Research

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Ectomycorrhizal fungi and soil enzymes exhibit contrasting patterns along elevation gradients in southern Patagonia

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Summary

- The biological and functional diversity of ectomycorrhizal (ECM) associations remain largely unknown in South America. In Patagonia, the ECM tree *Nothofagus pumilio* forms monospecific forests along mountain slopes without confounding effects of vegetation on plant–fungi interactions.
- To determine how fungal diversity and function are linked to elevation, we characterized fungal communities, edaphic variables, and eight extracellular enzyme activities along six elevation transects in Tierra del Fuego (Argentina and Chile). We also tested whether pairing ITS1 rDNA Illumina sequences generated taxonomic biases related to sequence length.
- Fungal community shifts across elevations were mediated primarily by soil pH with the most species-rich fungal families occurring mostly within a narrow pH range. By contrast, enzyme activities were minimally influenced by elevation but correlated with soil factors, especially total soil carbon. The activity of leucine aminopeptidase was positively correlated with ECM fungal richness and abundance, and acid phosphatase was correlated with nonECM fungal abundance. Several fungal lineages were undetected when using exclusively paired or unpaired forward ITS1 sequences, and these taxonomic biases need reconsideration for future studies.
- Our results suggest that soil fungi in *N. pumilio* forests are functionally similar across elevations and that these diverse communities help to maintain nutrient mobilization across the elevation gradient.

Introduction

The role of biodiversity in ecosystem functioning has been extensively studied in plants and animals, but similar questions have only recently been addressed for soil microbes (Fierer *et al.*, 2012; Wagg *et al.*, 2014). Ectomycorrhizal (ECM) fungi play critical roles in soil organic matter (SOM) recycling and plant nutrition by mobilizing essential nutrients to their hosts in exchange for a carbon (C) source (Smith & Read, 2008). Although ECM fungi mostly acquire C from their hosts, they contribute to SOM recycling by scavenging for nutrients, particularly organic nitrogen (N) that is trapped in SOM (Lindahl & Tunlid, 2015; Shah *et al.*, 2016). Taxonomically and functionally diverse ECM fungal communities associate with the root systems of individual trees and constitute a large proportion of the soil biota in temperate and boreal forests (Tedersoo *et al.*, 2014). Biotic and abiotic factors that affect the structure of these communities include host-plant diversity (Tedersoo *et al.*, 2013; Bonito *et al.*, 2014), competition (Kennedy *et al.*, 2011), edaphic variables (Peay *et al.*, 2015; Erlandson *et al.*, 2016) and climate (Talbot *et al.*, 2014; Miyamoto *et al.*, 2015). Changing environmental conditions can alter the balance between C allocation to ECM fungi and N and phosphorus (P) acquisition by ECM plants, thereby influencing global C storage and N/P cycles in forest ecosystems (Baskaran *et al.*, 2017; Corrales *et al.*, 2017). Ectomycorrhizal fungal communities can shift with the nutrient balance in soils while they compete with each other for nutrients in response to host demand for N and P (Franklin *et al.*, 2014). A reduction in ECM fungal species diversity may therefore impact plant nutrition and forest resilience in a changing environment (Sapsford *et al.*, 2017).

Microorganisms secrete extracellular enzymes to acquire C and nutrients from SOM, and, in the case of ECM fungi, to facilitate the nutrition of their host (Sinsabaugh *et al.*, 2008). Extracellular

enzyme activities (EEAs) can be used as traits to study the functional diversity of ECM fungal communities *in situ* and assess their impact on ecosystem functions (Courty *et al.*, 2010; Pritsch & Garbaye, 2011). Although some species are functionally redundant in enzyme production, ECM fungi generally exhibit functional complementarity in how they benefit their hosts (Jones *et al.*, 2010; Tedersoo *et al.*, 2012b). ECM fungal species differ in their enzymatic capacity, which may in turn vary according to environmental conditions such as edaphic variables, resource availability, climate or seasonality (Talbot *et al.*, 2014; Corrales *et al.*, 2017). Environmental variation (such as that occurring along an elevation gradient) might promote species that are better adapted to the local conditions but might also be functionally distinct. This, in turn, could alter EEAs, SOM cycling and, ultimately, plant nutrition (Talbot *et al.*, 2013; Schnecker *et al.*, 2014). The response of EEAs to elevation has seldom been studied (Nottingham *et al.*, 2016) and has never been linked to ECM fungal communities along an elevation gradient.

Elevation gradients have captured the interest of biologists for centuries (von Humboldt & Bonpland, 1807). Because they can be used to study the effects of global warming (Parmesan, 2006), elevation gradients are receiving considerable attention, including in microbial studies (Yang *et al.*, 2014; Geml, 2017). As multiple variables (i.e. climate, soil, vegetation) directly or indirectly change with elevation, mountain slopes are complex 'environmental' gradients (Körner, 2007). For example, shifts in temperature and moisture along elevation gradients can promote changes in the soil microbial community, which, in turn, can alter decomposition rates and nutrient mobilization (Looby *et al.*, 2016; Mayor *et al.*, 2017). Vegetation strongly affects the spatial structure of ECM fungi (Pellissier *et al.*, 2013; Tedersoo *et al.*, 2016) and fungal species turnover across elevation was correlated with the distribution and abundance of ECM plant species (Bahram *et al.*, 2012; Miyamoto *et al.*, 2014; Geml *et al.*, 2017). In combination with host-plant effects, factors such as seasonality (Looby *et al.*, 2016) and edaphic variables (Veach *et al.*, 2018) affected ECM fungal communities across elevations at the local scale. On the other hand, climate apparently had more impact at a regional to global scale (Talbot *et al.*, 2014; Miyamoto *et al.*, 2015). In the few cases where a single ECM host was examined along an elevation gradient, the effect of elevation on fungal beta-diversity correlated with both climatic and edaphic factors, especially temperature, soil moisture and pH (Coince *et al.*, 2014; Jarvis *et al.*, 2015; Rincón *et al.*, 2015).

In the presence of multiple host plants, ECM fungal richness declined monotonically with elevation (Bahram *et al.*, 2012; Nouhra *et al.*, 2012; Vašutová *et al.*, 2017), peaked at mid-elevation (Miyamoto *et al.*, 2014; Geml *et al.*, 2017), or, rarely, increased with elevation (Han *et al.*, 2017; Ni *et al.*, 2018). Decline in richness with elevation was explained by a decrease in rare species at high elevations under increased environmental stress (Geml, 2017). By contrast, the mid-elevation peak, or 'mid-domain effect', was interpreted as the geometric effect of overlapping distributions of species with broad elevation ranges (Colwell & Lees, 2000). Regardless, vegetation changes along the gradient strongly influenced ECM fungal richness (Geml *et al.*, 2014; Vašutová *et al.*, 2017; Nouhra *et al.*, 2018) and underlay the tight correlation between the diversity of co-occurring host plants and their ECM fungi (Tedersoo *et al.*, 2012a). Individual host-plant species might stimulate ECM fungal species that are more beneficial or suppress ECM fungal species which could represent a leak of plant resources depending on local environmental conditions (Tedersoo *et al.*, 2016). Accordingly, when only one ECM host was present along the altitudinal gradient, several studies found that elevation had no obvious effect on ECM fungal richness (Jarvis *et al.*, 2015; Rincón *et al.*, 2015; Bowman & Arnold, 2018), supporting the idea that host plants could be the main factor controlling ECM fungal community assembly (Saitta *et al.*, 2017).

