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ORIGINAL RESEARCH

Large-diameter trees disproportionately contribute to soil fungal diversity in a coniferous forest with one of oldest living trees on Earth

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Abstract

Soil fungi play major roles in ecosystem functioning and plant resilience to environmental stresses. However, they are often overlooked in ecosystem restoration plans. In the Coastal Range of southern Chile, the endangered conifer Alerce (*Fitzroya cupressoides*) is the second longest-lived tree species on Earth and one of the largest in South America. These Alerce forests are currently facing major threats due to habitat destruction and climate change. Using ITS2 and SSU soil metabarcoding, we characterized soil and mycorrhizal fungal communities in an Alerce forest comprising a millennial tree (the 'Alerce Abuelo') estimated to be over 2400 years old. We also compared the performance of reference DNA sequence databases (i.e. MaarjAM, UNITE and EUKARYOME) for the taxonomic identification of mycorrhizal fungi in metabarcoding data. Beneath the Alerce Abuelo, soil fungal richness was 2.25 higher than the mean richness per sample, harboring 361 unique fungal OTUs. Likewise, arbuscular mycorrhizal (AM) fungal richness was 1.75 times higher than the mean richness per sample. Soil and AM fungal richness positively correlated with diameter at breast height (DBH) and tree biomass, and negatively with available phosphorus, which was the best predictor of fungal community composition. Finally, EUKARYOME detected more mycorrhizal taxa compared to MaarjAM for SSU (AM fungi) and UNITE for ITS2 (ectomycorrhizal fungi). We therefore recommend to carefully evaluate the approaches used for assigning mycorrhizal guilds in comparative studies. Our study illustrates the importance of millennial trees in sustaining fungal diversity in forest soils. By accumulating fungal diversity as they age, large-diameter Alerce trees can act as umbrella species for soil and mycorrhizal fungi, thereby protecting fungal communities for future forest restoration efforts.

Camille Truong and Adriana Corrales contributed equally to this work.

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Keywords Conifers · Cupressaceae · Fitzroya · Millennial trees · Mycorrhizal symbiosis · Temperate rainforest · Tree biomass

Introduction

Old-growth temperate rainforests of Southern South America are among the most pristine and unpolluted ecosystems in the world (Perakis and Hedin 2002; Godoy et al. 2003). In particular, forests of the Chilean Coastal Range are considered a refuge for biodiversity and endemism (Olson and Dinerstein 2002; Scherson et al. 2017). These ecosystems are home to the endangered conifer species known as Alerce, the Cupressaceae species *Fitzroya cupressoides*. Alerce is considered one the largest tree species in South America, with individuals up to 50 m in height and 5 m in diameter (Veblen et al. 1976). Alerce is renowned for its extraordinary longevity. With individuals surviving for over 3600 years, it is the second oldest living tree species on Earth, surpassed only by the Great Basin bristlecone pine (*Pinus longaeva*) (Lara and Villalba 1993). These ecosystems are called 'slow-growth rainforests' that have the capacity to accumulate carbon for millennia (Urrutia-Jalabert et al. 2015b). Old-growth trees, such as Alerce, can play an overly large role for global carbon stocks while fostering biotic interactions (Lutz et al. 2018; Ali and Wang 2021; Chen et al. 2024). By disproportionately affecting aboveground biomass compared to younger trees, they could potentially act as key players in climate change mitigation strategies (Ali et al. 2019). Despite their vital ecological roles, old-growth trees such as Alerce are globally in decline due to deforestation, climate change and the resulting intensification of wildfires (Donoso et al. 1993; Lindenmayer and Laurance 2017).

Soil fungi are key elements of ecosystem health and functioning through their roles in organic matter decomposition, biogeochemical cycling, and plant productivity, among other processes (Delgado-Baquerizo et al. 2020; Runnel et al. 2025; Xu et al. 2025). Among the most influential groups are mycorrhizal fungi that form mutualistic symbiosis with plant roots to enhance plant nutrient acquisition, stabilize the physical soil environment and boost plant resistance to stressors (Powell and Rillig 2018; Sosa-Hernández et al. 2019; Wang et al. 2024). In forests, mycorrhizal fungi play key functional roles for tree growth, community dynamics and ecosystem processes, thereby impacting the trajectories of forest conservation and management (van der Heijden et al. 2015; Steidinger et al. 2019; Policelli et al. 2020). Alerce, and other southern hemisphere conifers, associate with communities of mycorrhizal fungi known as arbuscular mycorrhizal (AM) fungi (Marín et al. 2017, 2023; Godoy and Marín 2019). This contrasts sharply with the majority of temperate forest trees, including most northern hemisphere conifers, that form associations with another type of mycorrhizae called ectomycorrhizal (EcM) fungi (Soudzilovskaia et al. 2020). Both AM and EcM fungi contribute to plant nutrient acquisition, however they strongly differ in their morphology, physiology and evolutionary history (Tedersoo and Bahram 2019). While AM fungi establish intimate relations with plant hosts, by forming arbuscules that penetrate the root cells to exchange nutrients for carbon, EcM fungi develop in between epidermal cells and form a Hartig net that ensheathe the surface of fine roots, providing extra protection against root pathogens (van der Heijden et al. 2015). AM fungi primarily enhance nutrient acquisition from mineral sources, while some EcM fungal groups have enzymatic capabilities to mine organic forms of nutrients that are inaccessible to AM fungi (Chen et al. 2018; Pellitier and Zak 2018). Therefore, EcM associations tend to prevail in temperate forests exhibiting high carbon stocks, nutrient-poor soils and low decomposition rates (Steidinger et al. 2019; Yan et al. 2024). In these ecosystems, the importance of EcM fungi for tree growth, forest dynamics and conservation management has been repeatedly demonstrated (Policelli et al. 2020; Birch et al. 2023; Anthony 2025). On the other hand, AM fungi associating with forest trees are likely to exhibit unique diversity patterns and ecological functions that are currently overlooked (Zheng et al. 2023).

Despite the growing research on Alerce and other millennial tree species, how they have evolved such long lifespans remain poorly studied. Historically, research has focused on growth dynamics and biotic interactions aboveground rather than belowground (Lindenmayer and Laurance 2017; Tejo and Fontúrbel 2019; Ali and Wang 2021). A large body of research has shown that Alerce trees are highly dependent on AM fungi for their growth and survival (Godoy and Mayr 1989; Godoy et al. 1994; Marín et al. 2017, 2023). However, it is not well-understood how AM fungal communities contribute to tree growth during the lifespan of their host. These aspects have been studied more extensively in grasslands, where positive correlations of AM fungal richness with plant age and biomass are context-dependent (Šmilauer et al. 2021; Bryant and Bever 2024). Recent research also suggests that some AM plants can select which AM fungi they allow to colonize their roots (Frew et al. 2025). The few studies that have tested these relationships in temperate forests have mostly focused on EcM associations, and none of them was conducted in the southern hemisphere. They repeatedly showed that old-growth trees harbor unique EcM fungal communities in their rhizospheres that change as the tree or the stand mature (Boeraeve et al. 2018; Wasyliw and Karst 2020; Birch et al. 2021). Old, large-diameter trees can therefore act as drivers of belowground mycorrhizal diversity by accumulating adapted microbes in their rhizosphere during their long-life span (Birch et al. 2023). Alternatively, as trees mature, they may progressively select for stable, adapted root traits and mycorrhizal communities (Luo et al. 2024; Frew et al. 2025). Sometimes called the ‘second genome’, shifts in microbial community can therefore contribute to plant adaptation to environmental changes (Turner et al. 2013; Hao et al. 2024).

Alerce is currently listed as endangered in the IUCN Red list of threatened species (EN, under criteria A2acd) with only 40% of its distribution occurring within national protected areas (Urrutia-Jalabert, pers. comm.). The species is currently facing major threats in the Chilean Coastal Range due to habitat destruction from road development and increased wildfire due to climate change (Urrutia-Jalabert et al. 2023). Biodiversity loss generally has negative impacts on ecosystem functioning because many ecosystem processes depend on a high species diversity at multiple trophic levels (Soliveres et al. 2016). For example, a reduction of fungal diversity in soils can have adverse cascading effects on plant diversity and ecosystem multifunctionality, such as nutrient cycling and productivity (Delgado-Baquerizo et al. 2020; Xu et al. 2025). To effectively monitor and conserve these forests, it is therefore essential to understand how soil and mycorrhizal fungal communities change as Alerce individuals grow and age. Such knowledge is critical for protecting keystone mycorrhizal fungal communities that help millennial trees adapt to changing conditions as they age.

To address these knowledge gaps, we tested how soil and mycorrhizal fungal communities changed across different Alerce size classes, as a proxy for tree age. We had the opportunity to characterize soil fungi associated with a millennial renown tree called ‘the Alerce

Abuelo’. At over 2400 years old, this individual is considered to be one of the oldest living trees on Earth (Barichivich and Lara, pers. comm.). We hypothesized that (i) Alerce trees accumulate adapted microbes with time, resulting in positive correlations of soil and AM fungal richness with age class, diameter at breast height (DBH) and aboveground biomass; (ii) soil and AM fungal communities change during Alerce lifespan, resulting in shifts in community composition across age classes.

