Sapwood mycobiome vary across host, plant compartment and environments in *Nothofagus* forests from Northern Patagonia

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Abstract

Global forests are increasingly threatened by altered climatic conditions and increased attacks by pests and pathogens. The complex ecological interactions among pathogens, microbial communities, tree host, and environment are important drivers of forest dynamics. Little is known about the ecology of forest pathology and related microbial communities in temperate forests of the southern hemisphere. In this study, we used next-generation sequencing to characterize sapwood-inhabiting fungal communities in North Patagonian *Nothofagus* forests and assessed patterns of diversity of taxa and ecological guilds across climatic, site and host variables (health condition and compartment) as a contribution to *Nothofagus* autecology. The diversity patterns inferred through the metabarcoding analysis

were similar to those obtained through culture-dependent approaches. However, we detected additional heterogeneity and greater richness with culture-free methods. Host species was the strongest driver of fungal community structure and composition, while the host health status was the weakest. The relative impact of site, season, plant compartment and health status were different for each tree species; these differences can be interpreted as a matter of water availability. For *N. dombeyi*, which is distributed across a wide range of climatic conditions, site was the strongest driver of community composition. *Nothofagus pumilio*'s microbiome varied more with season and temperature, a relevant factor for forest conservation in the present climate change scenario. Both species carry a number of potential fungal pathogens in their sapwood, whether they exhibit symptoms or not. Our results provide an insight into the diversity of fungi associated with the complex pathobiome of the dominant *Nothofagus* species in southern south America.

***Keywords*:** temperate forests, forest decline, wood endophyte, latent pathogens, environmental DNA, metabarcoding

Introduction

The forest ecosystems have been affected by the climate change during the second half of the last century, altering the growth of trees and peripheral forest death, the distribution of native species, the increase of invasive species populations and the emergence of new pathogens (Seppälä, 2009). The disturbance regime has also been modified, producing mortality on a regional scale, decay and severe attacks by biotic agents (Desprez-Loustau et al., 2006; Seppälä et al., 2009; Suarez et al., 2004). The Andean Patagonian forests are valuable global reserves of Temperate Forests that have seen little anthropogenic alteration (Arroyo et al., 1996). Ninety percent of their area is occupied by species of *Nothofagus* (Donoso Zegers, 1993), which possess great socio-economic and ecological values (Donoso Zegers, 2006). Hereby, several studies have been conducted through the decades assessing its genetic diversity and ecology (Mathiasen & Premoli, 2010; McQueen, 1977). In

Northern Patagonia, *Nothofagus dombeyi* is the dominant species in the temperate-humid forests. *Nothofagus pumilio* is the dominant species in the high-altitude stands (Daniels & Veblen, 2004; Donoso Zegers, 1993). Both species are endemic and have non-overlapping altitudinal distribution. Fifty percent of their distribution areas are protected in natural reserves (World Heritage by UNESCO). Nevertheless, these forests are facing emerging threats related to climatic change that can be associated with pests and diseases along with other temperate forests worldwide (Fajardo et al., 2017; Kirkendall, 2011; Kitzberger et al., 2000; Suarez et al., 2004). In both species, progressive death has been observed in a grouped spatial pattern that is especially severe in their northern distribution and in Los Alerces National Park in particular (LANP) (Izquierdo & de Errasti, 2014; Tarabini et al., 2021). The phenomenon is characterized by the development of mortality patches up to hundreds of meters in diameter that grow radially through the years as new individuals become symptomatic first and eventually die (Izquierdo & de Errasti, 2014; Molina et al., 2020). A number of potential fungal pathogens have been isolated from symptomatic trees but the etiology of these mortality spots has not been attributed to a primary fungal pathogen (de Errasti et al., 2015; Molina et al., 2020; Pildain et al., 2010). There is a background, accrued during the last decades of research, concerning the fungal diversity associated with these trees in taxonomic groups such as polypores, ectomycorrhizae, ophiostomatoid fungi, and other decay fungi (Barroetaveña et al., 2019; de Errasti et al., 2016; Pildain et al., 2010; Rajchenberg, 2006).

Living trees in temperate forests have been demonstrated to be reservoirs of fungal diversity (Unterseher, 2011). It is known that these mycobiota play key roles in the fitness and functioning of the trees through complex dynamics (Baldrian, 2016) that fall along a continuum of mutualism, commensalism, and parasitism that can change through the same fungal organism's lifetime (Robinson et al., 2004; Saikkonen et al., 1998). Plant-associated mycobiota were shown to also contribute to large-scale patterns of plant diversity in forest ecosystems (Wang et al., 2019). However, there is a huge gap in the understanding of living-

wood-inhabiting fungal (LWIF) diversity, the drivers that modulate such communities, and the nature of the interactions they establish with plants (Suryanarayanan, 2020).

In the last decades, novel high-throughput sequencing technologies (HTS) became frequent and accessible, leading to progress in plant mycobiomes investigations, especially through metabarcoding approaches. They are cost and time-efficient and have allowed increasing sensitivity and rate at which biomes can be assessed (Terhonen et al., 2019)..

The advent of these methods has led to mycobiome research across a variety of plant compartments, geographical regions, and biomes. The majority of the efforts on describing the endophyte diversity has been developed for species under human impact and control, because of their economic value and/or the sampling advantages, while less attention has been given to wild and pristine ecosystems (Harrison & Griffin, 2020). As far as forest biomes are concerned, the endophyte studies have been focused mainly in tropical and subtropical wet forests, followed by temperate mixed forests. However, the studies on temperate forests have been mostly focused on foliar endophytes and in the Northern Hemisphere with a particular lack of studies in South America (Harrison & Griffin, 2020). The current background on tree endophyte diversity suggests the tropics as biodiversity hotspots (Arnold & Lutzoni, 2007; Unterseher, 2011). Within tree host species, plant compartment exhibits the strongest structuring effect on the endophyte community, compared with other variables such as site and season with the leaves harboring the highest diversity followed by the stems and roots woody tissues (Bahram et al., 2021; Küngas et al., 2020; Langer et al., 2021) although this trend is not always observed (Qian et al., 2019). The majority of the studies to date have been conducted on foliar endophytes, with much less attention on roots and stems (Harrison & Griffin, 2020). In the same way, most of the studies have been conducted on a single host taxon, with a minority of works targeting multiple hosts although these last have proven that host identity is a strong predictor of endophytic assemblage variation at a landscape scale (Arnold & Lutzoni, 2007; Hoffman & Arnold, 2008). The effect of host identity on endophytic fungal assemblies can be due to host specialistic fungal

strategies (i.e., related to host tissue chemistry) and/or the effect of environmental gradients between host distributions (Arnold & Lutzoni, 2007; Harrison & Griffin, 2020; Kivlin et al., 2019).

Recently, we described LWIF communities of *N. dombeyi and N. pumilio* in the context of grouped mortality with a culture-based approach (Molina et al., 2020). We described a rich and heterogeneous diversity, strongly driven by host variables such as holobiont identity, health condition, and plant compartment. We concluded that our results underestimated diversity of LWIF in *Nothofagus* trees and that such diversity was unable to be fully assessed through culture.

In this study, we aimed to assess how climatic, site and host variables structure the sapwood-inhabiting fungal communities of the North Patagonian *Nothofagus* forests *vis à vis* their health condition. We used HTS to characterize LWIF diversity across hosts, health conditions, plant compartments and seasons. We explored the taxonomic and functional structure of LWIF communities through a gradient of climatic, seasonal and site factors as a contribution to the autecology of *Nothofagus* species and to the understanding of how endophyte communities vary in space and time. We hypothesized that host identity and climatic factors act as the major drivers of *Nothofagus* fungal community structure due to their major effects on fungal dispersion and colonization (Vaz et al., 2014). We expected to find greater abundances of potentially pathogenic taxa in symptomatic than in asymptomatic trees based on previous findings in this system (Molina et al., 2020).

Materials and methods

Study area and sampling procedure

The study was conducted in Los Alerces National Park in Argentinian Patagonia (42° 58’ 27.075” S 71° 38’ 37.725” W), from November 2017 to May 2018. The sampling was performed seasonally at the beginning and the end of the growing season for each

Nothofagus species (referred to as austral spring and autumn, respectively). Three stands of *N. pumilio* and four of *N. dombeyi* were defined as sampling sites that all included areas with standing tree mortality (Fig. 1). Ten dominant trees were sampled in each site per sampling, per season: 5 symptomatic and 5 asymptomatic as defined in Molina et al., (2020). Trees in the first stage of decline were selected from the margins of the mortality patches, where radial growth of the patch is taking place as new individuals are affected. Symptomatic trees were defined as transparent crown, dry branches, chlorosis and defoliation in more than 25% of the crown; asymptomatic trees were healthy-looking, with a crown cover of 75 to 100% and were located at, at least, 80 m out from the mortality patch edge. Within each site, selected trees were of similar diameter at breast height (DBH). Each tree was sampled only once and a total of 140 trees were sampled during the study. Wood cores of 5 mm diameter and 20 mm length were extracted from vascular tissue using an increment borer, after removing the bark with a knife. The borer and the knife were sterilized with 70% ethanol (v/v) and flaming between each sample. Wood samples were collected from primary roots and from the stem at breast height for each individual sampled. For the root sampling, primary roots were discovered by using a shovel and cores were extracted 0.5-1 m from the stem before digging. Sapwood samples of 1.5 cm length were recovered, put into sterile 2 mL Eppendorf tubes, and kept at -20 °C until processing 48 hours later maximum.

DNA extraction, library preparation, and Illumina sequencing

About 50 mg of wood was ground to powder for each sample by combining the immersion in liquid nitrogen and 3 minutes shaking in a mixer mill according to Doyle (1990) methodology, adapted by Dumolin et al., (1995). Total DNA was extracted using DNeasy Power Plant Pro Kit (QIAGEN, Hilden, Germany) following the manufacturer's recommendations after 10 minutes of incubation at 65 °C.