The ECM tree family Nothofagaceae is a major component of temperate forests in the southern hemisphere. Fossil records suggest that Nothofagaceae have dominated Patagonian forests with no other confirmed ECM hosts for 70 million yr (Gandolfo *et al.*, 2011; Moreira-Muños, 2011). In northern Patagonia, one evergreen and two deciduous Nothofagaceae species had similar ECM fungal associations despite differences in leaf phenology (Nouhra *et al.*, 2013). On the other hand, elevation and precipitation influenced species richness and biomass of ECM fungal sporocarps with two Nothofagaceae species in the same region (Nouhra *et al.*, 2012). In both studies, various Nothofagaceae species co-occurred along the elevation gradient, whereas in southern Patagonia, *Nothofagus pumilio* forms monospecific forests along mountain slopes (*c.* 150–750 m elevation) without any co-occurring tree species (Frangi *et al.*, 2004). Elevation significantly influences tree growth, nutrient availability and litter degradation in these forests (Frangi *et al.*, 2005; Moretto & Martínez Pastur, 2014; Thébault *et al.*, 2014), but the response of ECM associations to elevation has never been studied.

Recent metagenomic studies have discovered extensive and cryptic diversity of soil fungi (Nilsson *et al.*, 2016). Nevertheless, dealing with 'big data' from next-generation sequencing (NGS) also generates methodological biases that must be addressed (Lindahl *et al.*, 2013). For example, on the Illumina MiSeq platform, paired-end sequencing can inadvertently discriminate against specific taxa when complementary sequences fail to pair (i.e. because of the poor quality of one of the complementary sequences) and therefore constitute a substantial data loss (Nguyen *et al.*, 2015; Tedersoo *et al.*, 2015). One common approach is to use exclusively unpaired forward sequences that are generally of higher quality (Taylor *et al.*, 2016) but which might not be long enough to generate complete ITS1 or ITS2 fragments for some taxa (Schoch *et al.*, 2012). Therefore, datasets generated from exclusively paired or unpaired forward sequences should be rigorously compared to test how different data-handling approaches might influence results.

To determine how community structure and function of ECM fungi are linked with elevation, we characterized fungal diversity and composition, edaphic variables and EEAs in 180 soil samples across six elevation transects in *N. pumilio* forests. We hypothesized that fungal communities would change drastically along elevation gradients with various groups peaking in richness and abundance at different elevations; that the effect of

elevation on fungal communities would be mediated by changes in edaphic variables along the gradient (e.g. indirect effects linked to slower recycling of SOM correlating with lower temperature at higher elevations); and that soil enzyme activities would vary across elevation and correlate with unique signatures of the different fungal communities. In addition, we developed a custom pipeline combining paired and unpaired ITS1 sequences to test how sequence length (the use of paired or unpaired forward sequences) influences diversity estimates.

Materials and Methods

Study area and sampling

The Tierra del Fuego archipelago (54°–55°S; Fig. 1a) is characterized by forested slopes and glacial valleys covered with peatlands. The climate is cold temperate with short summers and long winters with most precipitation as snow (Tuhkanen, 1992). Seasonality is low, with monthly mean air temperature ranging from –7 to 14°C and a mean annual precipitation of 492 mm at sea level (Moretto & Martínez Pastur, 2014). Precipitation increases with elevation, whereas temperature decreases. *N. pumilio* grows on well-drained, acidic, podzolic soils (Romanyà *et al.*, 2005). High-elevation soils have a thin organic horizon overlying decomposed rock, whereas mid- and low-elevation soils develop on sandy moraines. Tree cover is composed almost exclusively of *N. pumilio*, with a basal area of 80 $m^2\,ha^{-1}$ at lower elevation and 50 $m^2\,ha^{-1}$ at the treeline (Barrera *et al.*, 2000). The understory mainly comprises young *N. pumilio*, bryophytes and a few nonECM angiosperms (Martínez Pastur *et al.*, 2006). No ECM herbaceous plants have been documented from southern South America (Brundrett & Tedersoo, 2018). In February–March 2015, we established two transects (north- and south-facing slopes) in three locations (six elevation transects; Fig. 1a, b). Along each transect, we sampled three plots (18 plots in total) at 'lowland' (130–260 m), 'mid-elevation' (350–450 m) and 'treeline' (520–640 m) sites. At each plot we collected soil beneath one tree every 10 m at the same elevation (10 samples per plot, total of 180 samples). Each sample was composed of four soil cores (5 cm diameter × 10 cm deep) taken 1 m from the tree in cardinal directions. We measured the diameter at breast height (DBH) of each sampled tree and installed dataloggers (Trix16; LogTag Recorders, Auckland, New Zealand) beneath one sampled tree per plot to record air temperature every 2 h for > 1 yr.

Samples were maintained at < 10°C and processed within 24 h. Soils were sieved (2 mm mesh) and divided as follows: 0.15–0.25 g stored at –20°C in a Powersoil DNA Isolation tube (Mo Bio, Carlsbad, CA, USA); 5–10 g maintained at 4°C for < 1 wk before EEA measurements; and the remainder kept for the determination of edaphic variables.

Edaphic variables

We measured percentage soil moisture by drying 2.5–5 g of fresh soil at 60°C for 48 h. Air-dried soil was used to characterize the following: pH in KCl 1 M (1 : 10); percentage SOM by combustion at 500°C for 24 h; total C by dry combustion; total N by semi-micro Kjeldahl (Bremner, 1996); concentration of NO_3^- and NH_4^+ (Bremner, 1965); and available P with the Bray and Kurtz method 1 (Kuo, 1996). NO_3^- and NH_4^+ were summed into available N.

Soil enzymes

Eight EEAs were measured using fluorogenic substrates (Sigma-Aldrich, St Louis, MO, USA; Supporting Information Table S1): the C-acquiring α-glucosidase (AGLU), β-glucosidase (BGLU), β-glucuronidase (GLUCU), β-xylosidase (XIL) and cellobiohydrolase (CEL); the N-acquiring leucine aminopeptidase (LEU) and *N*-acetyl-glucosaminidase (NAG); and the P-acquiring acid phosphatase (PHOS). Briefly, we added 0.1 g of fresh soil to 10 ml of sterile 0.1 M MES buffer (2-(*N*-morpholino) ethanesulfonic acid) adjusted to pH 6.1, and homogenized the soil with a bead-beater for 120 s (Giacometti *et al.*, 2014). Soil slurry was immediately dispensed into 96-well microplates with buffer, sample, reference and substrate following a strict order and position on the plate (ISO-TS-22939, 2010). The final substrate concentration in each well was 40 μM. Fluorescence intensity was read with an excitation of 355 nm and an emission of 460 nm on a POLARstar Omega computerized microplate fluorimeter (BMG Labtech, Ortenberg, Germany) for 30 cycles of 60 s at 30°C. Enzyme activities were calculated based on three replicates by soil sample and expressed as nmol $h^{-1}\,g^{-1}$ (DeForest, 2009).

Identification of fungal communities

DNA was extracted using the Powersoil DNA Isolation kit (Mo Bio) following the manufacturer's protocol with one extra wash step. DNA purity was measured using a NanoDrop 2000 (ThermoFisher Scientific, Waltham, MA, USA). To ensure even PCR amplification, samples high in tannins were purified further with the PowerClean Pro Clean-Up kit (Mo Bio). ITS1 rDNA was amplified with fungal-specific primers ITS1f (Gardes & Bruns, 1993) and ITS2 (White *et al.*, 1990) using eight i5 (forward) and 20 i7 (reverse) TruSeq barcoded adapters (Illumina, San Diego, CA, USA). PCR conditions were as follows: 94°C (1 min) followed by 30 cycles at 94°C (30 s), 52°C (30 s), 68°C (30 s) and a final extension at 68°C (7 min), using 5–10 ng DNA and the Phusion High-Fidelity PCR Mix (New England Biolabs, Ipswich, MA, USA). Amplicons were verified on 1.5% agarose gels and normalized at equimolar concentration with the SequalPrep Normalization Plate Kit (ThermoFisher Scientific). The library was purified with the Agencourt AMPure XP kit (Beckman Coulter, Brea, CA, USA) before sequencing with a MiSeq 300 bp paired-end protocol (Illumina) at the University of Florida ICBR. Raw data are available at NCBI's Sequence Read Archive, #SRP150527.