To answer these questions, we characterized fungal communities in soil samples collected beneath Alerce individuals using ITS2 (for soil fungi in general) and SSU (for AM fungi) metabarcoding. Studies using metabarcoding to identify AM and EcM fungi from the environment typically rely on reference databases for DNA sequence identification. One inherent challenge of fungal metabarcoding lies in the incompleteness and/or fragmentation of sequence databases, depending on the taxonomic group studied and the genetic region targeted, which can lead to non-detections or incorrect inferences (Keck et al. 2023). This is particularly evident in studies whose hypotheses require high taxonomic resolution, at or above generic rank, to identify mycorrhizal fungal guilds (Põlme et al. 2020). Expansion of existing databases is essential, and the newly developed EUKARYOME may be a promising development in this area (Tedersoo et al. 2024a). EUKARYOME links the three ribosomal rDNA marker genes commonly used to identify AM fungi, i.e. SSU (Öpik et al. 2010), ITS (Abarenkov et al. 2024) and LSU (Delavaux et al. 2022), to improve taxonomic assignments. By doing so, EUKARYOME may also improve genera level identification of short-read ITS1 or ITS2 sequences, resulting in a higher number of OTUs assigned as EcM fungi. In addition to our ecological questions, we therefore assessed the performance of the EUKARYOME database compared to available reference databases for mycorrhizal fungi identification, i.e. the MaarjAM SSU database for AM fungi (Öpik et al. 2010) and the UNITE ITS database for EcM fungi (Abarenkov et al. 2024).

Methods

Site characteristics and soil sampling

We collected soil samples in an Alerce forest located in the Alerce Costero National Park, Los Rios region, Chile (40°11.777′S 73°26.129′W, Fig. 1). The climate of the area is characterized by high annual precipitation reaching up to 4800 mm, but with a Mediterranean influence and less than 20% falling in summer (Urrutia-Jalabert et al. 2015a). Mean annual temperature is around 7.5 °C, with minimum (winter) and maximum (summer) temperatures reaching around 3.5 °C and 11.7 °C, respectively. Shallow soils originate from metamorphic rocks and are severely podzolized (Urrutia-Jalabert et al. 2015a). Soils are very acidic and poor in nutrients (Table 1), with a high exchangeable aluminum content (Urrutia-Jalabert et al. 2015a; Marín et al. 2023). Water and nutrients fluxes showed that nutrient outputs through runoff (cations) and stream flows (organic and inorganic nitrogen) were much smaller than nutrient inputs through direct precipitation and soil infiltration (Oyarzun et al. 1998). Vegetation at the site is characterized by the dominance of Alerce with >95% of living biomass (González et al. 2022). The other most common vascular plants are shrubby species of *Chusquea montana*, *Ch. quila*, *Drimys winteri*, *Gleichenia cryptocarpa* and *Ugni candollei*, which associate with AM fungi, as well as *Gaultheria mucronata* and *G. insana*,

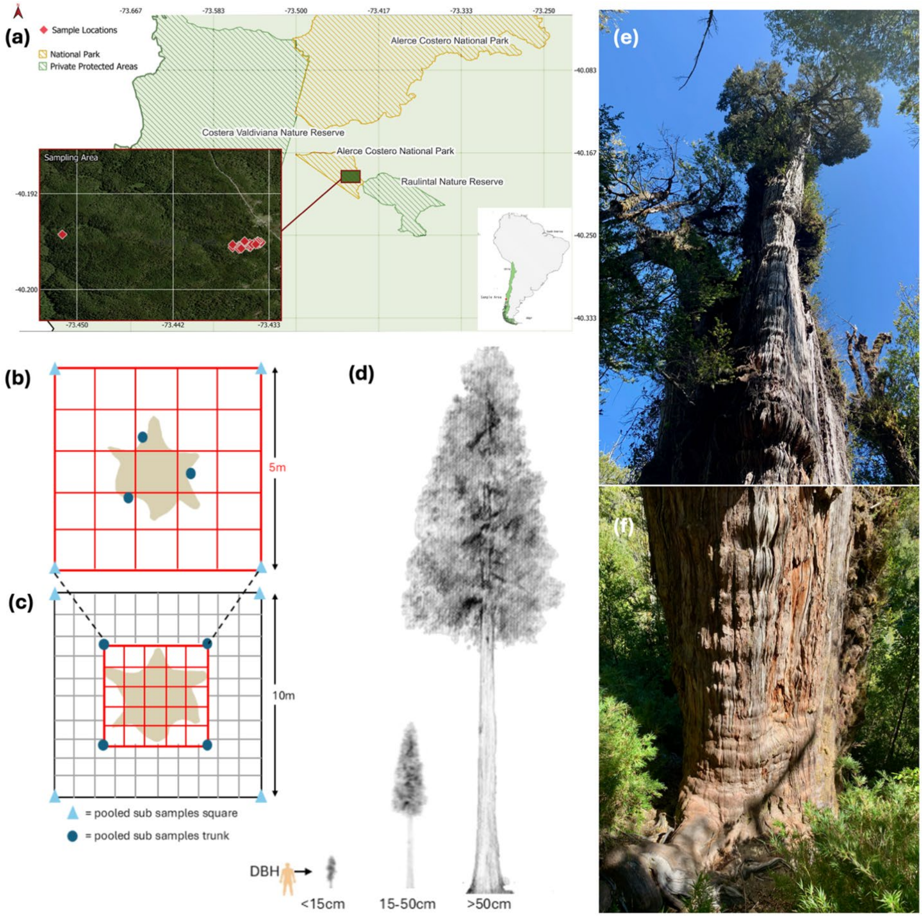

Fig. 1 **(a)** Map of the study area in the Alerce Costero National Park (Chile) showing the Alerce individuals sampled in red. Diagram of the soil sampling scheme **(b)** for each focal tree, one composite soil sample made of three soil cores around the base of the tree (trunk), and one composite soil sample made of four soil cores taken at each corner of a 5 m square around the tree (square); and **(c)** for the Alerce Abuelo tree, two composite soil samples made of four soil cores taken at each corner of a 5 m square and 10 m square, respectively. **(d)** Alerce age classes defined based on diameter by height (DBH): saplings < 15 cm, medium trees 15–50 cm, large trees > 50 cm. Cartoon by M. Woodward. **(e)–(f)** The Alerce Abuelo, a millennial tree of at least 2400 years old. Photos by R. Urrutia-Jalabert

which associate with ericoid mycorrhizal fungi (Urrutia-Jalabert et al. 2015a; Marín 2018; Godoy and Marín 2019). The EcM trees *Nothofagus nitida*, *N. betuloides* and their hybrids are also present at the site at abundances lower than 15% of the total tree density (Marín et al. 2023).

We collected topsoil beneath 31 Alerce individuals and grouped them into age classes based on tree DBH (Fig. 1; Table 1), i.e. nine saplings (DBH = 1.5–12.3 cm), twelve medium trees (DBH = 16.6–35.6 cm) and ten large trees (DBH = 62.8–470 cm, including the iconic Alerce Abuelo tree). Alerce individuals were selected along the park's main trail over an area of approximately 2 ha at 940 m of elevation, while the Alerce Abuelo tree was located

Table 1 Mean and standard error of observed fungal richness (SSU ASV for AM fungi, ITS2 OTUs for soil and ECM fungi), edaphic variables and tree measurements across Alerce age classes

	Saplings	Medium trees	Large trees
Soil fungal richness	239.83 ± 36.57*a*	235.04 ± 52.95*a*	326.15 ± 108.35*b*
AM fungal richness	43.94 ± 14.11*a*	47.76 ± 12.69*a*	54.17 ± 14.17*b*
ECM fungal richness	36.72 ± 10.75	39.74 ± 13.40	41.10 ± 12.57
Soil pH	4.12 ± 0.33	3.99 ± 0.22	4.01 ± 0.24
Available P [mg/kg]	7.09 ± 2.91	7.87 ± 5.01	6.75 ± 1.96
C: N ratio	20.44 ± 4.74	19.57 ± 3.89	20.35 ± 2.96
Tree DBH [cm]	5.86 ± 4.00*a*	27.02 ± 6.32*b*	131.86 ± 119.07*c*
Tree height [cm]	480.89 ± 390.48*a*	1873.91 ± 711.09*b*	2200.00 ± 707.85*c*
Biomass [ton]	N/A	0.14 ± 0.07*a*	8.56 ± 19.59*b*

Significant differences are indicated with letters in italic, based on generalized linear models with a Poisson (for richness) or gamma (for edaphic and tree variables) distribution, and pairwise comparisons using Tukey HSD post-hoc tests

1.3 km away at an elevation of 680 m. For each focal tree, we collected one composite soil sample made of three soil cores around the base of the tree, and one composite soil sample made of four soil cores taken at each corner of a 5 m square around the tree. Because the DBH of the Alerce Abuelo almost reached 5 m, the two Alerce Abuelo samples were collected as composite soil samples made of four soil cores taken at each corner of a 5 m square and 10 m square, respectively. Each soil core (5 cm diam. x 10 cm depth) was collected after removing the litter from the soil surface. A total of 62 samples were stored in plastic bags and kept refrigerated with ice packs in a cooler for a max. of 24 h before reaching the Soil Ecology Laboratory of the Universidad Santo Tomás in Máfil, Chile. Samples were stored at −80 °C until DNA extraction.