Internal Transcribed Spacer 1 (ITS1) library was prepared using the TrueSeq (two-step) dual indexing strategy. ITS1 amplification was performed by using the primers pair TS-ITS1-F (5'-

ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTTGGTCATTTAGAGGAAGTAA-3') and TS-ITS2-R (5'-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTGCGTTCTTCATCGATGC-3') (Gardes & Bruns, 1993; White et al., 1990) and MyTaq™ Mix (Bioline, USA, Inc., Memphis) in a total volume of 25 µL per reaction with the next cycling conditions: 94 °C for 5 min, 32 cycles of 94 °C for 45 sec, 50 °C for 45 sec, and 72 °C for 1 min, and a final extension at 72 °C for 7 min. PCR products were purified using ExoSap-IT (USB Corporation, Cleveland, OH) following the manufacturer's instructions. The purified PCR products were indexed by using sample-specific barcodes combinations of the TruSeq primers pairs i5-TS-DI-5xx (AATGATACGGCGACCACCGAGATCTACAC 8-nucleotide-barcode ACACTCTTTCCCTACACGAC) and i7-TS-DI-7xx (CAAGCAGAAGACGGCATACGAGAT 8-nucleotide-barcode GTGACTGGAGTTCAGACGTG) (Integrated DNA Technologies, Inc., Coralville, IA) with the following cycling conditions: 95 °C for 3 min, 8 cycles of 95 °C for 30 sec, 55 °C for 30 sec, and 72 °C for 30 sec, and a final extension at 72 °C for 5 min. PCR products were cleaned with ExoSAP-IT (Thermo Fisher Scientific) following manufacturer's instructions. DNA was quantified by using a NanoDrop spectrophotometer (ThermoFisher, Waltham, MA). Negative controls from PCR and DNA extraction as well as non-biological synthetic mock communities (Palmer et al., 2018) were included as samples and also amplified, included in the final pools, and sequenced (Palmer et al., 2018). Samples were randomly grouped into two groups and pooled in approximately equimolar ratios. ITS1 libraries were sequenced at the Purdue Genomics Core Facilities (Purdue University, West Lafayette, IN) with a MiSeq v2 Reagent kit of 500 cycles in the Illumina MiSeq platform (2 x 250 bp) .

Bioinformatic analysis

The resulting amplicon data set was analyzed and processed using the Amplicon toolkit (AMPtk) (v1.2.4; Palmer et al., 2018) which has been shown to perform better for fungal ITS amplicon analysis (Anslan et al., 2018; Nilsson et al., 2019). The pipeline first trimmed short

reads (Palmer et al., 2018), next, trimmed primer sequences from reads; then, merged the paired-end reads by using USEARCH (v9.2.64; Edgar, 2010) and performed the quality filtering of the assembled reads using expected error trimming (Edgar & Flyvbjerg, 2015).

The DADA2 pipeline (v1.6.0; Callahan et al., 2016) was performed in order to build amplicon sequence variants (ASVs) tables. The threshold was set at 97% identity. Additionally, cross-contamination error was corrected by recognizing the sequences of the SynMock community and their frequencies on the set and calculating, then, the tag-switching index (Palmer et al., 2018). Also, the LULU algorithm was ran (v0.1.0; Frøslev et al., 2017) as a post-clustering curation.

A “hybrid” approach was used to assign ASV taxonomy in the AMPtk platform against the UNITE database (Abarenkov et al., 2010). This approach combines classification from global alignment, with classification from the learning machine approach UTAX (RC Edgar, http://drive5.com/usearch/manual9.2/cmd_utax.html) and the SINTAX approach (Edgar, 2016). This method performs the taxonomy assignment by using the three approaches and then chooses the best taxonomy from the three by prioritizing the global alignment result (the top hit) if the threshold is higher than 97% or the higher confidence score from the other approaches. If there’s a conflict between the taxonomies from the different methods, the algorithm chooses the last common ancestor taxonomy (Palmer et al., 2018).

Finally, manual curation of the three pipeline outputs was performed by following Brown et al. (2015) recommendations. Non-fungal and kingdom-undefined ASVs, as well as ASVs represented by less than 10 reads, were removed from the set.

A set of common ASVs was defined, consisting of those present in at least 6 samples, which represents 2.5% of the samples. The taxonomy of common ASVs was manually reviewed and curated, and the ecological guild was assigned. Each ASV sequence was queried to the GenBank database (https://blast.ncbi.nlm.nih.gov/ accessed on November 2021) by using the BLASTn algorithm (Altschul et al., 1997). Identity from 90 to 98% was considered as a

genus-level match, identity above 98% was considered as a species-level match. When there was a conflict among the taxonomies of the multiple sequences queried, the last common ancestor was assigned.

Site and environmental variables

For each sampling site, site variables were registered at stem level in the field: exposition, elevation and slope and mean and median DBH calculated from 40 aleatory trees at each site (Table 1). Data from climatic variables was obtained from Terra Climate – Monthly database accessed on March 2022 through Climate Engine App (http://climateengine.org) (Huntington et al., 2017). Mean values of maximum and minimum temperatures and values of precipitation, climate water deficit, wind speed and soil moisture were collected for each site annually by taking average conditions during the two previous years to the study and at each season by taking average conditions during the three-month period before sampling date. Also, Trabucco & Zomer aridity index (2019) was considered at site level.

Statistical analyses

After manual curation, the ASV table was binarized to presence/absence tables. Downstream community analyses were performed with both abundance and presence-absence data. This decision relies on that reads' abundance ability to represent biological abundance from fungal HTS has been discussed (De Filippis et al., 2017; Palmer et al., 2018) and that ASV tables were corrected from tag-switching error which allows the confidence on presence-absence data. The effect of the host, plant compartment, site, health condition, and season over LWIF richness was evaluated at the sample level and each level of these variables by using the Kruskal-Wallis rank-sum test (Kruskal & Wallis, 1952) and Pearson's Chi-square test (Pearson, 1900), respectively ("kruskal.test" and "chisq.test" functions in the stats R package v4.1.2; R Core Team, 2021). Differences in community composition and structure between variables' levels were tested by performing perMANOVA (Anderson, 2017) with the "adonis" function in the vegan package (v2.5.7;

Oksanen et al., 2020). For the abundance-based ASV tables, perMANOVA was performed on Bray-Curtis dissimilarity matrices (Bray & Curtis, 1957) ("distance" function with "bray" as method argument in package vegan) of Hellinger-transformed matrices (Legendre & Gallagher, 2001) ("decostand" function with "hellinger" as method argument in package vegan). For the binary ASV tables, perMANOVA was performed on Raup-Crick metric matrices (Raup & Crick, 1979) build by using the "raupcrick" function in the vegan package. The assumption of multivariate homogeneity of dispersions was checked for each perMANOVA test by using the "betadisper" and the "permutest" functions in the vegan package (Anderson, 2006). The similarity patterns of wood samples' communities across host species, sites, plant compartments and seasons were explored with correspondence analysis (CA) (Hill, 1974) by using the "ordinate" function with "cca" as the method argument and plotting with the "plot_ordination" function in the phyloseq package (v1.38.0; McMurdie & Holmes, 2013).

Venn diagrams were drawn with eulerr package (v6.1.1; Larsson, 2020) to identify shared and unique ASVs for each variables' level. Species-site group associations were performed in order to detect indicator species, meaning those restricted to one or a few habitat types (Cáceres & Legendre, 2009). Indicator species analyses were performed for each of the variables assessed for the common ASVs dataset by using the "multipatt" function in the indicspecies package (v1.7.9; Cáceres & Legendre, 2009), in order to detect the species significantly associated to each variables' level.

The variance of climatic and stem variables for each site was summarized by principal component analysis (PCA) (Hotelling, 1933; Rao, 1961) by using the "PCA" function from the FactoMineR package (v2.4; Lê et al., 2008) and plotted by the "fviz_pca_biplot" function from the factoextra package (v1.0.7; Kassambara & Mundt, 2020). All variables were standardized to unit variance (O'Connor, 1988) by using the "decostand" function with the "standardize" method in the vegan package. The collinearity assumption between variables

was checked by using the “corrplot” function in the corrplot package (v0.92; Wei & Simko, 2021).

The variation of community structure along with climatic and stand factors was analyzed with canonical correspondence analysis (CCA) (Ter Braak, 1986) by using the “cca” function from package vegan on the Hellinger transformed ASVs abundances matrices. Stand and climatic variables were standardized as described above. The assumption of unimodality was checked with dip test (Hartigan & Hartigan, 1985) by using the “dip.test” function from the diptest package (v0.76.0; Maechler, 2021). The presence/absence community data set was not unimodal; thus, community structure variation along with climatic and stand factors was analyzed with distance-based redundancy analysis (db-RDA) (Anderson, 2006; Legendre & Anderson, 1999) on the Raup-Crick distance matrices by using the “capscale” function from vegan package.

The statistical analyses were run separately for the set of the total manually curated ASVs and for the common ASVs. The PCR run and the Illumina lane were included in the statistical analyses to avoid methodological biases on results interpretations.

Data analyses and graphics were performed in RStudio (v4.1.2; http://www.rstudio.com/) with the mentioned packages plus biomformat (v1.22.0; McMurdie & Paulson, 2021) and ggplot2 (v3.3.5; Wickham et al., 2016).

Results

General features of the high throughput sequencing experiment

The sequencing experiment had a mean depth of 133,855 reads per sample (paired-end raw reads) and a total depth of 38 million raw reads. The rarefaction curve approximated an asymptote when manually curated data was considered (not shown) indicating satisfactory sampling.