Quality filtering was performed in Trimmomatic (Bolger *et al.*, 2014) and chimeric sequences removed using 'usearch61' in Qiime 1.9.1 (Caporaso *et al.*, 2010). ITS1 fungal sequences were extracted with ITSx (Bengtsson-Palme *et al.*, 2013) and clustered

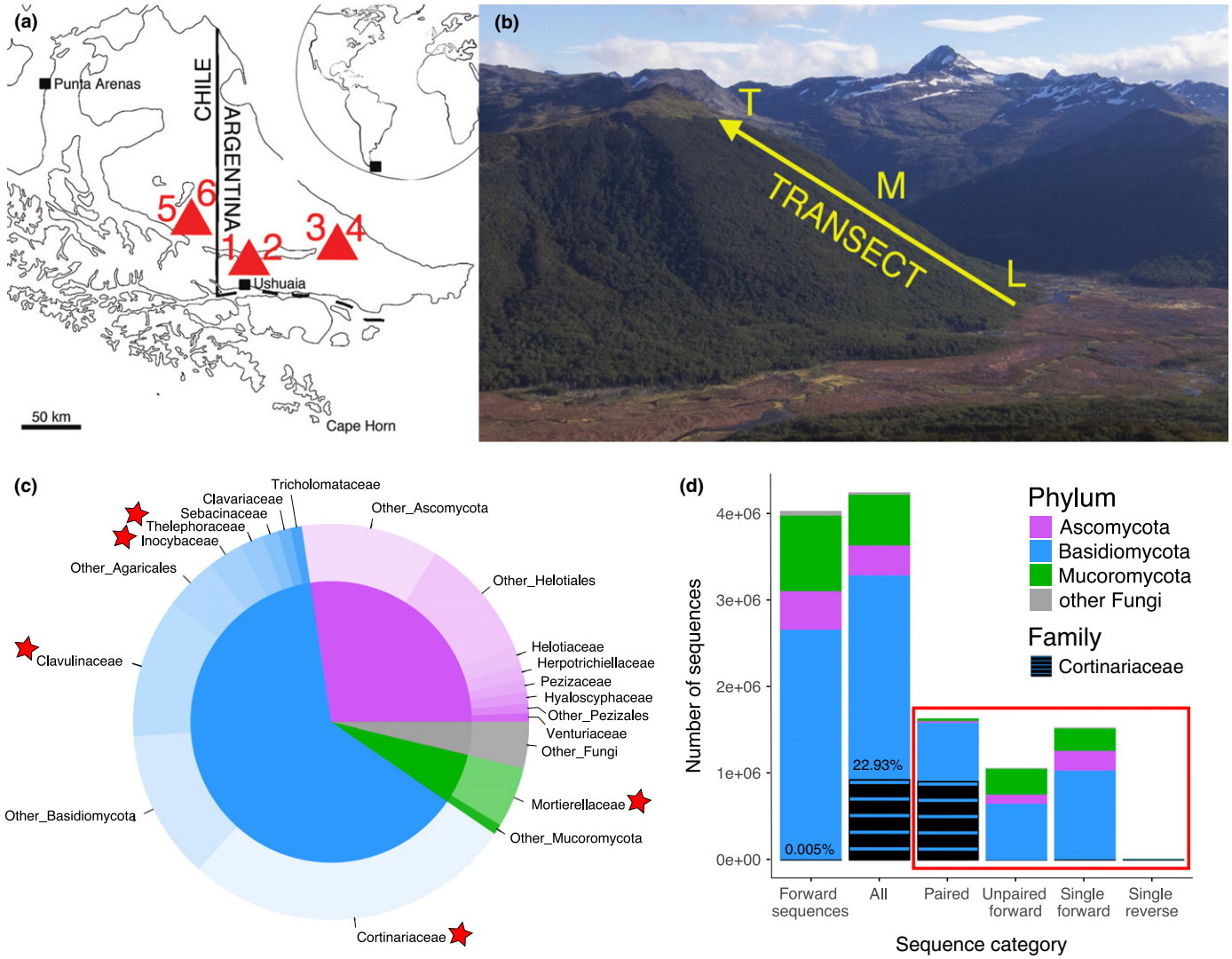

Fig. 1 (a) Tierra del Fuego archipelago with the three sampling locations (red triangles) and the six elevation transects on the north- and south-facing slopes in each location (red numbers). (b) Elevation transect five in Parque Karukinka (Chile) with three elevation plots (L, lowland; M, mid-elevation; T, treeline). (c) Soil fungi diversity detected by Illumina sequencing of ITS1 rDNA and represented by the relative number of operational taxonomic units (OTUs) per taxonomic group. The five most species-rich fungal families (Supporting Information Table S2) are indicated with red stars. (d) Number of sequences per fungal phyla for each sequence category. Paired sequences passed quality filtering with Trimmomatic and were joined in Qiime; unpaired forward sequences passed quality filtering but could not be joined because of the size of the ITS1 region; single forward/reverse sequences where those in which the complementary sequence did not pass the quality filter. These four sequence categories (red box) were combined into the dataset of all sequences, and compared with the dataset represented exclusively by forward sequences. Within the phylum Basidiomycota, the family Cortinariaceae (blue stripes) was represented mostly by paired sequences and was reduced to 0.005% in the dataset represented exclusively by forward sequences.

into operational taxonomic units (OTUs) at 97% similarity, a typical cutoff to approximate species in fungi (Kõljalg *et al.*, 2013). This conservative approach probably underestimates diversity because *Cortinarius* species are frequently clustered at >98% similarity (Garnica *et al.*, 2016). We performed OTU picking on sequences >150 bp using the 'sortmerna' algorithm and a custom database (Truong *et al.*, 2017) followed by *de novo* clustering in Qiime. Four sequence categories were clustered successively: paired sequences passing quality filtering and joined in Qiime; unpaired forward sequences passing quality filtering but not joined because ITS1 was too long; and single forward and reverse sequences in which the complementary sequence did not pass quality filtering. We used negative and positive controls (Nguyen *et al.*, 2015), and OTU occurrences that accounted for <0.5% of sequence counts per sample were removed to eliminate potential inflated diversity as a result of sequencing artifacts. Denoising and clustering parameters were calibrated based on a 20-species mock community with DNA from Nothofagaceae-associated ECM fungi. All scripts of our custom pipeline are available at https://github.com/camillethuyentruong/Illumina_paired_end. To evaluate the performance of this bioinformatic pipeline, we compared it directly to a similar pipeline that used exclusively forward sequences, as this is a common approach in published studies (Taylor *et al.*, 2016).

We assigned OTU taxonomy using Blastn searches in Qiime against our custom database. Owing to the paucity of Patagonian

fungi sequences, many OTUs could not be taxonomically identified. We therefore manually assigned OTU taxonomy based on similarity thresholds and coverage of > 90% by performing MegaBlast searches in PlutoF (Abarenkov *et al.*, 2010). We assigned fungal class with similarity > 80%, family > 90% and genus > 95% (Tedersoo *et al.*, 2015; Motooka *et al.*, 2017). The ECM fungal OTUs were identified using FUNGuild (Nguyen *et al.*, 2016). When FUNGuild failed to assign a guild as a result of taxonomic uncertainty at the genus level, we treated these OTUs as ECM fungi when the closest MegaBlast hits matched an ECM fungal species hypothesis with > 90% similarity and > 90% coverage in UNITE (Kõljalg *et al.*, 2005). When the FUNGuild assignment was not 'highly probable', for example when a genus contains both ECM and nonECM fungal taxa (i.e. *Entoloma*), we considered these OTUs as nonECM if their closest matches were nonECM fungi.