Tree measurements and edaphic variables

We measured diameter at breast height (DBH) using a diameter tape and tree height with a clinometer. Tree volume was calculated using an equation developed specifically for Alerce individuals from the Coastal Range of Chile, using DBH as the sole independent variable (Urrutia-Jalabert et al. 2015b):

$$Volume\ (m3) = 0.0002 \times\ DBH^{2.18308}$$

Biomass was then inferred as:

$$Biomass\ (ton) = Volume \times\ 411\frac{kg}{m^3}\,(wood\,density\,of\,trunk)$$
$$\times\ 1.066\ (biomass\,expansion\,factor\,for\,branches)$$

It is important to note that this equation is specific to the Coastal Range of Chile and differs for the Andean populations of Alerce. The Alerce Abuelo tree was a strong outlier in our dataset, with a DBH (470 cm) more than three times larger than the DBH of the second largest tree in our dataset (150 cm), and biomass (65.679 ton) more than twelve times larger than the biomass of the second largest tree in our dataset (5.428 ton). On the other hand, the Alerce Abuelo tree height (30 m) was within the range of other large trees that we measured (mean = 22 m, Table 1).

Soil pH was analyzed using a pH-meter in water, percentage organic carbon (C) by oxidation with sodium dichromate sulfuric acid, percentage total nitrogen (N) by Kjeldahl digestion, and available phosphorus (P) with Olsen extraction. These analyses were carried out in the Laboratorio de Nutrición y Suelos Forestales of the Austral University of Chile in Valdivia, Chile.

Soil metabarcoding

Genomic DNA was extracted from soil samples using the DNeasy PowerSoil Pro kit (Qiagen) according to the manufacturer's protocol. The ITS2 region was amplified with primer set ITS3/ITS4 (Tedersoo and Lindahl 2016) in a reaction volume containing: 10µL 2x KAPA HiFi HotStart DNA Polymerase Ready Mix (KAPA Biosystems), 0.75µL of each primer (10µM), 3.5µL nuclease free water, and 5µL DNA template. Following initial 2 min denaturation at 98 °C, thermocycler conditions for 30 cycles were as follows: denaturation at 98 °C for 20 s; annealing at 55 °C for 20 s; and extension at 72 °C for 30 s, followed by final extension at 72 °C for 5 min. The SSU region was amplified with AM fungi specific primer set WANDA/AML2 (Lee et al. 2008; Dumbrell et al. 2011) with the same conditions as for ITS2, except for 35 cycles with annealing at 55 °C for 30 s and extension at 72 °C for 20 s. Amplicons were cleaned up with 1x AMPure beads (Beckman Coulter), indexed with Illumina UD Indexes following the manufacturer's protocol, pooled at equimolar concentration, and sequenced using an Illumina NextSeq 2000 (2 × 300 paired-end chemistry) at Scripps Research, San Diego CA, USA.

ITS2 and SSU raw reads were processed into fungal operational taxonomic units (OTUs) at 97% sequence similarity for ITS2, and amplicon sequence variants (ASVs) for SSU, using LotuS2 v2.34 (Özkurt et al. 2022). For ITS2 data, VSEARCH was used for OTU clustering, and for SSU data DADA2 was used for the production of ASVs, both implemented within LotuS2. The taxonomy of ITS2 OTUs was assigned against UNITE v10 (Abarenkov et al. 2024) using *blastn* in LotuS2. The taxonomy of SSU ASVs was assigned by first discarding non-AM fungi ASVs using an edited version of SILVA v138.2 (Quast et al. 2013) with Glomeromycota sequences removed, then assigning taxonomy of the retained ASVs using MaarjAM (Öpik et al. 2010), with adjusted nomenclature based on 98% coverage and 97% similarity thresholds.

To compare how taxonomy assignments of AM and EcM fungi varied across databases, we also assigned the taxonomy of SSU ASVs and ITS2 OTUs using EUKARYOME v1.9.3 (Tedersoo et al. 2024a), with the custom database option in LotuS2. The resulting four datasets were used for downstream analyses: SSU dataset processed with MaarjAM, SSU dataset processed with EUKARYOME, ITS2 dataset processed with UNITE and ITS2 dataset processed with EUKARYOME. To assign mycorrhizal status, AM fungi were identified from the two SSU datasets by sub-setting ASVs with taxonomic assignment to the classes

Glomeromycetes, Archaeosporomycetes, and Paraglomeromycetes. EcM fungal OTUs were identified from the two ITS2 datasets based on generic level assignments in FungalTraits (Põlme et al. 2020).

Data analyses

All tests were carried out in R v4.4 (R Core Team 2024) with significance level indicated as follows: * ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001. Following initial processing in LotuS2 (Özkurt et al. 2022), ITS2 and SSU datasets were visualized in phyloseq (McMurdie and Holmes 2013) and putative contaminants were filtered out based on read occurrence in negative DNA extraction controls using decontam (Davis et al. 2018) with a cutoff threshold of 0.1. Following this step, OTUs and ASVs with less than 0.01% of the total read count per sample were removed to account for putative index switches, and samples with less than 500 reads were discarded.

We estimated fungal species richness by counting the number of ITS2 OTUs and SSU ASVs (observed richness) and visualized the number of recovered taxa per sample with species accumulation curves, using the 'random' method in vegan (Oksanen et al. 2025). We explored the number of ITS2 OTUs and SSU ASVs unique and shared between age classes (saplings, medium trees, large trees) with Euler diagrams. We correlated observed richness with age class, DBH, tree height, biomass, soil pH, C:N ratio and available P using generalized linear models (GLMs) with a Poisson (count data) distribution. We also compared edaphic variables across age classes using GLMs with a Gamma (continuous variables) distribution. Pairwise comparisons were performed using Tukey HSD post-hoc tests. To account for the fact that the Alerce Abuelo tree was a strong outlier in our dataset, all tests were performed both including and excluding the two soil samples collected beneath the Alerce Abuelo tree.

We calculated Bray-Curtis dissimilarity matrices, based on the relative abundances of soil fungal OTUs (ITS2 region), AM fungal ASVs (SSU region), and EcM fungal OTUs (ITS2 region) present in at least two samples. We plotted the ordination of fungal community composition according to soil pH, C:N ratio, available P, age class, DBH, tree height and biomass using distance-based redundancy analysis (dbRDA) in vegan (Oksanen et al. 2025). Significant predictors were selected based on p-values and adjusted coefficients of determination (R2adj). We tested the homogeneity of multivariate dispersions among age classes using beta-dispersion tests with 999 permutations and pairwise comparison according to Tukey's HSD tests. We also estimated positive and negative associations of DBH with soil fungal genera (ITS2 region) and AM fungal ASVs (SSU region) based on log fold changes of relative abundances in ANCOM-BC2 (Lin and Peddada 2024), after adjusting for covariates based on significant predictors from the dbRDA. Significant associations were tested by performing 100 bootstrap replicates and p-values adjusted using the Holm-Bonferroni method to account for multiple testing. All tests were performed both including and excluding the two outlier soil samples collected beneath the Alerce Abuelo tree.

Results

Taxonomy and mycorrhizal guild assignment

We obtained average per sample sequencing depths of roughly 383,000 reads for ITS2 amplicons and 234,000 reads for SSU. Species accumulation curves were generally saturated, suggesting that we adequately sampled fungal diversity (Fig. 2c–d).

The number of SSU ASVs identified as AM fungi varied depending on the database used for taxonomy assignment: We recovered 149 AM fungal ASVs and four genera using MaarjAM, while using EUKARYOME, we recovered 160 AM fungal ASVs and 6 genera, including three provisional genera within Glomeraceae (Fig. 2a). Specifically, the taxonomic differences between these two datasets depends on the nomenclature that was used in each case. Here, we used a version of MaarjAM with the nomenclature adjusted using the species list found at http://www.amf-phylogeny.com/, as carried out by the GlobalAMFungi