A total of 5,726 ASVs were obtained from 280 *Nothofagus* wood samples from which 359 (6%) were identified at the species level, 635 (11%) at the genus level, 280 (5%) at the family level, 447 (8%) at the order level, 318 (6%) at class level, 1663 (29%) at the phylum level and 2024 (35%) at the kingdom level. Richness per sample (unimodal distribution, mean=50.59, median=43) showed significant differences between host, site, and season (Table 2).

Differences in fungal assemblage across host and environmental variables for the entire data set

The total richness detected in the spring sampling was higher than in the autumn (4383 versus 3577). Total richness also varied between *Nothofagus* species and between sites (Table 2); *N. dombeyi* showed higher total richness than *N. pumilio*. The total richness of common ASVs remains significantly different between *Nothofagus* species and between season sampling for *N. pumilio* (Table 2), with spring showing higher wood fungal richness than in autumn.

The multivariate analysis of fungal community composition and structure indicated greater differences are due to the *Nothofagus* species (host). Nevertheless, the plant compartment the sampling season and the health condition also affected the community structure when the whole set of data was analyzed (Table 2, Suppl. Figure 1). Correspondence Analysis allows the representation of such hierarchical effects (Fig. 2). Samples from each *Nothofagus* species were ordinated into separated groups whose 95% confidence ellipse did not overlap. *Nothofagus pumilio* community structure showed a greater association with season and plant compartment when it was assessed separately. On the other hand, site Alerce River differed greatly in community structure for *N. dombeyi* (Fig. 2, Suppl. Figure 1). The health condition showed marginal differences.

Sapwood fungal communities of *N. dombeyi* and *N. pumilio* are strongly structured across stand and climatic variables (Fig. 4). Total ASV community structure of both species

separated in the db-RDA analysis according to climatic and stand variables, such as slope and elevation, and temperature and water availability, respectively (Fig. 4). Also, *N. pumilio* fungal communities were more variable within sites than *N. dombeyi* fungal communities. Such variability within *N. pumilio* sites is seasonal. It is structured along with the water availability gradient between spring and autumn (Fig. 4).

Site conditions of both *Nothofagus* species differed strongly. *Nothofagus pumilio* sites were less variable in climatic and stand features than *N. dombeyi* sites (Fig. 3). Both species' sites are separated in the PCA according to elevation gradient, DBH, slope, temperature and variables related with water availability. *Nothofagus pumilio* sites had a higher water deficit than *N. dombeyi*. The last had two dimensions that differentiated sites: water availability and stand DBH parameters. Alerce River differed strongly from other sites in water availability due to the higher values of annual precipitation; Lake Kruger and Alto el Petiso Mt. had higher DBH and temperatures due to the eastern exposition of their slopes (Table 1).

Differences between Nothofagus dombeyi and N. pumilio fungal diversity

Both communities are dominated by Ascomycota (70% and 64% occurrence in *N. dombeyi* and *N. pumilio* respectively) but *N. pumilio* exhibits a greater proportion of Basidiomycota (28% against 25%) due to higher abundance of the Orders Agaricales and Tremellales in *N. pumilio* than in *N. dombeyi* (Fig. 5). *Nothofagus pumilio* exhibits a greater proportion of other less common phyla such as Mortierellomycota and Mucoromycota (2% each against 1% in *N. dombeyi*) due to the high abundance of the Orders Umbelopsidales and Mortierellales, respectively, differentially located in their roots (Fig. 5).

When only common ASVs are considered, it is possible to identify a set of 79 ASVs that occurred exclusively in *N. dombeyi* and a set of 37 ASVs that occurred exclusively in *N. pumilio* (Fig. 6). Furthermore, indicator species analysis found 98 common ASVs significantly associated with *N. dombeyi* and 50 common ASVs associated with *N. pumilio* (Suppl. Table 1).

The functional structure differed between the two *Nothofagus* (Table 2). *Nothofagus pumilio* had a larger proportion of unassigned taxa and taxa with saprophytic strategies than *N. dombeyi*, which, in turn, was characterized by a larger proportion of taxa registered as endophytes and potential plant pathogens (Fig. 5).

On this basis, further statistical analyses were performed separately for fungal community data from *N. dombeyi* and *N. pumilio* in order to assess the other variables of interest more accurately.

Beta diversity patterns in N. dombeyi

Site was the strongest driver in the structure of *N. dombeyi* fungal communities (Table 2) although season and plant compartment were significant as well (Fig. 2, Suppl. Fig 1).

The site

The effect of site on community structure depended on season; in autumn site effect was stronger than in spring (CCA, goodness of fit R2= 74.5% and pvalue= 0.001 versus R2= 59.5%, pvalue= 0.001, respectively) and this was also observed for the functional structure (CCA, R2=17% and pvalue=0.002 in autumn versus R2= 12% and pvalue=0.006 in spring). Alto el Petiso Mt. and Alerce River had higher richness than other sites but Alerce River differed in community structure and composition at the order level (Table 2, Fig. 5). When only common ASVs were considered, Alerce River was the site with the highest number of exclusive ASVs (25) and indicator species (30), followed by Alto el Petiso Mt. (19 and 16, respectively), the two sites with the highest total richness, as mentioned above (Fig. 6. Supp. Table 1). Lake Menendez site was characterized by thirteen exclusive common ASVs and four indicator species. Lake Krugger site had the lowest richness (Table 2), a set of six indicator species (Suppl. Table 1), and the smallest set of exclusive common ASVs (Fig. 6). The functional structure of *N. dombeyi* communities showed significant differences between sites (Table 2) which can be explained by a greater proportion of xylophilous taxa in the

Alerce River site compared to other sites and, together with the Alto el Petiso site, a higher proportion of unassigned ASVs (Fig. 5).

The plant compartment

Fungal richness in *N. dombeyi* stems was higher than that found in roots but this difference did not prevail when common ASVs were assessed (Table 2). The difference in the community structure was observed at both ASV (Suppl. Fig. 1) and order levels (p=0.002, R2=0.025, F=3.368, perMANOVA, Fig. 5). *Nothofagus dombeyi* stem communities had 28 exclusive ASVs and 11 indicator species, whereas root communities had 44 and 6, respectively (Fig. 6, Suppl. Table 1). Functional structures differed significantly between stems and roots assemblages (Table 2). Stems exhibited a greater proportion of endophytes, while communities from roots contained more dark septate endophytes, taxa with saprophyte strategies, and mycorrhizal taxa (Fig. 5). The effect of the plant compartment in the functional structure differed according to season; in autumn, the effect of plant compartment was stronger than in spring (CCA, goodness of fit R2= 16.5% and pvalue= 0.001 versus R2= 12.6%, pvalue= 0.001, respectively).

The season

The total richness found in spring was higher than that found in autumn (Table 2). Community structure differed significantly between seasons and this was also true when assessing at the order level (p=0.002, R2=0.025, F=3.368, perMANOVA, Fig. 5) although the functional structure of the assemblages in autumn and spring did not show significant differences. When the set of common ASVs was assessed, richness differences between seasons were not significant (Table 2). The fungal community of spring could be characterized by a set of 54 exclusive common ASVs and 7 indicator species whereas the autumn community was characterized by 21 common ASVs and 17 indicator species (Fig. 6, Suppl. Table 1).

The health condition

Decayed *N. dombeyi* trees had higher richness than healthy ones whereas community structure did not differ significantly (Table 2, Suppl. Fig.1). Decayed *N. dombeyi* communities are characterized by a set of 42 exclusive ASVs when the common taxa set was considered and 3 indicator species, whereas healthy trees communities could be characterized by a set of 14 exclusive ASVs (Fig. 6, Suppl. Table 1).

*Beta diversity patterns in N. pumilio*Although as in *N. dombeyi* the site is the stronger driver in *N. pumilio* fungal communities' differentiation (Table 2), the plant compartment and the season were important driver variables as well (Fig. 2. Suppl. Fig. 1). The health condition of the tree was found to be significant in fungal community structure when rare ASVs were included in the analysis (Table 2).

The site

El Riscoso Mt. had the highest total richness and largest set of exclusive common ASVs (Table 2, Fig. 6). Nine indicator species were found associated with this site, among which there was a wood-rotting species of *Aotearoamyces* (Suppl. Table 1). Alto el Petiso Mt. had high richness as well and a large set of exclusive ASVs (Table 2, Fig. 6). Seven indicator species were found associated with this site (Suppl. Table 1). El Dedal Mt. had the lowest total richness with a set of 15 exclusive common ASVs (Table 2, Fig. 6). Seven ASVs were found associated with it, which are mainly taxa with saprophytic strategies and the mycorrhizal *Austropaxillus macnabii* (Suppl. Table 1).

The plant compartment

Roots exhibited greater fungal richness than stems (Table 2) although the number of exclusive common ASVs that described the two communities were similar (Fig. 6, Suppl. Table 1). Functional structure differed significantly between stems and roots (Table 2). Roots were dominated by saprophytic taxa and exhibited a greater proportion of xylophilous taxa.

Stems had a larger proportion of unassigned taxa, potentially pathogenic taxa, and endophytes (Fig. 5). In autumn, the effect of the plant compartment on the guild community structure (CCA, goodness of fit R2= 16.5%, pvalue= 0.001) is higher than in spring (CCA, goodness of fit R2= 12.5%, pvalue= 0.001). Wood rotting fungus, plant pathogens and endophytes were found to be associated with stems in autumn. In spring, stems were inhabited by fungi with unknown or unassignable guilds (Fig. 7. B and C).