Statistical analyses

Statistical tests were performed in R v.3.3.2 (R Core Development Team 2008) and results were considered significant at $P < 0.05$. Box-Cox transformations were applied to all soil and enzyme variables except soil moisture to improve normality. Pairwise Kendall rank correlations (Newson, 2002) were performed on the soil and enzyme datasets separately and visualized in scatterplot matrices. SOM was omitted from further analyses because it was highly correlated to total C (corr = 0.93, $P < 0.001$). We created three OTU datasets based on trophic modes: 'ECM fungi', 'nonECM fungi' and 'all fungi'. Because of concerns about rarefying NGS data (McMurdie & Holmes, 2014), we used the observed number of sequences normalized into relative abundance per sample. The relative abundance was then multiplied by 1000 and rounded to the nearest integer to be used as count data (Fernandez *et al.*, 2017). Fungal alpha-diversity was estimated in each sample (separately for each dataset) by counting the number of OTUs per sample (observed richness) and calculating Fisher's alpha-diversity index. Fisher's alpha is appropriate for microbial community studies because of its relative insensitivity to sampling size and robustness at low sample completeness (Beck & Schwanghart, 2010). Fisher's alpha gave similar results to tests using Shannon or Simpson diversity indices (data not shown). We tested the relationship between growing season air temperature (November to March), tree DBH, edaphic variables, EEAs and fungal alpha-diversity as dependent variables, and elevation and exposure as independent variables, by fitting linear mixed models (LMMs) with plot nested within transect as random effects, using the 'lmer' function in the R package lme4 (Bates *et al.*, 2015). Pairwise comparisons between elevations ('lowland', 'mid-elevation' and 'treeline') were performed using Tukey's honestly significant difference *post hoc* tests. We also modeled the correlation of fungal alpha-diversity (dependent variable) with edaphic variables and elevation (independent variables), after verifying the noncollinearity of variables based on a variance inflation factor (VIF) < 2.5 (Zuur *et al.*, 2010). Normality and homogeneity of residuals were verified with Shapiro–Wilk and Levene tests and linearity of the models was visualized with normal qq plots.

A Raup–Crick dissimilarity matrix (Chase *et al.*, 2011) was calculated based on the presence/absence of OTUs after removing OTUs present in only one sample. The relationship between fungal community composition and environmental predictors (elevation, exposure and edaphic variables) was visualized with distance-based redundancy analysis (dbRDA) using the 'capscale' function in vegan (Oksanen *et al.*, 2016) with transect partialed out before constraints. The best environmental predictors were selected based on P values < 0.05 and adjusted coefficients of determination (adjR2) with the 'ordiR2step' function. We repeated the analyses using Bray–Curtis dissimilarity distances calculated from OTU abundances after summing OTU abundances per plot ($n = 10$ trees) and using mean values of edaphic variables per plot, as plots predicted most of the variation arising from abundance data (i.e. almost no variation was left after partialing out the plots).

To further explore how the selected predictors affected individual fungal species in the community, we fitted generalized linear models (GLMs) with the 'manyglm' function in mvabund (Wang *et al.*, 2017) and performed univariate (analyzing each OTU independently) and multivariate (all OTUs simultaneously) analyses of deviance (i.e. ANOVA for models with nonnormal error distribution) on the 50 most abundant OTUs; by fungal families; and separately for the five most species-rich families in our dataset: Cortinariaceae (ECM), Clavulinaceae (ECM), Mortierellaceae (nonECM), Inocybaceae (ECM) and Thelephoraceae (ECM) (Fig. 1c; Table S2). We also included Russulaceae (ECM) because it was significant in the univariate family-level test. GLM models were fitted using a binomial distribution for presence/absence data and a negative binomial distribution after counting the number of OTUs per family in each sample. Permutation tests were restricted by transect to partial out variation among transects from our inferences.

Correlations between enzyme activities and environmental predictors were explored with redundancy analysis based on Euclidean distances among samples and transect partialed out before constraints. The best set of environmental predictors (edaphic variables, elevation and exposure) was selected using ordiR2step as described earlier. We also modeled the response of each enzyme to the richness (number of OTUs) and abundance (number of sequences) of ECM fungi, nonECM fungi and the six fungal families selected earlier (Cortinariaceae, Clavulinaceae, Mortierellaceae, Inocybaceae, Thelephoraceae and Russulaceae) using GLM models with a gamma distribution for continuous but nonnormal data. The significance level was adjusted with Bonferroni correction to account for multiple testing.

Finally, variation in the fungal community composition (Raup–Crick distances of ECM, nonECM and 'all fungi') and enzyme activities (Euclidian distances) explained by edaphic variables, elevation and transect was examined with variation partitioning with the 'varpart' function in vegan. In the case of enzymes, the richness and abundance of the six fungi families selected earlier (Cortinariaceae, Clavulinaceae, Mortierellaceae, Inocybaceae, Thelephoraceae and Russulaceae) were also examined using the same method.

Results

An overview of fungal diversity

We obtained 9324–112 673 sequences per sample and 128 053–386 232 per plot. Accumulation curves were generally saturated at the sample and plot level (data not shown), suggesting that we adequately sampled fungal diversity. The 4243 299 sequences that passed quality filtering clustered into 1452 OTUs, with ECM fungal taxa representing 50.0% of OTUs ($n = 726$) and 65.5% of sequences. We manually assigned the ECM guild to one of four *Entoloma*, 13 of 15 *Sebacina*, and 149 of 413 OTUs lacking generic identification. NonECM fungi were mostly saprobes with some endophytes and pathogens; they represented 29.2% of OTUs ($n = 424$) and 20.3% of sequences. The trophic mode of the remaining 20.8% of OTUs ($n = 302$) and 14.2% of sequences remained unresolved. ECM fungi dominated soil fungal communities at all elevations (Fig. S1). Out of the 50 most abundant OTUs in our dataset, 38 (76%) were ECM fungi, nine (18%) were nonECM fungi and three of unknown trophic mode.

Taxonomic biases as a result of pairing ITS1 sequences

We detected strong taxonomic biases among the different sequence categories (e.g. paired, unpaired forward, single forward and single reverse). Paired sequences were mostly Basidiomycota, whereas unpaired forward and single forward/reverse sequences contained more Ascomycota and Mucoromycota (Fig. 1d). After combining all sequence categories, Basidiomycota were still dominant and accounted for 62.3% of OTUs and 76.8% of sequences (Fig. 1c,d). Four of the five most species-rich fungal families were ECM (Cortinariaceae, Clavulinaceae, Inocybaceae, Thelephoraceae), whereas Mortierellaceae was the only nonECM fungal family that was abundant and species-rich (Fig. 1c; Table S2). Table S3 provides a complete list of OTUs.

When our bioinformatic pipeline was compared with a pipeline that used only forward sequences, we detected significant taxonomic biases against certain lineages. This was particularly problematic when short forward sequences did not allow recovery of the full ITS1 region. ITSx with default parameters selectively eliminated some taxonomic groups, excluding *c.* 1.5 million partial ITS1 sequences when using only forward sequences. For example, when using only forward sequences, ITSx reduced the Cortinariaceae (the most abundant and species-rich family in our dataset) from 389 to six OTUs and 23.7% to < 1% of all ITS1 sequences (Fig. 1d). Even when considerably reducing the stringency match to the SSU region (e.g. command: allow_single_domain), ITSx did not recognize these partial Cortinariaceae sequences as ITS1.

Environmental variables across elevation

Kendall correlations among soil variables were > 0.5 for soil moisture with C, and C with available P (Fig. S2). The soil variables were nevertheless considered independently as their VIF factors were < 2 in all models. pH was significantly higher in lowland plots compared with mid-elevation and treeline plots, and soil moisture was significantly higher in treeline than in lowland plots (Table 1). As expected, air temperature and tree DBH significantly decreased with elevation. All other correlation between soil variables and elevation or exposure remained nonsignificant.

Environmental predictors of fungal richness and composition

Fisher's alpha-diversity of ECM fungi peaked at mid-elevation (Table 1) and significantly increased with lower available N and lower soil moisture (Table 2). Fisher's alpha for nonECM fungi was significantly higher in lowland plots than in mid-elevation and treeline plots (Table 1). However, elevation remained nonsignificant when including edaphic variables in the model, where Fisher's alpha for nonECM fungi significantly increased with lower available P (Table 2). Fisher's alpha of 'all fungi' significantly increased with lower available N but was not correlated with elevation (Tables 1, 2). Models based on observed richness (number of OTUs per sample) yielded similar results (data not shown).