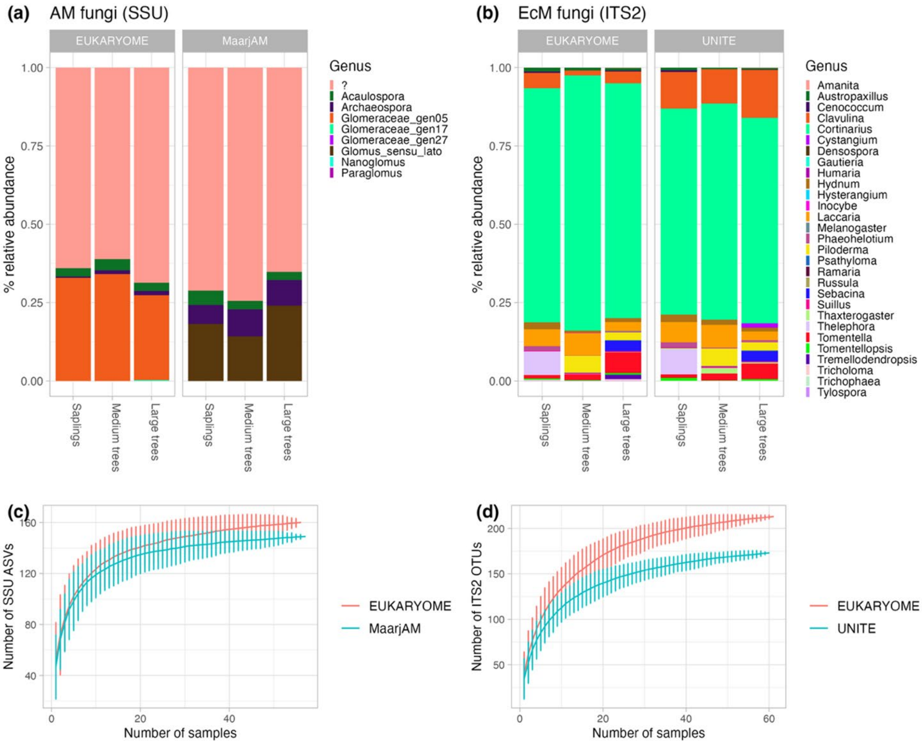

Fig. 2 Relative abundances of **(a)** arbuscular mycorrhizal (AM) fungal and **(b)** ectomycorrhizal (EcM) fungal genera per age class, with taxonomic classification using EUKARYOME, in comparison with MaarjAM (SSU) and UNITE (ITS2), respectively. Based on species accumulation curves representing the number of **(c)** AM fungi ASVs and **(d)** EcM fungal OTUs per sample, EUKARYOME retrieved a higher number of taxa and mycorrhizal fungal genera than MaarjAM (SSU) and UNITE (ITS2), respectively. The genera 'Glomeraceae_gen05, 17, and 27' are provisional genus-level taxa in EUKARYOME (Tedersoo et al. 2024a) while the taxonomy-adjusted version of MaarjAM uses '*Glomus_sensu_lato*' as a single genus-level category (Öpik et al. 2010; Větrovský et al. 2023). The recently described genus *Nanoglomus* (Corazon-Guivin et al. 2019) is included in EUKARYOME, but is absent from MaarjAM, as this database is not presently being updated

database (Schüßler and Walker 2010; Větrovský et al. 2023). This taxonomy merged all Glomus VTs with no species-level assignment (*n*=254 in our dataset) as '*Glomus_sensu_lato'*. On the other hand, EUKARYOME provides provisional genera *sensu* Tedersoo et al. *(2024)*, i.e. 'Glomeraceae_gen05, 17, and 27 (n=587 in our dataset), in addition to *Glomus sensu stricto*. Additionally, the recently described genus *Nanoglomus* (Corazon-Guivin et al. 2019) is included in EUKARYOME, but is absent from MaarjAM, as this database is not presently being updated.

The ITS2 dataset retrieved a similar number of fungal OTUs using UNITE or EUKARYOME (*n*=2522 and 2523, respectively). However, EUKARYOME detected 213 OTUs belonging to 28 genera as EcM fungi, compared to 173 OTUs belonging to 25 genera using UNITE (Fig. 2b). We therefore performed downstream ecological analyses based on taxonomic identification using EUKARYOME for both the SSU and ITS2 datasets. However, the relationship of fungal richness and community composition with Alerce age class, tree measurements and edaphic variables that we tested gave similar results when using MaarjAM (SSU dataset) and UNITE (ITS2 dataset) for taxonomy identification (data not shown).

Soil and mycorrhizal fungal richness

Out of 2523 soil fungal OTUs recovered using ITS2, 635 (25%) were shared among all age classes, while 761 (30%) were unique to large trees (Suppl. Fig. S1). The higher soil fungal diversity detected beneath large trees was strongly driven by the two Alerce Abuelo samples that harbored 361 unique fungal OTUs. When the two Alerce Abuelo samples were excluded, the number of fungal OTUs unique to large trees was reduced to 18% (400 out of 2162). Mycorrhizal diversity was in the most part shared between Alerce age classes, with 107 out of 160 (66%) AM fungal ASVs, and 120 out of 213 (56%) ECM OTUs shared between all Alerce age classes (Suppl. Fig. S1).

Based on GLMs and pairwise Tukey HSD post-hoc tests, we detected a significantly higher soil fungal richness (observed richness of ITS2 OTUs) beneath large trees than medium trees (z=17.811***) and saplings (z=15.668***, Fig. 3a). Notably, soil fungal richness was up to 2.26 times higher beneath the Alerce Abuelo tree than the mean richness per sample (Table 1; Fig. 3a). AM fungal richness (observed richness of SSU ASVs) was significantly higher beneath large trees than medium trees (z=2.798*) and saplings (z=4.230***, Table 1; Fig. 3a) and was up to 1.75 times higher beneath the Alerce Abuelo tree than the mean richness per sample (Table 1; Fig. 3b). On the other hand, EcM fungal richness (observed richness of ITS2 EcM OTUs) was not significantly different across Alerce age classes (Table 1; Fig. 3c). Tests excluding the two Alerce Abuelo outlier samples yield highly similar results, apart from the fact that AM fungal richness was significantly higher beneath large trees than saplings (z=3.093**), but not medium trees (Suppl. Fig. S2, Table S1).

Soil fungal richness (observed richness of ITS2 OTUs) positively and significantly correlated with tree DBH (z=31.36***), biomass (z=27.74***) and height (z=13.86***), and those correlations remained significant when excluding the two Alerce Abuelo outlier samples (Fig. 4a). Additionally, AM fungal richness (observed richness of SSU ASVs) positively and significantly correlated with tree DBH (z=6384***), biomass (z=5.331***) and height (z=2.593**), however, only DBH (z=3.342***) and biomass (z=2.794**)

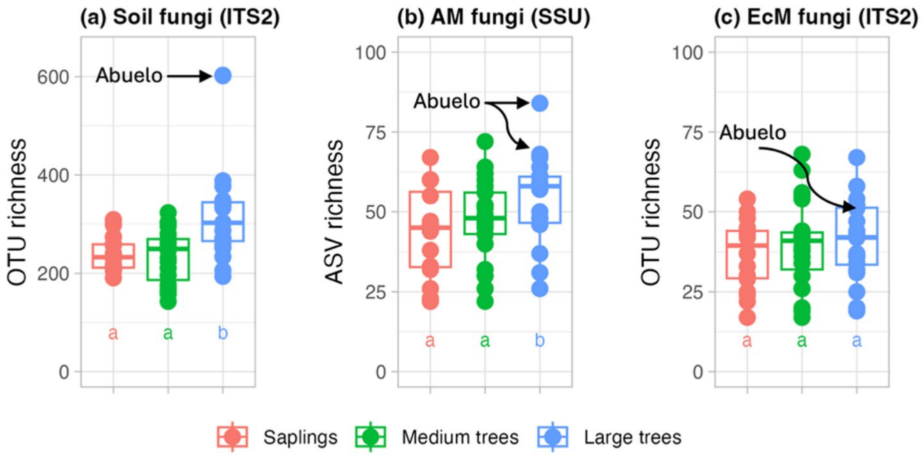

Fig. 3 Observed richness of **(a)** soil fungal OTUs, **(b)** arbuscular mycorrhizal (AM) fungal ASVs, and **(c)** ectomycorrhizal (EcM) fungal OTUs across Alerce age classes (saplings, medium trees and large trees). Based on generalized linear models with a Poisson distribution and pairwise comparisons with Tukey HSD post-hoc tests, large trees showed a significantly higher soil fungal richness than medium trees (z=17.811***) and saplings (z=15.668***), and a significantly higher AM fungal richness than medium trees (z=2.798*) and saplings (z=4.230***), while EcM fungal richness was not significantly different across Alerce age classes. Soil fungal richness was up to 2.26 times higher in soil samples collected beneath the Alerce Abuelo tree compared to the mean richness per sample, while AM fungal richness was up to 1.75 times higher (black arrows)

remained significant when excluding the two Alerce Abuelo outlier samples (Fig. 4b). The positive and significant correlation between the observed richness of ECM fungal OTUs (ITS2 region) with tree DBH (z=2.454*), biomass (z=2.118*) and height (z=2.494*) were clearly driven by the Alerce Abuelo tree, as none of these correlations remained significant when excluding the two Alerce Abuelo outlier samples (Suppl. Fig. S3).

Regarding edaphic variables, we didn't find any significant differences in soil pH, C:N ratio and available P across Alerce age classes, both when including (Table 1) and excluding (Table S1) the two Alerce Abuelo outlier samples. Soil fungal richness (observed richness of ITS2 OTUs) correlated positively with soil pH (z=3.433***) and negatively with available P (z=–8.886***), and those correlations remained significant when excluding the two Alerce Abuelo outlier samples, except that the relationship with soil pH became negatively correlated (Fig. 5a). AM fungal richness (observed richness of SSU ASVs) correlated negatively with soil available P both when including (z=–6.992***) or excluding (z=6.578***) the two Alerce Abuelo outlier samples, while a negative correlation with soil pH (z=2.818**) was only observed when two Alerce Abuelo outlier samples were excluded (Fig. 5b). We did not observe any correlation between fungal richness and C: N ratio, as well as between EcM fungal richness (observed richness of ITS2 EcM OTUs) and any edaphic variables (data not shown).

