collar diameter (mm).
[d] SDW: shoot dry weight (gr).
[e] RSLI: root specific length index (mm/gr).

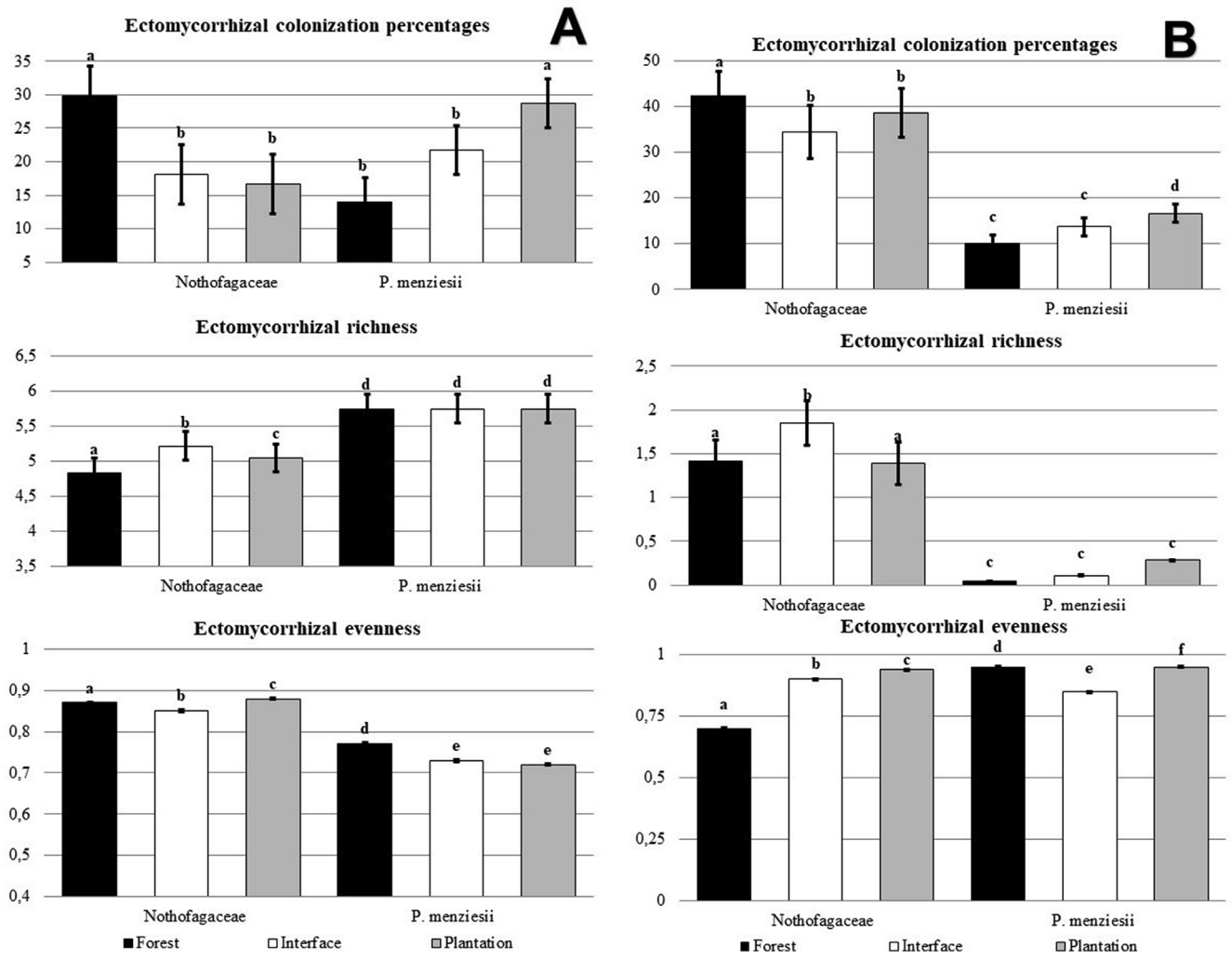

Fig. 2. A) Ectomycorrhizal colonization, EM richness and EM Eveness in *P. menziesii* and Nothofagaceae seedlings. Bars represent SE. Different letters indicate significant differences between treatments ($P < 0.05$, 3W-GLMM). B) Ectomycorrhizal colonization, EM richness and EM Eveness in *P. menziesii* and Nothofagaceae seedlings without OTUs presents in Control seedlings. Bars represent SE. Different letters indicate significant differences between treatments ($P < 0.05$, 3W-GLMM).

show a clear patron of EM species distribution regarding soil treatments or sites; only tree species clustered *P. menziesii* and Nothofagaceae differently ($R = 0.797$, $P = 0.001$; ANOSIM; Fig. 5).

4. Discussion

The fact that Nothofagaceae seedling growth features in this study were always lesser in INTERFACE, always associated to lower EM colonization rates, suggest that ectomycorrhizal maladaptation is occurring. Mycorrhizal maladaptation is defined as a relative decline in host fitness (survival, growth, nutrition) due to altered mycorrhizal communities from native settings (Kranabetter and Stoehr, 2015). Recent studies have alerted that the presence of invasive plants can decrease, sometimes drastically, the mycorrhizal colonization of native plants, causing the absence of new native seedlings in invaded sites (Inderjit et al., 2006; Mummey and Rillig, 2006; Rudgers and Orr, 2009; Weir, 2007; Zhang et al., 2007), a fact previously reported for Nothofagaceae forests in Patagonia (Salgado Salomón et al., 2013a). These findings suggest that *P. menziesii* invasion can seriously injure native Nothofagaceae forests ecosystems, while the presence of these species do not produce any detrimental effect on *P. menziesii* establishment.