Based on dbRDA ordinations of Raup–Crick distances, the best predictor of ECM, nonECM, and 'all fungi' community composition was pH (ECM, $F = 154.69$; nonECM, $F = 188.46$), followed by elevation (ECM, $F = 16.46$; nonECM, $F = 28.24$) (Fig. 2, data not shown for 'all fungi'). Exposure and other edaphic variables were nonsignificant. Models using Bray–Curtis distances from OTU abundances gave similar results to models using Raup–Crick distances. Furthermore, when controlling for elevation, pH and its interaction with elevation significantly predicted ECM, nonECM and 'all fungi' community composition (Table S4). However, when controlling for pH, elevation remained nonsignificant in all GLM models. Similarly, the composition of the four most species-rich families, Cortinariaceae (ECM), Clavulinaceae (ECM), Mortierellaceae (nonECM) and Inocybaceae (ECM), was significantly predicted by pH and its interaction with elevation, but not by elevation alone (Table S4). Only pH significantly explained the distribution of Thelephoraceae (ECM). Univariate tests yielded similar results with pH or its interaction with elevation, significantly predicting the distribution of the families Clavulinaceae and Russulaceae, several species of Clavulinaceae, Cortinariaceae, Russulaceae, and Thelephoraceae as well as three OTUs from the nonECM family Mortierellaceae, whereas elevation remained nonsignificant in all analyses (Table S5). Clavulinaceae (ECM) was most abundant and species-rich at pH < 3.5 and from mid-elevation to treeline, whereas Mortierellaceae (nonECM) was most abundant and species-rich at pH > 4.5 and in the lowland (Fig. 4). Cortinariaceae (ECM) was most species-rich at pH 3.5–4.5. Variation partitioning showed that elevation explained 7–10%, edaphic variables 15–18%, and their interactions 35–48% of the variation in ECM fungi, nonECM fungi and 'all fungi' community composition, whereas transect explained 8–12% of this variation (Fig. 3).

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Table 1 Means ± SE of environmental, enzyme and alpha-diversity variables across elevations.

	Lowland	Mid-elevation	Treeline
Air temperature (°C)	7.99 ± 0.04**a**	6.31 ± 0.06**b**	5.39 ± 0.04**c**
DBH (cm)	67.72 ± 2.68**a**	38.49 ± 1.50**b**	17.14 ± 0.89**c**
Soil moisture (%)	40.70 ± 1.80**a**	48.83 ± 1.20**ab**	54.13 ± 1.30**b**
pH	4.54 ± 0.06**a**	3.82 ± 0.07**b**	3.52 ± 0.06**b**
Total carbon (%)	15.21 ± 0.93	20.55 ± 1.54	24.91 ± 1.55
Total nitrogen (%)	0.95 ± 0.05	1.15 ± 0.05	1.04 ± 0.04
Available nitrogen (ppm)	36.97 ± 1.93	38.04 ± 2.52	34.20 ± 1.95
Available phosphorus (ppm)	37.62 ± 2.98	38.47 ± 2.94	57.81 ± 7.03
AGLU	4.29 ± 0.28	3.85 ± 0.28	5.04 ± 0.76
BGLU	30.50 ± 2.72	30.39 ± 2.84	30.78 ± 1.83
GLUCU	15.59 ± 1.11	29.31 ± 2.57	38.62 ± 7.24
CEL	2.66 ± 0.35	2.61 ± 0.23	3.56 ± 0.56
XIL	3.53 ± 0.26	5.09 ± 0.35	5.66 ± 0.43
LEU	34.94 ± 2.69	19.15 ± 1.88	22.48 ± 1.96
NAG	344.81 ± 20.95	420.92 ± 31.77	405.75 ± 37.36
PHOS	77.65 ± 6.55	96.40 ± 6.83	83.55 ± 8.05
ECM fungi, observed richness	32.90 ± 1.96**a**	47.29 ± 2.22**b**	39.11 ± 1.32**ab**
ECM fungi, Fisher's alpha	3.22 ± 0.21**a**	4.78 ± 0.25**b**	3.86 ± 0.15**ab**
NonECM fungi, observed richness	29.23 ± 1.23**a**	22.98 ± 1.38**b**	22.21 ± 1.23**b**
NonECM fungi, Fisher's alpha	2.81 ± 0.13**a**	2.16 ± 0.14**b**	2.08 ± 0.13**b**
'All fungi', observed richness	75.55 ± 2.86	79.69 ± 3.00	70.71 ± 1.88
'All fungi', Fisher's alpha	8.06 ± 0.34	8.54 ± 0.36	7.46 ± 0.22

Tukey's honestly significant difference *post hoc* tests with $P \leq 0.05$ are indicated with bold letters, based on linear mixed models with elevation as an independent variable and environmental, enzyme and alpha-diversity indices as dependent variables. Plot was nested within transect as random effects. AGLU, alpha-glucosidase; BGLU, beta-glucosidase; CEL, cellobiohydrolase; DBH, diameter at breast height; ECM, ectomycorrhizal; GLUCU, beta-glucuronidase; LEU, leucine aminopeptidase; NAG, *N*-acetyl-glucosaminidase; PHOS, acid phosphatase; richness, number of OTUs per sample; XIL, beta-xylosidase.

Table 2 Correlations of Fisher's alpha indices (dependent variable) with edaphic variables and elevation (independent variables) based on linear mixed models.

	ECM fungi		NonECM fungi		All fungi	
	Estimate	*t*-value	Estimate	*t*-value	Estimate	*t*-value
Soil moisture (%)	−0.03	**−2.30***	0.02	1.98	2.37	0.54
pH	−0.41	−1.45	0.32	1.66	0.15	0.03
Total carbon (%)	0.02	1.42	0.00	0.23	13.78	3.15
Total nitrogen (%)	−0.48	−1.12	−0.06	−0.21	1.50	0.34
Available nitrogen (ppm)	−0.02	**−2.65****	−0.01	−1.63	34.63	**7.92****
Available phosphorus (ppm)	−0.01	−1.74	−0.01	**−2.35***	17.14	3.92
Elevation						
M – L	1.54	**3.08****	−0.55	−1.66	0.56	0.73
T – L	0.54	0.96	−0.59	−1.56	−0.61	−0.69
T – M	−1.00	−2.16	−0.04	−0.12	−1.16	−1.63

Plot was nested within transect as random effects.
ECM, ectomycorrhizal.
t-values and honestly significant difference *post hoc* tests are indicated with significance level: *, $P \leq 0.05$; **, $P \leq 0.01$ (in bold). L, lowland; M, mid-altitude; T, treeline.

Soil enzymes across elevations

Kendall correlations among all EEAs except LEU were > 0.5 (Fig. S3). None of the EEAs responded significantly to elevation (Table 1) and exposure (data not shown) in the LMM models. Redundancy analysis of overall EEAs showed that total C ($F = 29.16$) was their best predictor, followed by pH ($F = 10.73$), total N ($F = 7.93$) and elevation ($F = 5.00$) (Fig. 2). Exposure and other edaphic variables were nonsignificant. In addition, LEU activity was positively correlated with ECM fungal richness and abundance and negatively correlated with nonECM fungal abundance (Table 3). LEU activity was also positively correlated with Cortinariaceae and Clavulinaceae richness (both ECM), and negatively correlated with Mortierellaceae richness and abundance (nonECM). By contrast, PHOS activity was negatively correlated with ECM fungal abundance and positively correlated