Fungal community composition

Available P (F=4.403***) and tree height (4.025***) significantly predicted the community composition of soil fungi (Fig. 6a). Available P (F=14.780***) was also the best predic-

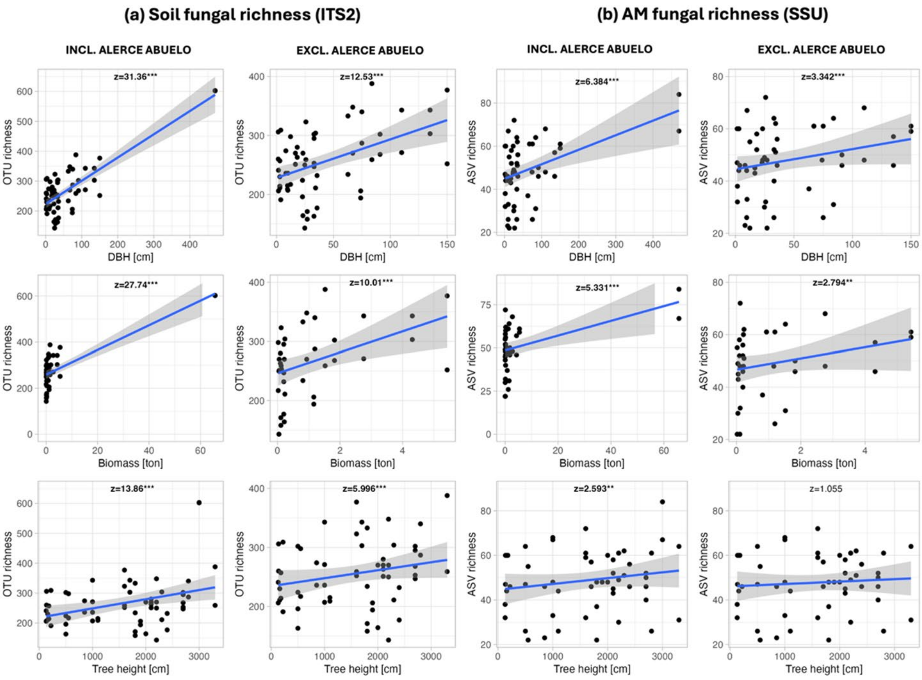

Fig. 4 Correlations between the observed richness of **(a)** soil fungal OTUs (ITS2 region) and **(b)** arbuscular mycorrhizal (AM) fungal ASVs (SSU region) with the tree's diameter at the breast height (DBH), biomass and height, based on generalized linear models (glm) with a Poisson distribution. Significant correlations are in bold. Soil and AM fungal richness significantly correlated with DBH and biomass, when including or excluding the two Alerce Abuelo outlier samples, while the correlation of AM fungi with tree height remained non-significant when excluding the two Alerce Abuelo samples. Gray bars in plots display the 95% confidence intervals from the glm and significant correlations are indicated in bold

tor of AM fungal community composition, followed by tree DBH (F = 4.144**) and height (F = 3.428*, Fig. 6b). None of the variables studied significantly correlated with EcM fungal community composition (Fig. 6c). Age class did not significantly predict fungal community composition and Beta-dispersion tests did not detect any significant differences in the homogeneity of multivariate dispersions among age classes, indicating that the variation in fungal beta-diversity was similar across age classes. Correlations excluding the two Alerce Abuelo outlier samples yield highly similar results, apart from the fact that AM fungal richness correlated significantly with available P (F = 14.816***) and tree height (F = 3.428*), but not DBH (Suppl. Fig. S4).

Out of a total of 207 fungal genera with OTUs present in at least two samples in the ITS2 dataset, 29 of them showed a differential abundance according to the tree's DBH (Fig. 7a), based on log fold changes after adjusting correlations for other significant predictors of AM fungal community composition, i.e. available P and tree height (Fig. 6a). When looking specifically at AM fungi (SSU dataset), 32 ASVs out of a total of 144 ASVs present in at least two samples, showed a differential abundance according to the tree's DBH (Fig. 7b), based on log fold changes after adjusting correlations for other significant predictors of AM fungal community composition, i.e. available P and tree height (Fig. 6b). However, only 7 fungal genera (ITS2 dataset) and 2 AM fungal ASVs (SSU dataset) showed a differen-

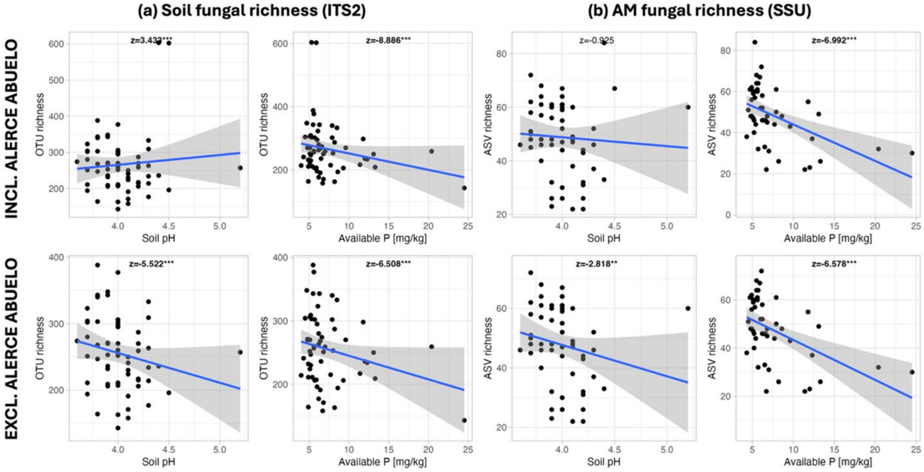

Fig. 5 Correlations between the observed richness of **(a)** soil fungal OTUs (ITS2 region) and **(b)** arbuscular mycorrhizal (AM) fungal ASVs (SSU region) soil pH and available phosphorus (P) based on generalized linear models (glm) with a Poisson distribution. Soil pH and available P negatively and significantly correlated with soil and AM fungal richness, except for a positive correlation of soil pH with soil fungal richness and a non-significant correlation with AM fungal richness when including the two Alerce Abuelo outlier samples. Gray bars in plots display the 95% confidence intervals from the glm and significant correlations are indicated in bold

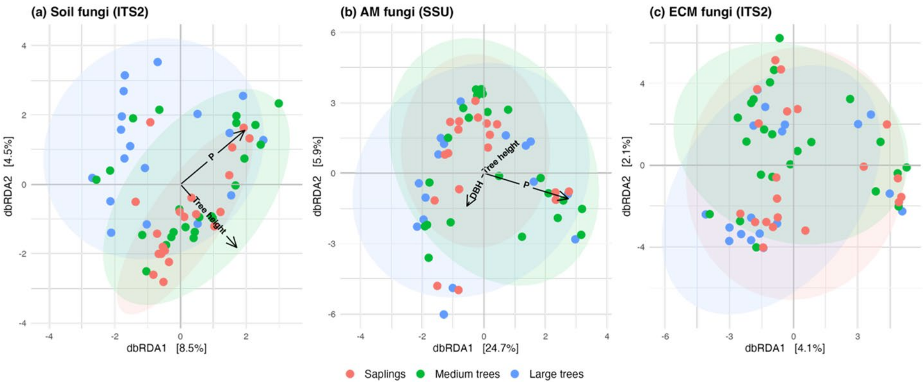

Fig. 6 Distance-based redundancy analysis (dbRDA) visualizing the community composition of **(a)** soil fungi, **(b)** arbuscular mycorrhizal (AM) fungi, and **(c)** ectomycorrhizal (EcM) fungi. Based on adjusted coefficients of determination (R2adj), available phosphorus (P, F=4.403***) and tree height (F=4.025***) significantly predicted soil fungal communities. Available P was also the best predictor of AM fungal community composition (F=14.780***), followed by the tree's diameter at breast height (DBH, F=4.144**) and tree height (F=3.428*). EcM fungi community composition did not correlate significantly with any of the variables studied

tial abundance according to the tree's DBH after excluding the two Alerce abuelo outlier samples (Suppl. Fig. S5).

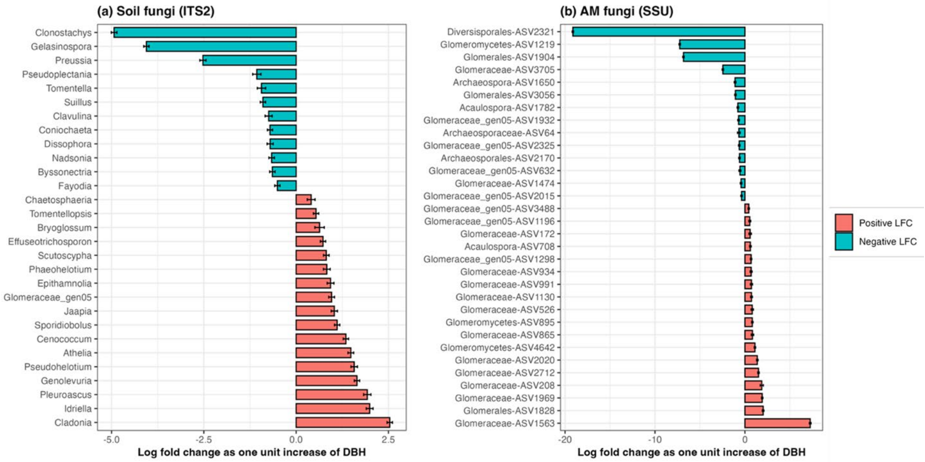

Fig. 7 Positive or negative association of **(a)** soil fungal genera and **(b)** arbuscular mycorrhizal fungal ASVs with the tree's diameter at breast height (DBH), based on log fold changes of relative abundances in ANCOM-BC2 (Lin and Peddada 2024) after adjusting for other significant predictors, i.e. available P and tree height (Fig. 6). Only significant associations are presented here based on 100 bootstrap replicates and adjusted p-values for multiple comparisons calculated using the Holm-Bonferroni method. Bars indicate standard errors

Discussion

The importance of millennial and large-diameter trees for soil fungal diversity in forests

