

The soil fungal community of native woodland in Andean Patagonian forest: A case study considering experimental forest management and seasonal effects



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ABSTRACT

Forest management can alter soil fungal communities which are important in the regulation of biogeochemical cycles and other ecosystem services. The current challenge of sustainable management is that management be carried out while preserving the bioecological aspects of ecosystems. Mixed Patagonian woodlands are subject to continuous disturbance (fire, wood extraction, and livestock), with unknown consequences for soil fungal communities. Our objective was to study the fungal community and the effects of an experimental forest management program, which combines harvesting and implantation, on the soil fungal communities in Patagonian woodland, in Argentina; considering contrasting seasons and soil parameters. At site level, the mixed woodlands appear to have greater soil cover with AM plants and soil chemical characteristics similar to other forest environments in the same region, with an intermediate fungal diversity value. When the seasonal effect was analyzed, no changes were observed in the fungal richness and diversity index, whereas modifications were recorded in the class level, in the ecological guilds, and in certain soil parameters; the inversion of phyla should be highlighted. Ascomycota and Basidiomycota were the predominant phyla, and presented a tendency to an inversion between seasons: Ascomycota fungi predominated in autumn, while Basidiomycota were more abundant in summer. The Glomeromycota were not well represented in this study. The most abundant classes were Agaricomycetes, followed by Leotiomycetes, Sordariomycetes, and Tremellomycetes, with some changes in the proportions between the two seasons, which could be related to certain soil characteristics. The most abundant orders in both seasons were Agaricales, Helotiales, Mortierellales, and Filobasidiales, correlated with some soil characteristics. The ordination analysis (NMDS) showed a correlation between taxonomic assignation and some soil characteristics, and only between class seasons. The most abundant ecological guild was saprotrophs (in both seasons: *Hygrocybe*, *Mortierella*, *Cryptococcus*), followed by plant pathogens (*Ilyonectria*, which increase in autumn); and the ectomycorrhizal symbiont (*Hygrocybe* in both seasons, while in summer *Cortinarius*, *Clitopilus*, and *Geoglossum*), all are related to C and N cycle. The only post-management changes observed in soil fungal community and chemical characteristics were in fungal richness (OTUs) and available phosphorus values; all the others (diversity indexes, taxonomy, and ecological guilds) were unaffected by this anthropic disturbance. It should be highlighted that available P and fungal species richness could be proposed as bioindicators. Future studies in native management programs, considering other aspects such as diversity of vegetation uses and system functions of the area will allow us to assess whether some of these changes are early bio-indicators of sustainable use and management.

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1. Introduction

In the twenty-first century, sustainable forest management is a principle that ensures the production of goods and services from forest ecosystems in an optimal, permanent manner; it constitutes a strategy for natural resource management that considers forestry activities in ecological, economic and social contexts (Aguirre-Calderón, 2015). Forest management modifies the original composition of forests through changes in the vegetation composition of tree species, the structure of age classes and the proportions of biomass contributed to the soil. It also influences diverse components of the ecosystem, directly and indirectly, including the availability and quality of resources for microbial development, which leads to alterations in the structure and succession of the microbial community (Goldmann et al., 2015; Purahong et al., 2015). The challenge of sustainable forest management and the basis of the new paradigms is responsible stewardship of the forests and forest lands; that is, regulation of their use in such a way as to preserve their biological diversity, productivity, capacity for regeneration, vitality, and ability to fulfil their ecological, economic and social functions, without causing damage to other ecosystems (Grossberg, 2009; Higman et al., 2000; Malmshheimer et al., 2008).

Despite new and more efficient approaches to biodiversity, there are still considerable gaps in geographical data in soil biodiversity (Tedersoo et al., 2014; Bueno et al., 2017; Cameron et al., 2018). South America holds one of the most diverse biodiversity hotspots, but little research has been carried out in the region, particularly on below-ground fundamental organisms and mycorrhizal symbiosis (Pagano and Lugo, 2019).

The Fungi kingdom is one of the most diverse Eukaryote lineages and is estimated to contain several million species (2.2–3.8 million species; Hawksworth and Lücking, 2017). Fungi are important saprophytic, symbiotic and pathogenic organisms that play critical roles in the function of terrestrial and aquatic ecosystems (Read and Perez-Moreno, 2003; Van Der Heijden et al., 2008; Taylor and Sinsabaugh, 2015; Grossart et al., 2016). Soil fungal communities represent the highest soil biomass and are essential for the maintenance of a large number of ecosystem services (diversity, abundance and plant productivity, soil health, water quality, biocontrol of pathogens, among others). Mycorrhizal (Brundrett, 2004) and free-living rhizospheric fungi (Raaijmakers et al., 2009) are associated with plant species, and they take part in nutrient fluxes from soil to plants (Van der Heijden et al., 2015). Symbiotic mycorrhizal fungi, such as arbuscular mycorrhizal (AM) fungi or ectomycorrhizal fungi (ECM), are ubiquitous components of most ecosystems throughout the world, and constitute a key component of soil microbiota (Van der Heijden et al., 1998; Brundrett and Tedersoo, 2018; Pagano and Lugo, 2019): they mobilize and transport nutrients, especially carbon (C), nitrogen (N), and phosphorus (P), and they also reduce water stress (Augé, 2001) and improve soil aggregation in eroded soils (Caravaca et al., 2002).

Environmental conditions have been shown to play an important role in determining the soil fungal community. Diverse biotic and abiotic factors could affect the structure and function of these communities, such as host-plant diversity (Tedersoo et al., 2013; Bonito et al., 2014), seasonal patterns (Berg et al., 1998; Mandyam and Jumpponen, 2005; Zinger et al., 2009; Dumbrell et al., 2011), weather and geographic factors (Talbot et al., 2013; Miyamoto et al., 2015; Truong et al., 2019), and edaphic variables (Zinger et al., 2009; Dumbrell et al., 2011; Kauserud et al., 2011; Davey, 2012; Peay et al., 2015; Erlandson et al., 2016). Changes in land use, such as forest management, can modify the fungal soil community and alter the balance between carbon (C) allocation to ECM fungi, and N and P acquisition by ECM plants, thereby influencing global C storage and N/P cycles in forest ecosystems (Baskaran et al., 2017; Corrales et al., 2017). Forest harvesting affects ECM fungi by changing the age structure and species composition of their host plants, and by modifying the physical, chemical and biological components of their soil habitat. Some ECM

fungi taxa appear to be primarily associated with mature forests, while others may persist as resistant soil propagules until disturbances occur such as fire or harvesting, providing an opportunity for colonization of seedling roots (Taylor et al., 2000; Jones et al., 2002, 2003). Management without extraction of residual trees plays a role in recently harvested forests by retaining a unique ECM fungal community, by influencing species composition and enhancing the diversity of ECM fungal communities on seedlings adjacent to trees (Cline et al., 2005). Another point of view was presented by Lauber et al. (2008) when considering the effects of forest management on soil fungal communities in boreal forests, suggesting that fungal community composition is more closely associated with changes in soil nutrient content than with different land uses.

In Argentina nowadays the sustainable management of native environments is regulated by National legislation (Law 26.331) and Provincial laws. These laws establish the need to implement sustainable wood extraction management (harvesting), and to encourage environmental restoration action such as the implantation of native plants in extraction areas. Andean Patagonian forests occupy an area of 3 million hectares, representing approximately 10% of the total forest area of Argentina (Laclau, 1997; Donoso and Lara, 1995; SAyDS, 2007). In these forests one of the principal dominant tree species is *Nothofagus* spp. (Cabrera, 1976; SAyDS, 2007). The mixed woodlands of this region are dominated by young arboreal plants and shrub species. From the XIX century the Northwestern Patagonian region has suffered a drastic reduction in vegetation cover due to indiscriminate burning and deforestation caused by settlements in the region (Kitzberger and Veblen, 1999). This kind of disturbance has significantly affected its conservation status (Donoso and Lara, 1995) and the ecological functions of these forests. The mixed woodlands (also called shrublands) are a common successional post-fire environment included in these forests, which are dominated by young arboreal plants, mainly the ECM *N. antarctica* and some AM tree and shrub species. The mixed woodlands have been subject to anthropic disturbance (Kitzberger and Veblen, 1999) due to their high productive capacity for livestock rearing (sheep and cattle) and wood products (poles and firewood) used in domestic heating and for sale on the timber market. It is estimated that more than 70% of mixed woodland in the Patagonian region is under some kind of forest management (Peri et al., 2005), with different intensities and extraction objectives (Veblen et al., 1992; Peri et al., 2005). Most of these woodlands lack a sustainable management program, causing a negative impact on the ecosystem and jeopardizing the sustainability of the system (Peri et al., 2005). A suitable management program is thus required for these native forest ecosystems (Tacón Clavaña, 2004).