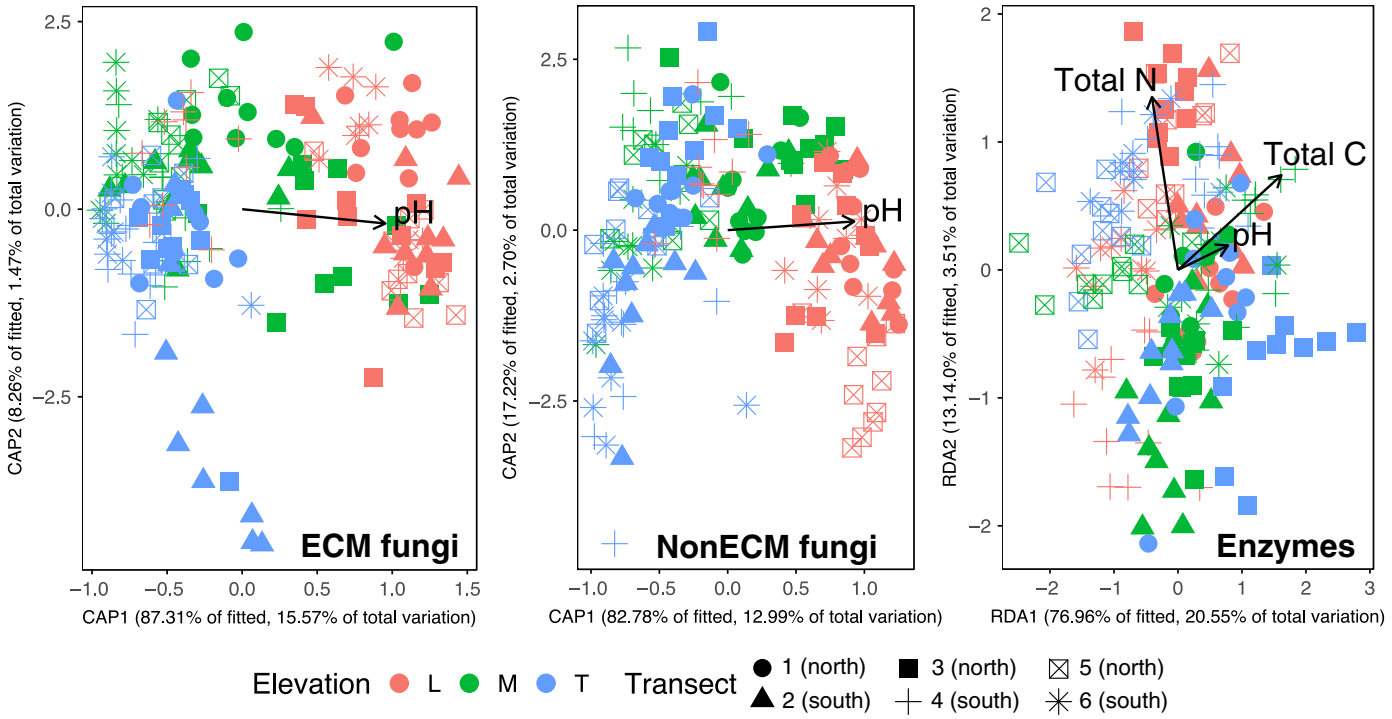

Fig. 2 Distanced-based redundancy analysis of ectomycorrhizal (ECM) and nonECM fungal community composition (Raup–Crick distances) and redundancy analysis of enzyme activities (Euclidean distances) with transect partialed out before constraints. Environmental predictors (elevation (L, lowland; M, mid-altitude; T, treeline), exposure and edaphic variables) were used to select the best model based on adjR2 and *P*-values. The best predictors of the ECM and nonECM fungal community composition were pH (ECM, $F = 154.69$***; nonECM, $F = 188.46$***) and elevation (ECM, $F = 16.46$***; nonECM, $F = 28.24$***), whereas enzyme activities were best correlated with total carbon (C; $F = 29.16$***), followed by pH ($F = 10.73$***), total nitrogen (N; $F = 7.93$***) and elevation ($F = 5.00$***). Significance level ***, ≤0.001.

with nonECM fungal abundance, as well as with Mortierellaceae richness and abundance (nonECM). Inocybaceae, Russulaceae and Thelephoraceae (ECM) were not significantly correlated with any EEAs (data not shown). Variation partitioning showed that, overall, EEAs were mostly explained by edaphic variables (17%) and transect (15%), whereas elevation (2%) and the richness/abundance of the fungal families Cortinariaceae, Clavulinaceae, Mortierellaceae, Inocybaceae, Thelephoraceae and Russulaceae (1%) minimally explained the variation in EEAs (Fig. 3).

Discussion

Fungal richness and species turnover across elevations

Soil pH was the strongest predictor of fungal community composition (both ECM and nonECM fungi) in *N. pumilio* forests (Fig. 2). Appropriate pH is crucial for mycelial growth (Yamanaka, 2003) and is considered the 'master' variable that links SOM recycling, plant nutrition and plant–microbial interactions in soils (Husson, 2013). Because it is linked with the composition of SOM and may be manipulated by plant and microbial communities (Cornelissen *et al.*, 2011), soil pH is both the driver and the outcome of these ecosystem processes. Previous studies demonstrated the importance of pH in shaping bacterial and soil fungi communities in various ecosystems (Lauber *et al.*, 2009; Rousk *et al.*, 2010), including elevation gradients (Geml *et al.*, 2014; Jarvis *et al.*, 2015; Rincón *et al.*, 2015). Yet, there is an ongoing debate regarding the comparative effect of pH and temperature on microbial communities (Zhou *et al.*, 2016; Tedersoo, 2017). We found that air temperature, soil moisture and pH were correlated with elevation (Table 1) and that both pH and elevation significantly predicted fungal community composition (Fig. 2). *In situ* experiments in arctic and boreal ecosystems demonstrated that warming induced compositional shifts in the soil community by favoring certain species while others may go locally extinct, with potential implications for nutrient cycling and C storage (Morgado *et al.*, 2015; Fernandez-Martinez *et al.*, 2016). In our case, GLM models (by fungal families or the 50 most abundant OTUs) showed that pH and its interaction with elevation were significant, but elevation alone remained nonsignificant (i.e. when controlling for pH, elevation did not significantly predict fungal community composition; Table S4). The importance of edaphic variables in shaping fungal communities is further illustrated with variation partitioning, where edaphic variables (15–18%) explained almost twice the variation explained by elevation (7–10%), and interactions between pH and edaphic variables explained 35–48% of the variation in fungal communities (Fig. 3). This clearly demonstrates how changes in edaphic variables linked to elevation are the main drivers of fungal community shifts in these forests. Climatic factors have been shown to affect fungal communities at the regional to global scale (Talbot *et al.*, 2014; Miyamoto *et al.*, 2015) and the small

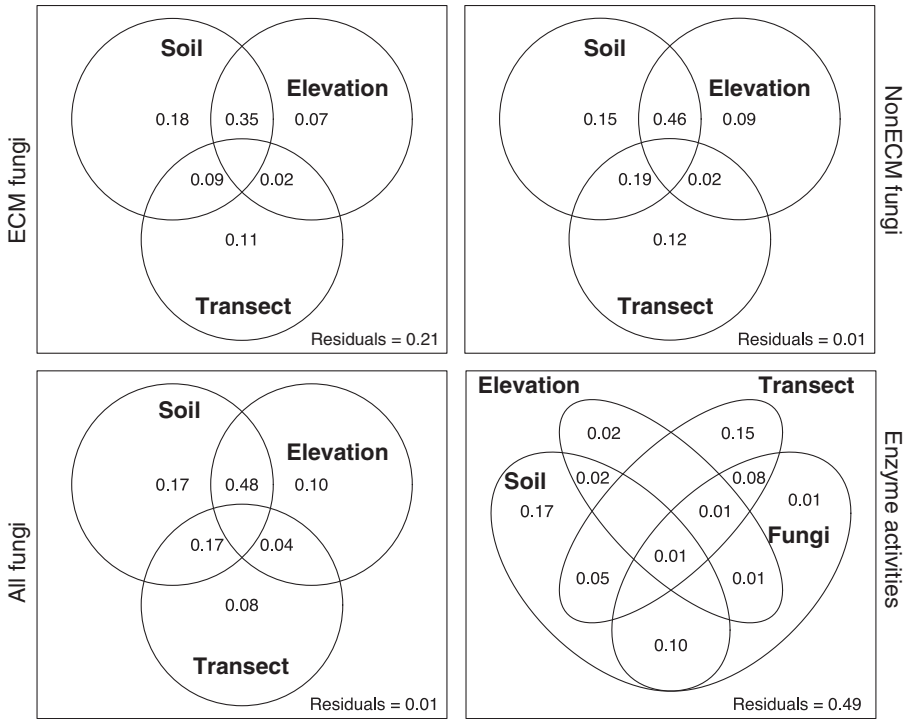

Fig. 3 Variation partitioning showing the proportion of fungal community composition (Raup–Crick distances of ectomycorrhizal (ECM), nonECM and 'all fungi' dataset) and enzyme activities (Euclidian distances) explained by edaphic variables (Soil), elevation, transect and, in the case of enzymes, the richness and abundance of the six fungal families Cortinariaceae, Clavulinaceae, Mortierellaceae, Inocybaceae, Thelephoraceae and Russulaceae (Fungi).