Our results present evidence that co-invasion with its fungal partners occurs in *P. menziesii* invading Nothofagaceae forests in Patagonia, as Dickie et al. (2010) proposed for *P. contorta* seedlings in *Nothofagus* forests in New Zealand, Hynson et al. (2013) for *Pinus radiata* D. Don in the Hawaiian Islands, Moeller et al. (2015) for *P. menziesii* in *Fuscospora* (*Nothofagus*) *cliffortioides* (Hook. f.) forests in New Zealand and Hayward et al. (2015) for exotic Pinaceae in Isla Victoria (Neuquén, Argentina). Also, we got evidence that soil environments located beyond invading *P. menziesii* seedlings hold EM exotic and shared inoculum capable to associate with incoming new plants. This supports the concept that invasion occurs first by the mycorrhizal inoculum and then by their plant symbionts. The same was observed in *Pinus ponderosa* Dougl. ex Laws. plantations in Patagonian steppe systems and in *Pinus elliottii* Engelm. in grassland in the Sierras Grandes Mountains, Córdoba, in Argentina, where it was shown that conifer plantations produce an EM spore bank in surrounding environments that is ready to colonize and support invasive conifers, even 9000 m from plantations borders for Sierras Grandes Mountains (Salgado Salomón et al., 2011; Urcelay et al., 2017).

Three EM generalist species were found to be shared by *P. menziesii* and Nothofagaceae seedlings. From the previously reported shared species, only *Hebeloma mesophaeum* was detected in *P. menziesii* and *N. dombeyi*. This species was reported from Nothofagaceae forests (Singer, 1969) and also widely distributed in *P. menziesii* nurseries and plantations from Patagonian Argentina (Barroetaveña et al., 2006, 2007). Additionally, *Wilcoxina* sp. 1, was found shared by *P. menziesii* and *N. antarctica*. It has been reported as a pioneering EM species in nursery

Table 3
EM determined by morphotyping and ITS sequences, associated hosts and treatment.