The season

The total richness in spring was significantly higher than that in autumn (Table 2). In spring the *N. pumilio* community could be characterized by 64 exclusives common ASVs and six indicator species, whereas autumn communities had 27 common ASVs and ten indicator species (Fig. 6, Suppl. Table 1). The functional structure differed significantly between autumn and spring (Table 2, Fig. 7.A). Assemblages from samples collected in autumn exhibited a greater proportion of ASVs with unassigned ecological guilds. In autumn greater proportions of endophytes, wood-rotting fungi, and potential plant pathogens were detected in association with stems (Fig. 5, Fig. 7.B). On the other hand, spring assemblages were dominated by taxa with saprophytic strategies in a wide sense and dark septate endophytes, which are associated with roots (Fig. 5, Fig. 7.A and C).

The health condition

Richer assemblages were found in healthy trees compared to decayed ones in *N. pumilio* (Table 2). The number of exclusive common ASVs for decayed and healthy-looking *N. pumilio* were similar, 30 and 33 respectively (Fig. 6, Suppl. Table 1). Two ASVs were found associated with symptomatic trees, the mycorrhizal *Austropaxillus macnabbii,* and a *Penicillium* species. Four ASVs were found associated with asymptomatic trees, such as the saprophytic *Helotium* sp. and *Pseudoeurotium* sp. The effect of health condition on guild community structure differed according to season; in autumn, health condition effect was

significant (Fig. 7.B. CCA, goodness of fit R2= 7.4%, pvalue= 0.03) whereas in spring it was not (Fig. 7.B. CCA, goodness of fit R2= 1.4%, pvalue= 0.4).

Discussion

There is a growing understanding that eukaryotes are assemblages of organisms rather than autonomous entities. They function as consortiums composed by the host and its associated microbiota, developing complex biomolecular networks (Bordenstein & Theis, 2015). Holobiont is the term that conceptualizes these findings which are transforming the way that life is comprehended and studied (Blaser, 2014). In this sense, the research frameworks are being challenged in terms of accounting for these constituent associations in plant biology and ecology models (Wingfield et al., 2017).

In this study, we aim to present the reader information focused on *Nothofagus* grouped mortality LWIF communities and predisposition drivers of their diversity. *Nothofagus* decline is an emerging complex disease not yet fully understood. This is not a recent phenomenon, it has been reported by previous studies and reports and variously attributed to insects, fungi, earthquake and climate change (de Errasti, 2015; Fajardo et al., 2017; Izquierdo & de Errasti, 2014; Kirkendall, 2011; Suarez et al., 2004). Historically, where no major disturbances were obvious, the research is commonly focus on the insects and diseases directly associated with dying trees and urged research into these causes of decline. The idea that tree health condition shapes microbial diversity from a balanced, 'healthy' microbiome into a pathobiome was observed for example in olive knot disease (Gomes et al., 2019). Here we demonstrated that this factor was significantly related to *N. pumilio* but not for *N. dombeyi*. The host species identity is the stronger driver of LWIF communities than site or time.

In this study, we describe the LWIF communities of the two major *Nothofagus* species from the North Andean Patagonian Forests, complementing culture-based findings with results from culture-free approaches. Our previous studies from culture prospection found a great

variability of fungi across tre individuals, and a huge richness of rare fungal taxa (Molina et al., 2020). Also, they estimated a great and hidden diversity of LWI fungi, unable to be detected by culture approaches. This study goes further by detecting additional variables driving the community structure, by ranking their impacts at different scales, and by explaining the patterns at a functional level. Such patterns were robust, on the other hand, because they were consistent when both including and excluding the rare taxa. The community structure of LWIF is the result of the combined effect of biotic and abiotic variables acting at site and individual level (Schröter et al., 2019); the relative effects of the variables tested were different for each *Nothofagus* species.

The sequence depth of the HTS experiment was higher than that of similar wood fungal endophytes studies (Küngas et al., 2020; Migliorini et al., 2021; Onufrak et al., 2020). Sequence depth is the more important variable in HTS experimental design that aims to assess beta diversity (Smith & Peay, 2014). However, high sequencing depth increases the potential for cross-contamination and errors during sequencing (Baldrian et al., 2021). In this study, we combine a high sequence depth with an approach to correct cross-contamination errors by using a synthetic mock community.

The taxonomy assignment lacked mycological accuracy for the system under study: up to 35% of total manually curated ASVs could not go further than the Kingdom Fungi determination. In general, sequence-based identification depends on informative sequence databases (Costello et al., 2013). In particular, bioinformatic methods for taxonomy assignment, and especially the learning machine approaches, are sensitive to the incompleteness of the reference databases because the algorithms perform better when there are multiple representatives for each group (Gdanetz et al., 2017). There is a current lack of knowledge about fungal diversity in certain environments and about entire fungal lineages that keep the public databases incomplete (Halwachs et al., 2017). This is a common issue in metabarcoding studies from diverse environments (Kirker et al., 2017; Purahong et al., 2019), especially when plant endophyte mycobiota are being assessed

(James et al., 2020) because next-generation sequencing technologies permit the discovery of hidden diversity. *Nothofagus pumilio* communities had the greatest relative abundance of unassignable taxa, indicating the understudied nature of its LWIF communities. For *N. dombeyi*, the highest relative abundances of unassignable taxa were found in the richer sites.

Previous culture-based study in the same sites showed that differences in wood fungal community composition across health conditions in both *Nothofagus* species could be explained by species turnover towards more potential pathogens in symptomatic trees, especially in *N. dombeyi* (Molina et al., 2020). Although we have observed marginal community differences in structure between symptomatic and asymptomatic trees, this study found that both *Nothofagus* species harbor latent potentially pathogenic fungal taxa as wood-inhabiting mycobiota which can be there whether the tree exhibits symptoms or not. The next question is whether differential detection through both approaches mirrors fungal growth development (and, thus, differential impacts on plant-fitness) or accounts for limitations in the detection capacities of the culture-based and culture-independent approaches. In other words, fungal inoculum can be there and does not trigger any decay because of other factors acting at the individual level as health promoters (i.e., cohabiting mycobiota, plant genetics, host defense mechanisms). Nevertheless, many of these potentially pathogenic species pointed out by culture prospection were taxa difficult to detect in HTS approaches targeting partial ITS regions, such as species from the order Ophiostomatales and the Hymenochaetaceae family (Ceballos-Escalera et al., 2022; Skelton et al., 2019).

Host identity is the major driver for LWIF communities' composition and structure. Both *N. dombeyi* and *N. pumilio* account for very dissimilar non-overlapping fungal assemblages. The effect of the host in LWIF communities has been reported as strong, low, and even non-significant in different studies (Bahram et al., 2021; Küngas et al., 2020; Rim et al., 2021; U'Ren et al., 2019, respectively), suggesting that the complexity of plant endobiome and the

roles of host, biota, and environment need to be achieved for each case at a medium spatial scale. This study coincides with previous findings for *Nothofagus* in New Zealand native forests that have found the greatest dissimilarities between the hosts and have linked similarity hierarchy to phylogenetic distances between the three *Nothofagus* species assessed (Johnston et al., 2012, 2017). The divergence of *N. dombeyi* and *N. pumilio* LWIF communities has been discussed as a reflection of nonadjacent altitudinal distributions in Northern Patagonia, water availability, and stand age (Molina et al., 2020) although it is, indeed, an interesting question whether co-occurrence or phylogenetic distances of the tree species inhabiting Northern Patagonian Forests are relevant drivers of its LWIF communities. More studies in further tree species, and in pure and mixed stands, are needed to assess such questions.

As found previously, LWIF communities in both *Nothofagus* are composed of a majority of Ascomycota taxa but with an important presence of Basidiomycota, whose taxa exhibits greater relative abundances in *N. pumilio.* Further, this species exhibited a higher relative abundance of Mucoromycota and Mortierellomycota than in *N. dombeyi.* Taxa from these phyla were known as soil inhabitants but recently were discovered as root and stem endophytes suggesting their capacity for systemic plant colonization from soil (Rim et al., 2021; Vélez et al., 2020). Overall, there is an abundance of taxa with xylophilous and saprophytic strategies in *Nothofagus* LWIF communities. The dominance of LWIF communities by these guilds has been explained as strategic for further dominating the late decomposing succession of fallen wood (Song et al., 2017; Viotti et al., 2021). *Nothofagus pumilio* exhibits greater relative abundances of wood-rotting fungi and taxa with saprophytic strategies than *N. dombeyi*, whereas the latter exhibits greater relative abundances of taxa registered as endophytes, entomopathogens, foliar fungi, and plant pathogens.

In *N. dombeyi*, site had a major impact on community structure at both taxonomic and functional levels compared to other variables tested. This mirrors the greater heterogeneity of climatic and edaphic conditions in which the species is distributed compared to *N. pumilio*.

Geographic location structures endophyte communities due to dispersal limitations and because strong gradients of climatic factors affect the viability of fungal inocula (Hubbell, 2001; Schulz & Boyle, 2005; Vaz et al., 2014). We have found a gradient of fungal richness that can be accounted for by water availability across *N. dombeyi* sites. It has been demonstrated that endophytic frequency increases with precipitation in vascular plants (Arnold & Herre, 2003; Carroll, 1995; Singh et al., 2017) and that environmental factors that shape plant communities also drive diversity of fungal endophytes of trees (Oita et al., 2021; U'Ren et al., 2012; Zimmerman & Vitousek, 2012). Alerce River was the richest across *N. dombeyi* sites, had the highest abundances of rare taxa, the most numerous groups of exclusive taxa, high relative abundance of xylophilous taxa (such as Polyporales and Agaricales orders) and low relative abundance of mycorrhizal taxa. Alerce River is a mixed stand with *Fitzroya cupressoides*. It is located at the bottom of a fluvial valley that floods several months per year. These features, combined with a high annual precipitation, provide this site with greater water availability than the others. Hereby, it can be placed as a Valdivian rainforest biome because of the increased plant diversity in both species and structure (Orellana Ibáñez et al., 2018). Furthermore, the relative abundances of several fungal functional guilds can vary according to the pure or mixed nature of the stand due to density dependence of conspecific hosts in such a way, for example, that higher plant richness accounts for lower ectomycorrhizal and higher pathogen or saprophytic abundances (P. Wang et al., 2019).