Authors such as Peri et al. (2005) and Reque et al. (2007) have worked in forests where *N. antarctica* predominates, and have characterized and proposed sustainable management guidelines for these ecosystems. However, data concerning the effects of different management practices on these native forests is limited, and almost no information is available regarding their effect on soil microorganism communities. In the Northwest Patagonian forest (including woodlands) some authors (La Manna and Barroetaveña, 2011; Berrios et al., 2014) indicated that the soil under *N. antarctica* presented higher N content than under other shrubs, and they suggested that uncontrolled productive use or the replacement of this native species could reduce the N content of the soil with possible negative consequences for the environment. However, knowledge is lacking on the effects of different management practices on this extensively-used woodland. Furthermore, almost no information is available regarding forest management effects on the soil microorganism communities.

Our objective was to study the fungal community and the effects of an experimental forest management program, which combines harvesting and implantation, on these communities in an Argentinian Patagonian mixed woodland. The study was carried out at one year of installation, in two contrasting seasons, and soil chemical characteristics were considered.

2. Materials and methods

2.1. Study area and sampling site

This work was carried out in a site called El Foyel (41°38'48.44"S, 71°29'59.06"W), a representative area of native mixed woodland located in the homonymous community (Río Negro Province, Argentina), in Northwest Patagonia, Argentina. This site has a slope and an average altitude of 850 m above sea level. It is a mixed high woodland, mainly covered by low trees of *Embotrium coccineum*, *Austrocedrus chilensis*, *Lomatia hirsuta*, *Maytenus* sp., and *N. antarctica*, and by mixed shrubs such as *Berberis* sp., *Discarya chacaye*, *Diostea juncea*, and *Schinus patagonicus*. Phytogeographically, the site is located in the Subantarctic Domain, Andean-Patagonian region, Subantarctic Province, and the deciduous forest district (Cabrera, 1971). Average rainfall is 1100 mm annually, occurring principally during autumn and winter, due to the humid winds of the Pacific. Winter precipitation is mainly in the form of snow. Summer is the driest season. The climate in the area is temperate cold, with an average annual maximum temperature of 17 °C; the minimum temperature is 2.5 °C, and the annual average is 8.4 °C.

On the El Foyel site the *Universidad Nacional de Río Negro* group (led by Dr. L. Garibaldi) established a 11340 m² experimental program consisting of eight plots (each 31.5 × 45 m) which simultaneously underwent an experimental forest management program that combined harvesting and implantation factors (Table 1). The eight plots were located consecutively in the site (numbered from 1 to 8, Table 1) and each was submitted to a certain level of harvesting (four levels), and one implantation condition [two levels: implanted (I) with native forest species of ecological interest, and non-implanted (NI)]. Table 1 shows that each harvesting treatment had two plots, one with implanted native species and the other without implanted species.

Harvesting was performed as follows: three intensities were installed (with 0.3, 0.5 and 0.7 of the basal area removed, corresponding to 30, 50, and 70% respectively), and a control plot that was not harvested (0%, Table 1). In each plot harvesting was carried out in six transects of different widths, according to the harvesting intensity. In plots with 0.3 harvesting intensity, six transects of 1.5 m width were cut; in plots with 0.5 harvesting six transects 2.5 m wide were cut, and for plots with 0.7 harvesting the transects cut were 3.5 m wide. Within transects the vegetation was cut at ground level (except for *A. chilensis* trees, which were not cut). In each plot all the larger firewood was extracted, while the smaller plant material was left scattered on the harvesting intensity strip as soil cover.

Implantation: six native species of high forest value were planted: *A. chilensis*, which are colonized by AM, and five ECM Nothofagus species (*N. antarctica*, *N. dombeyi*, *N. nervosa*, *N. obliqua*, and *N. pumilio*) (Fontenla et al., 1998; Diehl et al., 2003, 2008). The seedlings used were between four and six years old at the time of planting. They came from three different geographical origins and were cultivated in greenhouses in the region. Each species was planted in equal quantities, 30 plants per plot, resulting in a planting density of approximately 1269 plants ha⁻¹, per species.

For the intra-annual seasonality analysis, sampling was carried out in autumn (May of 2014) and summer (January of 2015), one year after the forest management treatment had been established (May of 2013).

Table 1

Experimental block assay detailing the distribution of plots according to the forest management established: harvesting (4 levels) and implanted (I) with native forest species or non-implanted (NI).

Plot number	1	2	3	4	5	6	7	8
Harvesting	0.3	0.7	0	0.5	0.7	0.3	0	0.5
Implantation	NI	I	NI	I	NI	I	I	NI

¹Harvesting intensity expressed as basal area removed.

One soil sample was collected per plot, composed of 25 equidistantly distributed sub-samples, thus forming a total of eight samples per season. The "pooling" of these 25 samples enabled us to properly estimate the average value of each plot, but did not allow analysis of within-plot variability, which can be important in fungal communities. The samples were stored at 4 °C for one day. Under aseptic conditions in the laboratory each sample was homogenized and fractionated for the different determinations: gravimetric humidity, soil chemical analyses, and DNA extraction and amplification.

2.2. Description of plot vegetation and mycorrhizal behavior

Before establishment of the experimental program, M. Goldemberg (per.com.) described some particular characteristics of the experimental site where the plot was established; he determined the percentage cover of dead organic matter (leaf litter, detritus and branches), bare soil and abundant plant species. Using bibliographical data on the region (Fernández et al., 2012; Fontenla et al., 1998, 2001), each plant species used for determination of cover was theoretically assigned a mycorrhizal behavior. Both types of result, cover and mycorrhizal behavior of the species were analyzed together.

2.3. Soil characteristics

Chemical characteristics were determined in a soil sample from each plot, in both seasons. Percentage of gravimetric humidity was determined from the fresh samples (three repeats per sample) after drying at 105 °C until constant weight (48 h approx.). The pH in deionized water (2:5 w:v) and conductivity (EC = 1:5 w:v - $\mu\text{cimen cm}^{-1}$) were measured (at room temperature) from dry soil samples sieved through a 2 mm sieve, using a pH-meter and an electric conductivity meter, respectively. The remaining determinations were made with dried soil samples at room temperature (20–25 °C) sieved through a 0.5 mm sieve. Percentages of total carbon (C) and total nitrogen (N) were determined by combustion at 900 °C (Nelson and Sommers, 1996) using the NC Soil analyzer Flash EA 1112 carbon–nitrogen analyzer (Thermo Fisher Scientific, USA). Available phosphorus (Olsen P - $\mu\text{g g}^{-1}$) was determined by extraction with a 0.5 N NaHCO₃ solution and quantified by colorimetry with ascorbic acid, according to Kuos (1996). Percentage Calcium (Ca) was determined by extraction with 1 N ammonium acetate, followed by atomic absorption (Suarez, 1996) using AAnalyst 100 (Perkin-Elmer, USA). Plots 3 and 7 were not analyzed in autumn.

2.4. Molecular and bioinformatic analysis

The fungal internal transcribed spacer (ITS) region was sequenced for each soil sample. The ITS fungal region was amplified by genomic soil DNA, using a PowerSoil DNA isolation Kit (Mo Bio Laboratories, USA), following the manufacturer's instructions. DNA concentration was quantified on a QuantiTTM PicoGreen[®] dsDNA Assay Kit (Invitrogen, USA). Genomic DNA was used as a template for PCR amplification of the ITS ribosomal region using primers: ITS1F (5'- CTTGGTCATTTAG AGGAATAA-3'; Gardes and Bruns, 1996) and ITS4 (5'- TCCTCGCT TATTGATATGC-3'; White et al., 1990) with TAG extensions. Each PCR mix (Inbio Highway, Argentina) consisted of 15.8 μl ddH₂O, 0.2 μl Taq polymerase, 2.5 μl 10x PCR buffer, 2 μl MgCl₂ (25 mM), 0.5 μl dNTP mixture (2 mM), 0.5 μl of each primer (10 μM), 1 μl BSA (20 mg ml⁻¹; Sigma, USA) and 2 μl of a diluted DNA template. The parameters of the first PCR were as follows: an initial denaturation step at 95 °C for 5 min was followed by 35 cycles consisting of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 60 s, followed by the final extension at 72 °C for 10 min. A second amplification of the PCR products was performed to include sequencing adapters and barcodes to identify each of the samples (Manual FLX + Roche, jun 2013, Instituto de Agrobiotecnología Rosario, INDEAR, Rosario, Argentina).