EMF determined by morphotyping	Gen bank/unite blast	Host	Treatment where the morphotype was most abundant
P[a]1	*Rhizopogon villosulus* AF058310 (I: 98%; Q: 36%)	*P. menziesii*	INTERFACE and PLATATION
P2/N9	*Hebeloma mesophaeum* JQ724062 (I. 99%; Q: 46%); *Hebeloma mesophaeum* UDB018001 (98,86%)/JQ724062 (I: 97%; Q: 95%); *Hebeloma mesophaeum* UDB018001 (96,72%)	*P. menziesii; N. dombeyi*	PLANTATION and FOREST/FOREST
P3/N10	*Wilcoxina* sp. 1 GU181904 (I: 99%; Q: 32%); Pezizales UDB027213 (99,27%)/ GU181904 (I: 92%; Q: 80%)	*P. menziesii; N. antarctica*	INTERFACE/FOREST
P4	*Tomentella* sp. 1, AJ534914 (I: 92%; Q: 81%); Thelephoraceae UBD014398 (99.26%) and *Hebeloma mesophaeum* EF644106 (I: 99%; Q: 95%)	*P. menziesii*	PLANTATION
P5	*Wilcoxina* sp. 2 EU668269 (I: 98%; Q: 97%)	*P. menziesii*	PLANTATION
P6	*Wilcoxina* sp. 3 FJ553828 (I: 99%; Q: 96%)	*P. menziesii*	INTERFACE and FOREST
P7	*Sebacina* sp. JX630403 (I: 99%; Q: 41%); SebacinalesUDB008509 (93.30%)	*P. menziesii*	PLANTATION and INTERFACE
P8	*Wilcoxina mikolae* DQ069000 (I: 99%; Q: 37%)	*P. menziesii*	INTERFACE and PLATATION
P9	*Wilcoxina* sp.4 FJ553829 (I: 99%; Q: 85%)	*P. menziesii*	FOREST and INTERFACE
P10/N1	Uncultured fungus (I: 99%; Q: 78%) JX898976; Sordariales UDB026499 (98.68%)/Unculture fungus (I: 99%; Q: 95%) JX898976; Sordariales UDB026499 (99.64%)	*P. menziesii; N. dombeyi; N. antarctica; L. alpina; L. obliqua*	FOREST/FOREST and INTERFACE
P11	*Sebacina* aff. *vermifera* JQ711843 (I: 92%; Q: 96%); Sebacinales UDB008509 (99.38%)	*P. menziesii*	PLANTATION
N2	*Tomentella patagonica*, KT032091 (I: 98%; Q: 97%); Thelephoraceae UDB014400 (98.89%)	*N. dombeyi; N. antarctica; L. alpina; L. obliqua*	FOREST
N3	Not determined	*N. dombeyi; N. antarctica; L. alpina; L. obliqua*	FOREST
N4	Not determined	*N. dombeyi; N. antarctica; L. alpina; L. obliqua*	FOREST
N5	Not determined	*N. dombeyi; N. antarctica; L alpina; L. obliqua*	INTERFACE
N6	*Descolea* aff. *antarctica* AF325646 (I: 92%; Q: 74%); *Descolea* sp. UDB007113 (99,08%)	*N. dombeyi; N. antarctica*	PLANTATION
N7	*Tuber* sp. JQ723995 (I: 99%; Q: 96%)	*N. dombeyi*	PLANTATION
N8	*Tomentella ellisii* HQ406823 (I: 99%; Q: 89%); *Tomentella ellisii* UDB016490 (99.06%)	*N. antarctica*	PLANTATION
N11	*Cortinarius* sp. KF727362 (I: 90%; Q: 73%); *Cortinarius badiovinaceus* UDB002221 (90.57%)	*N. antarctica*	FOREST
N12	*Hebeloma hiemale* JF908033 (I: 98%; Q: 92%), *Hebeloma cavipes* UDB003187 (99.70%)	*N. antarctica*	PLANTATION

