Plant Soil
https://doi.org/10.1007/s11104-022-05820-8

RESEARCH ARTICLE

Co-invasive ectomycorrhizal fungi alter native soil fungal communities

Alija Bajro Mujic · Nahuel Policelli · Martin A. Nuñez · Camille Truong · Matthew E. Smith

Received: 16 July 2022 / Accepted: 24 November 2022

Abstract

Purpose Pinaceae (pine family) trees are native to the Northern Hemisphere and their invasion into the Southern Hemisphere is a growing problem threatening biological diversity. Pinaceae are ectomycorrhizal (ECM) and their invasions are facilitated by non-native and co-invasive ECM fungi. Nothofagaceae species (southern beeches) are dominant overstory trees across large swaths of the Southern Hemisphere and are the only widespread ECM trees native to southern South America (SSA). This observational study investigates the in situ impact of Pinaceae invasions upon native soil fungi associated with Nothofagaceae hosts in SSA.

Methods We performed soil nutrient testing and metabarcode sequencing of fungi in the rhizosphere of *Nothofagus antarctica* and *Nothofagus dombeyi* invaded by Pinaceae trees to determine whether co-invasive fungi might impact native soil fungi. Sampling transects extended from invasions into adjacent *Nothofagus* stands without invasive Pinaceae.

Results The fungal community composition of the Nothofagaceae rhizosphere was dominated by plant-associated Mortierellaceae OTUs in metabarcode data. Mortierellaceae OTU relative abundance was significantly reduced near invasions of *Pinus contorta* (Pinaceae). Invasions of *Pseudotsuga menziesii* (Pinaceae) and *Pinus contorta* were associated with reduced relative abundance of *Nothofagus*-associated

Responsible Editor: Janusz J Zwiazek.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11104-022-05820-8.

A. B. Mujic (✉) · C. Truong · M. E. Smith
Department of Plant Pathology, University of Florida, Gainesville, FL, USA
e-mail: amujic@mail.fresnostate.edu

Present Address:
A. B. Mujic
Department of Biology, California State University, Fresno, Fresno, CA, USA

N. Policelli · M. A. Nuñez
Grupo de Ecología de Invasiones - Instituto de Investigaciones en Biodiversidad Y Medio Ambiente-CONICET- Universidad Nacional del Comahue, San Carlos de Bariloche, Río Negro, Argentina

Present Address:
N. Policelli
Department of Biology, Boston University, Boston, MA, USA

M. A. Nuñez
Department of Biology and Biochemistry, University of Houston, Houston, TX 77204, USA

Present Address:
C. Truong
Royal Botanic Gardens Victoria, Birdwood Avenue, Melbourne, VIC 3004, Australia

Published online: 13 January 2023

ECM OTUS in the *Nothofagus* rhizosphere. *Pinus contorta* invasions were also associated with reduced soil organic matter, total carbon, total phosphorus, and total nitrogen.

Conclusion Further empirical study is warranted to investigate the hypothesis that Mortierellaceae and Pinaceae-specific /suillus-rhizopogon ECM fungi compete for nutrients bound in soil organic matter. Such competition may have potential long-term legacy effects upon post-invasion restoration efforts and implications for Pinaceae invasions globally.

Keywords Invasive Species · Fungi · *Pinus* · Environmental DNA · Ectomycorrhiza · *Nothofagus*

Introduction

Biological invasions of Pinaceae (pine family) trees are a growing issue of conservation, social, and economic concern, especially in the Southern Hemisphere where Pinaceae is not native (Nuñez et al. 2017). Pinaceae trees are obligately ectomycorrhizal (ECM) and co-invade with a relatively small subset of non-native ECM fungi (Dickie et al. 2010, 2017; Hayward et al. 2015a, b; Nuñez et al. 2013). Some of these co-introduced ECM fungi are also invasive (Policelli et al. 2022c), and are likely strong competitors for space and soil resources, yet little is known about their interactions with native ECM fungal communities (Grove et al. 2017). Invasive ECM fungi could lead to replacement of native ECM fungi or coexist with the local soil community with potential impacts in soil biogeochemistry (García et al. 2018). Even when there is some experimental evidence on the effects of ECM plant hosts invasions upon native ECM plant hosts (Baohanta et al. 2012; Bahram et al. 2013; Dehlin et al. 2008; Dickie et al. 2014; Salomón et al. 2018; Ning et al. 2021; Policelli et al. 2020), the lack of high throughput in situ studies make it hard to fully understand the native fungal community's response to invading ECM fungi.

The invasion of Pinaceae trees in Southern South America (SSA) is an ideal scenario to study the belowground effects of invasive ECM fungi (Hoeksema et al. 2020; Policelli et al. 2022b). Native ECM forests occur along both sides of the Andean Cordillera in Patagonia and are dominated by trees in the genus *Nothofagus* (Nothofagales, Nothofagaceae). Nothofagaceae trees are endemic to the Southern Hemisphere, obligate ECM symbionts, and form monodominant or mixed forests in SSA, Australia, New Zealand, New Guinea, and New Caledonia (Heenan and Smissen 2013; Hill 2001; Horak 1983). Humboldt's willow (*Salix humboldtiana*) is the only other extant ECM host tree which co-occurs natively in SSA with Nothofagaceae trees, but their co-occurrence at the same locality is rare (Budde et al. 2011; Thomas and Leyer 2014; Thomas et al. 2015). ECM *Eucalyptus* hosts were present in SSA until the late Oligocene but have been absent since that time (Gandolfo et al. 2011; Hermsen et al. 2012; Hill et al. 2017). The geographic isolation of Nothofagaceae forests from other ECM species in SSA for more than 25 MY (Marchelli et al. 2021), and the unique community structure of *Nothofagus* ECM fungal communities in SSA (Tedersoo et al. 2012), likely make these communities particularly vulnerable to the effects of ECM fungi invasions.

The Nothofagaceae-associated ECM fungal communities in SSA are dominated by Basidiomycota (/cortinarius, /inocybe, /tomentella-thelephora, and /tricholoma ECM lineages) and naturally lack many of the ECM lineages (/suillus-rhizopogon, /russula-lactarius, /boletus, and /pisolithus-scleroderma) typically found in Pinaceae forests and other ECM biomes (Nouhra et al. 2012, 2013; Tedersoo et al. 2010; Truong et al. 2017). ECM fungal symbionts in SSA have coevolved with their Nothofagaceae hosts and have been relatively isolated from other ECM systems since continental drift separated the component landmasses of Gondwana (Kuhar et al. 2017; Skrede et al. 2011). A novel ECM community was introduced to this system together with the introduction of Pinaceae trees as part of Argentinian forestry trials between 1900 and 1934. Almost a century later, multiple Pinaceae species have been planted elsewhere as economically important sources of timber and wood pulp and some of them have established invasive populations throughout Patagonia (Hayward et al. 2015a; Simberloff et al. 2003).

To address the belowground effects of invasive ECM fungi introduced in native ECM forests, we characterized the rhizosphere soil of native *Nothofagus* species in Pinaceae-invaded forests of SSA using fungal DNA metabarcoding. We predict that: 1) co-invasion of exotic Pinaceae and their associated ECM

fungi alter the community structure of native fungi associated with *Nothofagus* species, and 2) the effects of co-invasion on native Nothofagacaeae-associated fungi are directly correlated with the percent cover and proximity of invasive Pinaceae trees.

Materials and methods

Field localities and sampling

Field localities were chosen near San Carlos de Bariloche, Argentina, where invasions of *Pseudotsuga menziesii* (Douglas fir) and *Pinus contorta* (lodgepole pine) occur in native Nothofagaceae forests (Table 1). Both non-native Pinaceae species are amongst the most invasive and best studied Pinaceae species in the Southern Hemisphere (Dickie et al. 2017; Nuñez et al. 2017). While *Pseudotsuga menziesii* is shade tolerant and tends to invade the understory of mesic forests dominated by the native ECM species *Nothofagus dombeyi* (coihue), *Pinus contorta* is shade intolerant and invades open and xeric grassland habitats as well as mattoral (mixed xeric shrubland), which are often dominated by the native ECM species *Nothofagus antarctica* (ñire). *Nothofagus dombeyi* favors moist conditions and forms forests along waterways and on gentle slopes at middle elevation. In contrast, *N. antarctica* grows in extreme conditions such as poorly drained soil, low temperature, or xeric sites, and forms stands in middle to low elevation mattoral shrublands, marshes, as well as in steep, high elevation sites (Gut 2008). The non-native Pinaceae species were introduced from Western North America together with ECM fungi from their native ranges, and have been able to establish novel interactions with other non-native ECM fungi from Europe and Asia (e.g. European *Suillus luteus*) (Farjon 2010; Hayward et al. 2015a, b). Less is known regarding the composition of ECM fungal communities that colonize the two native *Nothofagus* hosts. Ex situ bioassay experiments of field-collected soil have demonstrated that a few ECM fungal species commonly form ECM structures with both *N. dombeyi* and *N. antarctica* (Salomón et al. 2018).

Sampling design

Invasions of *Pi. contorta* were characterized at three localities where *Nothofagus antarctica* grows in a shrub-like form as part of native xeric mattoral vegetation communities. Invasions of *P. menziesii* were characterized at four localities. At three of them, *N. dombeyi* grows as the dominant overstory tree in mesic mixed temperate forest. The fourth locality was a mesic floodplain where *P. menziesii* invades

Table 1 Geographic coordinates and properties of sampling localities

Locality Code	Native ECM Host	Invasive ECM Host	Locality Name	Ambient Moisture	Habitat Type	Coordinates	Elevation (meters)
A1	*Nothofagus antarctica*	*Pinus contorta*	Cerro Catedral	Xeric	Mattoral shrubland	41 8 19.9 S, 71 25 52.1 W	855
A2.1	*Nothofagus antarctica*	*Pinus contorta*	Reserva el Foyel	Xeric	Mattoral shrubland	41 40 34.9 S, 71 27 23.7 W	813
A2.2	*Nothofagus antarctica*	*Pinus contorta*	Reserva el Foyel	Xeric	Mattoral shrubland	41 40 35.5 S, 71 27 22.2 W	812
A3	*Nothofagus antarctica*	*Pseudotsuga menziesii*	Villa la Angostura	Mesic	Wetland edge	40 46 59.7 S, 71 36 23.8 W	829
D1	*Nothofagus dombeyi*	*Pseudotsuga menziesii*	Arroyo Goye	Mesic	Humid forest	41 6 24.1 S, 71 31 9.3 W	965
D2	*Nothofagus dombeyi*	*Pseudotsuga menziesii*	Lago Mascardi	Mesic	Humid forest	41 21 8.3 S, 71 31 1.3 W	875
D3.1	*Nothofagus dombeyi*	*Pseudotsuga menziesii*	Isla Victoria	Mesic	Humid forest	40 58 47.7 S, 71 31 29.8 W	832
D3.2	*Nothofagus dombeyi*	*Pseudotsuga menziesii*	Isla Victoria	Mesic	Humid forest	40 58 54.5 S, 71 31 34.2 W	824

stands of *N. antarctica* growing in a tree-like form (Table 1). At each locality we sampled *Nothofagus* rhizosphere soil from three independent replicate transects, each of them spanning four distinct Pinaceae invasion densities: Invaded (I) = Cover of invasive Pinaceae greater than 70%; Invasion Front (IF) = Cover of invasive Pinaceae approximately 50%; Near Invasion (NI) = Nearest invasive Pinaceae individual approximately 7 m from sample; Uninvaded (U) = Nearest cover of invasive Pinaceae at least 10 m from sample. The transects originated within patches of invasive Pinaceae and terminated within nearby, uninvaded, stands of *Nothofagus.* Sampling locations for each invasion site type were identified along a transect based upon the described criteria (Fig. 1). Different *Nothofagus* individuals were chosen for each sample point and transects themselves were located at least 60 m apart from one another to avoid pseudoreplication. Percent cover for non-native *P. menziesii* was measured as total canopy coverage of the tree within a 5 X 5 m quadrat centered upon a single *Nothofagus* individual. For non-native invasive *Pi. contorta* percent cover was measured as the total canopy coverage of *Pi. contorta* at the dripline of the *N. antarctica* individual sampled. The differences in the percent cover measurements between both non-native invasive species are due to the differences in the vegetation structure of the habitats they invade, as previously described.