In *N. pumilio* stands, site affects community structure and composition, although its relative impact was lower than in *N. dombeyi* compared to other variables considered and was related to DBH due to site differences in stand age. Mount El Riscoso was the site with the highest LWIF richness, due to the greatest abundance of wood-rotting fungi and xylophilous taxa. It was the oldest stand among *N. pumilio* sites. Wood-rotting fungi are positively associated with tree age in several species (Berry & Lombard, 1978; Lesica et al., 1985). The greatest effects upon community structure at taxa and guild levels in *N. pumilio* were

season and plant compartment. Studied stands of *N. pumilio* are distributed up to the timberline, at about 1300 meters above sea level. The climatic conditions for this extreme altitudinal distribution led to late and short growing seasons. The spring sampling represents the start of the growing season and provides a picture of the effect of colder periods, which are also the wettest. Conversely, at the end of the growing season, we can observe the effect of the warmer and dryer period following a delayed pattern such as in soils (Kunkel et al., 2016; Voříšková et al., 2014). In spring, we found higher taxonomic richness and higher relative abundances of taxa with saprophytic strategies, lichens, and dark septate endophytes than in autumn, especially in roots. On the other hand, autumn accounted for lower taxonomic richness but higher relative abundances of wood-rotting fungi, plant-pathogens, and other endophytes, mainly located in stems. Similar patterns, according to seasonality, have been observed for root-associated mycobiota of native coniferous forests in China (H. H. Wang et al., 2021). They reflect the higher competitiveness of Basidiomycota taxa at warmer temperatures than that of Ascomycota, and, additionally, the best adaptability of Ascomycota species facing colder temperatures. That is why we can observe an increment of richness and rare taxa richness during the cold season. Ascomycota taxa increment their infection rate at higher humidity conditions and lower temperatures as observed in many tree species and organs (Banerjee, 2011; Rhoden et al., 2012; Tedersoo et al., 2010; Vaz et al., 2014). Also, Basidiomycota shows low stress-tolerance exposed to functional sapwood (Chapela & Boddy, 1988; Giordano et al., 2009); hereby, the decrease in water content in living tissues during the dry season might weaken plant defense against infection allowing the development of decay fungi, as proposed by the latent infection model (Boddy & Rayner, 1983). Indeed, it was during the dryer period when the fungal functional structures between symptomatic and asymptomatic trees of *N. pumilio* became significant. This demonstrates the higher predisposition of affected individuals to colonization by wood-rotting fungi when water availability decreases in extreme environments.

Similarly, a stronger differentiation of LWIF communities was observed across plant compartments in *N. pumilio* than in *N. dombeyi*, where the roots harbored the richest assemblages with more saprophytic taxa and the stems with the lowest richness and high wood-rotting and pathogen abundances. This is contrary to the trend observed in other tree species that exhibited higher endophyte richness in stems and which has been explained as the result of longer periods of exposure to fungal inoculum in aerial plant compartments than in belowground ones (Harrison & Griffin, 2020). In *N. pumilio* the compartmentalization of fungal assemblages interacts with seasonality; the root acts as a diversity reservoir of fungal diversity because belowground the seasonality is attenuated (Bahram et al., 2021). Plus, the roots might be inducing factors that promote the recruitment of fungal colonization, as suggested before for temperate and tropical tree species (Qian et al., 2019; Schröter et al., 2019). Hence, *N. pumilio*'s holobiome features a higher susceptibility to seasonality and temperature changes, compared to *N. dombeyi*', which distributes in less extreme environments. The important effects of climatic and seasonal factors on functional and taxonomic diversity of the microbiome has implications for forest conservation in a climate change scenario.

Conclusions

Both *Nothofagus* species studied carry a wide diversity of potential fungal pathogens on their sapwood, both on trees showing visible symptoms and those that appear healthy. The tree health condition did not show a strong effect on the sapwood mycobiome. The most important driver of the wood endophyte community structure is the host identity. The relative impact of tree health condition and compartimentalization, togheter with climatic and site variables are different for each tree species, however those impacts can be interpreted as a matter of water availability. In *N. dombeyi*, the main driver of wood endophytic communities is the site, mirroring the water availability gradient along which the species is distributed. Conversely, for *N. pumilio*, the major drivers of the wood fungal community structure are season and plant compartment. The warmer periods, which are also the driers, result in a

greater abundance of wood-degrading fungi, which are more efficient at colonizing water-stressed plant tissues. During cooler periods, the roots act as reservoirs of diversity for taxa with other types of trophic strategies. In this scenario, *Nothofagus* grouped mortality evidenced as a decline phenomenon, in which we have a synergy between climatic and biotic variables.

The Patagonian Forest holobiome has not been extensively studied and is poorly represented in the reference databases. It is imperative to continue and deepen study of this biome in order to increase the explanatory power of culture-independent technologies. The results of our study of the *Nothofagus* mycobiome through HTS approaches provide an insight into the diversity of microorganisms associated with southern temperate forests.

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Author contributions

M.P.A.C., M.B.P., and L.M. designed the research. M.B.P., A.dE. and L.M. performed the research. M.C.A., M.B.P., and M.R. contributed reagents/tools. L.M., M.B.P., M.R. and B.V. analyzed the data. L.M. wrote the paper.

Data availability statement

Raw sequence reads are deposited in the Short Read Archive of the National Center for Biotechnology Information (BioProject ID: PRJNA785007).

Sanger DNA sequences from culture are available on GenBank (accessions MT076081-MT07685).

Benefit-Sharing Statement

Benefits Generated: The project counts with the research permissions from the National Park Administration and the National Ministry of Environment and Sustainable Development according to Argentinian laws for the compliance of the Nagoya Protocol. The main results were communicated to the Los Alerces National Park Administration and rangers during workshops carried out in December 2019 and 2021.

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Figures and Tables Captions

Figure 1. A) Study area and sampling sites. *N. dombeyi* sites are circles, *N. pumilio* sites are triangles. MS: Alerce River. ME: Lake Menéndez. AC: *N. dombeyi* stand in Alto el Petiso Mt. K: Lake Kruger. AL: *N. pumilio* stand in Alto el Petiso Mt. Ri: El Riscoso Mt. D: El Dedal Mt. The geodetic geographic coordinate system used for the grid is WGS 84. B) Altitudinal distribution of both *Nothofagus* species. Decidous *N. pumilio* occupies altitudes up to the timberline. *N. dombeyi* is an evergreen species. Photo taken on April 2018. C) *N. pumilio* grouped standing mortality in El Dedal Mt.

Figure 2. Correspondence Analysis (CA) plots illustrating the association between the variables tested -host, site, plant compartment, season, and health condition- and samples according to their community composition for the whole set of data (above), *N. dombeyi* data (middle) and *N. pumilio* data (below). AC: Mt. Alto El Petiso *N. dombeyi*; K: Lake Kruger; ME: Lake Menendez; MS Alerce River; AL: Mt Alto El Petiso *N. pumilio*; D Mt. El Dedal; Ri: Mt. El Riscoso.

Figure 3. Principal components analysis (PCA) biplot illustrating correlations between stand and climatic variables in the first and second axes. The percentage of variance explained by the axis is showed in parentheses. Arrows are the variables analysed. Each point is a site and are shape-given according to *Nothofagus* species. Nd: *N. dombeyi* (triangle). Np: *N. pumilio* (circles). AC: *N. dombeyi* stand in Alto el Petiso Mt. K: Lake Kruger. ME: Lake Menéndez. MS: Alerce River. Ri: El Riscoso Mt. AL: *N. pumilio* stand in Alto el Petiso Mt. D: El Dedal Mt. DBH: diameter at breast height. ppt: precipitation. Max: maximum temperature. Min: minimum temperature. The variables' definitions and units are detailed in Table 1.

Figure 4. Distance-based redundancy analysis (db-RDA) of Raup-Crick distances showing community structure variations for the whole set of wood samples along with site and seasonal factors. Standard deviation ellipses are drawn for each site and for each site at each season. CAP1 and CAP2 axis explained 14.4% and 13.5% of the variance, respectively (CAP1 F=1.2110, p=0.001 y CAP2 F= 1.1410, p= 0.001, ANOVA). The climatic data are average condition values for each seasonal sampling season and was collected from Terra Climate – Monthly database (Abatzoglou et al. 2018) by using Climate Engine App (climateengine.com). Nd: *N. dombeyi* (triangle). Np: *N. pumilio* (circles). AC: *N. dombeyi* stand in Alto el Petiso Mt. K: Lake Kruger. ME: Lake Menéndez. MS: Alerce River. Ri: El Riscoso Mt. AL: *N. pumilio* stand in Alto el Petiso Mt. D: El Dedal Mt. ppt: precipitation.

Figure 5. Relative abundances at Order level (above) and guild level (above). Comparison between hosts (a), and between variables' levels for *N. dombeyi* (b) and *N. pumilio* (c) separated datasets. Nd: *N. dombeyi*. Np: *N. pumilio*. St: symptomatic. At: asymptomatic. F: stem. R: root. O: autumn. P: spring. AC: *N. dombeyi* site Alto el Petiso Mt. K: Lake Kruger. ME: Lake Menéndez. MS: Alerce River. AL: *N. pumilio* site Alto el Petiso Mt. D: site El Dedal Mt. Ri: site El Riscoso Mt.