[a] P: Ectomycorrhizal morphotypes from *P. menziesii* seedlings; N: Ectomycorrhizal morphotypes from Nothofagaceae seedlings.

seedlings (Yu et al., 2001; Barroetaveña et al., 2010; Kohout et al., 2011), and in invasive *P. menziesii* seedlings in grasslands and *F. cliffortioides* forests in New Zealand (Moeller et al., 2015). Also, an uncultured fungus (GenBank JX898976) was found shared between all tree species involved in this work. Hynson et al. (2013) associated this taxon with a species in the Pyronemataceae (Pezizales, Ascomycota). This EM fungus was more abundantly found in Nothofagaceae seedlings from FOREST and INTERFACE treatments, and scarcely so in *P. menziesii* from FOREST treatment. Jones et al. (2010) reported abundant presence of Pyronemataceae EM for *P. menziesii* in sites degraded by severe fires or clear cuts. Due to the high abundance of this species in FOREST and INTERFACE treatments and that it was widely found associated with Nothofagaceae spp., it is likely that it is a native EM species that is captured by *P. menziesii*. Anyway, even three taxa were found shared, similarity analysis did not showed mixed clusters between *P. menziesii* and Nothofagaceae seedlings.

Arbuscular mycorrhizal colonization rates in *P. menziesii* seedlings were not as expected. Contrary to our expectations, the lowest value

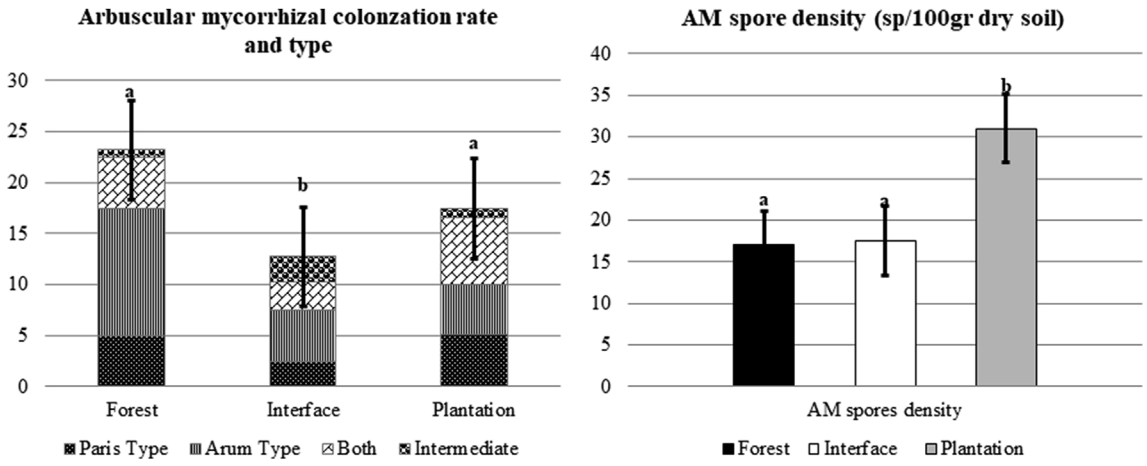

Fig. 3. Arbuscular mycorrhizal colonization in *P. menziesii* and Nothofagaceae seedlings and AM spore soil density for each treatment. Bars represent SE. Different letters indicate significant differences between treatments ($P < 0.05$, 3W-GLMM).