While sampling, we noticed that *Pinus contorta* invasions were most common and successful in disturbed areas at our field localities, which is a known trait of such invasions (Nuñez et al. 2017). Disturbance could have an effect on the native ECM community associated with native *Nothofagus* trees that would be independent of the Pinaceae invasion. For that reason, we also sampled *N. antarctica* rhizosphere soil from a total of nine "disturbed ñire" (DN) sites at two of the localities (A1 and A2). The DN site type had no *Pi. contorta* invasion and but were located near roads or *Pi. contorta* plantation at collection localities. All DN sites had experienced clearing of most vegetation, soil grading/compaction, and soil disturbance from the use of heavy equipment. Finally, we sampled rhizosphere soil of three *N. antarctica* individuals growing within *Pi. contorta* plantations at locality A2 (site type denoted as "ñire in pine plantation" (NP)) to determine the effect of *Pi. contorta*

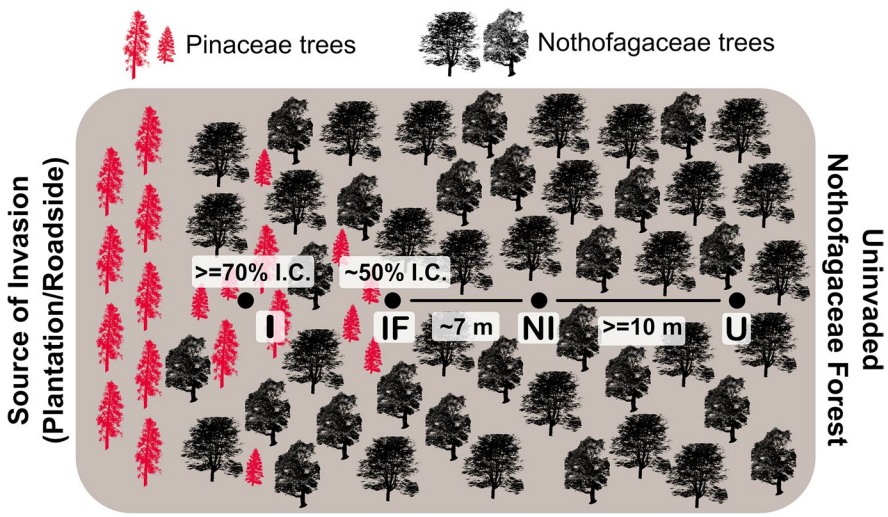

Fig. 1 Transect design and the four invasion site types sampled along each invasion transect. Black points show generalized sampling locations of the four invasion site types: "invaded" (I), "invasion front" (IF), "near invasion" (NI), and "uninvaded" (U). Percent invasive cover (I.C.) of Pinaceae trees was used to determine sampling location for I and IF site types. For detailed descriptions of invasive cover calculations, see materials and methods. Nothofagaceae trees sampled for NI site type replicates were located approximately 7 m from the nearest Pinaceae tree while Nothofagaceae trees sampled for U site type replicates were located at least 10 m from the nearest Pinaceae tree. Black lines drawn between IF, NI, and U site types indicate these distances and are not drawn to scale

monodominance upon *N. antarctica* rhizosphere fungi.

Rhizosphere soil collection

At every sampling point, we extracted four soil cores measuring 7.5 X 10 cm (diameter X depth) from within the drip line of a *N. dombeyi* or *N. antarctica* individual. These four cores were pooled and loose soil was sieved through a 2 mm screen (Forestry suppliers, Jackson, MS, U.S.A.) and discarded. *Nothofagus* roots remaining in the screen were separated into a plastic bag. Roots were separated enough to distinguish tree species but they were not cleaned in the field, leaving the rhizosphere soil adhering to fine roots. All rhizosphere soil adhering to *Nothofagus* roots was collected, sieved, and 0.25 g of each sieved soil sample was stored in a MoBio Powersoil DNA extraction tube (MoBio, Carlsbad, CA, U.S.A.). All remaining rhizosphere soil was dried in a forced air dryer for 48 h at 37º C in preparation for soil nutrient testing. An additional core was taken at each "uninvaded" site type replicate and *Nothofagus* roots were collected from these cores. These roots were pooled, cleaned of adhering soil with tap water, and then examined for ECM root tips using a dissecting microscope. Up to 110 ECM root tips from each pool of "uninvaded" *Nothofagus* roots were saved in 2X CTAB buffer for DNA extraction. Finally, four to six soil cores were collected at each locality from within a pure stand of the invasive Pinaceae species. Pinaceae roots were separated from cores and adhering rhizosphere soil was collected for DNA extraction and nutrient testing.

All root/soil samples were stored at 4º C after collection and processed within 24 h from the time of collection. Soil and roots preserved in MoBio DNA extraction tubes and CTAB buffer were stored at 4º C for 1 to 4 weeks and then at -20º C until DNA extraction was performed. Dried soil samples were stored at room temperature until nutrient testing could be performed at the Laboratory of Soils, Plants, and the Environment (LABSPA) at the Universidad Nacional del Sur in Bahía Blanca, Argentina. These tests were performed using LABSPA protocols and included measurements of pH, organic material (24 h at 500º C), available nitrate (NO_3^-) and ammonium (NH_4^+) (Bremner 1965), available phosphorus with the Bray Kurtz method (Kuo 1996), total carbon by dry combustion, and total nitrogen by semi-micro Kjeldhal (Bremner 1996).

DNA extraction, illumina library preparation, and sequencing

Environmental DNA (eDNA) extractions were made from soil following MoBio Powersoil DNA extraction protocols with additional wash steps. Sample quality and concentration of eDNA elutions were determined using a Nanodrop 2000 spectrophotometer (ThermoFisher Scientific, Waltham, MA, U.S.A.). Any eDNA sample with an A260/A280 ratio < 1.5 or an A260/A230 ratio < 1 were processed using the MoBio Powerclean DNA extraction cleanup kit. eDNA extractions were standardized to a concentration of 5 – 10 ng/μL by dilution with sterile deionized water. ECM root tip samples were processed by draining CTAB buffer from roots, rinsing with deionized water, and removing excess water by blotting with laboratory wipes. DNA was extracted from rinsed and dried ECM roots using the MoBio Powersoil DNA extraction kit as described.

Illumina amplicon libraries of the Internal Transcribed Spacer 1 (ITS1) were generated for all eDNA and root tip DNA extractions by PCR amplification using the fungal-specific primers ITS1F (Gardes and Bruns 1993) and ITS2 (White et al. 1990). Primers were synthesized by Integrated DNA technologies (Skokie, IL, U.S.A.) to contain Illumina sequencing adaptors, an eight nucleotide DNA barcode sequence, and the target primer sequence. PCR reactions were performed using Phusion DNA polymerase Master Mix (New England Biolabs, Ipswich, MA, U.S.A.) in 25 μL reactions composed of 2 μL DNA template, 12.5 μL Phusion Master Mix, 5.5 μL deionized water, and 2.5 μL (5 μM) of each Illumina-barcoded primer. Thermocycling conditions were 30 s at 95 C; 30–35 cycles of 10 s at 95 C, 20 s at 60 C, and 20 s at 72 C; and a final polymerase extension step of 7 min at 72 C. PCR products were stained with SYBR green (Molecular Probes, Inc., Eugene, OR, U.S.A.), subjected to gel electrophoresis, and visually inspected under ultraviolet illumination to confirm the presence of amplicons in the expected size range. Amplicon libraries were cleaned and standardized for concentration using the SequalPrep Standardization kit

(Thermo Fisher Scientific, Waltham, MA, U.S.A.) following the manufacturer's protocol with 10 µL of PCR product as input. 5 µL of each standardized amplicon library was pooled into a single multiplexed library, cleaned of residual primers by Ampure Bead Size selection (Beckman Coulter, Brea, California, USA), and submitted for 300 bp paired-end sequencing on an Illumina MiSeq device at the Interdisciplinary Center for Biotechnology Research (University of Florida, Gainesville, FL, U.S.A.).

DNA sequence clustering and curation

Illumina sequence data were quality screened following the methods of Truong et al. (2019). Reference-based components of operational taxonomic unit (OTU) clustering were performed with a custom ITS database of South American fungi (Truong et al. 2017), concatenated with version 7.2 of the UNITE ITS database (Kõljalg et al. 2005, released June 28, 2017), and the unique ITS OTUs generated by Truong et al. (2019). OTU clustering was performed using QIIME (Caporaso et al. 2010). Taxon-specific clustering biases and data denoising were performed by including both positive and negative Illumina sequencing controls as suggested by Nguyen et al. (2015). The negative control was an Illumina library generated by using water for the PCR reaction instead of eDNA template. The positive control was a 20-species biological mock community that contained equal-molar concentrations of DNA extracts from Nothofagaceae-associated fungi. OTUs that occurred in high read count in the negative control sample were determined to either be environmental contaminants, barcode index-bleed, or physical cross-contamination of samples. OTUs determined to be environmental contaminants were removed entirely from further analysis (20 OTUs with 5882 total reads). Read counts of 54 OTUs determined to have barcode index-bleed or physical cross-contamination of samples were reduced in all samples by the number of reads present in the negative control sample (235 total reads detected in negative control). Read counts of remaining OTUs were reduced to zero in any sample where they were less than 0.05% of the total read count for this sample. Inconclusive BLASTn taxonomy assignment performed by QIIME was corrected through megaBLAST searches of the UNITE and International Sequence Databases (INSD) using the PlutoF platform (Abarenkov et al. 2010). We assigned taxonomy at the generic level when BLASTn sequence identity was > 95%, family level at > 90%, and order level at > 80%.

Assignment of OTUs to ecological guilds was performed using a combination of automated assignment in FUNGuild (Nguyen et al. 2016) as well as manual assignment using BLASTn searches of UNITE, NCBI sequence database, and a custom ITS database (Truong et al. 2017). We manually assigned ECM status to an OTU when BLASTn searches revealed > 90% sequence identity to a known ECM fungus, a threshold that falls between the established thresholds for generic and familial level taxonomic distinction and is expected to coincide with ECM lineage (Vu et al. 2019; Tedersoo et al. 2010; Tedersoo and Smith 2013). OTUs assigned to the ECM ecological guild were further categorized into "Nothofagaceae-associated", "Pinaceae-specific", and "naturalized Pinaceae" ECM taxa. ECM taxa were designated as Nothofagaceae-associated or Pinaceae-specific if they belonged to ECM lineages that are known to occur only with those hosts (Tedersoo et al. 2010; Tedersoo and Smith 2013) and/or that produced significant BLAST matches (identity > = 95%) only with database records identified from those host trees. Our "naturalized Pinaceae" taxa fall into the category of 'introduced species that spread to local hosts' as defined by Vellinga et al. (2009). This category was established because exotic Pinaceae-associated ECM fungi, such as *Hebeloma hiemale, Sistotrema sp.,* and *Wilcoxina sp*., are able to form ECM associations with *Nothofagus* species (Policelli et al. 2020; Salomón et al. 2018). We identified "naturalized Pinaceae" OTUs as those meeting all three of the following criteria: 1) they were present in rhizosphere soil samples from both Pinaceae plantations and undisturbed *Nothofagus* forests, 2) they were present in cleaned *Nothofagus* ECM root samples, and 3) they had high BLAST identity (> 97%) with OTUs in the UNITE database identified only from Pinaceae trees in their native ranges.

Statistical analyses

Raw read OTU tables were transformed after the curatorial steps described above into two independent

matrices for downstream community structure analysis. First, we transformed data into a presence/absence table (read counts coded as 1 or 0, representing presence or absence, respectively) for qualitative analysis of community composition. Second, we normalized sample read counts by transforming the data into a relative abundance table for quantitative analysis of community composition. All statistical analyses were performed using the R statistical computing environment (version 4.0.3). Plots of multivariate statistical data were created using the R library ggplot2 (version 3.3.2, Wickham 2016) and plots of community assemblage data were created using the R library phyloseq (version 1.32.0, McMurdie and Holmes 2013). Multivariate statistics were performed using the R library vegan (version 2.6–2, Oksanen et al. 2018). Non-metric multidimentional scaling (NMDS) was used to visualize qualitative (presence/absence) community composition in multidimentional space and significance of differences in multidimentional community composition (centroid location) were tested using permutational multivariate analysis of variance (PERMANOVA) as implemented in the adonis2() function of vegan upon Raup-Crick distance matrices calculated using the raupcrick() function of vegan. The primary assumption of PERMANOVA is homogeneity of variance in multivariate space around the centroid of site type groups and this was tested using Anderson's (2006) test as implemented in the betadisper() function of vegan. A subsequent permutation test for F-statistic significance was performed upon betadisper() results as implemented in the permutest() function of vegan.