Figure 6. Venn diagrams showing common ASVs that are shared and exclusive from each variable's level. The whole set of samples (above) is analysed, as well as *N. dombeyi* (Nd) and *N.pumilio* (Np) datasets separately. Nd: *N. dombeyi*, Np: *N. pumilio*, ME: Lake Menendez, MS: Alerce River, K: Lake Krugger, AC: Mount Alto el Petiso *N. dombeyi*, Ri: Mount El Riscoso, D: Mount El Dedal, AL: Mount Alto el Petiso *N. pumilio*, St: symptomatic, At: asymptomatic, F: stem, R: root, O: autumn, P: spring.

Figure 7. Guild community structure in *Nothofagus pumilio*. Canonical correspondence analysis (CCA) of the Hellinger's transformed abundances of the common ASVs agglomerated at the ecological guild level. Each point is a wood sample. Arrows indicate the direction and the magnitude of each stand or climatic variable associated with guild

community structure. A) Guild community structure along seasonality; standard deviation ellipses are drawn for autumn (gray) and spring samples (pink). Total inertia proportion of the model 16%. B) Guild community structure across plant compartments in autumn. Standard deviation ellipses are drawn for stem (blue) and root samples (brown)Total inertia proportion of the model 13%. C) Guild community structure across plant compartment in spring; standard deviation ellipses are drawn. Total inertia proportion of the model 13%. ppt: precipitation. Max: maximum temperature. Min: Minimum temperature. DBH: diameter at breast height. F: stem. R: root. At: asymptomatic. St: symptomatic.

Table 1. Stand and climatic metadata for each site.

Table 2. Diversity structure of sapwood fungal assemblages in *Nothofagus* forests. Alpha and Beta diversity analysis for the abundance-based and presence-absence datasets (above). Constrained analyses of functional guilds including environmental and host variables (below).

Table 1. Stand and climatic metadata for each site.

	Alerce River	Lake Menéndez	Lake Kruger	Alto el Petiso Mt. (*Nd*)	Alto el Petiso Mt. (*Np*)	El Riscoso Mt.	El Dedal Mt.
coordinates	-42,740618	-42,663181	-42,863615	-42,717178	-42,693054	-42,745421	-42,896524
	-72,007092	-71,821488	-71,773073	-71,751453	-71,745784	-71,71048	-71,648931
elevation (m)	550	750	670	730	1350	1350	1330
average slope (%)	3	31	30	45	49	42	52
exposure	E	SO	SSE	SSE	SO	NO	SSO
mean DBH (cm)	33	56	71	55	15	19	12
median DBH (cm)	27	51	70	56	15	17	12
* minimum temperature (°C)	1,2	-0,3333	0,6	1,8	-0,4667	1,0333	-0,7333
* maximum temperature (°C)	9,9333	8,8333	9,7667	11	8,9	10,4	8,6333
* precipitation (mm)	2902	2165	2234	2390	2073	2217	1812
* climate water deficit index (mm)	1,8667	4,0667	2,1	1,2	3,9667	1,8667	4,5
* soil moisture (mm)	122,1333	87,7667	86,3333	95,7	77,7333	87,0333	58,4
* wind speed (m/sec)	3,7167	4,3633	3,5733	2,8967	4,33	3,23	4,4733
** aridity index	1,65	1,19	1,17	1,12	1,02	0,99	0,84

Nd: *Nothofagus dombeyi* . Np: *Nothofagus pumilio* . DBH: diameter at breast height.

* Terra Climate - Monthly database (Abatzoglou et al. 2018) for May 2016 / May 2018 period accessed from https//climateengine.com/ on March 2022

Temperature, climate water deficit , soil moisture and wind speed are the average values for the period, precipitation is the mean annual value (Huntinghton et al. 2017)

** Trabuco & Zomer (2019)

Table 2. Diversity structure of sapwood fungal assemblages in *Nothofagus* forests. Alpha and Beta diversity analysis for the abundance-based and presence-absence datasets (above). Constrained analyses of functional guilds including environmental and host variables (below).

			Total ASVs									Common ASVs									Functional Guilds								
						perMANOVA									perMANOVA							perMANOVA							
			Richness	χ2	K-W	abundance			P/A			Richness	χ2	K-W	abundance			P/A			K-W	abundance			P/A			
						F.Model	R2		F.Model	R2					F.Model	R2		F.Model	R2			F.Model	R2		F.Model	R2		
		N. dombeyi	3920	***	*	2,4176	0,0101	**	43,5890	0,1554	**	247	*		6,2740	0,0264	**	29,0010	0,1115	**	***	14,0850	0,0575	**	14,3700	0,0586	**
		N. pumilio	2637									205															
N. dombeyi dataset	site	Alto el Petiso Mt.	1461									169															
		Alerce River	1685	***	***	1,4760	0,0338	**	13,3414	0,2091	**	146		***	2,6012	0,0590	**	8,4054	0,1557	**	*	2,3158	0,0521	**	2,9861	0,0627	*
		Lake Kruger	1025									136															
		Lake Menéndez	1080									137															
	health condition	Symptomatic	2634	***		1,0659	0,0081	*	1,8504	0,0097		233			1,0860	0,0082		1,0002	0,0062			0,6551	0,0049		0,1765	0,0012	
		Asymptomatic	2309									205															
	plant compartment	Stem	2567	***		1,2797	0,0098	**	11,7063	0,0612	**	203			1,8220	0,0138	**	6,1338	0,0379	**		4,6783	0,0351	**	11,8312	0,0829	**
		Root	2301									219															
	season	Autumn	2244			1,4417	0,0110	**	14,8315	0,0775	**	193			2,6110	0,0197	**	9,4512	0,0584	**		2,2356	0,0168	*	0,1617	0,0011	
		Spring	2546									226															
N. pumilio dataset	site	Alto el Petiso Mt.	1022									131															
		El Dedal Mt.	941	***	**	1,4313	0,0260	**	9,3061	0,1193	**	118			2,0686	0,0369	**	6,5584	0,0937	**		1,9155	0,0326	*	2,4852	0,0398	
		El Riscoso Mt.	1446									152															
	health condition	Symptomatic	1548	*		1,1865	0,0108	**	4,8848	0,0313	**	172			1,1733	0,0105		2,8299	0,0202	*		1,3214	0,0113		1,2377	0,0099	
		Asymptomatic	1706									175															
	plant compartment	Stem	1545	**								166															
		Root	1696			1,4773	0,0134	**	15,1977	0,0974	**	168			2,9084	0,0260	**	12,7236	0,0909	**		5,0132	0,0427	**	4,9613	0,0397	*
	season	Autumn	1333	***		1,5411	0,0140	**	14,2965	0,0917	**	141	*		2,7987	0,0250	**	10,2863	0,0735	**		6,0354	0,0514	**	13,2830	0,1064	**
		Spring	1837									178															

		Total ASVs	*N. pumilio* dataset		
				autumn	spring
		db-RDA	CCA	CCA	CCA
		F.Model	F.Model	F.Model	F.Model
environmental variables	slope	1,1156 **			
	elevation	1,1564 ***			
	wind speed	1,0862 *	1,9191		
	climate water deficit	1,1037 **	1,8741 *	1,6539	2,0414 *
	Min	1,1009 **	1,7003		
	Max	1,0405	1,7346		
	ppt	1,0451 *	4,1726 ***		
	soil moisture	1,1674 **			
	mean DBH			0,9760	1,6007
	plant compartment		4,1985 ***	3,5320 ***	3,2009 **
	health condition		1,7718	1,1273	0,8438

P/A: presence-absence dataset. χ2: Pearson's Chi-Square test significance for the total richness on each variable's level. K-W: Kruskal-Wallis sum rank test significance for the richness per sample. db-RDA: distance-based redundancy analysis. CCA: canonical constrained analysis. DBH: diameter at breast height.

* significance at 0.01 level; ** significance at 0.001 level; *** significance at 0.0001 level

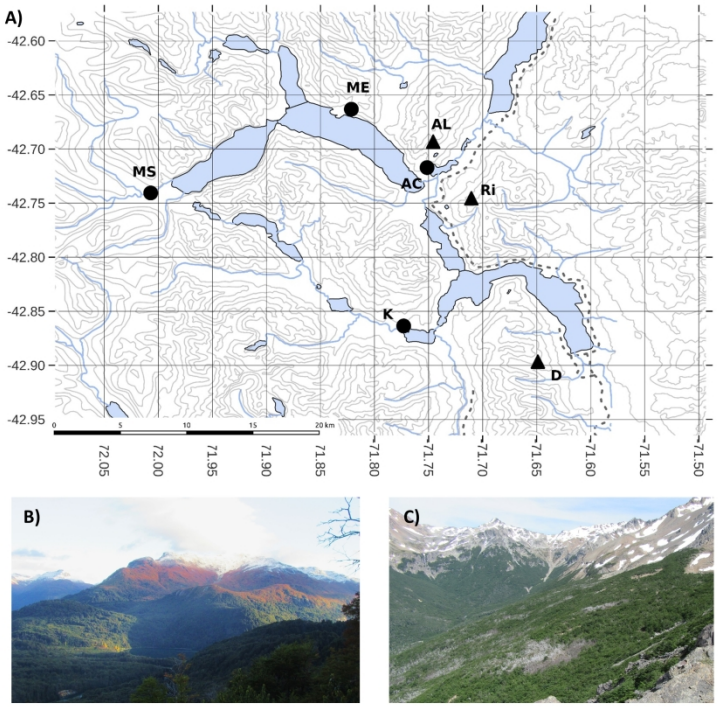

Figure 1. A) Study area and sampling sites. N. dombeyi sites are circles, N. pumilio sites are triangles. MS: Alerce River. ME: Lake Menéndez. AC: N. dombeyi stand in Alto el Petiso Mt. K: Lake Kruger. AL: N. pumilio stand in Alto el Petiso Mt. Ri: El Riscoso Mt. D: El Dedal Mt. The geodetic geographic coordinate system used for the grid is WGS 84. B) Altitudinal distribution of both Nothofagus species. Decidous N. pumilio occupies altitudes up to the timberline. N. dombeyi is an evergreen species. Photo taken on April 2018. C) N. pumilio grouped standing mortality in El Dedal Mt.