The amplicon libraries were then purified with Agencourt AMPure XP according to the Amplicon Library Preparation Method Manual - GS FLX Titanium Series (Roche, USA). Purified amplicons were quantified using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen, USA). The amplicon pool was prepared with all the equimolar libraries, according to the corresponding concentration (ng ul^{-1}). This pool was brought to work concentration ($1\text{e}7$ molecules ul^{-1}). Sequencing was performed with the FLX + Roche Sequencing platform (INDEAR 2015).

The sequences were quality-filtered and pooled using Qiime v. 1.3.0 (Caporaso et al., 2010). The filtration criteria were: ambiguous bases that exceeded 6 bases, with an average quality score below the minimum of 25; homopolymer maximum exceeding 6 bases; inequalities in the primer exceeding 10 bases; and absence of a discernible reverse primer. The aligner was made using the MUSCLE v38 method (Edgar, 2004). OTU mapping was done with Uclust v1.2.22 (Caporaso et al., 2010) parameters at 97% similarity. OTUs represented by a single sequence were discarded from the data set, according to Tedersoo et al. (2010). The number of raw input sequences was 119235, giving a total of 55,345 sequences at the end of the process.

2.5. Fungal community analysis

Diversity analyses: these were carried out using indices: OTU richness, expected richness (Chao1), evenness as equitability ($= 1-D$, where D is Simpson diversity index), community diversity (Shannon-Weaver index, H), and similarity between communities (Bray-Curtis). The diversity indices were calculated using the *vegan* 2.5–6 (Oksanen et al., 2019) and *fossil* 0.3.0 packages for expected richness (Vavrek, 2011), in R 3.3.1 (R Core Team, 2019). Estimated species richness was also calculated with rarefaction curves using the OTU numbers, and curves were drawn using the *vegan* package *rarefy* function (Oksanen et al., 2019). This was carried out for the entire data set ($n = 16$ samples) and for each season: autumn (A) and summer (S) ($n = 8$ samples/season).

Taxonomic fungal classification: the OTUs were classified carrying out the RAPD classifier application (Manual FLX + Roche, jun 2013, Instituto de Agrobiotecnología Rosario, INDEAR, Rosario, Argentina), with a confidence of 0.5 (Wang et al., 2007), using the UNITE 12_11 ITS database. Fungal taxonomy was considered at different levels: the abundance of phyla, classes, orders, genera, and species. For each plot, the number of sequences for each taxon was standardized by rarefaction using the *vegan* package *rarefy* function (Oksanen et al., 2019).

Ecological guilds: OTUs were assigned an ecological guild using FUNGuild database (Nguyen et al., 2016). As the authors suggest, we filtered the assignments according to their confidence ranking (“highly probable”, “probable”, and “possible”; reflecting the likelihood that a taxon belongs to a given guild). Only assignments with highly probable and probable confidence were considered, and the number of sequences for each guild was standardized by rarefaction. The arbuscular mycorrhizal fungi were not analyzed since the primers used cannot

identify the community of Glomeromycetes well (Stockinger et al., 2010; Schoch et al., 2012).

2.6. Statistical analysis

To evaluate forest management (harvesting and implantation factors), seasonal effects, soil chemical characteristics (humidity, pH, conductivity, C, N, P, and Ca) and fungal community diversity (observed and expected richness, Simpson equitability index, Shannon - Weaver diversity index) were estimated using general mixed-effect models. Fixed effects included harvesting intensity (quantitative variable modeled with a second order polynomial function), implantation (I vs. NI), season (autumn vs. summer), and their two-way interactions. The hierarchical data structure (two measures in each plot over time) was accounted for by including plot as a random effect. In particular, our model estimated different intercepts for each plot. A different model was estimated for each soil and fungal community characteristic as a response variable, and normal distribution was assumed for all models. Model assumptions were met in all cases, and homogeneity of variances and normality were evaluated with residual and predicted graphs, Q-Q plots, and Shapiro-Wilk tests. We used the corrected Akaike Information Criterion (AICc) to select the model that best described the behavior of the fungal community with respect to management and season variables. The AICc values and estimation of the parameters for the final models were obtained from the maximum likelihood estimates (Zuur et al., 2009).

Structure of the fungal community: In order to represent the structure of the fungal communities at plot level, we performed a non-metric multidimensional scaling analysis (NMDS). The abundance of phyla, classes, orders, and ecological guilds (rarefied) was considered in correlation with forest management, season, and soil characteristics. The parameters used were the Bray-Curtis dissimilarity, with 999 free permutations. The variables that had a significant effect on the ordering of the fungal communities were plotted using *envfit* function, and were also evaluated with *distance-based RDA*.

All statistical analyses were performed using the *vegan* 2.5–6 (Oksanen et al., 2019), *nlme* 3.1–140 (Pinheiro et al., 2019) and *MuMIn* 1.43.6 (Barton, 2019) packages in R (R Core Team, 2019).

3. Results

3.1. Vegetation and soil characteristics

The El Foyel site is a high woodland (as previously mentioned); however, Goldemberg (per.com.) reported that in this area the experimental program plots had certain particular vegetation characteristics: the soil was covered by a significant amount of dead organic matter (leaf litter, detritus and branches) over 75% in all the plots and the percentages of bare soil were between 0 and 9.5%, the highest value

Table 2

Description of El Foyel plots before establishment of the experimental program: percentage cover categorized into bare soil, fallen woody material and plants grouped by their mycorrhizal types.

Cover (%) / Plot	1 (0.3 ¹ NI)	2 (0.7 I)	3 (0 NI)	4 (0.5 I)	5 (0.7 NI)	6 (0.3 I)	7 (0.0 I)	8 (0.5 NI)
Bare soil	9.5	0.0	0.0	2.0	0.0	3.3	0.5	2.0
Leaf litter, branches and plant detritus	88.8	76.3	98.8	100.0	98.0	97.5	100.0	98.8
AM plants	98.8	30.5	19.5	14.0	25.0	18.8	17.5	51.0
ECM <i>Nothofagus antartica</i>	1.3	0.0	0.8	0.0	0.0	0.0	0.0	0.0
MER <i>Gaultheria mucronata</i>	0.0	0.0	0.0	0.5	3.0	0.0	0.0	0.0
ND mycorrhizal species	0.0	0.0	1.0	0.0	3.5	3.0	0.0	0.0
NH <i>Embothrium coccineum</i>	0.0	6.0	1.5	7.3	2.8	6.0	6.3	2.8

¹ Harvesting intensity expressed as basal area removed, and implanted (I) with native forest species or non-implanted (NI). AM: arbuscular mycorrhizal symbiosis, ECM: ectomycorrhizal symbiosis, MER: ericoide mycorrhizal, NH: non host mycorrhizal plants, ND: mycorrhizal types non-determined in the region.

being for plot 1 and the lowest for plots 2, 3 and 5 (Table 2). The plants associated with AM symbiosis were *Acaena* sp., *Azara micriphylla*, *Berberis buxifolia*, *Chusquea culeou*, *Cynanchum descrolei*, *Galium hypocarpium*, *Geranium magellanicum*, *Lomatia hirsute*, *Maytenus boaria*, *Maytenus chubutensis*, *Mutisia spinosa*, *Ribes cucullatum*, *Schinus patagonicus*, *Vicia nigricans*, and *Blechnum* sp. These AM species presented the highest soil cover in each plot (Table 2). The highest values were observed in plot 1 (98.8%), principally associated with the presence of *Maytenus chubutensis* and *Berberis buxifoli*; in plot 8 (51%) the value was related to *Blechnum* sp. and *Acaena* sp. These species also contributed to the intermediate cover values (between 30 and 10%) with the presence also of *Berberis buxifolia* and *Ribes magellanica*. The ECM *Nothofagus antarctica* and ERM *Gaultheria mucronata* showed lower cover values. The ECM had 1.3 and 0.8% in plots 1 and 3, and the remaining plots had undetectable cover (0%). *Gaultheria mucronata*, an ERM species, had cover values of 0.5 and 3.0% in plots 4 and 5 respectively.