Table 3 Response of each enzyme to the richness (number of operational taxonomic units (OTCs)) and abundance (number of sequences) of ectomycorrhizal (ECM) fungi, nonECM fungi and the fungal families Cortinariaceae (ECM), Clavulinaceae (ECM) and Mortierellaceae (nonECM).

	AGLU	BGLU	GLUCU	CEL	XIL	LEU	NAG	PHOS
Richness ECM fungi						5.83***		
Abundance ECM fungi						4.55***		−3.41***
Richness nonECM fungi								
Abundance nonECM fungi			3.17*			−4.33***		3.66***
Richness CORT						4.35***		
Abundance CORT								
Richness CLAV						3.81***		
Abundance CLAV					−3.08*		−3.37***	
Richness MORT	3.65***		4.05***			−3.19*	3.72*	3.46***
Abundance MORT						−4.71***	3.09*	4.37***

AGLU, alpha-glucosidase; BGLU, beta-glucosidase; CEL, cellobiohydrolase; CLAV, Clavulinaceae; CORT, Cortinariaceae; GLUCU, beta-glucuronidase; LEU, leucine aminopeptidase; MORT, Mortierellaceae; NAG, *N*-acetyl-glucosaminidase; PHOS, acid phosphatase; XIL, beta-xylosidase.
t-values from generalized linear models with a gamma distribution are indicated with significance level (*, $P \leq 0.005$; ***, $P \leq 0.001$) adjusted using Bonferroni correction.

range of our gradient (*c.* 400 m) might explain the tighter link with edaphic variables.

Species richness of ECM fungi peaked at mid-elevation and correlated negatively with both available N and soil moisture (Tables 1, 2). Previous studies in which a single ECM plant species was present along the gradient demonstrated the predominant effect of edaphic variables on ECM fungal richness (Rincón *et al.*, 2015). Soil moisture has been shown to affect both ECM fungal richness and *Nothofagus* seedling recruitment (Hiiesalu *et al.*, 2017; Hewitt *et al.*, 2018), and increased soil moisture with elevation (Table 1) could explain some of the observed patterns. We also detected a shift between nonECM and ECM fungi, with ECM fungal richness increasing from lowland to mid-elevation plots while nonECM fungal richness decreased (Fig. 1c; Table 1). Warming experiments in the Arctic showed that ECM fungi richness generally decreased with temperature, whereas richness of saprotrophic, pathogenic and root-endophytic fungi increased, although the responses varied between fungi with different foraging strategies (Geml *et al.*, 2015). Niche partitioning across the elevation gradient could structure the diversity of soil microbial communities in response to nutrient demand (Moeller *et al.*, 2014). We found that ECM fungal richness was negatively correlated with available N and positively correlated with the activity of LEU (Tables 2, 3). Higher N demand at higher elevation may favor C allocation to ECM fungi that are able to access recalcitrant forms of organic N, enhancing their diversity and

competitiveness towards non-ECM fungi (Clemmensen *et al.*, 2015; Baskaran *et al.*, 2017).

Enzyme activities across elevations

Despite the high species turnover across elevations and pH ranges, overall EEAs displayed similar rates across elevations. By contrast, EEAs were strongly affected by soil variables, especially total C (Fig. 2). Previous studies clarified the role of SOM composition (i.e. C and N content in organic horizons) as the main driver of EEAs at high latitudes (Schnecker *et al.*, 2014; Waldrop *et al.*, 2017), whereas EEAs seemed relatively insensitive to temperature in a tropical nonECM Peruvian forest (Nottingham *et al.*, 2016). The contribution of ECM fungi to SOM recycling has been demonstrated (Lindahl & Tunlid, 2015) and, in some cases, was comparable to saprotrophic fungi (Phillips *et al.*, 2014). In boreal forests, ECM fungi act as C and N regulators, transferring small fractions of N to trees under N-limited conditions, but larger amounts when more inorganic N is available (Näsholm *et al.*, 2013). We found that LEU activity was positively correlated with ECM fungal richness and abundance, as well as the richness of the two most species-rich ECM fungal families, Cortinariaceae and Clavulinaceae (Table 3). This is similar to previous studies in northern hemisphere forests (Talbot *et al.*, 2013; Phillips *et al.*, 2014), although laboratory assays did not find this pattern (Talbot *et al.*, 2015). LEU is a proteolytic enzyme that mobilizes N by hydrolyzing amino acids of proteins and polypeptides (Matsui *et al.*, 2006). Our results support the hypothesis that ECM fungi are involved in N mobilization by degrading recalcitrant forms of organic N in soils. Increased N-mineralization rate and turnover at the treeline compared with adjacent *N. pumilio* forests downslope were attributed to the 'krummholz' tree morphology of *N. pumilio* at the treeline (Barrera *et al.*, 2000; Frangi *et al.*, 2005). These dwarfed, multistem trees exhibited higher leaf nutrient content coupled with increased rates of nutrient movement between roots and shoots. Our results support previous evidence that ECM associations adapted to high elevations and low soil pH may facilitate N accessibility at high elevations (Moeller *et al.*, 2014; Marín *et al.*, 2018). Both Clavulinaceae and Cortinariaceae were particularly abundant at the treeline (Fig. 4) where nutrient demands and environmental stress are high. Cortinariaceae are known to use long-distance exploration strategies and access organic N sources (Lilleskov *et al.*, 2011; Bodeker *et al.*, 2014). This group is hyperdiverse in Patagonia with > 150 species based on sporocarps (Truong *et al.*, 2017). Clavulinaceae, however, have been reported in Nothofagaceae forests almost exclusively from soil or roots (Tedersoo *et al.*, 2008; Nouhra *et al.*, 2013). Clavulinaceae was the most abundant and species-rich group at very low pH (Fig. 4) and the role of these fungi in N mobilization in acidic soils needs further investigation.

At high latitudes, high richness and abundance of Mortierellaceae has been detected in acidic forest soils rich in humic acids produced from lignin degradation (Nouhra *et al.*, 2013; Phillips *et al.*, 2014). Mortierellaceae was the only species-rich nonECM fungal family in our dataset (Fig. 1c; Table S2) and was most abundant in lowland plots and at higher pH (Fig. 4). These saprobes are involved in lignin and chitin degradation (Burns & Dick, 2002; Allison *et al.*, 2009), as illustrated by positive correlations with the activity of AGLU, GLUCU and NAG (Table 3). Mortierellaceae richness and abundance were also positively correlated with PHOS. Mortierellaceae putatively perform rock weathering, thereby mobilizing essential lithophilic plant nutrients, particularly P (Alori *et al.*, 2017). P uptake involving ECM fungi has been demonstrated (Landeweert *et al.*, 2001; Köhler *et al.*, 2018), but the contribution of Mortierellaceae to P availability in the rhizosphere is unknown. Further investigation is needed to demonstrate the role of Mortierellaceae in plant P nutrition.