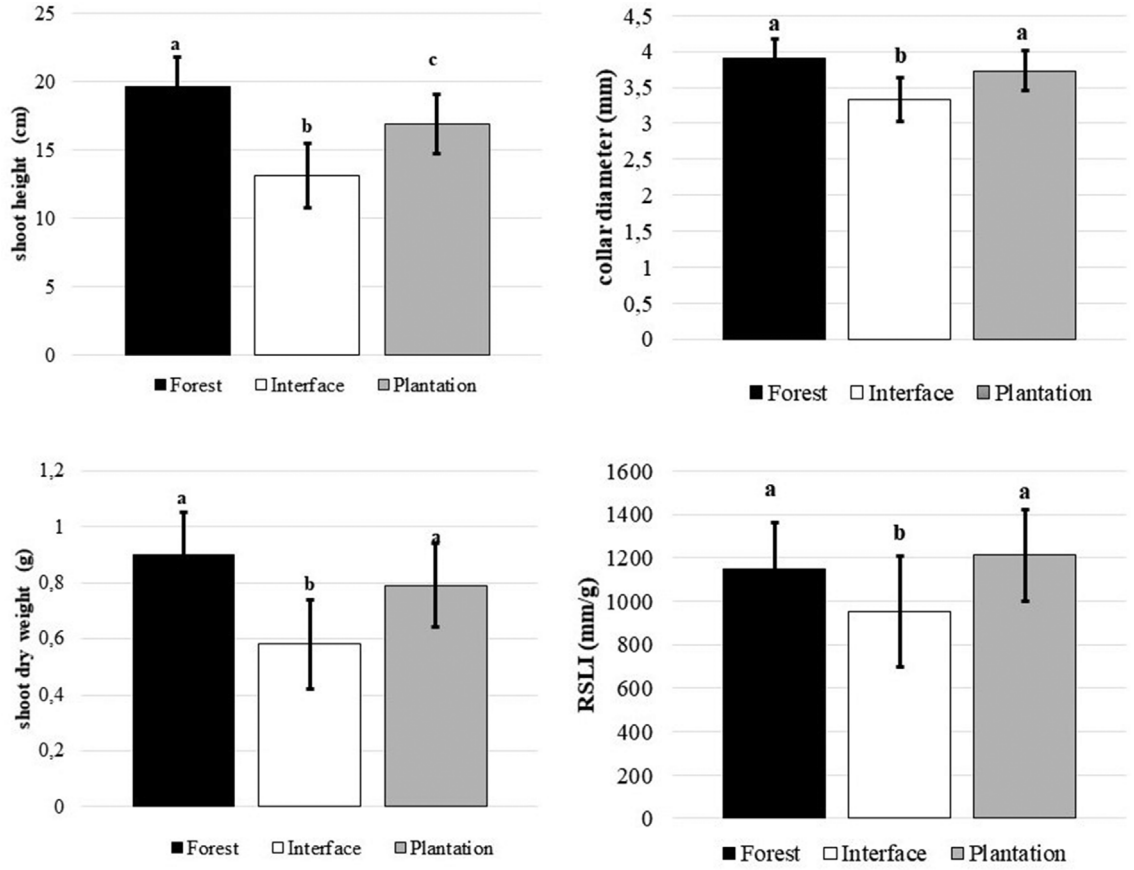

Fig. 4. Nothofagaceae seedlings features. Bars represent SE. Different letters indicate significant differences between treatments ($P < 0.05$, 3W-GLMM).