Results

OTU clustering and fungal community composition

After trimming and quality filtering, 3,931,007 Illumina reads were clustered at 97% similarity into 1135 OTUs by QIIME. Reads were overwhelmingly (71%) distributed in OTUs classified as the saprotrophic plant-associated genus *Mortierella* (Mortierellales: Mortierellaceae). Only 18% of reads clustered into OTUs classified as ECM taxa and the final 11% of reads clustered into OTUs classified as non-ECM (mostly saprotrophic) taxa other than *Mortierella*. A complete OTU table listing all read relative abundances as well as taxonomic and ecological determinations of OTUs can be found in Online Resource 1. Representative sequences of all fungal OTUs can be found in Online Resource 2. Of the 18% of reads clustered into ECM taxa, the majority (60%) were clustered into OTUs determined to be native Nothofagaceae-associated ECM fungi, while 30% clustered into OTUs identified as Pinaceae-specific ECM taxa, and the final 10% into OTUs identified as "naturalized Pinaceae" ECM taxa (Online Resource 3).

The relative abundance of native Nothofagaceae-associated ECM sequence reads in *N. antarctica* (xeric) rhizosphere was significantly higher in "uninvaded" (U) and "near invasion" (NI) site types than it was in "invasion" (I), "invasion front" (IF), "ñire in pine plantation" (NP), "disturbed ñire" (DN) site types (Kruskal–Wallis test, $p = 0.001$, Dunn post-hoc test, $p < 0.05$). There was no significant difference in the relative abundance of "naturalized Pinaceae" ECM sequence reads in the rhizosphere of plantation-grown *Pi. contorta* and all *N. antarctica* (xeric) site types (Kruskal–Wallis test, $p = 0.22$). Similarly, no significant difference was observed in the relative abundance of "naturalized Pinaceae" ECM sequence reads in the rhizosphere of plantation-grown *P. menziesii* and *N. dombeyi* (Kruskal–Wallis test, $p = 0.12$) or *N. antarctica* (mesic) (Kruskal–Wallis test, $p = 0.36$). There was no significant difference in the relative abundance of Native Nothofagaceae-associated ECM sequence reads in the *N. dombeyi* rhizosphere in all *P. menziesii* invasion site types (I, IF, NI, U) (ANOVA, $p = 0.16$, Bartlett's test of homogeneity of variances, $p = 0.24$).

The relative abundance of Pinaceae-specific sequence reads was significantly greater in the *N. antarctica* (xeric) rhizosphere of "invaded" (I) and "invasion front" (IF) site types than in "near invasion" (NI) and "uninvaded" (U) site types (Fig. 2a, Kruskal–Wallis test, $p = 0.0003$, Dunn post-hoc test, $p < 0.05$). This trend was particularly striking in "ñire in pine plantation" (NP) site type where invasive Pinaceae-specific ECM sequence reads made up, on average, 96.7% ($n = 3$) of the ECM reads in the *N. antarctica* (xeric) rhizosphere. Samples from the *N. antarctica* (xeric) disturbance control site type ("disturbed ñire" (DN)) and "uninvaded" (U) site type had no reads assigned to Pinaceae-specific ECM OTUs. We observed a similar pattern of increased relative

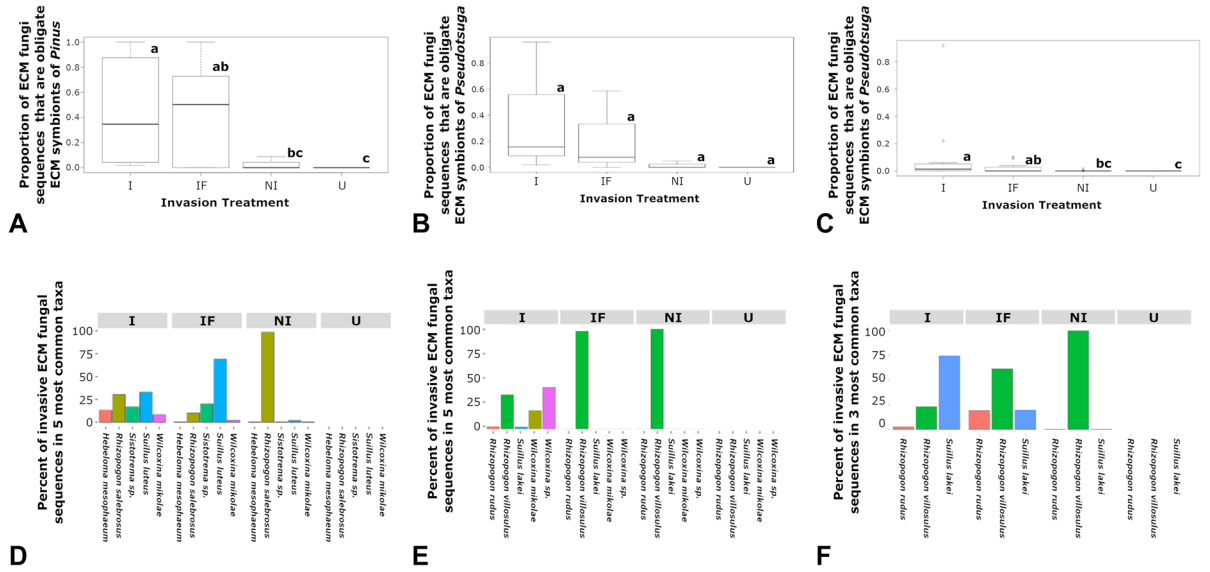

Fig. 2 The percentage of ECM fungi sequences that are obligate ECM symbionts of Pinaceae and the community composition of these Pinaceae-obligate ECM fungi based upon invasion site type. **A** Relative proportion of Pinaceae-associated ECM fungi across site types in the rhizosphere of *Nothofagus antarctica* invaded by *Pinus contorta* (localities A1 and A2). **B** Relative proportion of Pinaceae-associated ECM fungi across site types in the rhizosphere of *Nothofagus antarctica* invaded by *Pseudotsuga menziesii* (locality A3). **C** Relative proportion of *Pinaceae*-associated ECM fungi across site types in the rhizosphere of *Nothofagus dombeyi* invaded by *Pseudotsuga menziesii*. (localities D1, D2, and D3). **D** Species identity of invading *Pinaceae*-obligate ECM fungi across site types in the rhizosphere of *Nothofagus antarctica* invaded by *Pinus contorta* (localities A1 and A2). **E** Species identity of invading *Pinaceae*-obligate ECM fungi across site types in the rhizosphere of *Nothofagus antarctica* invaded by *Pseudotsuga menziesii* (locality A3). **F** Species identity of invading *Pinaceae*-obligate ECM fungi across site types in the rhizosphere of *Nothofagus dombeyi* invaded by *Pseudotsuga menziesii* (localities D1, D2, and D3). Note: image Fig. 2f shows only 3 Pinaceae-obligate taxa because only three such OTUs were present in *Nothofagus dombeyi* invasion site types (I, IF, NI, U)

abundance of Pinaceae-specific sequence reads in the *N. dombeyi* rhizosphere across invasion site types (I, IF, NI, U) (Fig. 2b, Kruskal–Wallis test, $p = 0.006$, Dunn post-hoc test, $p < 0.05$). Relative abundance of Pinaceae-specific ECM sequence reads in the *N. antarctica* (mesic) rhizosphere was not found to be significantly different across site types (Fig. 2c, ANOVA, $p = 0.69$, Bartlett's test of homogeneity of variances, $p = 0.091$). A greater OTU richness of Pinaceae-specific ECM sequence reads was seen in "invaded" (I) and "invasion front" (IF) site types than in "near invasion" (NI) and "uninvaded" (U) site types (Fig. 2d, e, and f). *Rhizopogon spp.* made up the majority of the Pinaceae-specific ECM sequence reads in site types more distant from Pinaceae trees.

Community composition and richness of Nothofagaceae-associated ECM OTUs for all locality types can be found in Fig. 3. OTU richness was roughly equivalent for *N. antarctica* (xeric) (119 OTUs) and *N. dombeyi*-associated ECM sequence reads (116 OTUs) with many OTUs shared between these forest types. In comparison, the richness of ECM OTUs associated with *N. antarctica* (mesic) was notably reduced (42 OTUs). ECM sequence read pools of *N. antarctica* (xeric) and *N. dombeyi* forest types was largely dominated by the ECM lineages /tomentella-thelephora, /cortinarius, /inocybe, /clavulina, and /sebacina. The ECM sequence read pool of *N. antarctica* (mesic) was also dominated by /tomentella-thelephora and /cortinarius lineages, but has a much higher proportion of /descolea and /terfezia-peziza depressa (*Ruhlandiella spp.*) ECM lineages.

A total of 81 Pinaceae-specific OTUs were identified in our samples and they belong to the following ECM lineages: /amphinema-tylospora, /cantharellus, /clavulina, /cortinarius, /hebeloma-alnicola, /inocybe, /pisolithus-scleroderma, /sebacina, /suillus-rhizopogon, /tomentella-thelephora, /tricholoma, /

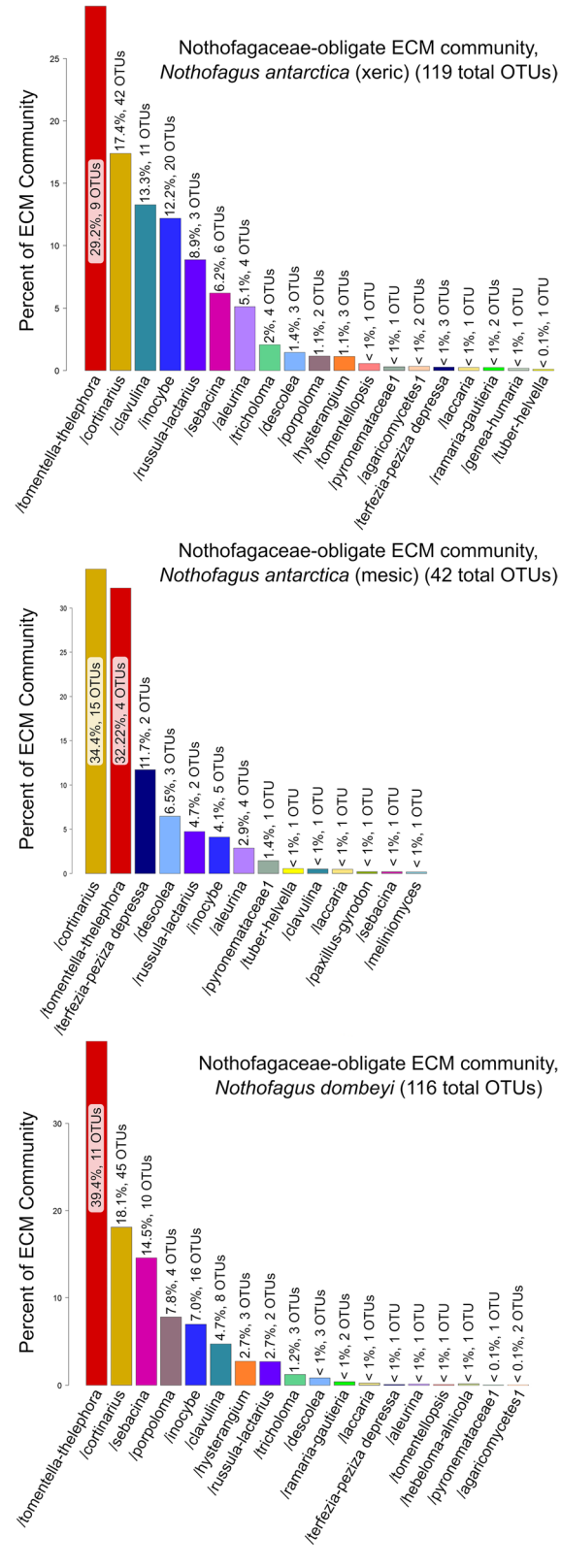

Fig. 3 Community composition of Nothofagaceae-associated ectomycorrhizal fungi colonizing the rhizospheres of *Nothofagus antarctica* (xeric) invaded by *Pinus contorta* (localities A1 & A2), *Nothofagus antarctica* (mesic) invaded by *Pseudotsuga menziesii* (locality A3), and *Nothofagus dombeyi* invaded by *Pseudotsuga menziesii* (localities D1, D2, D3). Taxa are represented by their ECM linages sensu Tedersoo et al. (2010) and Tedersoo and Smith (2013). The percentage of total sequences as well as the number of OTUs assigned to each ECM lineage are shown

tuber-helvella, and /wilcoxina. Only 24 Pinaceae-specific OTUS were associated with *P. menziesii* while 70 were associated with *Pi. contorta.* Some Pinaceae-specific ECM OTUs were shared between *Pi. contorta* and *P. menziesii*, primarily OTUs in the genera *Cortinarius, Hebeloma, Tylospora*, and *Wilcoxina*. Rhizosphere soil of *Pi. contorta* in plantations harbored 64 Pinaceae-specific ECM OTUs, while rhizosphere soil of *N. antarctica* (xeric) invaded by *Pi. contorta* contained only 21. The rhizosphere soil of *P. menziesii* in plantations contained 24 Pinaceae-specific ECM OTUs. In contrast, the richness of Pinaceae-specific ECM OTUs in the rhizosphere of *N. antarctica* (mesic) was only 8 OTUs and the richness in the rhizosphere of *N. dombeyi* was only 3 OTUs (Fig. 2f).