Our study highlights the importance of large-diameter trees for structuring and conserving fungal diversity in forest soils, including arbuscular mycorrhizal fungi. We found that soil fungal richness was up to 2.26 times higher than the mean richness per sample in soil collected beneath the Alerce Abuelo tree, with 361 fungal OTUs unique to those samples, while AM fungal richness was up to 1.75 times higher (Fig. 3, Suppl. Fig. S1). The Alerce Abuelo is a monumental tree with a DBH of 470 cm that is considered as one of the oldest living trees in the world (Lara and Villalba 1993; Barichivich and Lara, pers. comm.). Its unique soil and AM fungal communities were further illustrated by the 29 fungal genera and 32 AM fungal ASVs showing a differential abundance according to tree DBH (Fig. 7) that were drastically reduced to 7 fungal genera and 2 AM fungal ASVs after excluding the two Alerce Abuelo samples (Suppl. Fig. S5). Despite the limitation of our sampling size due to the rarity of millennial Alerce trees, however, most of the relationships that we tested remained significant when we excluded the two Alerce Abuelo outlier samples from our dataset. For example, soil and AM fungal richness remained positively correlated with age class, DBH and tree biomass (Figs. 3 and 4, Suppl. Fig. S2), and negatively correlated with available P (Fig. 5) both when including and excluding the Alerce Abuelo samples. These results further demonstrate the critical role of millennial Alerce as an umbrella species for the conservation of soil and mycorrhizal fungal biodiversity in the forests of the Chilean Coastal Range, emphasizing the importance for their urgent priority conservation.

Previous studies showed that large-diameter trees can disproportionally contribute to aboveground and belowground carbon stocks in forests (Urrutia-Jalabert et al. 2015b; Lutz et al. 2018; Ali et al. 2019; González et al. 2022) and promote microbial diversity in soils (Carey et al. 2020). This is further supported by our study that found a positive correlation of soil and AM fungal richness with tree DBH and biomass (Fig. 4). In EcM dominated forests, large-diameter ECM trees can disproportionately impact mycorrhizal fungal diversity, with distance to the nearest large-diameter tree being a strong predictor of EcM fungal richness (Birch et al. 2023), and EcM fungal community composition varying with tree age (Birch et al. 2021). Our study substantiates that large-diameter trees play similar roles in the AM-dominated forests in Southern Chile. Tree height was likewise a significant predictor of soil and AM fungal community composition, in addition to available P and tree DBH in the case of AM fungi (Fig. 5). Large trees tend to invest more in enhancing their root system than small-diameter trees (Li et al. 2019), resulting in a higher density of active fine roots for mycorrhizal diversity and root colonization. This was demonstrated by the higher soil and AM fungal richness beneath large trees (Fig. 3). These results illustrate how tree size can affect AM fungal diversity and structure in similar ways to EcM systems. Future research tracking the trajectories of AM fungal communities in fine root systems of forest trees as they age would provide additional evidence for host-driven habitat filtering, as previously observed in crop systems (Frew et al. 2025).

Biodiversity loss can strongly impair ecosystem functioning, since many ecological processes rely on high species diversity across multiple trophic levels (Soliveres et al. 2016). In particular, decline in soil fungal diversity can trigger cascading negative effects on multiple ecosystem functions, including nutrient cycling and primary productivity (Delgado-Baquerizo et al. 2020; Xu et al. 2025). Forest restoration strategies that consider mycorrhizal fungi have so far focused mostly on EcM associations and growth effects in the early life stage of trees (Policelli et al. 2020; Anthony 2025). Our results illustrate how the selective preservation of large-diameter individuals, that act as ‘umbrella’ of soil and mycorrhizal fungal biodiversity, can potentially contribute to forest regeneration in AM-dominated forests. Many AM fungi are generalist species that can associate with a broad range of host plants, including understory plants (Marín 2018). This is in contrast to EcM fungi that associate more specifically with trees in forests. Approximately 2/3 of the vascular plant diversity of Alerce forests associates with AM fungi (Godoy and Marín 2019). A loss of AM fungal richness, for example due to nutrient enrichment, is likely to negatively impact plant diversity and ecosystem multifunctionality (Ma et al. 2021). Therefore, conserving AM fungal diversity, by preserving old and large-diameter individuals, may benefit understory plant communities by giving them access to a more diverse pool of AM fungal partners. Soil inoculation can increase the abundance of mycorrhizal fungi in degraded ecosystems (Maltz and Treseder 2015), although these approaches can be difficult to manipulate at large scale. AMF inoculations from native soils have shown promising results for the restoration of other endangered native Chilean conifers, such as *Araucaria araucana* (Godoy et al. 1993, 2023), and similar strategies could be tested in Alerce forests. Nevertheless, it is important to evaluate the impact of such approaches on the native site before translocating soil, and more viable alternatives that preserve existing millennial trees, or islands of old-growth trees, should be considered in priority. For example, in productive tree species, keeping patches of mature trees from logging can favor the establishment of new seedlings near those patches, therefore reducing logging impacts (Sterkenburg et al. 2019). The disproportionately large role played by old and large-diameter trees for maintaining biodiversity and species interactions above and belowground (Lindenmayer and Laurance 2017; Tejo and Fontúrbel 2019; Ali and Wang 2021) emphasize the need for priority conservation of millennial trees, as we demonstrated here. Particularly, old-growth Alerce forests should be prioritized for protection as part of the 30 × 30 goals of the Kunming–Montreal Global Biodiversity Framework. Most of Alerce populations are not currently lying within protected areas and are threatened by illegal logging, a high degree of habitat fragmentation due to projected infrastructure and land subdivision, as well as threats from climate change and subsequent wildfires (Bannister et al. 2020; Urrutia-Jalabert et al. 2023).

Available phosphorus negatively correlates with AM fungal diversity and best predicts AM fungal community composition in Alerce forest soils

Soils in Alerce forests of the Coastal Range are very poor in nutrients due to heavy weathering from the high rainfall (Godoy et al. 2013). Therefore, this ecosystem is particularly prone to P limitations. We found that samples with lower soil available P showed a significantly higher richness of AM fungi at our study site (Fig. 5). AM fungi are well-known to alleviate the effect of soil nutrient deficiencies by enhancing plant P uptake, with profound effects on plant performance (Johnson et al. 2010; Augusto et al. 2017; Qiu et al. 2022; Wu et al. 2024). Available P was also the best predictor of AM fungal community composition (Fig. 6), illustrating the importance of P availability in shaping mycorrhizal interactions with Alerce, and the putative roles AM fungi may play for P uptake in the temperate rainforests of southern Chile. Available P also negatively correlated with the richness of the total soil fungal community (Fig. 5) and was the best predictor of soil fungal community composition (Fig. 6). This suggests that other fungal guilds besides AM fungi likely contribute to alleviate P limitations in this system. Native conifer forests in southern Chile harbor a high diversity and abundance of saprotrophic fungal communities (Marín et al. 2023) that generally tend to be shaped by microbial interactions, litter and substrate quality (Bödeker et al. 2016; Fernandez et al. 2020; Fang et al. 2020). These results highlight the complex relationship between mycorrhizal and non-mycorrhizal fungal communities, and the way these interactions are shaped by environmental conditions or the presence of neighboring hosts (Fernández et al. 2022). AM trees generally tend to have more rapid C and nutrient turnover rates than EcM trees, but the underlying mechanisms driving soil processes in AM-dominated coniferous forests are not fully understood (Zheng et al. 2023). It was recently proposed to include soil environmental parameters, such as soil fertility, when evaluating the conservation value of forests, to better take into account soil fungal diversity in management plans (Kyaschenko et al. 2025). Similarly, our results show that soil fertility, such as available P levels, is a valuable predictor of soil and mycorrhizal fungal diversity that could be used in conservation value assessments of Chilean temperate rainforests. Future experimental studies testing the relative effects of nutrient enrichment on soil microbes would also enable to better predict the adaptability of Alerce to environmental change.

EUKARYOME identifies more mycorrhizal genera and ASVs/OTUs than MaarjAM and UNITE

The EUKARYOME database represents a significant development for fungal metabarcoding studies, by combining the three commonly used rDNA markers, SSU, ITS, and LSU, as well as morphological data and environmental DNA (eDNA) sequences in a phylogenetic framework (Tedersoo et al. 2024a; 2024b). The MaarjAM database has the advantage of being highly curated, but is not currently being updated, yet EUKARYOME provides regular updates that include newly described AM fungal taxa, as well as long read eDNA sequences, which can result useful for identifying 'dark' AM fungi from the soil.

Our study aimed to examine whether the EUKARYOME and MaarjAM databases performed similarly for the taxonomic assignment of AM fungi. Based on our SSU dataset, EUKARYOME recovered a slightly larger number of AM fungal ASVs compared to MaarjAM (7% increase). EUKARYOME also provided updated taxonomic assignment for ASVs belonging to unnamed, provisional genera *sensu* Tedersoo et al. (2024b) within Glomeraceae, as well as recently described species and genera that are absent from MaarjAM (Fig. 2a). It should be noted that ITS and LSU rDNA sequences can be highly variable within and between closely related AM species, which may result in phylogenetic noise and species being split as distinct novel taxa, due to their infra-specific genetic variability (Stefani et al. 2025). As increasing evidence becomes available from eDNA sequencing to assess AM fungal diversity, these provisional genera may therefore need reassessment for a more accurate taxonomic assignment of AM fungi.