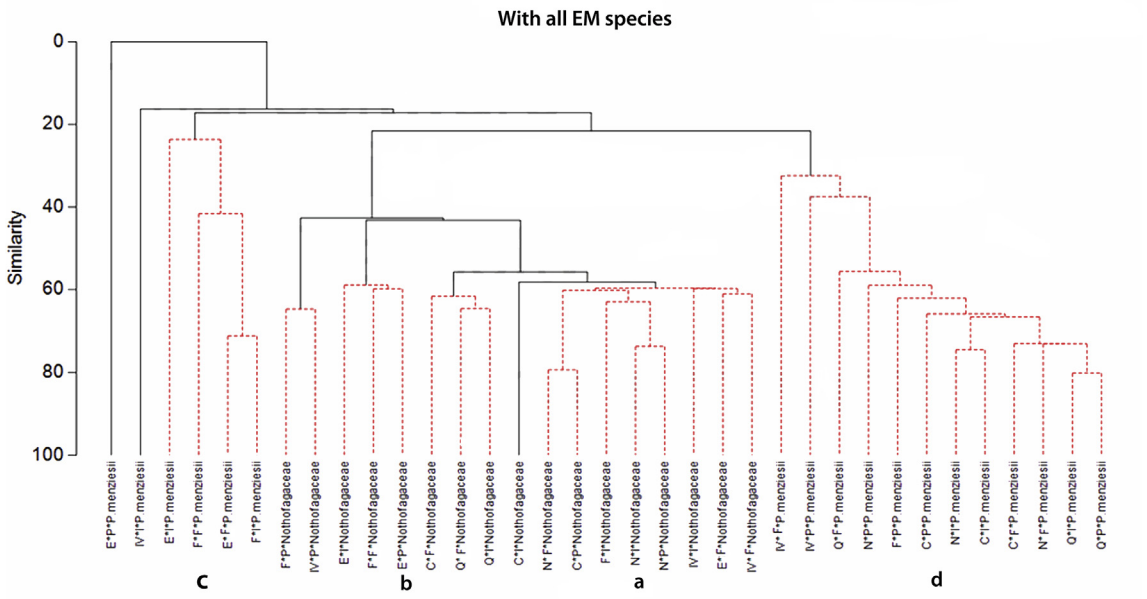

Fig. 5. Hierarchical clustering analysis of ectomycorrhizal (EM) fungal community structure similarity. Different letters indicate significant differences between treatments ($P < 0.05$, ANOSIM).

was found in INTERFACE treatment, due possibly to the fact that *P. menziesii* seedlings were highly colonized with their own EM, and also with three EM shared species. Although AM of native herbs and shrubs constitute an inoculum source for *P. menziesii*, it can be said that AM spore bank abundance is not driving seedling colonization preference, as was observed in grasses and shrubs in European forests (Clapp et al., 1995; Sykorova et al., 2007). Worth to point out is that *P. menziesii* seedlings seem to have the ability to form different AM colonization types (*Paris*-, *Arum*- Both-, Intermediate-type) depending on the situation (*v. gr.* Treatment). Significant higher presence of *Arum*-type was

found in seedlings grown in FOREST treatment, a type that has been consistently found in pioneer situations, abundant in cultivated plants (Brundrett and Kendrick, 1990a, 1990b; Smith and Smith, 1997; van Aarle et al., 2005). Intermediate-type has shown possible differences in P uptake, increasing efficiency and plant growth-related responses (Peterson and Massicotte, 2004). Interestingly, this type showed high incidence in INTERFACE treatment in this study, where the AM colonization was less abundant. Anyway, AM symbiont taxonomic elucidation is still needed as molecular techniques have revealed higher AM infrageneric diversity and host specificity than previously believed (Schüßler and Walker, 2010; Oehl et al., 2011; Öpik et al., 2013).

Most of the EM and ECM species found in this study for both forests types correspond to *Rhizopogon, Hebeloma* (2 spp.), *Wilcoxina* (5 spp.), *Tomentella* (3 spp.), *Tuber* and *Descolea*, all genera reported as pioneers, abundant in nurseries (Baar et al., 1999; Barroetaveña et al., 2006; Izzo et al., 2006; Lu et al., 1999; Marmeisse et al., 1999; Taylor and Bruns, 1999; Tedersoo et al., 2008, 2009; Yu et al., 2001), commonly present in disturbed sites (Mah et al., 2001; Molina et al., 1999; Palfner et al., 2008) and also reported in invaded matrices (Hayward et al., 2015; Moeller et al., 2015). Moreover, *Rhizopogon villosulus* Zeller was described as *Pseudotsuga*'s mostly exclusive ectomycorrhizal partner (Mujic et al., 2014), associated with *P. menziesii* in invasive processes (Hayward et al., 2015; Moeller et al., 2015). Contrary to our findings, Hayward et al. (2015) and Moeller at al. (2015) reported *Suillus* spp. as one of the most abundant EM OTUs, which was not detected in our sampling. Undoubtedly, EM and ECM pioneer genera are a co-adjuvant factor in *P. menziesii* seedling invasion. On the other hand, *Cortinarius* sp. and *Sebacina* spp. were scarce in seedlings in Nothofagaceae and *P. menziesii*, respectively. Both fungi genera have been reported as *late-stage* species, with a poor competitive ability in disturbed sites (Horton, 2011; Nguyen et al., 2012) and were only detected for each species growing in their own soil.