In general, El Foyel soil was slightly acidic (pH 5.5), with the following average values: electrical conductivity of $110 \mu\text{cm cm}^{-1}$, gravimetric humidity 25.13%, 0.46% of N, and 7.51% of C. Available P was $7.02 \mu\text{g g}^{-1}$ soil, and the Ca quantity was 0.31%. When comparing soil parameters between seasons, autumn values for gravimetric humidity, pH, EC, and N were all higher than in summer (humidity: 32.71–19.44%; $F = 119.8$, $p < 0.0001$; pH: 5.94–5.17; $F = 43.3$, $p = 0.0001$; EC: 138.22–89 $\mu\text{cm cm}^{-1}$; $F = 48.6$, $p = 0.0001$; N: 0.5–0.43%; $F = 7.5$, $p = 0.02$; Table 3). There were no differences between season in the amount of C, P and Ca (C: 8.2–7%; $F = 4.5$, $p = 0.06$; P: 7.87–6.39 $\mu\text{g g}^{-1}$; $F = 4.42$, $p = 0.06$; Ca: 0.33–0.29%; $F = 4.7$, $p = 0.06$ autumn and summer respectively, Table 3). The only soil variable that showed differences related to forest management was available P (harvesting intensity: $F = 0.003$, $p = 0.9$; implantation: $F = 0.5$, $p = 0.5$; harvesting intensity: implantation: $F = 9.2$, $p = 0.01$). It was observed that an increase in harvesting intensity in implanted plots led to an increase in P, while in non-implanted plots P values diminished when harvesting was intensified (Supplementary Fig. 1).

3.2. Community analysis in relation to seasonality and forest management

3.2.1. Diversity indices

A total of 119,235 fungal sequences were generated from the soil, which after the filtering process and removal of singletons constituted 55,345 sequences. These sequences were grouped into 1848 OTUs. The average sequences and number of OTUs per plot ($n = 16$) were 3459 and 485 respectively, without considering the difference between seasons.

Considering the diversity indices, the global Simpson equitability was 0.98, the Shannon-Weaver diversity was 4.98, and Chao1 estimated richness was 659 (Fig. 1 and Table 4). The OTU rarefaction curves for all data sets reached the asymptote (Fig. 1). Species richness estimates were similar for both seasons and presented superposition of the estimated curves (Fig. 1).

Considering the total dataset (1848 OTUs), over 50% (1047) were shared between the two seasons, while 385 were found exclusively in autumn and 416 only in summer. The between-season Bray-Curtis similarity index was 0.66 (Table 4).

Analysis using the statistical models showed that neither fungal richness parameters (expected or estimated) were influenced by the intra-annual seasonality factor. However, an effect was observed when interaction between the two forest management factors was analyzed (implantation: harvesting intensity: harvesting intensity²) (Fig. 2 and Supplementary Table 1). In the implanted plots, richness (OTU observed) was similar for 0 and 0.3 harvesting intensities, and these values were lower than the other harvesting intensities. A similar tendency was observed for estimated richness, with a high number of OTUs in the 0.5 and 0.7 harvesting intensities. In non-implanted plots, the estimated and expected richness values behaved in the same way, with the highest values found for 0.3 harvesting intensity (Fig. 2). The

statistical analysis of Simpson equitability and Shannon-Weaver diversity indices showed that there was no effect on fungal richness due to season or forest management (harvesting and implantation) (Fig. 2 and Supplementary Table 1).

3.2.2. Taxonomic classification

Of the 1848 OTUs obtained, 3 (0.1%) were not assigned to kingdom level and 245 (13%) were classified only to Fungi kingdom level. The remaining 1600 OTUs and 51,667 sequences were classified into 5 phyla (87% of OTUs and 93% of sequences), 18 classes (72% of OTUs and 79% of sequences), 61 orders (62% of OTUs and 68% of sequences), 103 families (49% of OTUs and 57% of sequences), 207 genera (37% of OTUs and 44% of sequences) and 194 species (17% of OTUs and 32% of sequences).

Analysis of the effects of intra-annual seasonality on the fungal community at site level revealed that at phylum level an inversion of dominance was observed between seasons: Ascomycota fungi predominated in autumn, increasing from 42% in summer to 49% in autumn, while Basidiomycota showed an increase in summer, rising from 36% in autumn to 45% in summer. The third most abundant phylum in both seasons was Zygomycota (7 and 5% in autumn and summer, respectively) (Fig. 3a). The most abundant classes in both seasons were Agaricomycetes (Basidiomycetes), followed by Leotiomycetes, Sordariomycetes (both Ascomycetes), and Tremellomycetes (Basidiomycetes). In autumn there was an increase in the abundance of Leotiomycetes, Sordariomycetes, and Tremellomycetes. In contrast, Agaricomycetes displayed an increase in abundance in summer (Fig. 3b). The most abundant orders in both seasons were Agaricales (Agaricomycetes) and Helotiales (Leotiomycetes) with sequence abundances of 12–22% for Agaricales in autumn and summer respectively, and 13–11% for Helotiales in autumn and summer, respectively. In autumn the orders Mortierellales (Mortierellomycetes) and Filobasidiales (Tremellomycetes) presented sequence abundances of 7 and 6%, respectively, which decreased to values < 5% in summer (Fig. 3c).

Community structure: At plot level, the ordering study (NMDS analyses) between phylum assignment, forest management, season, and soil characteristics showed no correlation between phylum and season, phylum and harvesting intensity or implantation. However, a statistical relationship was observed between phylum and soil chemical properties (Fig. 4a). The increase in Ascomycota was related to the increase in C ($p = 0.032^*$), N ($p = 0.02^*$), and EC ($p = 0.015^*$), while the increase in Zygomycota was associated with higher humidity ($p = 0.02^*$). When considering plot and class level, correlations were observed only with season ($p = 0.004^{**}$), humidity ($p = 0.001^{**}$), EC ($p = 0.007^{**}$), and pH ($p = 0.03^*$; Fig. 4a); there was no correlation with harvesting or implantation (Fig. 4b). In autumn the plots had higher soil humidity, EC, and pH, and these plots were correlated with Leotiomycetes, Sordariomycetes, and Tremellomycetes. In contrast, the summer plots were associated with Dothideomycetes, Eurotiomycetes and Agaricomycetes (Fig. 4b). The NMDS analysis of the orders at plot level showed correlations between some soil chemical characteristics, but no correlations were evidenced for the season and management variables (harvesting and implantation; Fig. 4c). The chemical characteristics of the soil that showed correlations were: EC ($p = 0.013^*$), humidity ($p = 0.025^*$), N ($p = 0.002^{**}$), C ($p = 0.003^{**}$), and available P ($p = 0.026^*$; Fig. 4c). The results obtained with distance-based RDA were similar to envfit (not shown).