Regarding the taxonomic assignment of EcM fungi, we recovered a larger number of EcM fungal OTUs using EUKARYOME in our ITS2 dataset (19% increase) compared to UNITE (Fig. 2b). This is likely due to the fact that EUKARYOME assigned a higher number of ITS2 OTUs at generic level in our ITS2 dataset, including OTUs from the genera *Gautieria*, *Melanogaster*, *Tremellodendropsis*, and *Tylospora* that were not recovered using UNITE, therefore enabling the guild assignment of more OTUs as EcM fungi using FungalTraits (Põlme et al. 2020). These promising results indicate that future metabarcoding studies should consider the use of EUKARYOME for the taxonomic assignment of mycorrhizal taxa.

Conclusions

Large-diameter, monumental trees, such as those found in Alerce forests, are among the most iconic biota on Earth. They significantly impact aboveground and belowground C stocks, nutrient and water fluxes, as well as entire communities of organisms, including fungi (Tejo and Fontúrbel 2019; Yuan et al. 2020; Ali and Wang 2021; Birch et al. 2023). Our study demonstrated that the richness and community composition of soil and AM fungi correlated significantly with Alerce DBH, height and/or biomass in the forests of the Chilean Coastal Range. Soil beneath the iconic Alerce Abuelo tree, one of the oldest trees on Earth, hosted a unique and particularly diverse soil fungi and mycorrhizal fungal richness, emphasizing the role of millennial trees as umbrella species for fungal diversity in soils. Available P was a strong predictor of soil and AM fungal richness and community composition that could be used for conservation value assessments of Chilean temperate forests.

Taken together, our findings underscore the importance of preserving large-diameter and millennial trees as crucial contributors to soil biodiversity and functions in temperate rainforests. This is particularly relevant under the current threats that the species is facing due to illegal logging and habitat destruction (Urrutia-Jalabert et al. 2023), emphasizing its urgent priority conservation. Our study also emphasizes the need to consider AM and other soil fungi in forest management plans, comparably to efforts that have been conducted in EcM-dominated forests.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10531-026-03277-0.

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Author contributions All authors contributed to the study conception and design. Material preparation and data collection were performed by AC, MVN, JDS, RUJ, RG, MS, GF, ETK and CM. Analyses of data were performed by CT, AC and BM. The first draft of the manuscript was written by CT and AC. All authors commented on previous versions of the manuscript and approved the final manuscript.

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Data availability Raw sequence data are deposited in the NCBI Sequence Read Archive (SRA). All scripts used for bioinformatics analyses are available at https://github.com/SocietyProtectionUndergroundNetworks/alerce_amf_diversity, including custom database files, with adjusted nomenclature.

Declarations

Competing interests The authors declare no competing interests.

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Supplementary material for:

Large-diameter trees disproportionately contribute to soil fungal diversity in a coniferous forest with one of oldest living trees on Earth.

Submitted to Biodiversity and Conservation

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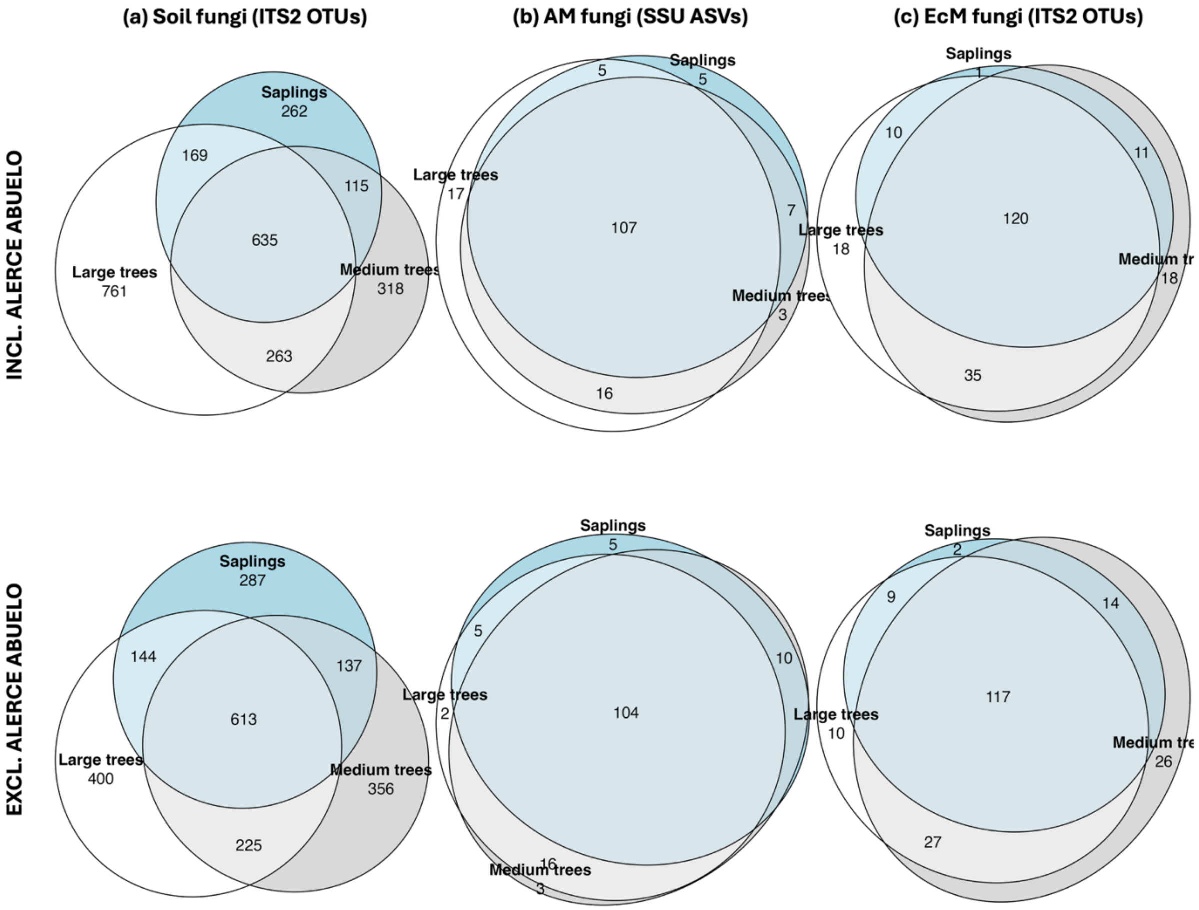
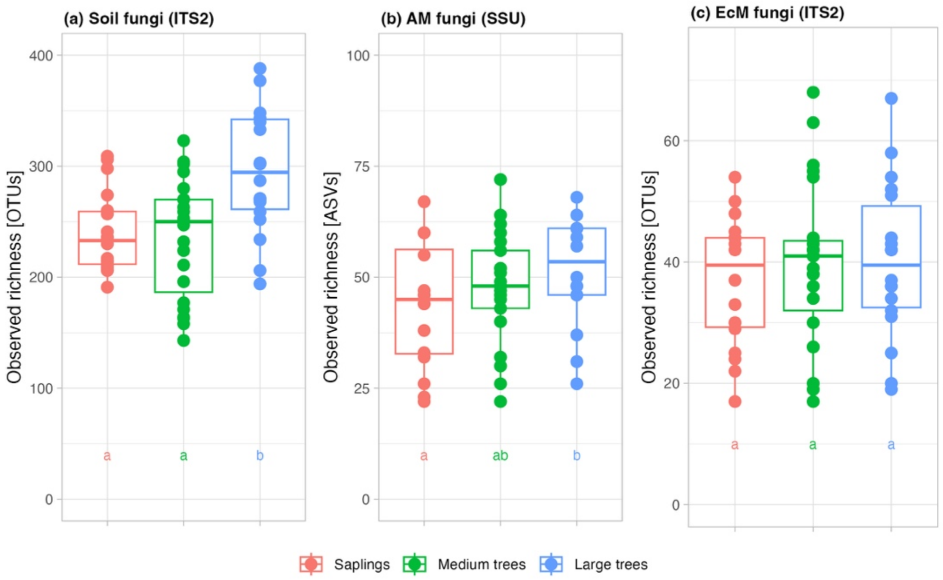
Table S1 Mean and standard error of observed fungal richness (SSU ASV for AM fungi, ITS2 OTUs for soil and ECM fungi), edaphic variables and tree measurements across Alerce age classes, after excluding the two Alerce Abuelo outlier samples. Significant differences are indicated with letters in italic, based on generalized linear models with a Poisson (for richness) or Gamma (for edaphic and tree variables) distribution, and pairwise comparisons using Tukey HSD post-hoc tests.