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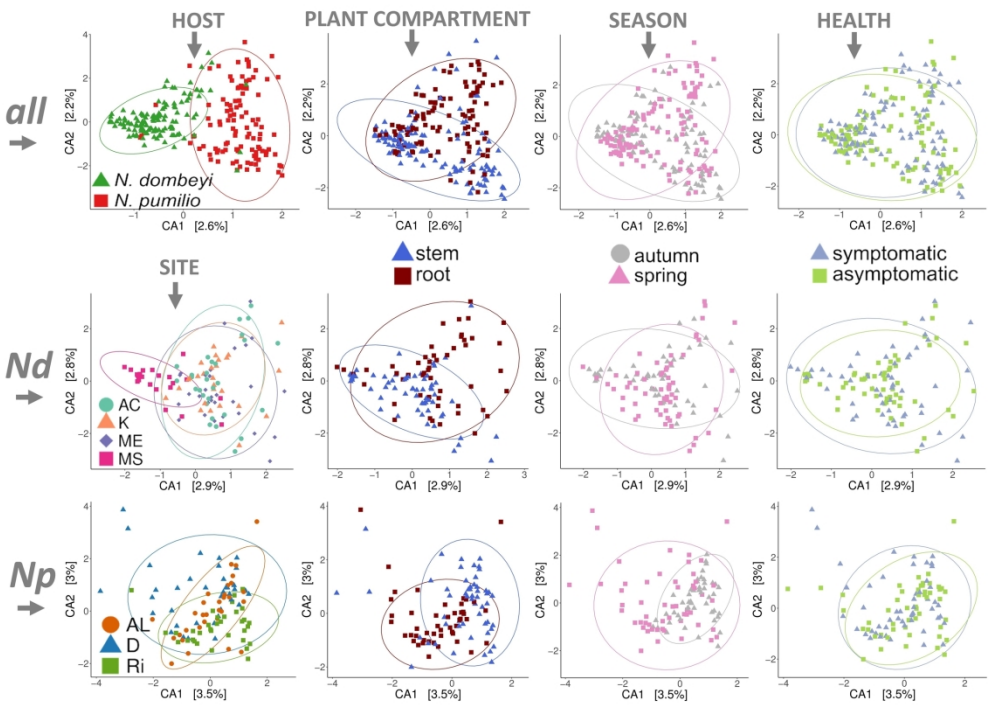

Figure 2. Correspondence Analysis (CA) plots illustrating the association between the variables tested -host, site, plant compartment, season, and health condition- and samples according to their community composition for the whole set of data (above), N. dombeyi data (middle) and N. pumilio data (below). AC: Mt. Alto El Petiso N. dombeyi; K: Lake Kruger; ME: Lake Menendez; MS Alerce River; AL: Mt Alto El Petiso N. pumilio; D Mt. El Dedal; Ri: Mt. El Riscoso

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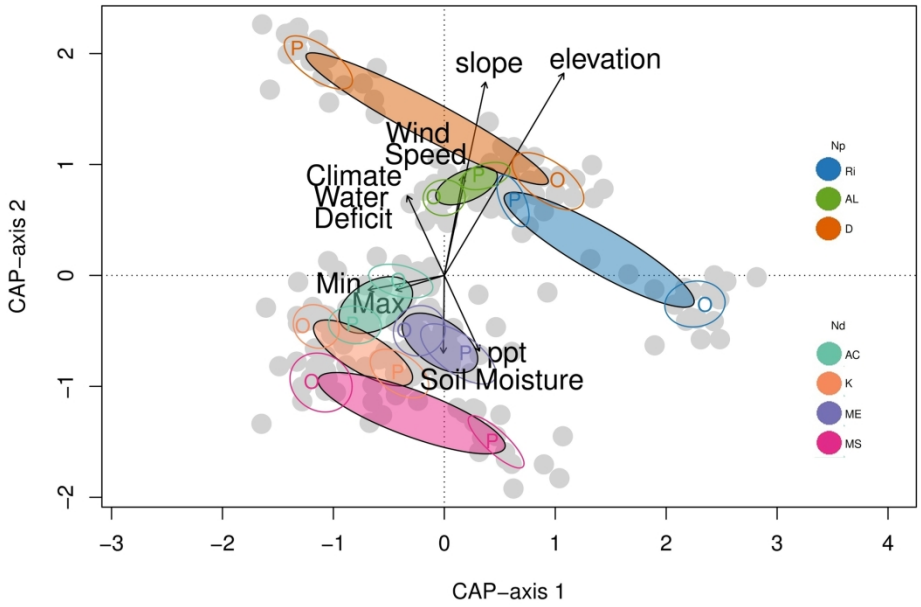

Figure 3. Distance-based redundancy analysis (db-RDA) of Raup-Crick distances showing community structure variations for the whole set of wood samples along with site and seasonal factors. Standard deviation ellipses are drawn for each site and for each site at each season. CAP1 and CAP2 axis explained 14.4% and 13.5% of the variance, respectively (CAP1 F=1.2110, p=0.001 y CAP2 F= 1.1410, p= 0.001, ANOVA). The climatic data are average condition values for each seasonal sampling season and was collected from Terra Climate – Monthly database (Abatzoglou et al. 2018) by using Climate Engine App (climateengine.com). Nd: N. dombeyi (triangle). Np: N. pumilio (circles). AC: N. dombeyi stand in Alto el Petiso Mt. K: Lake Kruger. ME: Lake Menéndez. MS: Alerce River. Ri: El Riscoso Mt. AL: N. pumilio stand in Alto el Petiso Mt. D: El Dedal Mt. ppt: precipitation.

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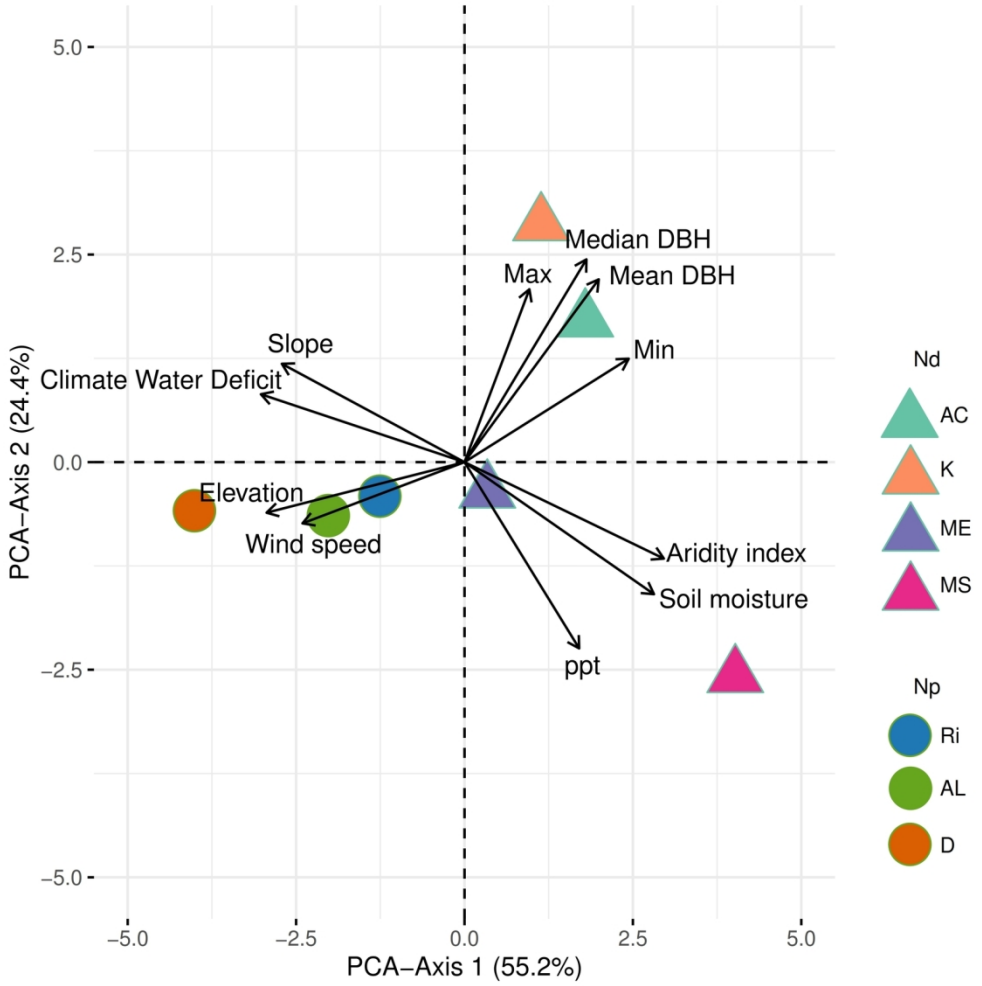

Figure 4. Principal components analysis (PCA) biplot illustrating correlations between stand and climatic variables in the first and second axes. The percentage of variance explained by the axis is showed in parentheses. Arrows are the variables analysed. Each point is a site and are shape-given according to Nothofagus species. Nd: N. dombeyi (triangle). Np: N. pumilio (circles). AC: N. dombeyi stand in Alto el Petiso Mt. K: Lake Kruger. ME: Lake Menéndez. MS: Alerce River. Ri: El Riscoso Mt. AL: N. pumilio stand in Alto el Petiso Mt. D: El Dedal Mt. DBH: diameter at breast height. Ppt: precipitation. Max: maximum temperature. Min: minimum temperature. The variables' definitions and units are detailed in Table 1.