3.2.3. Ecological guild characterization at species level

The genera and species frequencies were poorly represented with respect to the total data (as mentioned in the previous section). However, in order to analyze the ecological guilds of the assigned species, the distribution of genera and species followed these patterns: in autumn the most numerous genera (more than 500 sequences) were: *Ilyonectria* (1694 sequences), *Mortierella* (1597), *Cryptococcus* (1479), *Hygrocybe* (1321), *Lecythophora* (607), and *Trichocladium* (607),

Table 3
Physical-chemical soil characteristics of the El Foyel site in Andean Patagonian woodland, expressed per season.¹

Plot number	Forest management	pH		EC ($\mu\text{cm cm}^{-1}$)		H (%)		C (%)		N (%)		P ($\mu\text{g g}^{-1}$)		Ca (%)		
		A	S	A	S	A	S	A	S	A	S	A	S	A	S	
1	0.3 NI	6.32	5.27	95.7	56.7	24.75	13.74	7.4	5.2	0.4	0.31	13.8	9.8	0.3	0.2	
2	0.7 I	5.87	5.3	108.5	71	26.68	17.16	7.2	6	0.4	0.34	12.9	9.4	0.3	0.3	
3	0 NI	na	5.24	na	72.3	na	20.15	na	7.1	Na	0.42	na	10	na	0.3	
4	0.5 I	5.46	5.14	130.4	103.3	35.89	20.97	9	7	0.55	0.43	6.5	6.8	0.4	0.3	
5	0.7 NI	6.16	5.17	139.8	72	36.55	18.66	8.4	6.7	0.53	0.43	5.4	4.4	0.4	0.3	
6	0.3 I	6.15	5.12	165	95	36.42	21.01	9.5	7.5	0.56	0.48	4.5	4.1	0.3	0.3	
7	0 I	na	5.06	na	103.9	na	20.82	na	7.1	Na	0.45	na	3	na	0.3	
8	0.5 NI	5.68	5.08	189.9	137.8	35.97	23.03	7.7	9.4	0.53	0.58	4.1	3.6	0.3	0.3	
Average per season ²		5.9- 4 \pm -	5.1- 7 \pm -	138.2- 2 \pm -	89.0- 0 \pm -	32.7- 1 \pm -	19.4- 4 \pm -	8.2- 0 \pm -	7.0- 0 \pm -	0.5- 0 \pm -	0.4- 3 \pm -	7.8- 7 \pm -	6.3- 9 \pm -	0.3- 3 \pm -	0.3- 0.05 ^a	0.2- 0.05 ^a
9		0.33 ^a	0.09 ^b	35.05	26.13 ^b	5.46 ^a	2.89 ^b	0.92 ^a	1.22 ^a	0.07 ^a	0.08 ^b	4.34 ^a	2.98 ^a	0.05 ^a		

9 \pm 0.04^a

Average per site El Foyel

5.5 \pm 0.45

110.09 \pm 38.46

25.13 \pm 7.9

7.51 \pm 1.23

0.46 \pm 0.08

7.02 \pm 3.55

0.31 \pm 0.05

¹ Data from two seasons: A: autumn and S: summer; EC: electrical conductivity; H: humidity; C: Carbon; N: Nitrogen; P: available Phosphorus; na: not analysed.

² Letters indicate differences between seasons using the gls model.

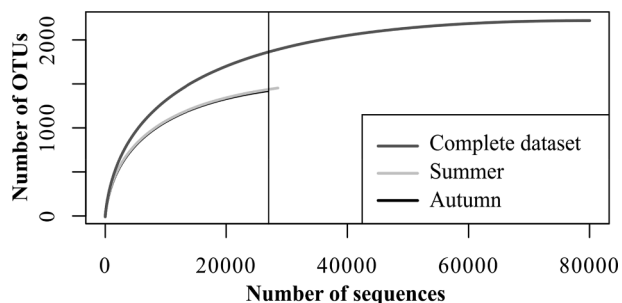


Fig. 1. Rarefaction curve for the complete OTU dataset (n = 16) and for each season (n = 8), summer and autumn. The rarefied OTU richness values were for the complete dataset = 1868, summer = 1445 and autumn = 1432.

Table 4
Average values for total and seasonal soil fungal data and the diversity index of El Foyel Patagonian woodland.

	Autumn + Summer	Autumn	Summer
Abundance ¹	3459 ± 361	3350 ± 453	3568 ± 219
Number of shared OTUs	1047 (56.75%)		
Number of non-shared OTUs		385 (20.8%)	416 (22.5%)
Expected richness ²	485 ± 43	475 ± 40	495 ± 47
Estimated richness ³	659 ± 59	661 ± 57	658 ± 67
Equitability Index	0.98 ± 0.006	0.98 ± 0.006	0.98 ± 0.007
Diversity Index	4.98 ± 0.2	4.95 ± 0.16	5.01 ± 0.24
Similarity Index		0.66	

¹ Number of sequences.

² Observed OTUs.

³ Estimated OTUs.

represented principally by the species *Ilyonectria rufa*, *Hygrocybe conica*, *Mortierella verticillata*, *Cryptococcus terreus*, *Lecythophora mutabilis*, *Mortierella* spp., *Trichocladium opacum*, and *Cryptococcus terricola*

(Supplementary Table 2). In summer the most numerous genera were *Hygrocybe* (1983), *Mortierella* (1237), *Cryptococcus* (830), *Cortinarius* (699), *Ilyonectria* (653), *Clitopilus* (638), *Lecythophora* (593), and *Geoglossum* (527), and the species were *Hygrocybe conica*, *Mortierella verticillata*, *Cortinarius* spp., *Clitopilus hobsonii*, *Ilyonectria rufa*, *Lecythophora mutabilis*, and *Cryptococcus terreus* (Supplementary Table 2).

Of the 207 genera, 170 were assigned to ecological guilds (with highly probable and probable confidence). The saprotroph ecological guild presented the highest sequence abundance (49 and 59% in autumn and summer respectively), followed by plant pathogens (22–12% in autumn and summer respectively), other symbionts (12–11% in autumn and summer respectively), and ectomycorrhizal fungi (10–13% in autumn and summer respectively; Fig. 5a). Since the primers used were not able to identify the community of Glomeromycetes well, and the assignment to AM fungi represented < 1% of the total phyla and of ecological guild assignment in autumn and summer respectively, they were not included in this analysis (see Materials and methods, taxonomic fungal classification).

At plot level, the NMDS study was performed between ecological guilds, forest management, season, and soil chemical characteristics. Correlations were found between seasons ($p = 0.016^*$) and soil chemical characteristics: humidity ($p = 0.002^{**}$), EC ($p = 0.002^{**}$), C ($p = 0.001^{***}$) and N ($p = 0.001^{***}$; Fig. 5 b). Autumn was related to increased values for plant pathogens and an increase in humidity, EC, C, and N, while summer was associated with saprotroph, other symbionts, and ectomycorrhizal fungi, and a decrease in soil characteristics (Fig. 5 b).

4. Discussion

The mixed woodland of the experimental program presented vegetation typical of this environment (as mentioned in the introduction) with low and young trees and high shrubs. Although each plot had certain particular characteristics with respect to the abundance of vegetation, in general the higher cover levels were determined by more or

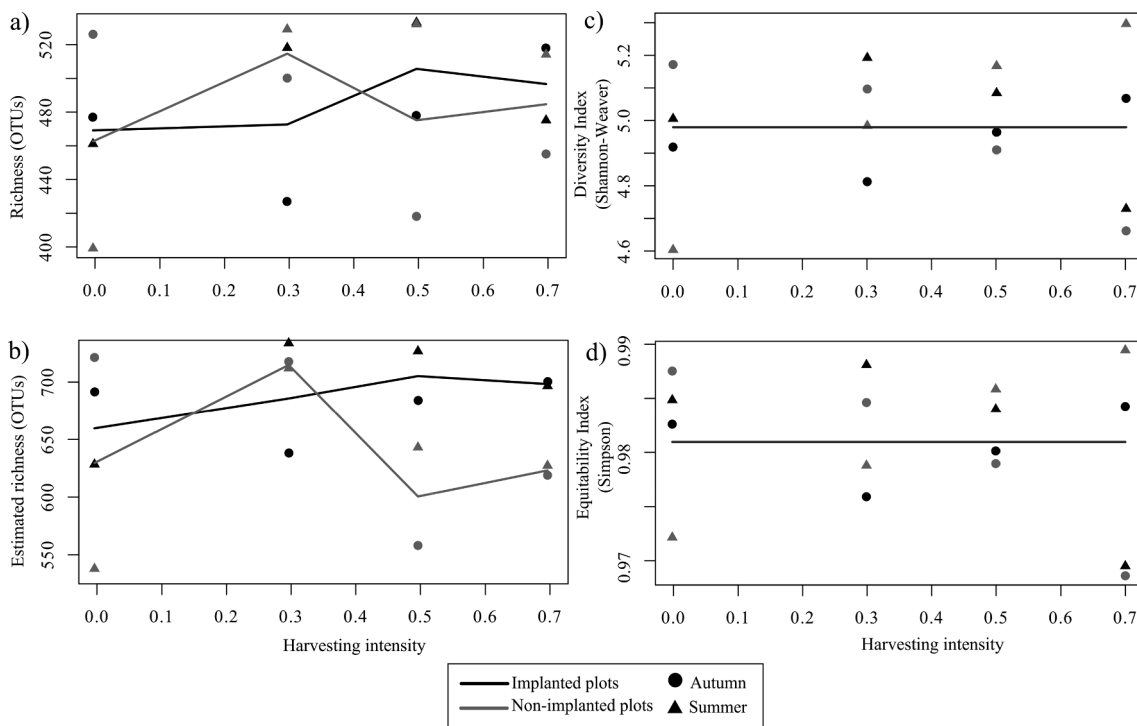


Fig. 2. Richness of OTUs (observed -a- and Chao1 expected -b-), Shannon-Weaver diversity index (c) and Simpson equitability index (d) in relation to harvesting. The prediction of the model with the best fit is shown for each comparison. The black plots and lines represent implanted plots and the gray non-implanted; circles represent autumn data and triangles represent summer.