We found that *Hebeloma hiemale* and *Wilcoxina* sp. 1, common mycorrhizal partners for *P. menziesii* in Patagonia (Barroetaveña et al., 2007; Cline et al., 2005) and not previously reported from Nothofagaceae forest, were associated with *N. antarctica* seedlings grown in PLANTATION and FOREST soils, respectively. Considering both taxa have been reported as cosmopolitan, present either in conifers and broadleaf hosts (Bacher et al., 2010; Bergemann and Garbelotto, 2006; Marmeisse et al., 1999), future studies should elucidate if these species have been introduced with *P. menziesii* or are part of the EM Nothofagaceae biota not previously detected.

The presence of dark septate endophytes (DSE) in *P. menziesii* seedlings have already been reported (Cázares and Smith, 1996; Moeller et al., 2015). *Phialocephala fortinii* sensu lato is the dominant species of the DSE complex (Ahlich-Schlegel, 1997; Sieber, 2002). Despite the non-categorical evidence in favor of DSEs being involved in nutrient acquisition and gain of dry matter, indirect evidence supports this view (Addy et al., 2005; Alberton et al., 2010; Newsham, 2011; Vohník et al., 2003, 2005). Volcanic soils of the Andean Patagonian forests are rich in nutrients but low in P, given their allophanic characteristics (Rivas et al., 2007). Therefore, those DSE abilities could improve the nutritional status of invasive seedlings.

Control seedlings showed fortuitous EM colonization, with the lowest diversity (1–3 morphotypes per seedling) and abundance. The most probable source of contamination could have been not complete soil sterilization, as 7 from 11 EM types for *P. menz*iesii and 9 from 12 for Nothofagaceae seedling were at least once detected in controls. Also air dispersed spores from nearby fruiting bodies [*Hebeloma* sp. was found fruiting in bioassay seedlings from Corcovado, Foyel and ENFORSA (data not shown)] or blown soil inoculum could have also contributed. Ectomycorrhizal species found in control seedlings are common in *P. menziesii* nurseries/plantations and/or Nothofagaceae forests (Barroetaveña et al., 2006, 2007; Palfner, 2001; Singer and Morello, 1960).

5. Conclusions

In Patagonia, *P. menziesii* plantations have a “nursery” effect with respect to EM inoculum, which spreads out from plantations into native forests and is already available upon the arrival of seeds. This strategy is highly beneficial and is certainly a contributory factor in their invasion on Nothofagaceae forests. Notwithstanding the taxonomic distance between the two trees species, two not previously reported generalist EM species were found in common, and one EM taxa from *P. menziesii*, not previously reported for Nothofagaceae, was detected only in *N. antarctica*, indicating that EM communities' interaction may flow in both directions. The stable morphometric parameters detected in *P. menziesii* across treatments evidenced its plasticity. The presence of several mutualistic associations (EM, AM and DSE) with different arrangement in each situation reflects this fact which implies better possibilities for development and establishment in invasion process. The adjuvant effect of mycorrhizae has been consistently observed in different environments; therefore, this fact should be considered in the management of fast growing exotic plantations in Patagonia.

Author contributions

Experimental design: MESS, MR, CB. Sampling and field-lab-nursery work: MESS, MBP, EWS. Data analyses: MESS, CB, MBP. Manuscript writing: MESS, CB, MR, MBP.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.actao.2018.07.003.

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