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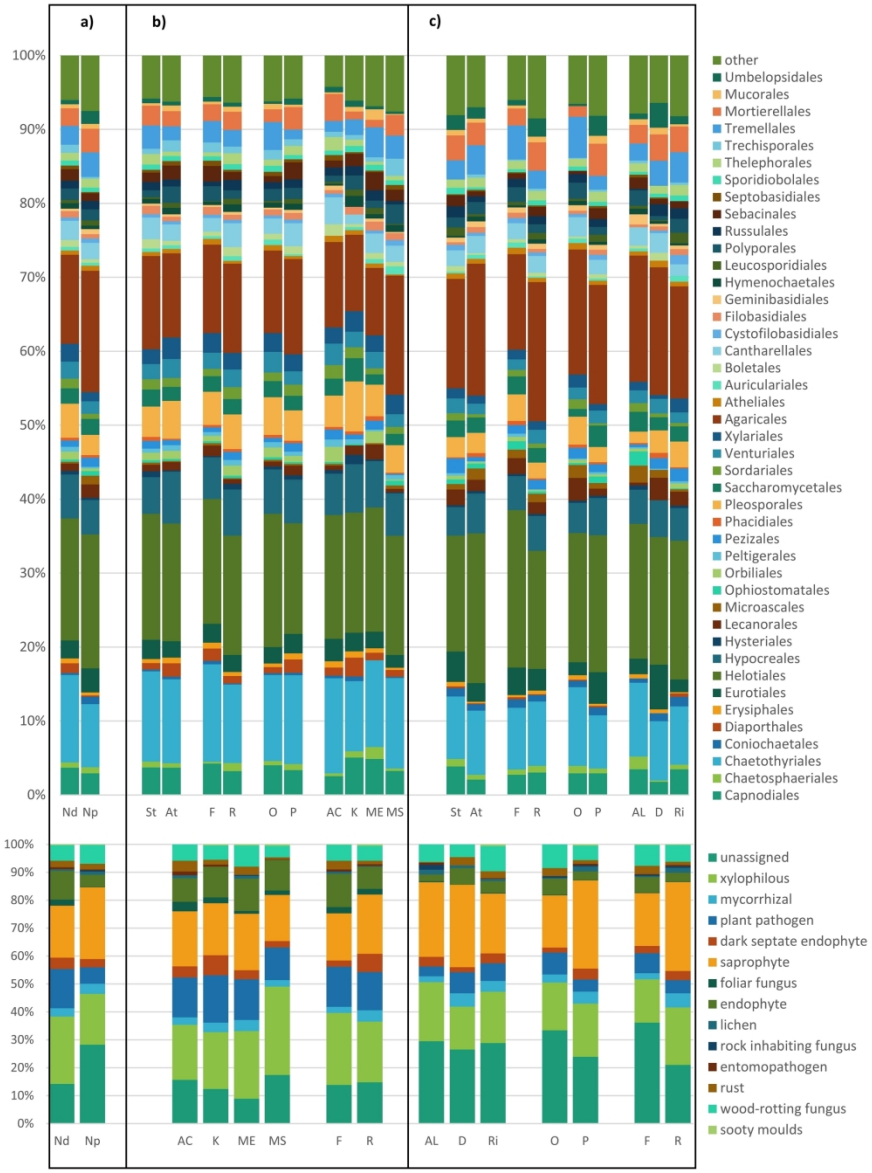

Figure 5. Relative abundances at Order level (above) and guild level (above). Comparison between hosts (a), and between variables' levels for N. dombeyi (b) and N. pumilio (c) separated datasets. Nd: N. dombeyi. Np: N. pumilio. St: symptomatic. At: asymptomatic. F: stem. R: root. O: autumn. P: spring. AC: N. dombeyi site Alto el Petiso Mt. K: Lake Kruger. ME: Lake Menéndez. MS: Alerce River. AL: N. pumilio site Alto el Petiso Mt. D: site El Dedal Mt. Ri: site El Riscoso Mt.

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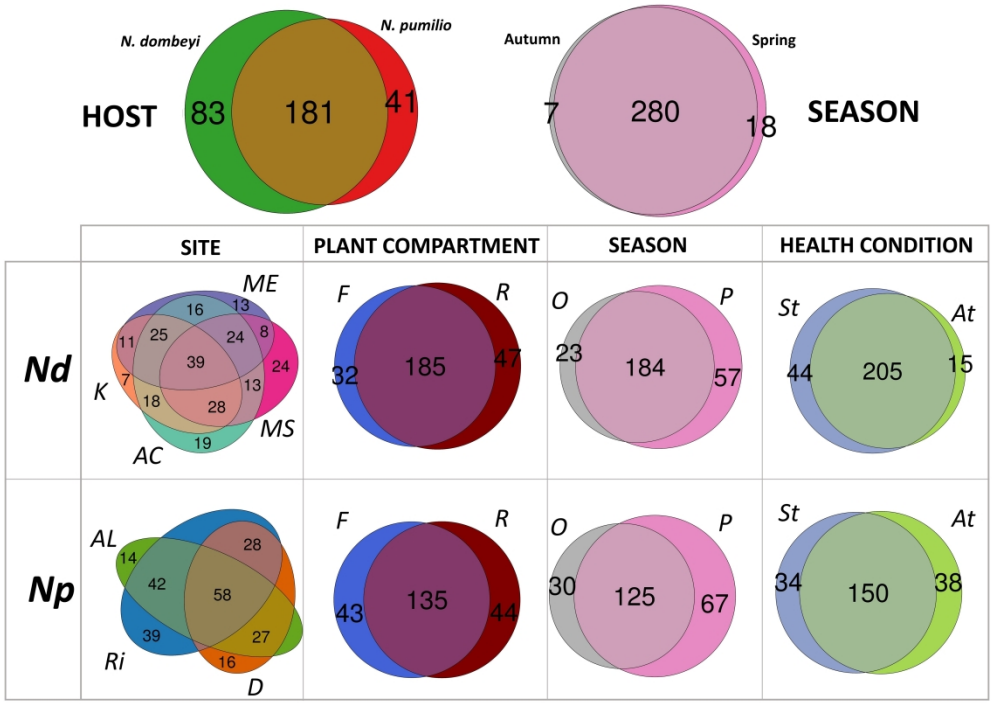

Figure 6. Venn diagrams showing common ASVs that are shared and exclusive from each variable's level. The whole set of samples (above) is analysed, as well as N. dombeyi (Nd) and N.pumilio (Np) datasets separately. Nd: N. dombeyi, Np: N. pumilio, ME: Lake Menendez, MS: Alerce River, K: Lake Krugger, AC: Mount Alto el Petiso N. dombeyi, Ri: Mount El Riscoso, D: Mount El Dedal, AL: Mount Alto el Petiso N. pumilio, St: symptomatic, At: asymptomatic, F: stem, R: root, O: autumn, P: spring.

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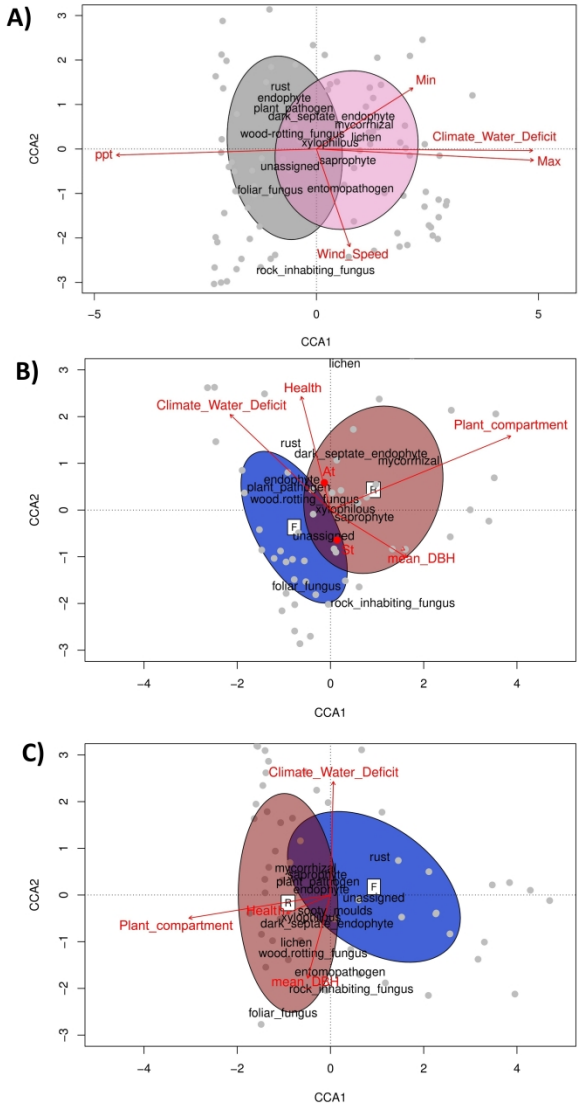

Figure 7. Guild community structure in Nothofagus pumilio. Canonical correspondence analysis (CCA) of the Hellinger's transformed abundances of the common ASVs agglomerated at the ecological guild level. Each point is a wood sample. Arrows indicate the direction and the magnitude of each stand or climatic variable associated with guild community structure. A) Guild community structure along seasonality; standard deviation ellipses are drawn for autumn (gray) and spring samples (pink). Total inertia proportion of the model 16%. B) Guild community structure across plant compartments in autumn. Standard deviation ellipses are drawn for stem (blue) and root samples (brown)Total inertia proportion of the model 13%. C) Guild community structure across plant compartment in spring; standard deviation ellipses are drawn. Total inertia proportion of the model 13%. ppt: precipitation. Max: maximum temperature. Min: Minimum temperature. DBH: diameter at breast height. F: stem. R: root. At: asymptomatic. St: symptomatic.

190x275mm (600 x 600 DPI)