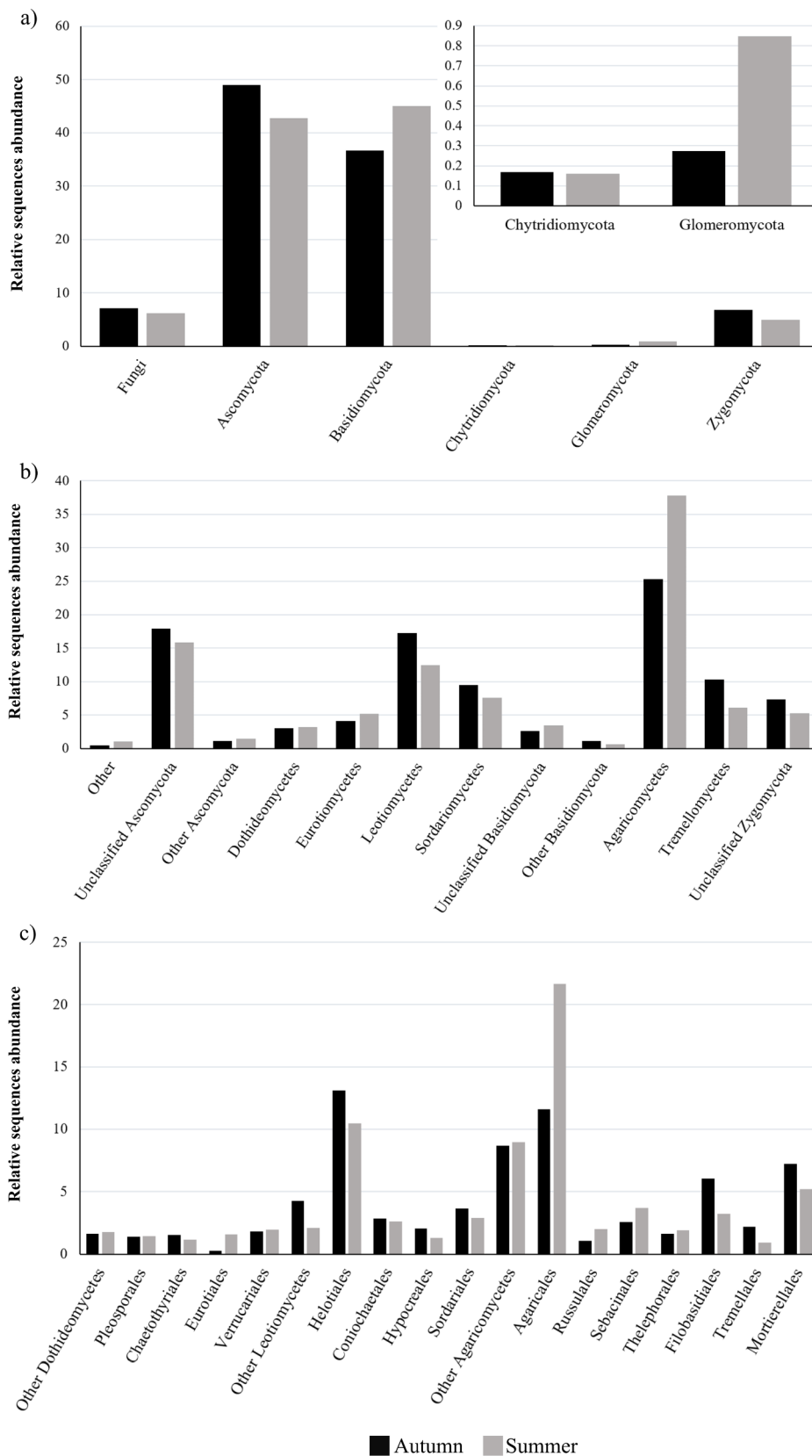


Fig. 3. Relative abundance of sequences assigned to phyla (a), classes (b), and orders (c), in both seasons. Unclassified classes included sequences grouped into their respective phyla, as they were not identified to class level. “Others” includes classes and orders with an abundance less than 1%. In (b) “Others” include: Chytridiomycetes, Monoblepharidomycetes, and Glomeromycetes; “Other Ascomycota” include: Archaeorhizomycetes, Lecanoromycetes, Orbiliomycetes, Pezizomycetes, and Saccharomycetes; “Other Basidiomycota” include: Agaricostilbomycetes, Dacrymycetes, Entorrhizomycetes, and Microbotryomycetes. In (c) “Other Dothideomycetes” include: Dothideales and Capnodiales; “Other Leotiomycetes” include: Erysiphales, Leotiales, and Thelebolales; “Other Agaricomycetes” include: Atheliales, Auriculariales, Boletales, Cantharellales, Corticiales, Geastrales, Gomphales, Hysterangiales, and Polyporales.

less the same species, with a predominance of *Maytenus chubutensis* and shrub species. It has previously been reported (Fernández et al., 2012; Fontenla et al., 1998, 2001; Velázquez et al., 2016; Godoy and Marín, 2019) that AM plants are the most abundant in the native forest of North Patagonia, not only in forest where the dominant tree species had

AM, such as those dominated by ECM native species, *Nothofagus spp.* In the mixed woodland analyzed here, the species with highest cover were associated with AM symbiosis. In another study, carried out in the same experimental management program between October 2014 and January 2015, it was found that the most abundant floral plant species