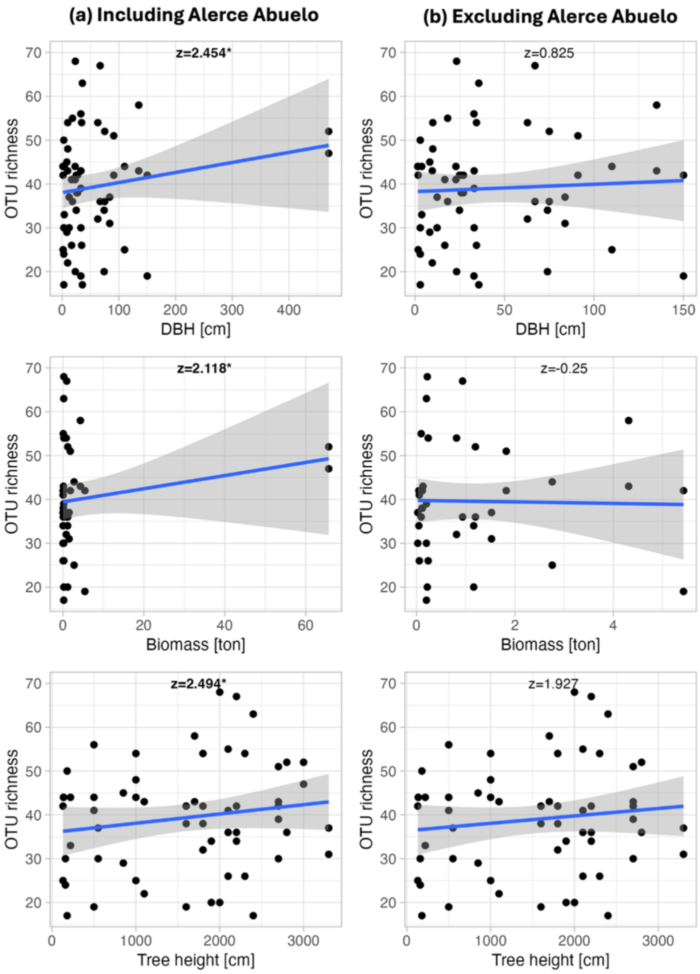
	SAPLINGS		MEDIUM TREES		LARGE TREES	
Soil fungal richness	239.83 ± 36.57	*a*	235.04 ± 52.95	*a*	295.44 ± 56.02	*b*
AM fungal richness	43.94 ± 14.11	*a*	47.76 ± 12.69	*ab*	51.50 ± 12.23	*b*
ECM fungal richness	36.72 ± 10.75		39.74 ± 13.40		40.17 ± 12.91	
Soil pH	4.12 ± 0.33		3.99 ± 0.22		3.96 ± 0.20	
Available P [mg/kg]	7.09 ± 2.91		7.87 ± 5.01		6.87 ± 2.03	
C:N ratio	20.44 ± 4.74		19.57 ± 3.89		20.22 ± 3.06	
Tree DBH [cm]	5.86 ± 4.00	*a*	27.02 ± 6.32	*b*	94.29 ± 30.00	*c*
Tree height [cm]	480.89 ± 390.48	*a*	1873.91 ± 711.09	*b*	2111.11 ± 690.17	*c*
Biomass [ton]	N/A		0.14 ± 0.07	*a*	2.22 ± 1.59	*b*



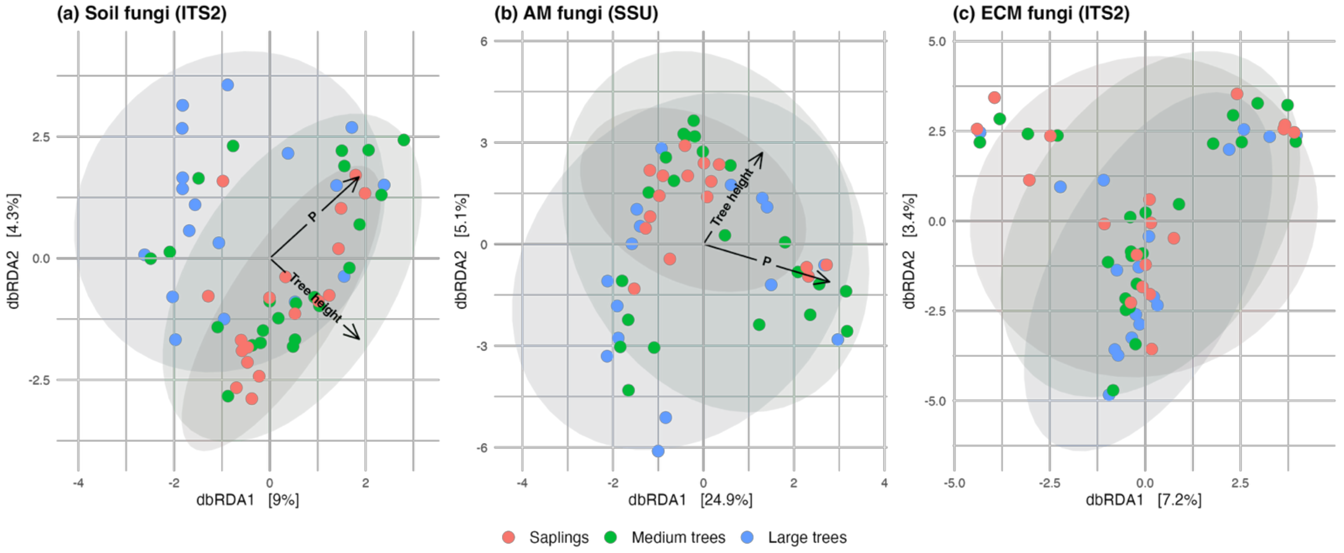
Suppl. Fig. S1 Euler diagrams showing the number of unique and shared **(a)** soil fungal OTUs, **(b)** arbuscular mycorrhizal (AM) fungal ASVs, and **(c)** ectomycorrhizal (EcM) fungal OTUs between Alerce age classes (saplings, medium trees and large trees) when including (above) or excluding (below) the two Alerce abuelo outlier samples.



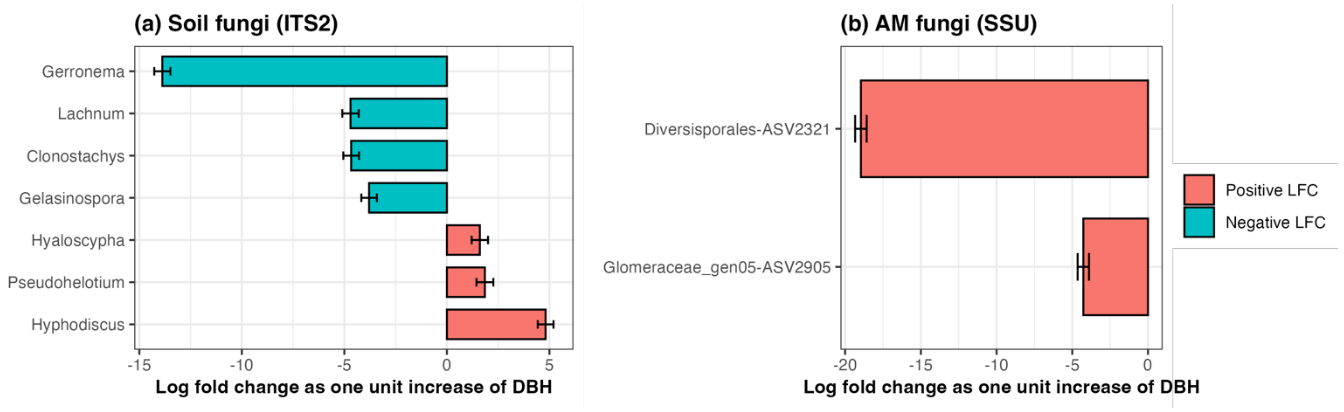
Suppl. Fig. S2 Observed richness of **(a)** soil fungal OTUs, **(b)** arbuscular mycorrhizal (AM) fungal ASVs, and **(c)** ectomycorrhizal (EcM) fungal OTUs across Alerce age classes (saplings, medium trees and large trees) after excluding the two Alerce abuelo outlier samples. Based on generalized linear models with a Poisson distribution and pairwise comparisons with Tukey HSD post-hoc tests, large trees showed a significantly higher soil fungal richness than medium trees (z=11.842***) and saplings (z=10.179***), and a significantly higher AM fungal richness than saplings (z=3.093**), while EcM fungal richness was not significantly different across age classes.



Suppl. Fig. S3 Correlations between the observed richness ECM fungal OTUs (ITS2 region) with the tree's diameter at the breast height (DBH), biomass and height, based on generalized linear models (glm) with a Poisson distribution, when **(a)** including and **(b)** excluding the two outlier Abuelo tree samples. Significant correlations are indicated in bold, showing that ECM fungal richness significantly correlated with DBH, biomass and height only when including the two Abuelo tree samples. Gray bars in plots display the 95% confidence intervals from the glm and significant correlations are indicated in bold.



Suppl. Fig. S4 Distance-based redundancy analysis (dbRDA) visualizing the community composition of **(a)** soil fungi, **(b)** arbuscular mycorrhizal (AM) fungi, and **(c)** ectomycorrhizal (EcM) fungi, after excluding the two Alerce Abuelo outlier samples. Based on adjusted coefficients of determination (R2adj), tree height (F=4.220***) and available P (P, F=4.104***) significantly predicted soil fungal communities. P (F=14.816***) was also the best predictor of AM fungal community composition, followed by tree height (F=3.428*). EcM fungi community composition did not correlate significantly with any of the variables studied.



Suppl. Fig. S5 Positive or a negative association of **(a)** soil fungal genera and **(b)** arbuscular mycorrhizal fungal ASVs with the tree’s diameter at breast height (DBH), based on log fold changes of relative abundances in ANCOM-BC2 (Lin and Peddada 2024), after excluding the two Alerce Abuelo outlier samples. Only significant associations are presented here based on 100 bootstrap replicates and adjusted p-values for multiple comparisons calculated using the Holm-Bonferroni methods, after adjusting for other significant predictors, i.e. available P and tree height (Fig. S3). Bars indicate standard errors.