To pair or not to pair: biases in compiling and interpreting metagenomic sequences

Our custom pipeline combining paired and unpaired ITS1 sequences allowed us to detect taxonomic biases linked to sequence quality and length. Many sequences failed to pair as a result of the poor quality of one of the two complementary sequences (mostly reverse sequences). This was particularly the case for many Ascomycota (Fig. 1d), which may indicate that the reverse primer matched poorly for this group (Nguyen *et al.*, 2015). On the other hand, unpaired forward sequences (that failed to pair because ITS1 was too long) contained disproportionately more Mucoromycota (Fig. 1d). Some zygomycetes have very long ITS rDNA sequences, which may impede their detection in environmental DNA studies (Gottlieb & Lichtwardt, 2001; Lazarus *et al.*, 2017).

Using exclusively forward sequences was even more problematic. It reduced sequence accuracy, because paired reads decrease the risk of sequencing errors, but also generated biases against certain taxa in which the shorter forward sequence length prohibited recovery of the full ITS1 region. ITSx is a Perl-based tool that extracts and verifies the identity of ITS sequences (Bengtsson-Palme *et al.*, 2013). It is widely used and recommended for fungal meta-barcoding studies (Lindahl *et al.*, 2013; Balint *et al.*, 2014). ITSx recognizes ITS1 sequences by detecting small flanking domains within 18S and 5.8S rDNA using hidden Markov models (HMMs; Bengtsson-Palme *et al.*, 2013). If only 18S is detected, for example when the 5.8S flanking region is missing (Fig. S4), the stringency match with a unique domain needs to be higher for the sequence to be recognized as ITS1. When using our dataset with exclusively forward sequences, some taxonomic groups with partial ITS1 sequences were selectively eliminated because the flanking 18S domain was too divergent from the HMM profiles. As a striking example, Cortinariaceae is hyperdiverse in Patagonia (Truong *et al.*, 2017) and was the most abundant and species-rich family in our dataset (Fig. 1c; Table S2). When using exclusively forward sequences, Cortinariaceae were drastically reduced from 389 to six OTUs and 23.73% to < 1% of sequences (Fig. 1d), even though only a short region was missing at the end of ITS1 (Fig. S4). Reducing the stringency match to one domain did not allow us to recover these partial

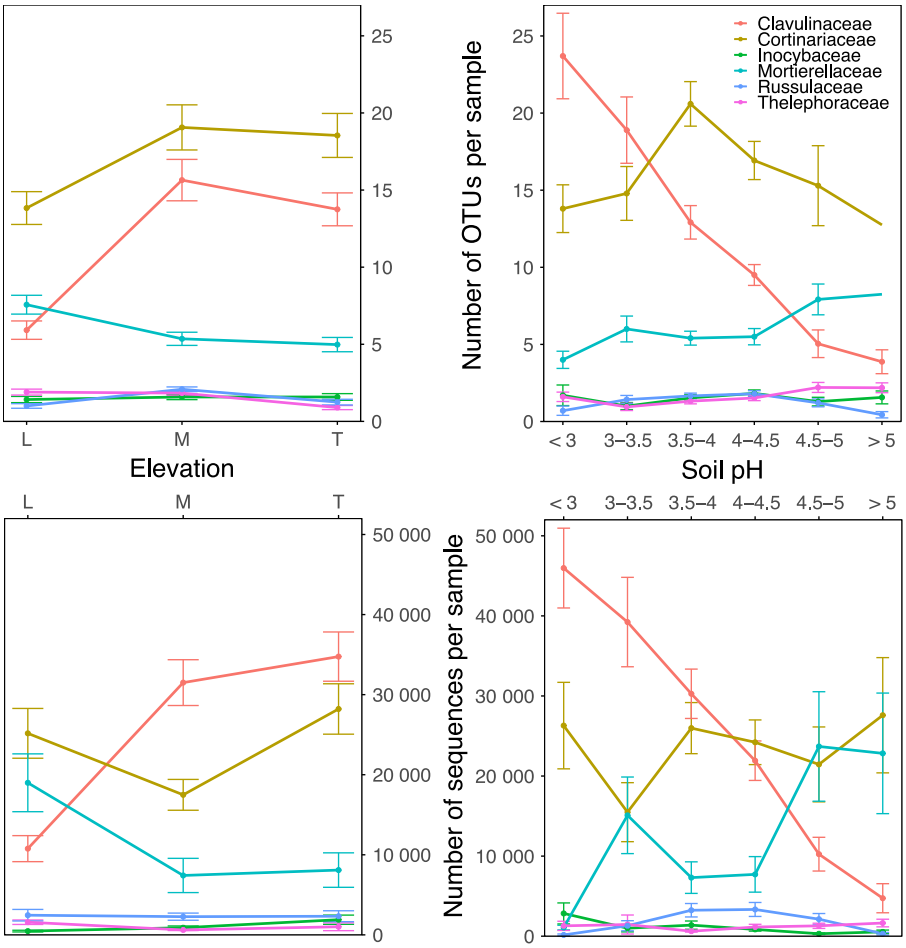

Fig. 4 Number of operational taxonomic units (OTUs) and sequences per sample across elevations (L, lowland; M, mid-altitude; T, treeline) (left) and soil pH values (right) for the five most species-rich fungal families in our dataset, as well as the family Russulaceae. Error bars represent ± SE per elevation and pH categories.

ITS1 sequences, and changing this parameter undoubtedly increases the risk of obtaining nonfungal and nonribosomal sequences.

In light of our results, great care should be taken when dealing with Illumina Miseq data, specifically when using exclusively paired or forward sequences. Our custom pipeline combining high-quality paired and unpaired ITS1 sequences allowed us to recover fungal diversity across taxonomic groups, including the full ITS1 region from paired sequences of all 387 Cortinariaceae OTUs (Fig. S4) using the default parameters of ITSx.

Conclusions

Our findings from Patagonia are consistent with the role of ECM fungi as N regulators at high latitudes and elevations (Näsholm *et al.*, 2013; Phillips *et al.*, 2014). Soils in *N. pumilio* forests are usually shallow and have high P content, whereas N is limiting, especially at high elevations (Moretto & Martínez Pastur, 2014). Our data provide evidence that, in these monodominant *N. pumilio* forests, ECM fungi dominate the soil communities across elevations in terms of both species richness and fungal biomass. The high ECM fungal species richness associated with the higher activity of N-acquiring enzyme LEU and coupled with low variation in other EEAs across elevations suggests that the diversity of ECM fungi associated with *N. pumilio* helps to maintain nutrient mobilization in environments with strong N limitation. Increased atmospheric CO_2 and N deposition associated with climate change are predicted to affect C allocation from plants to mycorrhizal fungi and to induce ECM fungal community shifts in response to changes in plant nutrient demands (Corrales *et al.*, 2017; Fernandez *et al.*, 2017). Our results emphasize how ECM fungal biodiversity contributes to the stability of ecosystem services along environmental gradients (Schnecker *et al.*, 2014). This stabilizing effect is considered among the most important roles of mycorrhizal symbionts in forests facing increasing environmental stress as a result of climate change (Simard & Austin, 2010).

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Author contributions

CT and MES planned and designed the research. CT, JME and AM conducted fieldwork. CT and LAG performed laboratory work. CT, ABM and AC analyzed the data. CT, AC and MES wrote the manuscript. All authors contributed to revising the manuscript.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Number of OTUs and sequences of each fungal guild (ECM, nonECM and unknown) per sample at each elevation.

Fig. S2 Scatterplot matrix (pairwise comparisons) of edaphic variables.

Fig. S3 Scatterplot matrix (pairwise comparisons) of extracellular enzyme activities.

Fig. S4 Representative ITS1 sequences from each of the 389 Cortinariaceae OTUs detected in our dataset.

Table S1 Enzymes and substrates used in the fluorogenic assays.

Table S2 Fungi families comprising 10 or more OTUs detected by Illumina sequencing of the ribosomal ITS1 amplicon.

Table S3 List of operational taxonomic units (OTUs) detected by Illumina sequencing of ITS1 rDNA.

Table S4 Analysis of deviance estimating the significance of pH, elevation, and their interactions on fungal community composition of the 50 most abundant OTUs, OTUs pooled by family, and the five most species-rich fungal families.

Table S5 Univariate tests from the analysis of deviance estimating the significance of pH, elevation, and their interactions on fungal community composition.

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