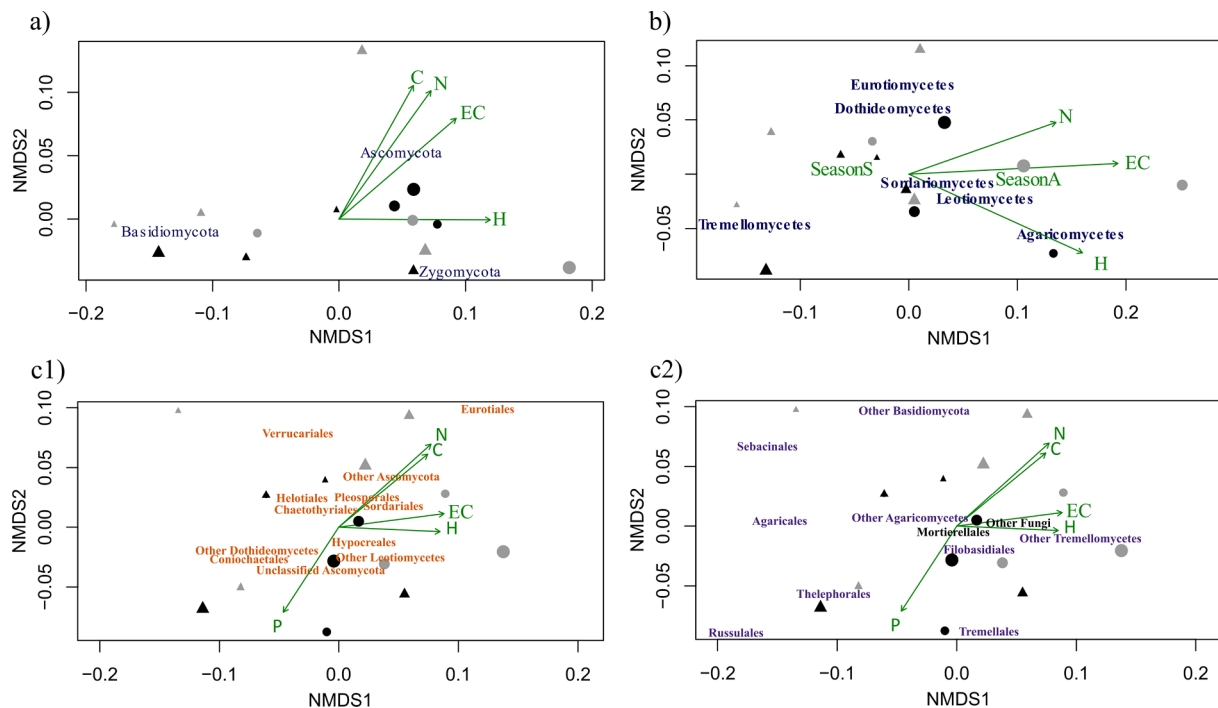


Fig. 4. Non-metric multidimensional scaling plot from phyla (a), classes (b), and orders (c1 and c2), using the Bray-Curtis differences as parameters. The order data were presented in two complementary graphs for visual clarity: Ascomycota orders (c1); Basidiomycota and Zygomycota orders (c2). The plots are represented by circles: autumn, triangles: summer, black: implanted plots, gray: non-implanted plots. Size represents harvesting intensity, an increase in size indicating an increase in environmental level (smaller control plots). Environmental variables that were significant in the analysis are presented; SeasonA: autumn; SeasonS: summer; C: carbon; N: nitrogen; H: humidity; P: phosphorus; EC: electric conductivity.

were woody, including *D. chacaye*, *S. patagonicus*, and *Maytenus* sp., and that plant density decreased in the plots with increasing harvesting intensity, while plant richness increased with an increase in harvesting level (Coulin et al., 2019).

In general, the site had slightly acidic soil, with values of N and C consistent with those obtained in different native forests from similar regions in Northwest Patagonia (Diehl et al., 2003; Alauzis et al., 2004; Chaia et al., 2006; Satti et al., 2007). When the seasonal effect was analyzed, differences were revealed in some soil chemical characteristics: gravimetric humidity, mainly related to the increase in rainfall at autumn, and pH, EC, and N (Table 2). Ca and available P did not show statistically significant seasonal changes. Previous studies in forest soil of the region reported statistical differences in available P between seasons (Satti et al. 2007; Diehl et al., 2008), which may constitute another difference between this mixed woodland and forest dominated by other *Nothofagus* species.

Several studies have shown that soil chemical and physical characteristics are modified with changes in land use (McDowell and Stewart, 2006; Klein et al., 2008; Lauber et al., 2008; Dube et al., 2009; Redel et al., 2015). When analyzing the effect of forest management, available P was the only soil parameter that showed significant differences (Supplementary Fig. 1). This was in accordance with studies in *Nothofagus* forests in southern Chile, where a decrease in different phosphorus fractions was found, in particular available P, with the application of forest management (Redel et al., 2008) and other land-use changes (Redel et al., 2015).

Taxonomic classification of the community showed a high number of unidentified sequences (Buée et al., 2009; Truong et al., 2017), suggesting a considerable number of undescribed fungi for these environments, as previously mentioned by other authors in the region (de Garcia et al., 2012; Duo Saito et al., 2018). It is also important to consider that nowadays new NGS methods are used, which could produce novel or more detailed analyses than considered here using 454-pyrosequencing (Roche) platforms (Henrik Nilsson et al., 2011; Lindahl

et al., 2013; Oulas et al., 2015). The Shannon-Weaver diversity index presented values close to 4.5 in both seasons (Table 4). These values were lower than those found by Fernández et al. (2018), with a diversity value of 0.84 for the rhizosphere of total fungi in *N. pumilio* forests, and around 0.95 for the area affected by the tephra fall. The number of total fungal genomic studies in the Northwest Patagonia is small, with very few that address subject of forest management, but these suggest that mixed woodland could have an intermediate diversity value.

The community analysis (richness, Shannon-Weaver, and Simpson indexes) showed that these factors were similar between seasons. More than half of the OTUs (57%) were shared between the two seasons, and the Bray-Curtis similarity index was 0.66. These results are comparable to those cited by Purahong et al. (2015) in temperate *Fagus sylvatica* forests of the central region of Germany (studied over a complete year, in each season) where they found that approximately 44–55% of the fungal OTUs were shared between the different types of forest cutting.

At a fungal community level, neither the equity nor diversity indices showed a significant management effect. When considering richness, a significant effect was observed in the interaction between harvest intensity and implantation (Fig. 2). Our results partly contradict Goldmann et al. (2015) who studied different forest management strategies of *Fagus sylvatica*, *Picea abies*, and *Pinus sylvestris* in Germany, and found that in general the study site and the type of forest management had no effect on fungal diversity and richness.

In our work, when analyzing the taxa between seasons, a tendency of an inversion of dominance was observed: Ascomycota increase in autumn, and Basidiomycota in summer. This data partially overlaps with studies in cold regions in both hemispheres, in spring and summer, where it has been reported that Ascomycetes were the dominant phylum in soils (Goldmann et al., 2015; Duo Saito et al., 2018). It has been documented also that Basidiomycota, and to a lesser extent Ascomycota fungi, are predominant in forest soils in different regions of the world (Tedersoo et al., 2014; Voriskova et al., 2014) and in similar

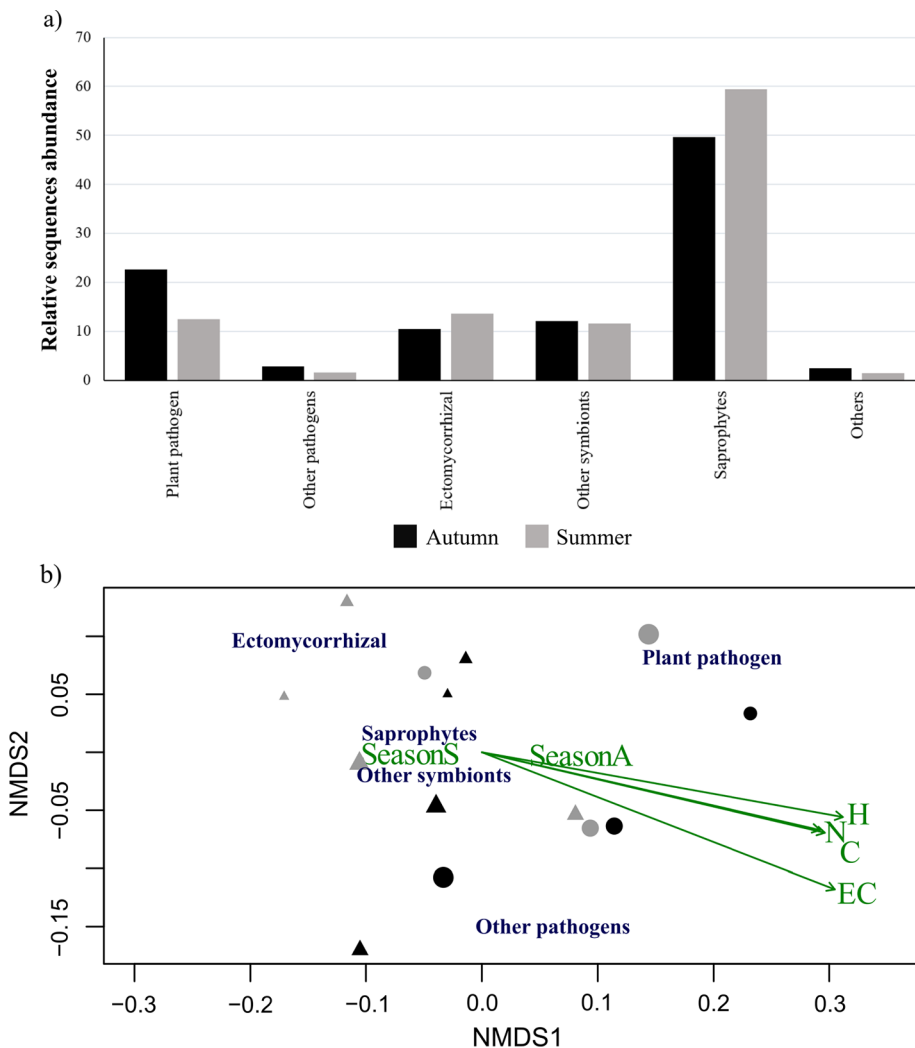


Fig. 5. Relative abundance of sequences (a) and non-metric multidimensional scaling (b) assigned to ecological guilds. In (a) “Others” includes: Animal endosymbiont-Undefined saprotroph, Endophyte-Wood saprotroph, Animal pathogen-Endophyte-Fungal parasite-Lichen parasite-Plant pathogen-Wood saprotroph, Fungal parasite, and Lichen. In (b) the plots are represented by circles: autumn, triangles: summer, black: implanted plots, gray: non-implanted plots. Size represents the harvesting intensity, an increase in size indicating an increase in harvesting level (smaller control plots). Environmental variables that were significant in the analysis are presented; SeasonA: autumn; SeasonS: summer; C: carbon; N: nitrogen; H: humidity; EC: electric conductivity.