A total of 28 "naturalized Pinaceae" ECM OTUs were identified in this study that are resolved in the /helotiales1, /cantharellus, /sebacina, /pseudotomentella, and /tomentella-thelephora ECM lineages. We also found some evidence to support the colonization of Pinaceae trees by Nothofagaceae-associated ECM taxa. OTUs identified as a Thelephoraceae species (SH184540.07FU_UDB014400_reps), *Sebacina sp.* (SH263696.07FU_UDB008428_refs), and *Inocybe sp.* (nov2015.0.ReferenceOTU54, a BLAST match to *Inocybe* OTU 7 Hayward et al. (2015a)) were detected from the rhizosphere of plantation-grown *P. menziesii* and are apparently native Nothofagaceae-associated ECM taxa (Truong et al. 2017). A complete list of all OTUs identified as Pinaceae-specific, Nothofagaceae-associated, and "naturalized Pinaceae" ECM taxa are provided in Online Resource 4.

Effects of Pinaceae invasion on Nothofagaceae rhizosphere fungal community structure

Mortierella OTUs were the most abundant fungal OTU detected in all of our *Nothofagus* rhizosphere

samples and 83 OTUs were assigned to this taxon (Online Resources 1, 2, and 3). Despite their high relative abundance in rhizosphere soil, *Mortierella* OTUs were completely absent in samples of cleaned *Nothofagus* ECM roots. Relative abundance of *Mortierella* sequence reads was significantly reduced in rhizosphere soil of *N. antarctica* growing in *Pi. contorta* plantations ("ñire in pine plantation" (NP)) compared with uninvaded *N. antarctica* (xeric) rhizosphere and are nearly absent from rhizosphere soil of plantation-grown *Pi. contorta* ("PICO RS") (Fig. 4, ANOVA, $p=0.0005$, Barlett's test of homogeneity of variances, $p=0.19$, Tukey's HSD $p<0.05$). Further investigation revealed a positive linear correlation between the relative abundance of rhizosphere *Mortierella* sequence reads and the distance to the nearest *Pi. contorta* individual (Fig. 5, $p=0.0003$, $R^2=0.28$). In contrast, relative abundance of *Mortierella* sequence reads in the *P. menziesii* rhizosphere showed no significant difference from the rhizosphere of *N. antarctica* (mesic) (site types I, IF, NI, U) (ANOVA, $p=0.8$, Bartlett's test of homogeneity of variances, $p=0.09$) or the rhizosphere of *N. dombeyi* (site types I, IF, NI, U) (ANOVA, $p=0.76$, Bartlett's test of homogeneity of variances, $p=0.07$).

We analyzed both the qualitative (presence/absence) as well as quantitative (relative abundance) community composition of fungal OTUs in rhizosphere soil samples. These analyses were largely in agreement, and we present the qualitative analyses

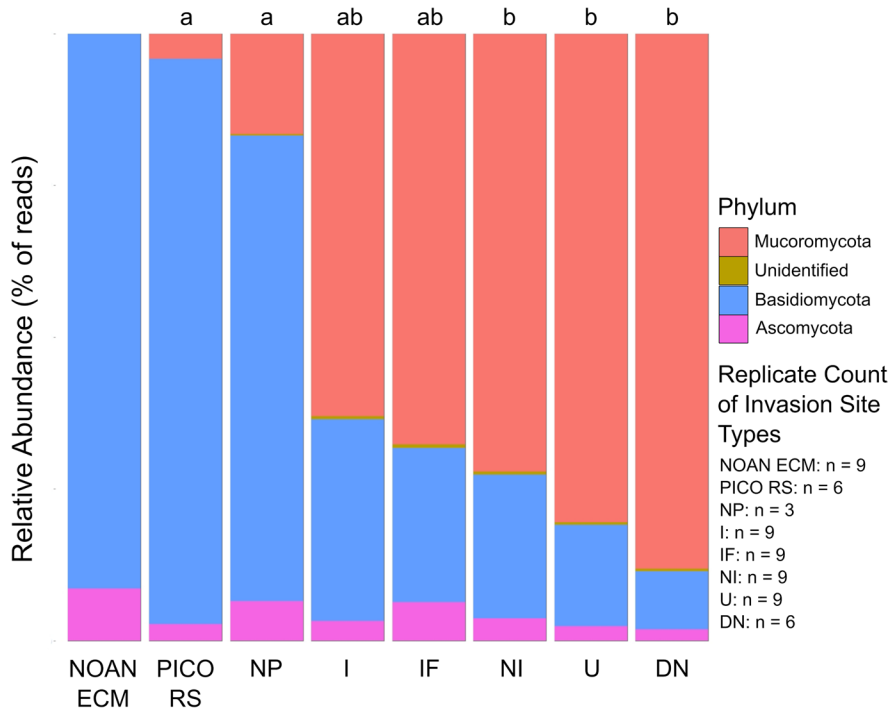

Fig. 4 Community composition for all invasion site types collected in stands of *Nothofagus antarctica* (xeric) being invaded by *Pinus contorta* (localities A1 & A2). Replicate counts (n) refer to the number of individual trees sampled (*N. antarctica* or *Pi. contorta*, 4 soil cores each) for that site type. "Invaded" (I), "invasion front" (IF), "near invasion" (NI), and "uninvaded" (U) are communities from the rhizosphere of *N. antarctica* sampled under the invasion site types outlined in Fig. 1. The "NOAN ECM" site type ("*Nothofagus antarctica* ectomycorrhizal roots") are sequences from pooled and cleaned ECM root tips of *N. antarctica* (xeric). The PICO RS site type ("*Pinus contorta* rhizosphere") are sequences from rhizosphere soil of *Pi. contorta* growing in plantations adjacent to invasion localities. The NP site type ("ñire in pine plantation") are sequences from rhizosphere soil of *N. antarctica* individuals growing in the understory of *Pi. contorta* plantations. The DN site type ("disturbed ñire") are sequences from the rhizosphere of *N. antarctica* individuals growing in the same level of disturbance as the "invasion" (I) site type but lacking invasion by *Pi. contorta.* Note that > 99% of all Mucoromycota sequences are from *Mortierella* species. Lowercase letters indicate groups that are significantly different in the relative abundance of *Mortierella* OTUs in the fungal community (ANOVA, $p=0.0005$, Bartlett's test for homogeneity of variances, $p=0.188$, Tukey's honest significant difference, $p<=0.05$)

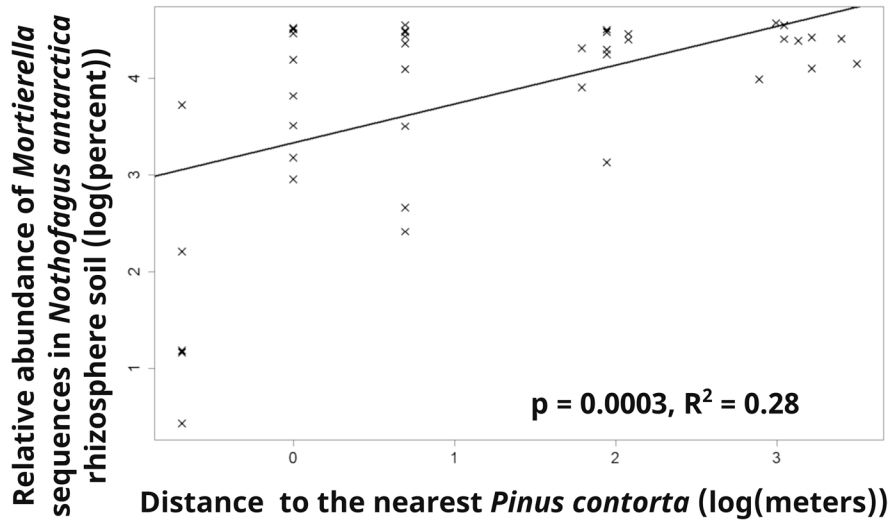

Fig. 5 Linear correlation plot showing the log relative abundance of *Mortierella* OTUs in *Nothofagus antarctica* rhizosphere soil versus the log distance (meters) of the soil core from the nearest *Pinus contorta* individual. This analysis includes samples taken from all localities where *Pi. contorta* was invading *Nothofagus antarctica* (xeric) stands (localities A1 and A2). These samples include *N. antarctica* rhizosphere soil from invasion site types (I, IF, NI, U), *Pi. contorta* rhizosphere soil from individuals in the nearby plantation (PICO), and *N. antarctica* rhizosphere soil from individuals growing inside *Pi. contorta* plantations (NP). Where the samples were derived from *Pi. contorta* roots (PICO) or rhizosphere soil of *N. antarctica* growing in the understory of *Pi. contorta* (NP) we designated a distance of 0 m from the nearest *Pi. contorta* individual. Such distance values were adjusted + 0.1 m to allow for log transformation

here. A summary of quantitative community composition analyses can be found in Online Resource 3. We found significant difference in qualitative (presence/absence) community composition of total fungal OTUs in *N. antarctica* (xeric) rhizosphere between invasion site types (I, IF, NI, U) (PERMANOVA $p = 0.052$, Permutation test for homogeneity of multivariate dispersions, $p = 0.302$). The statistical significance of these differences was increased by the addition of the disturbance control site type ("disturbed ñire", DN) to this analysis (site types I, IF, NI, U, DN) (Fig. 6a, PERMANOVA $p = 0.036$, Permutation test for homogeneity of multivariate dispersions, $p = 0.555$). NMDS ordination clustered the I, IF, and NI site types as the most similar with the U and DN site types shifting toward the negative NMDS2 axis. We found no significant difference in the qualitative community composition of total fungal OTUs in the rhizosphere of *N. dombeyi* invasion site types (I, IF, NI, U) (PERMANOVA $p = 0.171$). There was no significant difference between the qualitative community composition of native Nothofagaceae-associated ECM OTUs within the rhizosphere of *N. antarctica* (xeric) (Fig. 6b, Site types: I, IF, NI, U, DN, NP, PERMANOVA $p = 0.094$) or *N. dombeyi* (Site types: I, IF, NI, U) (PERMANOVA $p = 0.336$). No significant difference was found in the qualitative community composition of total fungal OTUs (PERMANOVA $p = 0.761$) or Nothofagaceae-associated ECM OTUs (PERMANOVA $p = 0.366$) in invasion site types (I, IF, NI, U) of *N. antarctica* (mesic) invaded by *P. menziesii*.

Qualitative community composition of *Mortierella* OTUs in the *N. antarctica* (xeric) rhizosphere did not differ significantly between invasion site types (I, IF, NI, U) (PERMANOVA, $p = 0.327$). However, qualitative community composition of *Mortierella* OTUs in the *N. antarctica* (xeric) rhizosphere differed significantly between the "disturbed ñire" (DN) sites and the invasion sites (I, IF, NI, U) (PERMANOVA = 0.051, Permutation test for homogeneity of multivariate dispersions, $p = 0.99$). When the "ñire in pine plantation" (NP) site type was included in this analysis, we observed even more significant differences in the composition of *Mortierella* OTUs, with different compositional changes observed in DN and NP site types compared with invasion site types (I, IF, NI, U) (Fig. 6c,

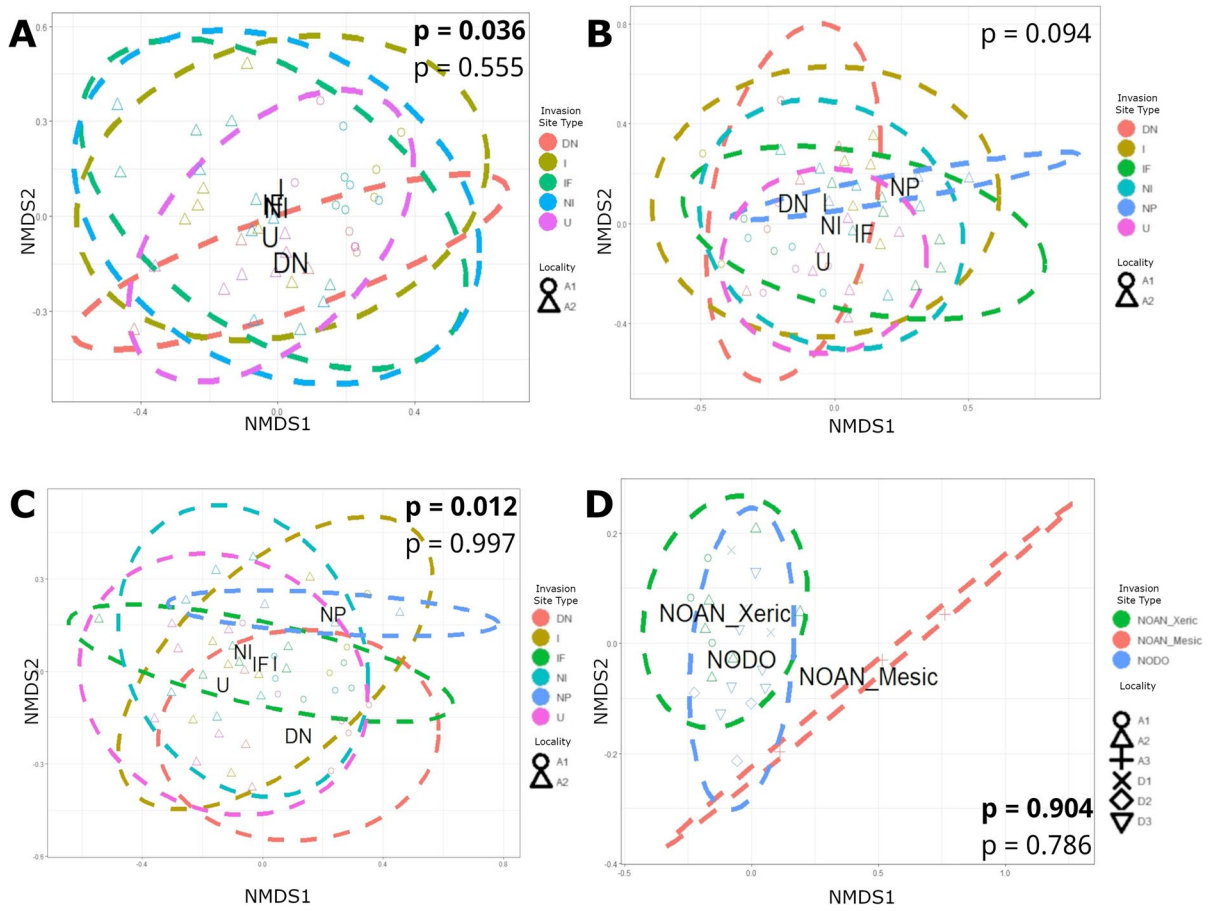

Fig. 6 NMDS ordination plots contrasting the qualitative fungal community composition (presence/absence) of the Nothofagaceae rhizosphere. **A** A comparison of all fungal OTUs in the rhizosphere of *Nothofagus antarctica* (xeric) invaded by *Pinus contorta* (localities A1 & A2) for disturbance control site types (DN) and invasion site types (I, IF, NI, U). **B** A comparison of native Nothofagaceae-associated ECM fungal OTUs in the rhizosphere of *N. antarctica* invaded by *Pi. contorta* (localities A1 &A2) in all *N. antarctica* (xeric) site types. **C** A comparison of all Mortierellaceae OTUs in the rhizosphere of *Nothofagus antarctica* (xeric) invaded by *Pi. contorta* (localities A1, A2). **D** A comparison between the "uninvaded" (U) site type of native Nothofagaceae-associated ECM fungi in the rhizosphere of *Nothofagus dombeyi* (NODO), *N. antarctica* (mesic) (NOAN_mesic), and *N. antarctica* (xeric) (NOAN_xeric). Invasion site type abbreviations: I = "invasion", IF = "invasion front", NI = "near invasion", U = "uninvaded", DN = "disturbed ñire" (*N. antarctica* in disturbed, but uninvaded, sites), and NP = "ñire in pine plantations" (*N. antarctica* in the understory of *Pi. contorta* plantation). Invasion site type and taxon labels in each plot are mapped to the centroid of their multivariate dispersions. P-values are results from PERMANOVA tests and are shown in boldface when representing statistically significant results ($p < = 0.05$). Significant PERMANOVA p-values are followed by the p-values of their corresponding Anderson's test for homogeneity of multivariate dispersion (Anderson 2006)

PERMANOVA, $p = 0.012$, Permutation test for homogeneity of multivariate dispersions, $p = 0.997$). *Mortierella* OTU community composition in the rhizosphere of plantation-grown *P. menziesii* was not significantly different from *N. dombeyi* invasion site types (I, IF, NI, U) (PERMANOVA, $p = 0.129$). The qualitative community composition of *Mortierella* OTUs in the rhizosphere of *N. antarctica* (xeric) and *N. dombeyi* "uninvaded" (U) site types were statistically similar (PERMANOVA, $p = 0.277$), but both of these *Mortierella* OTU communities varied significantly from those colonizing the rhizosphere of the *N. antarctica* (mesic) "uninvaded" (U) site type (PERMANOVA, $p = 0.013$, Permutation test for homogeneity of multivariate dispersions, $p = 0.084$).

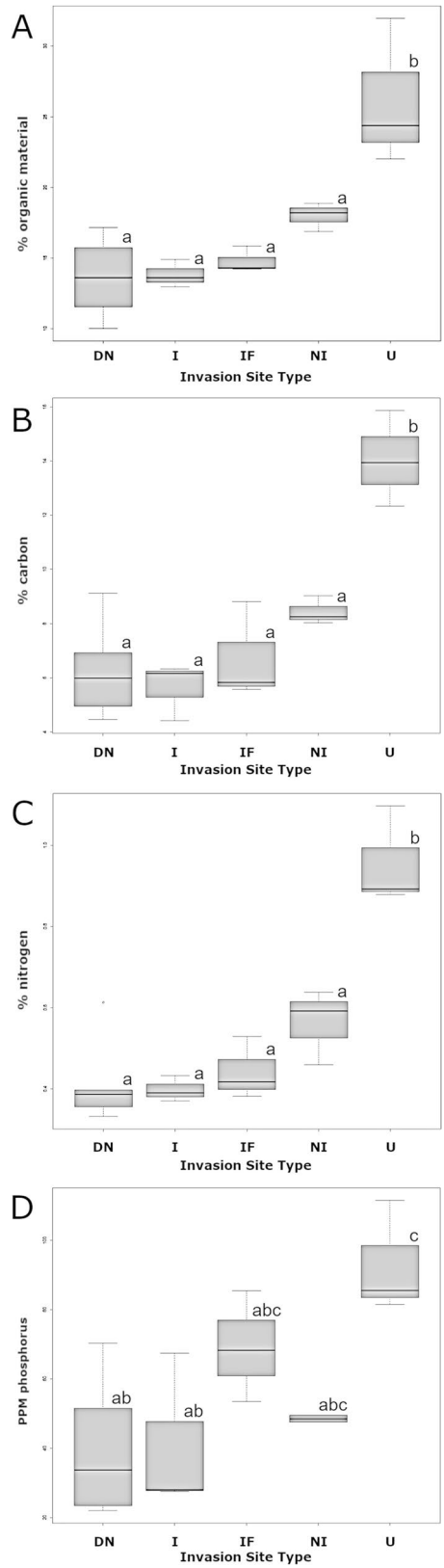

Fig. 7 Boxplots showing **A** organic material, **B** total carbon, **C** total nitrogen, and **D** total phosphorus comparisons of invasion site types in the rhizosphere of *Nothofagus antarctica* (xeric) invaded by *Pinus contorta* in xeric mattoral shrubland habitat (localities A1 and A2). Significantly different site types are indicated by differing lowercase letters (Tukey's Honestly Significant Difference Tests ($p <= 0.05$)). No significant difference between soil parameters were observed for any site types outside of *N. antarctica* (xeric) localities

To determine if the qualitative community composition of ECM OTUs differed between *Nothofagus* species we pooled all of the Nothofagaceae-associated ECM OTUs from "uninvaded" (U) site types by host tree type: *N. dombeyi, N. antarctica* (mesic) (locality A3), and *N. antarctica* (xeric) (localities A1 and A2). NMDS ordination of these pooled qualitative ECM communities revealed significant differences between them (Fig. 6d, PERMANOVA, $p = 0.001$, Permutation test for homogeneity of multivariate dispersions, $p = 0.786$). Significant differences were also observed when comparisons were made strictly between the pooled ECM OTU composition of *N. antarctica* (xeric) and *N. dombeyi* (PERMANOVA $p = 0.002$, Permutation test for homogeneity of multivariate dispersions, $p = 0.663$). The qualitative community composition of Nothofagaceae-associated ECM OTUS was statistically similar between localities of the same host tree type: *N. dombeyi* (localities D1, D2, D3, PERMANOVA, $p = 0.092$) and *N. antarctica* (xeric) (localities A1 and A2, PERMANOVA, $p = 0.296$).

Soil chemistry analyses

Significant differences were observed between invasion site types (I, IF, NI, U) and "disturbed ñire" (DN) site types at sites where *Pi. contorta* was invading *N. antarctica* (xeric) in mattoral shrubland, with significantly greater organic matter (Fig. 7a, ANOVA, $p = 0.0002$), total C (Fig. 7b, ANOVA, $p = 0.00006$), total N (Fig. 7c, ANOVA, $p = 0.00002$), and phosphorus (Fig. 7D, ANOVA, $p = 0.009$) in the "uninvaded" (U) site type compared with site types closer to *Pi. contorta* invaders (I, IF, NI) or in disturbed sites (DN). Phosphorus differed from other soil chemical parameters in that it was only significantly lower in "invasion" (I) and "disturbed ñire" (DN) site types compared to the "uninvaded" (U) site type (Fig. 7D). At sites where *P. menziesii* was invading *N. dombeyi* (localities D1, D2, D3) or *N. antarctica* (mesic)

(locality A3) there were no significant differences in any soil chemical parameter between any of the invasion site types (I, IF, NI, U in Online Resource 5) or Nothofagaceae reference sites with no Pinaceae present ("REF" in Online Resource 5). Similarly, no correlations were detected between soil variables and fungal community structure in NMDS analyses. Results of all soil chemical analyses are shown in Online Resource 5.

Discussion

Effects of Pinaceae Invasions on fungal community structure in the Nothofagaceae Rhizosphere

Our ITS metabarcoding analyses focused on invasion gradients of *P. menziesii* and *Pi. contorta* in native *Nothofagus* forests in SSA (Fig. 1, Table 1). *Pinus contorta* invasions (localities A1 and A2) had significant effects on native Nothofagaceae-associated fungal community composition and these effects were not driven by changes in the community of Nothofagaceae-associated ECM fungi (Fig. 6A & B). These effects were consistent with our prediction of a proportional response to the proximity and cover of Pinaceae trees, with site types closer to *Pi. contorta* invasions (I, IF, NI) clustering in NMDS ordination while site types further from invasion clustered separately (U, DN) (Fig. 6). In accordance with this finding, the relative abundance of invasive Pinaceae-specific ECM fungal OTUs in the *N. antarctica* rhizosphere increased significantly in site types with proximity to invading *Pi. contorta* (I, IF, NI) as compared to "uninvaded" (U) site types (Fig. 3A). Given these results, we hypothesize that invasive Pinaceae-associated ECM fungi displace native soil fungi in the *N. antarctica* rhizosphere.

A striking result of our study is the reduced relative abundance of *Mortierella* sequence reads in response to *Pi. contorta* invasion. We found that the relative abundance of *Mortierella* sequence reads in the rhizosphere of *N. antarctica* (xeric)(localities A1 and A2) was proportional to the distance of the nearest *Pi. contorta* individual (Figs. 4 and 5). This agrees with our predictions and is worth special note given the count number of *Mortierella* reads in our metabarcoding analysis (71% of all reads recovered). Mortierellaceae fungi are a common component of the soil fungal community in SSA Nothofagaceae forests. A metabarcoding study conducted on bulk soil in *N. antarctica* forests near locality A2 found that *Mortierella* species were the most abundant nonECM fungus and accounted for 5–7% of the fungal OTU community in metabarcode analyses (Carron et al. 2020). Another metabarcoding study from *Nothofagus pumilio* forests in southern Patagonia found that Mortierellaceae was the only nonECM taxon that was both speciose and abundant (Truong et al. 2019). High relative abundance of *Mortierella* sequence reads in metabarcode analyses is also common in high latitudes of the northern hemisphere where they can comprise up to 44% of the soil fungal community of ECM forest soil (Phillips et al. 2014) and reach particularly high relative abundances of up to 60% following soil disturbance (Sukdeo et al. 2019).

The relative abundance of Mortierellaceae OTUs in *N. antarctica* (xeric) rhizosphere was high compared with these other studies, with an average relative abundance in the *N. antarctica* rhizosphere of 67% in invasion site types (I, IF, NI, U). It is possible that the high relative abundance of Mortierellaceae OTUs represents a PCR bias for Mortierellaceae taxa because these taxa typically have shorter ITS1 amplicon fragments (< 300 bp) and short fragments are systematically inflated in metabarcode relative abundance (Reynolds et al. 2022). We believe that PCR bias alone is unlikely to have produced such high relative abundance, however, because the Agaricales lineages also possess similarly sized ITS1 amplicon fragments and have been observed with inflated relative abundance in metabarcode analysis (Reynolds et al. 2022). Agaricales fungi are both abundant and speciose in Nothofagaceae forests in SSA (Carron et al. 2020; Nouhra et al. 2013; Truong et al. 2017; Truong et al. 2019, this study) but showed much lower relative abundance than Mortierellaceae OTUs in our samples. While PCR bias may have produced some inflation of relative abundance in Mortierellaceae OTUs, this bias would be expected to produce inflation of other taxa such as Agaricales if it were the only driver of observed relative abundance and this pattern was not observed.

While *Mortierella* species are common in high latitude ECM forests, their ecological role in these forests is poorly understood. *Mortierella* species are considered to be soil saprotrophs but some species are also

common as root endophytes of ECM trees (Bonito et al. 2016). Mortierellaceae are frequently detected by Sanger sequencing of Nothofagaceae ECM root tips in SSA, indicating that these fungi can inhabit roots of Nothofagaceae as endophytes (Nouhra et al. 2013). However, we did not detect any *Mortierella* sequences from pooled samples of cleaned ECM root tips. This suggesting that endophytic Mortierellaceae were either absent from ECM root samples or below the detection limits of metabarcoding methods when common ECM sequence reads occupied the majority of the Illumina flowcell. Metatranscriptomic analyses have demonstrated that rhizosphere interactions with *Mortierella elongata* enhance the growth of the ECM tree *Populus trichocarpa* through upregulation of plant genes associated with lipid signaling, nutrient uptake, and growth, while also facilitating plant-fungal association through downregulation of genes associated with plant immune response (Liao et al. 2019). Similarly, greenhouse studies of the arbuscular mycorrhizal (AM) plant *Arabidopsis thaliana* have shown that colonization by *Mortierella hyalina* as a root endophyte increased aboveground growth and biomass while also suppressing plant immune response (Johnson et al. 2018). *Mortierella spp.* seem capable of benefiting both AM as well as ECM host plants and their molecular interactions with these hosts are similar to those between ECM fungi and their host plants (Plett et al. 2014).

Interactions of soil disturbance, Pinaceae invasions, and their potential influence upon soil nutrient dynamics

Significant reductions of total soil nitrogen, organic matter, and total carbon of *N. antarctica* rhizosphere soil were correlated with disturbed sites as well as *Pi. contorta* invasions of *N. antarctica* in xeric mattoral shrubland (Fig. 7). Soil phosphorous at these localities was significantly reduced in *Pi. contorta* "invasion" (I) and "disturbed ñire" (DN) site types as compared to "uninvaded" (U) site types (Fig. 7D). DN site types possessed the highest relative abundance of Mortierellaceae sequence reads of any *N. antarctica* (xeric) site type (Fig. 4). This is not surprising given that other metabarcode studies have found that *Mortierella* OTUs increase in richness and relative abundance (up to 60% of the community) in ECM forest soils in association with the decomposition of plant and fungal necromass following soil disturbance (Sukdeo et al. 2019). Phosphatase activity of Mortierellaceae fungi is a possible explanation for reduced P in DN site types (Fig. 7D) because Mortierellaceae relative abundance is negatively correlated with inorganic P abundance and positively correlated with high pH, low inorganic P abundance, soil clearing/disturbance, and high phosphatase activity in *Nothofagus* forest soils (Carron et al. 2020; Truong et al. 2019). The decreased total N, total C, and P in the DN site type corroborates previous research and suggest that increased abundance of Mortierellaceae OTUs in these sites may be associated with the reduced nutrient levels observed. We found significant differences in the total fungal OTU community composition between *N. antarctica* (xeric) rhizosphere from DN and *Pi. contorta* invasion site types (I, IF, NI, U) (PERMANOVA, $p = 0.036$) but observed no such differences in ECM fungal OTU community composition between the same site types (I, IF, NI, U, DN) (Fig. 6b). This indicates that effects of disturbance affect the overall fungal community composition, but the community composition of ECM fungal OTUs in the *N. antarctica* (xeric) rhizosphere is not significantly altered by land clearing practices as has previously been observed in this system (Carron et al. 2020). However, such disturbance provides conditions that are highly favorable for *Pi. contorta* invasions (Langdon et al. 2010; Nunez et al. 2017) and we demonstrate here that these may have dramatic effects on saprotrophic soil fungal communities.

The potential role of /suillus-rhizopogon fungi in observed patterns of nutrient dynamics and Mortierellaceae abundance

The dominant ECM taxa that coinvade with *Pi. contorta* are /suillus-rhizopogon lineage fungi (Dickie et al. 2014; Policelli et al. 2019, 2020) and these fungi can facilitate Pinaceae invasions as the sole member of a coinvading ECM community (Ashkannejhad and Horton 2006; Hayward et al. 2015a, b). In accordance with this expectation, /suillus-rhizopogon fungi were the most abundant ECM OTUs in the *N. antarctica* rhizosphere at sites with active *Pi. contorta* invasions (site types I and IF) (Fig. 2A and D). Invasive Pinaceae-associated ECM taxa were undetectable in the "uninvaded" (U) site type and *Rhizopogon* species

persisted as the only Pinaceae-specific ECM taxon present in *Nothofagus* rhizosphere soil as distance from the nearest invasive Pinaceae host increased (Fig. 2D, E, and F). This result conflicts with previous reports that detected high inoculum potential of invasive Pinaceae-specific ECM /suillus-rhizopogon fungi in undisturbed Nothofagaceae forest as much as 600 m from the nearest Pinaceae invasion front (Hayward et al. 2015a, b; Policelli et al. 2020, 2022a). *Rhizopogon* and *Suillus* basidiospores can survive and persist in soil and are able to germinate and colonize ECM hosts even at low spore abundance (Bruns et al. 2009; Nguyen et al. 2012). Our failure to detect either *Suillus* or *Rhizopogon* OTUs in the *Nothofagus* rhizosphere of the undisturbed (U) site type indicates that these fungi might effectively colonize hosts even when their abundance is below the detection limit of ITS metabarcoding methods or that basidiospores of these fungi were not effectively lysed by our DNA extraction method.

Competition between native Mortierellaceae fungi and exotic /suillus-rhizopogon ECM lineage fungi to decompose organic matter may drive the pattern of reduced Mortierellaceae read counts seen in *Pi. contorta* invasions. Ectomycorrhizal fungi mobilize soil nutrients for their plant hosts and this is well documented for nitrogen, carbon, and phosphorus (Brundrett and Tedersoo 2018). Fungi in the /suillus-rhizopogon lineage reach high abundance in nutrient poor systems by secreting enzymes that degrade cellulose and nitrogen-rich compounds, thereby allowing them to access otherwise inaccessible nutrients (Ning et al. 2021). This nutrient scavenging behavior in *Suillus luteus* can cause up to 30% soil carbon loss in *Pinus* plantations where they subsidize host-provided C with soil C to produce large numbers of sporocarps (Chapela et al. 2001). /Suillus-rhizopogon lineage fungi are capable of enzymatic digestion and liberation of both inorganic (Durall et al. 1994) and organic (Abuzinadah et al. 1986) soil N pools. The Kjeldahl nitrogen (total N) extraction used in our study assays the total inorganic ammonium as well as organic N pools present in the soil (Bremner 1996) and this measurement was reduced in the *N. antarctica* rhizosphere near *Pi. contorta* invasions (Fig. 7). In contrast, available inorganic nitrate and ammonium assays revealed no significant reductions associated with *Pi. contorta* invasions. Our data suggest that observed total N reduction was due to digestion of the organic soil N fraction by /suillus-rhizopogon fungi common in *Pi. contorta* invasions (Fig. 2).

This total N reduction of the *N. antarctica* rhizosphere is striking because *N. antarctica* significantly increases nitrogen content of soil in xeric mattoral habitats (Carron et al. 2020) where the available N:P ratio is positively correlated with plant species richness (Blanck et al. 2011). Nitrogen is also a limiting factor in the production of carbohydrate-degrading enzymes by fungi and an alteration of the soil nitrogen economy by ECM fungi accessing organically bound N can reduce saprotrophic fungal species abundance and subsequently affect plant community structure (Corrales et al. 2016). Digestion of soil organic matter and organic N by /suillus-rhizopogon lineage fungi in *Pi. contorta* invasions may allow these fungi to monopolize soil C, N, and P and subsequently reduce the relative abundance of saprotrophic Mortierellaceae fungi. The effects of *Pi. contorta* invasion upon fungal community structure and biogeochemical cycling could have significant implications upon management and conservation of the xeric mattoral ecosystem. Such detrimental legacy effects of biogeochemical alterations by *Pi. contorta* invasion are already known from non-ECM grassland and shrubland habitats (Chapela et al. 2001; Dickie et al. 2014).

It should also be considered that metabarcode data is compositional in nature and cannot be fully representative of true species abundances in samples due to PCR bias and physical limitations of next-generation sequencing technologies (Gloor et al. 2017). For example, the increased relative abundance of Pinaceae-associated ECM OTUs and decreased relative abundance of Mortierellaceae OTUs in proximity to *Pi. contorta* may, in fact, represent no real change in the actual relative abundance of Mortierellaceae species. Rather, it may be that Pinaceae-associated ECM OTUs became more abundant due to proximity with their obligate host and Mortierellaceae OTUs remained in the same actual abundances, only appearing to be less abundant in metabarcode data due to competition for physical space on the Illumina sequencing flow cell. However, we do not believe that Mortierellaceae species would be unaffected by invasion of Pinaceae-associated ECM taxa. There is ample evidence that both Mortierellaceae and the Pinaceae-associated /suillus-rhizopogon ECM lineage access nutrients locked in soil organic matter and this

ability grants an ecological advantage that both taxa leverage to reach high abundance (Abuzinadah et al. 1986; Chapela et al. 2001; Ning et al. 2021; Sukdeo et al. 2019; Truong et al. 2019). Given this evidence, we hypothesize that reductions in Mortierellaceae relative abundance are due to direct competition with invasive /suillus-rhizpogon ECM lineage fungi for nutrients bound in soil organic matter.

Disparate effects of invasion between *Pseudotsuga menziesii* and *Pinus contorta*

In contrast to the results seen in *Pi. contorta* invasions, we found no effect upon the total fungal or ECM fungal community structures in the *N. dombeyi* (localities D1, D2, and D3) or *N. antarctica* (mesic, locality A3) rhizospheres where *P. menziesii* was invading (Fig. 6C and D). Thus, our predictions are not supported for invasions of *P. menziesii.* Similarly, we found no significant differences in the soil chemical parameters at sites invaded by *P. menziesii*. However, we did find that the percent of invading Pinaceae-specific ECM fungi in the rhizosphere soil of *N. dombeyi* was significantly increased by proximity to *P. menziesii* individuals (Fig. 2C). The relative abundance of invasive Pinaceae-specific taxa in the *N. antarctica* (xeric) rhizosphere was much greater than observed for the *N. dombeyi* rhizosphere (Fig. 2A and C). This might indicate that *Pi. contorta*–associated ECM taxa are more successful competitors of Nothofagaceae-associated ECM or that they disperse more efficiently than *P. menziesii*–associated ECM taxa. This may also be an effect of the xeric habitat inhabited by *N. antarctica* as plants growing in xeric environments have a greater dependence upon their ECM fungal partners than plants growing in mesic habitats (Gehring et al. 2017). A greater dependence of *Pi. contorta* on ECM fungi for growth might also be reflected in the greater richness of OTUS identified as Pinaceae-specific ECM fungi in its rhizosphere soil (74 OTUs in *Pi. contorta* vs 24 OTUs in *P. menziesii*).

Nothofagaceae-associated ECM fungal community composition

We detected significant differences between *Nothofagus* rhizosphere ECM fungal community composition between the three distinct habitat types sampled in this study: *N. antarctica* in xeric mattoral shrubland, *N. antarctica* in mesic wetland edge, and *N. dombeyi* in mesic forest with well-drained soils (Fig. 6D, Table 1). This finding conflicts with the surveys of Nouhra et al. (2012, 2013) that found no significant difference between the ECM communities of the SSA Nothofagaceae hosts *N. dombeyi, Lophozonia alpina,* and *Lophozonia obliqua*, albeit at a much lower sample size derived from Sanger Sequencing of ECM roots. These three trees often occur in the same mesic forest habitat type (Gut 2008) and this shared habitat could explain the similarities in ECM community composition observed by Nouhra et al. (2012, 2013). Hydrologic gradients have significant effects upon ECM fungal community structure (Erlandson et al. 2016) and the variation in water regimes amongst habitat types is a likely explanation for the observed differences in *Nothofagus* ECM community composition. We rule out local geographic effects as drivers of significant variation in ECM communities of *N. antarctica* and *N. dombeyi* because we observed no significant differences in the composition of ECM communities between geographically distant localities of the same habitat type.

The fungal community assemblage of Nothofagaceae-associated fungi we observed had high abundance and richness of OTUs in the /clavulina, /cortinarius, /inocybe, /porpoloma, /tomentella-thelephora, and /tricholoma ECM lineages in both rhizosphere soil and *Nothofagus* ECM root samples and this agrees with previous surveys (Carron et al. 2020; Nouhra et al. 2012, 2013; Truong et al. 2017, 2019). We also found high richness of taxa in the /sebacina ECM lineage, particularly in mesic *N. dombeyi* forests (Fig. 3). These results corroborate previous observations that ECM communities of SSA Nothofagaceae possess relatively few taxa from relatively few ECM lineages compared with ECM communities from other continents (Kuhar et al. 2017; Tedersoo et al. 2012; Truong et al. 2017). The low diversity of naturally occurring ECM hosts in SSA is a likely factor contributing to this reduced ECM fungal diversity.

Conclusions

We found that *Pseudotsuga menziesii* invasions of both *Nothofagus antarctica* and *Nothofagus dombeyi* did not affect the community composition of native fungi colonizing the Nothofagaceae

rhizosphere. However, *Pinus contorta* caused significant change in the community composition and relative abundance of Mortierellaceae OTUs, the overall community composition of all fungal OTUs, and reduced levels of soil P, N, C, and organic matter in the rhizosphere of *N. antarctica* in xeric mattoral shrublands. These observations indicate that co-invasion of /suillus-rhizopogon ECM lineage fungi with *Pi. contorta* is correlated with reductions to *Mortierella* OTU relative abundance and that this effect may be due to nutrient competition between /suillus-rhizopogon fungi and *Mortierella* species. The ecological interactions of *Mortierella* species with Nothofagaceae species is unclear, but the findings of our study and others indicate that they are an important component of the Nothofagaceae rhizosphere microbiome (Carron et al. 2020; Nouhra et al. 2013; Truong et al. 2019). We expect that study of *Mortierella* species from SSA Nothofagaceae forests will increase understanding of their ecological roles in the ECM rhizosphere and provide insight into the impacts of ECM plant invasions globally.

Acknowledgements The authors would like to thank Viviana Pangare and Jorge Greco of La Reserva El Foyel for their kind assistance and for permitting research access to the reserve. We would also like to thank Parques Nacionales de Argentina for issuing research and collection permits (projects 720, 1444, and 1543) and Dr. Eduardo Nouhra for his assistance in coordinating the permit for project 720.

Author contributions The Research was designed by Alija Bajro Mujic, Martin Nuñez, and Matthew Smith. Field locality selection and field collection was performed by Alija Bajro Mujic and Nahuel Policelli. All analyses were performed by Alija Bajro Mujic and Camille Truong. The manuscript was written by Alija Bajro Mujic with significant contributions from all co-authors.

Funding This work was supported by National Science Foundation grant DEB 1354802 (M.E.S.) and an Advanced Postdoc Mobility Fellowship (P300P3_158523) from the Swiss National Science Foundation (C.T.).

Data availability Genetic data: Raw sequence reads presented in this manuscript are deposited in the GenBank Short Read Archive (SRA) (BioProject number: PRJNA916756).

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

References

Abarenkov K, Tedersoo L, Nilsson RH, Vellak K, Saar I, Veldre V, … Kõljalg U (2010) PlutoF—a Web Based Workbench for Ecological and Taxonomic Research, with an Online Implementation for Fungal ITS Sequences. Evol Bioinform Online 6:189–196. https://doi.org/10.4137/EBO.S6271

Abuzinadah RA, Finlay RD, Read DJ (1986) The role of proteins in the nitrogen nutrition of ectomycorrhizal plants. New Phytologist 103(3):495–506. https://doi.org/10.1111/j.1469-8137.1986.tb02887.x

Anderson MJ (2006) Distance-Based Tests for Homogeneity of Multivariate Dispersions. Biometrics 62(1):245–253

Ashkannejhad S, Horton TR (2006) Ectomycorrhizal ecology under primary succession on coastal sand dunes: interactions involving *Pinus contorta*, suilloid fungi and deer. New Phytol 169:345–354

Bahram M, Kõljalg U, Kohout P, Mirshahvaladi S, Tedersoo T (2013) Ectomycorrhizal fungi of exotic pine plantations in relation to native host trees in Iran: evidence of host range expansion by local symbionts to distantly related host taxa. Mycorrhiza 23(1):11–19. https://doi.org/10.1007/s00572-012-0445-z

Baohanta R, Thioulouse J, Ramanankierana H, Prin Y, Rasolomampianina R, Baudoin E, Rakotoarimanga N et al (2012) Restoring native forest ecosystems after exotic tree plantation in Madagascar: combination of the local ectotrophic species *Leptolena bojeriana* and *Uapaca bojeri* mitigates the negative influence of the exotic species *Eucalyptus camaldulensis* and *Pinus patula*. Biol Invasions 14(11):2407–2421. https://doi.org/10.1007/s10530-012-0238-5

Blanck YL, Gowda J, Mårtensson LM, Sandberg J, Fransson AM (2011) Plant species richness in a natural Argentinian matorral shrub-land correlates negatively with levels of plant phosphorus. Plant Soil 345(1–2):11–21. https://doi.org/10.1007/s11104-010-0671-0

Bonito G, Hameed K, Ventura R, Krishnan J, Schadt CW, Vilgalys R (2016) Isolating a functionally relevant guild of fungi from the root microbiome of *Populus*. Fungal Ecol 22:35–42. https://doi.org/10.1016/j.funeco.2016.04.007

Bremner JM (1965) Inorganic forms of nitrogen. In: Black CA, et al. Eds. Methods of soil analysis, Part 2. Chemical and Microbiological Properties. Agronomy Monograph No. 9, ASA and SSSA, Madison, 1179–1237

Bremner JM (1996) Nitrogen—Total. In: Sparks, D.L., et al. Eds. Methods of Soil Analysis. Part 3. Chemical Methods. SSSA Book Ser. 5. SSSA and ASA, Madison, WI. p. 1085–1089

Brundrett MC, Tedersoo L (2018) Evolutionary history of mycorrhizal symbioses and global host plant diversity. New Phytol 220(4):1108–1115. https://doi.org/10.1111/nph.14976

Bruns TD, Peay KG, Boynton PJ, Grubisha LC, Hynson NA, Nguyen NH, Rosenstock NP (2009) Inoculum potential of Rhizopogon spores increases with time over the first 4 yr of a 99-yr spore burial experiment. New Phytol 181(2):463–470. https://doi.org/10.1111/j.1469-8137.2008.02652.x

Budde KB, Gallo L, Marchelli P, Mosner E, Liepelt S, Ziegenhagen B, Leyer I (2011) Wide spread invasion without sexual reproduction? A case study on European willows in Patagonia, Argentina. Biol Invasions 13(1):45–54. https://doi.org/10.1007/s10530-010-9785-9

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, … Knight R (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7(5):335–336. https://doi.org/10.1038/nmeth.f.303

Carron AI, Garibaldi LA, Marquez S, Fontenla S (2020) The soil fungal community of native woodland in Andean Patagonian forest: A case study considering experimental forest management and seasonal effects. For Ecol Manag 461:117955. https://doi.org/10.1016/j.foreco.2020.117955

Chapela IH, Osher LJ, Horton TR, Henn MR (2001) Ectomycorrhizal fungi introduced with exotic pine plantations induce soil carbon depletion. Soil Biol Biochem 33(12):1733–1740. https://doi.org/10.1016/S0038-0717(01)00098-0

Corrales A, Mangan SA, Turner BL, Dalling JW, Chave J (2016) An ectomycorrhizal nitrogen economy facilitates monodominance in a neotropical forest. Ecol Lett 19(4):383–392. https://doi.org/10.1111/ele.12570

Dehlin H, Peltzer DA, Allison VJ, Yeates GW, Nilsson MC, Wardle DA (2008) Tree Seedling Performance and below-ground properties in stands of invasive and native tree species. N Z J Ecol 32(1):67–79

Dickie IA, Bolstridge N, Cooper JA, Peltzer DA (2010) Co-invasion by *Pinus* and its mycorrhizal fungi. New Phytol 187(2):475–484. https://doi.org/10.1111/j.1469-8137.2010.03277.x

Dickie IA, St John MG, Yeates GW, Morse CW, Bonner KI, Orwin K, Peltzer DA (2014) Belowground legacies of *Pinus contorta* invasion and removal result in multiple mechanisms of invasional meltdown. AoB Plants 6(1):plu056. https://doi.org/10.1093/aobpla/plu05

Dickie IA, Bufford JL, Cobb RC, Desprez-Loustau M-L, Grelet G, Hulme PE, Klironomos J, Makiola A, Nuñez MA, Pringle A, Thrall PH, Tourtellot SG, Waller L, Williams NM (2017) The emerging science of linked plant-fungal invasions. New Phytol 215(4):1314–1332. https://doi.org/10.1111/nph.14657

Durall DM, Todd AW, Trappe JM (1994) Decomposition of 14C-labelled substrates by ectomycorrhizal fungi in association with Douglas fir. New Phytologist 127(4):725–729. https://doi.org/10.1111/j.1469-8137.1994.tb02976.x

Erlandson SR, Savage JA, Cavender-Bares JM, Peay KG (2016) Soil moisture and chemistry influence diversity of ectomycorrhizal fungal communities associating with willow along an hydrologic gradient. FEMS Microbiol Ecol 92(1). https://doi.org/10.1093/femsec/fiv148

Farjon A (2010) A Handbook of the World's Conifers. BRILL, Leiden, Netherlands

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes application to the identification of mycorrhizae and rusts. Mol Ecol 2(2):113–118

Gandolfo MA, Hermsen EJ, Zamaloa MC, Nixon KC, González CC, Wilf P, Cúneo NR, Johnson KR (2011) Oldest known *Eucalyptus* macrofossils are from South America. PLoS One 6(6). https://doi.org/10.1371/journal.pone.0021084

García RA, Franzese J, Policelli N, Sasal Y, Zenni RD, Nuñez MA, Taylor K, Pauchard A (2018) Non-native Pines Are Homogenizing the Ecosystems of South America. In Rozzi R, May Jr RH, Chapin III FS, Massardo F, Gavin MC, Klaver IJ, Pauchard A, Nuñez MA, Simberloff D (Eds.), From Biocultural Homogenization to Biocultural Conservation (pp. 245–263). Springer International Publishing. https://doi.org/10.1007/978-3-319-99513-7_15

Gehring CA, Swaty RL, Deckert RJ (2017) Chapter 16—Mycorrhizas, Drought, and Host-Plant Mortality. In Johnson NC et al. (Eds.) Mycorrhizal Mediation of Soil pp. 279–298. Elsevier. Amsterdam, Netherlands. https://doi.org/10.1016/B978-0-12-804312-7.00016-4

Gloor GB, Macklaim JM, Pawlowsky-Glahn V, Egozcue JJ (2017) Microbiome datasets are compositional: and this is not optional. Front Microbiol 8:2224. https://doi.org/10.3389/fmicb.2017.02224

Grove S, Haubensak KA, Gehring C, Parker IM (2017) Mycorrhizae, invasions, and the temporal dynamics of mutualism disruption. J Ecol 105(6):1496–1508. https://doi.org/10.1111/1365-2745.12853

Gut B (2008) Trees in Patagonia. Birkhäuser Verlag AG, Basel, Switzerland

Hayward J, Horton TR, Nuñez MA (2015a) Ectomycorrhizal fungal communities coinvading with Pinaceae host plants in Argentina: Gringos bajo el bosque. New Phytol 208(2):497–506. https://doi.org/10.1111/nph.13453

Hayward J, Horton TR, Pauchard A, Nuñez MA (2015b) A single ectomycorrhizal fungal species can enable a *Pinus* invasion. Ecology 96(5):1438–1444. https://doi.org/10.1890/14-1100.1

Heenan PB, Smissen RD (2013) Revised circumscription of Nothofagus and recognition of the segregate genera Fuscospora, Lophozonia, and Trisyngyne (Nothofagaceae). Phytotaxa 146(1):1–31. https://doi.org/10.11646/phytotaxa.146.1.1

Hill RS (2001) Biogeography, evolution and palaeoecology of Nothofagus (Nothofagaceae): the contribution of the fossil record. Aust J Bot 49:321–332

Hill RS, Beer YK, Hill KE, Maciunas E, Tarran MA, Wainman CC (2017) Evolution of the eucalypts – an interpretation from the macrofossil record. Aust J Bot 64(8):600–608. https://doi.org/10.1071/BT16117

Hermsen EJ, Gandolfo MA, Zamaloa MD (2012) The fossil record of *Eucalyptus* in patagonia. Am J Bot 99(8):1356–1374. https://doi.org/10.3732/ajb.1200025

Hoeksema JD, Averill C, Bhatnagar JM, Brzostek E, Buscardo E, Chen K-H, Liao H-L, Nagy L, Policelli N, Ridgeway J, Rojas JA, Vilgalys R (2020) Ectomycorrhizal Plant-Fungal Co-invasions as Natural Experiments for Connecting Plant and Fungal Traits to Their Ecosystem Consequences. Front For Glob Change 3:84. https://doi.org/10.3389/ffgc.2020.00084

Horak E (1983) Mycogeography in the south Pacific region: Agaricales, Boletales. Aust J Bot Suppl Ser 10:1–41

Johnson JM, Ludwig A, Furch ACU, Mithöfer A, Scholz S, Reichelt M, Oelmüller R (2018) The Beneficial Root-Colonizing Fungus *Mortierella hyalina* Promotes the Aerial Growth of *Arabidopsis* and Activates Calcium-Dependent

Responses That Restrict *Alternaria brassicae*–Induced Disease Development in Roots. Mol Plant Microbe Interact 32(3):351–363. https://doi.org/10.1094/MPMI-05-18-0115-R

Kõljalg U, Larsson K-H, Abarenkov K, Nilsson RH, Alexander IJ, Eberhardt U, Ursing BM (2005) UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. New Phytol 166(3):1063–1068. https://doi.org/10.1111/j.1469-8137.2005.01376.x

Kuhar F, Smith ME, Mujic A, Truong C, Nouhra E (2017) A systematic overview of *Descolea* (Agaricales) in the Nothofagaceae forests of Patagonia. Fungal Biol 121(10):876–889. https://doi.org/10.1016/j.funbio.2017.06.006

Kuo S (1996) Phosphorus. In: Sparks DL, et al. (Eds) Methods of Soil Analysis. Part 3. Chemical Methods. SSSA Book Ser. 5. SSSA and ASA, Madison, WI 869–920

Langdon B, Pauchard A, Aguayo M (2010) *Pinus contorta* invasion in the Chilean Patagonia: Local patterns in a global context. Biol Invasions 12(12):3961–3971. https://doi.org/10.1007/s10530-010-9817-5

Liao H-L, Bonito G, Rojas JA, Hameed K, Wu S, Schadt CW, Labbé J, Tuskan GA, Martin F, Grigoriev IV, Vilgalys R (2019) Fungal Endophytes of *Populus trichocarpa* Alter Host Phenotype, Gene Expression, and Rhizobiome Composition. Mol Plant-Microbe Interact 32(7). https://doi.org/10.1094/MPMI-05-18-0133-R

Marchelli P, Pastorino MJ, Gallo LA (2021) Temperate subantarctic forests: A huge natural laboratory. In: Marchelli P, Pastorino M, Gallo L (eds) Low intensity breeding of native forest trees in Argentina. Springer, Cham, pp 27–54

McMurdie PJ, Holmes S (2013) phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLoS One 8(4):e61217. https://doi.org/10.1371/journal.pone.0061217

Nguyen NH, Hynson NA, Bruns TD (2012) Stayin' alive: Survival of mycorrhizal fungal propagules from 6-yr-old forest soil. Fungal Ecol 5(6):741–746. https://doi.org/10.1016/j.funeco.2012.05.006

Nguyen NH, Smith D, Peay K, Kennedy P (2015) Parsing ecological signal from noise in next generation amplicon sequencing. New Phytol 205(4):1389–1393. https://doi.org/10.1111/nph.12923

Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, Schilling JS, Kennedy PG (2016) FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecol 20:241–248. https://doi.org/10.1016/j.funeco.2015.06.006

Ning C, Egerton-Warburton LM, Mueller GM, Xiang W, Yan W, Liu S (2021) Shifts in ectomycorrhizal fungal community composition during the early establishment of native and exotic pine seedlings. Appl Soil Ecol 157:103722. https://doi.org/10.1016/j.apsoil.2020.103722

Nouhra ER, Urcelay C, Longo MS, Fontenla S (2012) Differential hypogeous sporocarp production from *Nothofagus dombeyi* and *N. pumilio* forests in southern Argentina. Mycologia 104(1):45–52. https://doi.org/10.3852/11-098

Nouhra E, Urcelay C, Longo S, Tedersoo L (2013) Ectomycorrhizal fungal communities associated to *Nothofagus* species in Northern Patagonia. Mycorrhiza 23(6):487–496. https://doi.org/10.1007/s00572-013-0490-2

Nuñez MA, Hayward J, Horton TR, Amico GC, Dimarco RD, Barrios-Garcia MN, Simberloff D (2013) Exotic Mammals Disperse Exotic Fungi That Promote Invasion by Exotic Trees. PLoS One 8(6):e66832. https://doi.org/10.1371/journal.pone.0066832

Nuñez MA, Chiuffo MC, Torres A, Paul T, Dimarco RD, Raal P, Policelli N, Moyano J, García RA, van Wilgen BW, Pauchard A, Richardson DM (2017) Ecology and management of invasive Pinaceae around the world: progress and challenges. Biol Invasions 19(11):3099–3120. https://doi.org/10.1007/s10530-017-1483-4

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H (2018) vegan: Community Ecology Package. R package version 2.4–6. Retrieved November 11, 2022, from https://CRAN.R-project.org/package=vegan

Phillips LA, Ward V, Jones MD (2014) Ectomycorrhizal fungi contribute to soil organic matter cycling in sub-boreal forests. ISME J 8(3):699–713. https://doi.org/10.1038/ismej.2013.195

Plett JM, Daguerre Y, Wittulsky S, Vayssières A, Deveau A, Melton SJ, Kohler A, Morrell-Falvey JL, Brun A, Veneault-Fourrey C, Martin F (2014) Effector MiSSP7 of the mutualistic fungus Laccaria bicolor stabilizes the Populus JAZ6 protein and represses jasmonic acid (JA) responsive genes. Proc Nat Acad Sci 111(22):8299–304. https://doi.org/10.1073/pnas.1322671111

Policelli N, Bruns TD, Vilgalys R, Nuñez MA (2019) Suilloid fungi as global drivers of pine invasions. New Phytol 222(2):714–725. https://doi.org/10.1111/nph.15660

Policelli N, Horton TR, García RA, Naour M, Pauchard A, Nuñez MA (2020) Native and non-native trees can find compatible mycorrhizal partners in each other's dominated areas. Plant Soil 454(1):285–297. https://doi.org/10.1007/s11104-020-04609-x

Policelli N, Horton TR, Kitzberger T, Nuñez MA (2022a) Invasive ectomycorrhizal fungi can disperse in the absence of their known vectors. Fungal Ecol 55:101124. https://doi.org/10.1016/j.funeco.2021.101124

Policelli N, Vietorisz C, Bhatnagar JM, Nuñez MA (2022b) Ectomycorrhizal fungi invasions in Southern South America. In: Lugo MA, Pagano MC (eds) Mycorrhizal fungi in South America: biodiversity, conservation, and sustainable food production. Springer International Publishing, Cham, pp 25–46. https://doi.org/10.1007/978-3-031-12994-0_2

Policelli N, Hoeksema JD, Moyano J, Vilgalys R, Vivelo S, Bhatnagar JM (2022c) Global pine tree invasions are linked to invasive root symbionts. New Phytol 237(1):16–21. https://doi.org/10.1111/nph.18527

Reynolds NK, Jusino MA, Stajich JE, Smith ME (2022) Understudied, underrepresented, and unknown: Methodological biases that limit detection of early diverging fungi from environmental samples. Mol Ecol Resour 22(3):1065–1085. https://doi.org/10.1111/1755-0998.13540

Salomón MES, Barroetaveña C, Pildain MB, Williams EA, Rajchenberg M (2018) What happens to the mycorrhizal communities of native and exotic seedlings when *Pseudotsuga menziesii* invades Nothofagaceae forests in

Patagonia, Argentina? Acta Oecol 91:108–119. https://doi.org/10.1016/j.actao.2018.07.003

Simberloff D, Relva MA, Nunez M (2003) Introduced species and management of a *Nothofagus/Austrocedrus* forest. Environ Manag 31(2):263–275. https://doi.org/10.1007/s00267-002-2794-4

Skrede I, Engh IB, Binder M, Carlsen T, Kauserud H, Bendiksby M (2011) Evolutionary history of Serpulaceae (Basidiomycota): molecular phylogeny, historical biogeography and evidence for a single transition of nutritional mode. BMC Evol Biol 11(1):1–13

Sukdeo N, Teen E, Rutherford PM, Massicotte HB, Egger KN (2019) Bacterial and fungal saprotrophs are strongly stimulated weeks to months after forest soil profile reconstruction. Pedobiologia 73:29–41. https://doi.org/10.1016/j.pedobi.2019.01.001

Thomas LK, Leyer I (2014) Age structure, growth performance and composition of native and invasive Salicaceae in Patagonia. Plant Ecol 215(9):1047–1056. https://doi.org/10.1007/s11258-014-0362-7

Thomas LK, Mosner E, Leyer I (2015) River dynamics and invasion: Distribution patterns of native and invasive woody vegetation at the Río Negro, Argentina. Riparian Ecol Conserv 2(1):45–57. https://doi.org/10.1515/remc-2015-0001

Tedersoo L, May TW, Smith ME (2010) Ectomycorrhizal lifestyle in fungi: Global diversity, distribution, and evolution of phylogenetic lineages. Mycorrhiza 20(4):217–263. https://doi.org/10.1007/s00572-009-0274-x

Tedersoo L, Bahram M, Toots M, Diédhiou AG, Henkel TW, Kjøller R, Kõljalg U (2012) Towards global patterns in the diversity and community structure of ectomycorrhizal fungi. Mol Ecol 21(17):4160–4170. https://doi.org/10.1111/j.1365-294X.2012.05602.x

Tedersoo L, Smith ME (2013) Lineages of ectomycorrhizal fungi revisited: Foraging strategies and novel lineages revealed by sequences from belowground. Fungal Biol Rev 27(3):83–99. https://doi.org/10.1016/j.fbr.2013.09.001

Truong C, Mujic AB, Healy R, Kuhar F, Furci G, Torres D, Niskanen T, Sandoval-Leiva PA, Fernández N, Escobar JM, Moretto A, Palfner G, Pfister D, Nouhra E, Swenie R, Sánchez-García M, Matheny PB, Smith ME (2017) How to know the fungi: combining field inventories and DNA-barcoding to document fungal diversity. New Phytologist 214(3):913–919. https://doi.org/10.1111/nph.14509

Truong C, Gabbarini LA, Corrales A, Mujic AB, Escobar JM, Moretto A, Smith ME (2019) Ectomycorrhizal fungi and soil enzymes exhibit contrasting patterns along elevation gradients in southern Patagonia. New Phytologist 222(4):1936–1950. https://doi.org/10.1111/nph.15714

Vu D, Groenewald M, de Vries M, Gehrmann T, Stielow B, Eberhardt U, Al-Hatmi A, Groenewald JZ, Cardinali G, Houbraken J, Boekhout T, Crous PW, Robert V, Verkley GJM (2019) Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom fungi and reveals thresholds for fungal species and higher taxon delimitation. Stud Mycol 92:135–154. https://doi.org/10.1016/j.simyco.2018.05.001

White TJ, Bruns TD, Lee SB, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA et al (eds) PCR protocols: a guide to methods and applications. Academic Press, United States, pp 315–322

Wickham H (2016) ggplot2: elegant graphics for data analysis. Springer-Verlag, New York