regions to that studied in this work (Marín et al., 2017). In addition, yeasts from these groups have also been cited in soil (Mestre et al., 2014), in aquatic environments (Libkind, 2006), and in *N. nervosa* fruits (Fernández et al., 2012) in the region. The Glomeromycota showed very little representation of sequences and OTUs in this study and were not analyzed, since the primers used (ITS1F and ITS2) are not effective for amplification of this group of fungi (Stockinger et al., 2010; Schoch et al., 2012).

The most abundant classes in both seasons were the Agaricomycetes, followed by Leotiomycetes, Sordariomycetes, and Tremellomycetes. These results could partially overlap with the work of Marín et al. (2017) and Duo Saito et al. (2018). Marín et al. (2017) found that in general Agaricomycetes, Dothideomycetes, and Sordariomycetes were the most abundant soil fungus classes in *Nothofagus* forests of Chile. Furthermore, Duo Saito et al. (2018) found that in naked soil near glaciers the Leotiomycetes were the most common taxon, followed by Sordariomycetes, Dothideomycetes, and Eurotiomycetes. In their global study, Tedersoo et al. (2014) found that Agaricomycetes and Tremellomycetes presented peaks of richness at mid-latitudes, whereas Sordariomycetes presented a maximum peak in tropical ecosystems and Leotiomycetes did so towards the poles. Our results could partially overlap with this global behavior, considering the latitude and climatic conditions of the area. In our work, the mixed woodland in autumn presented an increase in the number of sequences of Leotiomycetes, Sordariomycetes, and Tremellomycetes, and these were correlated with an increase in humidity, conductivity, and pH (Fig. 4b). In the summer, Agaricomycetes, Dothideomycetes and

Eurotiomycetes showed an increase in number of sequences. These results are in agreement with the work done in *N. betuloides* forests in Chile (Marín et al., 2017).

The most abundant orders in both seasons were Agaricales (Agaricomycetes) and Helotiales (Leotiomycetes), followed by Mortierellales (Mortierellomycetes) and Filobasidiales (Tremellomycetes). Agaricales, Helotiales and Mortierellales presented high percentages of abundances in other works (Lim et al., 2010; Sterkenburg et al., 2015; Zhang et al., 2016; Truong et al., 2019), and Agaricales have high diversity in the Patagonian *Nothofagus* forests when compared with European *Fagus* forests (Marín et al., 2017; Godoy and Marín, 2019). Chemical properties of the soil, such as gravimetric humidity, EC, C, N, and available P, were modelers of the fungal community at different taxonomic levels (Fig. 4).

If we consider the genera and species assigned instead of the low representative values, the most numerous genera shared in both seasons were: *Hygrocybe*, *Mortierella*, *Cryptococcus*, *Ilyonectria*, and *Lecytophora*; in autumn the genus *Trichocladium* was also important, as were *Cortinarius* and *Clitopilus* in summer. The most numerous species in autumn were *Hygrocybe conica*, *Ilyonectria rufa*, *Mortierella verticillata*, and *Cryptococcus terreus*, and in summer *Hygrocybe conica*, *Mortierella verticillata*, *Cortinarius* spp., *Clitopilus hobsonii*, and *Ilyonectria rufa*. The genus *Hygrocybe* was described as a saprotroph-symbiotroph, and its carbon signature suggests plant biotrophy, including bryophytes (Seitzman et al., 2011; Halbwachs et al., 2013; Tello et al., 2013; Lodge et al., 2014). The genera *Clitopilus*, *Geoglossum*, *Mortierella*, and *Trichocladium* are classified as saprophytes (Tedersoo et al., 2014; Truong

et al., 2019). It has been found that the yeasts of the genus *Cryptococcus* were associated with soil nutrient cycles and with plants, acting as promoters of plant growth (Cloete et al., 2009; Mestre et al., 2016). The species of genus *Cortinarius* and *Lecytophora* are symbionts, the former being ectomycorrhizal fungi (Rinaldi et al., 2008) and the latter dark septate endophytes (Taylor et al., 2014). Finally, species of the genus *Ilyonectria* have been reported as pathogenic, causing the disease of root rot (Chaverri et al., 2011; Cabral et al., 2012) and have been found as an endophyte in asymptomatic plants (White et al., 1962). With the limitations of the low representative values of these two taxa, and until further identification is made or new identification methodologies are used, it seems that most of the abundant sequences were principally related to saprotrophs and symbiotrophs related to summer and lower soil values of C and N, maybe associated, and pathotrophs. Also, for definitive conclusions it would be necessary to consider the important AM fungi group, particularly because all the plots had higher soil cover associated AM plants.

In our work we found a correlation between soil characteristics and ecological guilds (Fig. 5 b), but not with management. The NMDS analysis revealed that the ecological guilds, in autumn there was an increase in the sequences pathogens (i.e. plant pathogens such as *Ilyonectria*) and other symbionts, related with high values of C and N. In summer there was an increase in the sequences of saprotrophs and ectomycorrhizal fungi, which could be related to the increase in sequences of *Cortinarius*, one of the most abundant genera in *Nothofagaceae* forest (Hewitt et al., 2018; Truong et al., 2019). Marín et al. (2017) saw an inverse relationship between pathotrophs and symbiotrophs, related to the use of land; in plots with intense use (=total removal of vegetation) pathogens were more abundant, and the pristine, managed (=partial removal of vegetation) plots had higher mycorrhizal fungal abundance. Tedersoo et al. (2014) recorded the effect of pH and the plant community on ectomycorrhizal fungi, and the effect of the distance from the equator and soil C/N ratio for pathogens, and suggested that the richness of fungi and functional groups is unrelated to plant diversity, with the exception of ectomycorrhizal fungi, since plant-soil feedbacks does not influence the diversity of soil fungi on a global scale. In these work we also could bear in mind that the FUNGuild platform depends, to a large extent, on precise taxonomic identification and many OTUs were placed in the unassigned group for the incomplete database (Nguyen et al., 2016).

The mixed woodlands of Andean-Patagonian forests constitute an important habitat characterized by representative plant species of this region and some of the Patagonian steppe, with a high level of biodiversity and ecosystem services (Larreta et al., 2017; Rusch et al., 2017). However, these, mixed woodlands are considered usable zones for current forest activities (Gowda et al., 2014; Grosfeld et al., 2019), and are underrepresented in terms of protection measures (for example, they are underrepresented in the National Parks system of Argentina). These attributes represent an opportunity for social and economic development of forestry activities in the region, but these activities must be regulated because of the high ecological value of this environment (Rusch et al., 2017; Grosfeld et al., 2019). For this reason, further research should be carried out to establish sustainable management plans that guarantee the best use and maintenance of these resources. These results suggest that diverse factors, such as those analyzed here, modify the fungal community structure at different taxonomic levels and ecological guild, but not the management at one year of done. Further research, with other sequencing technology and an expanded database of the ecological guilds, is required to clarify how these microbial groups are affected over time by management of this environment.

5. Conclusion

This study contributes to the knowledge of woodland fungal communities in the Northwest Patagonian region, and is one of the first basal studies to show the effect of forest management on the soil fungal

community in a mixed woodland. The work is novel due to its experimental approach in a native environment. At site level, the mixed woodlands appear to have soil chemical characteristics similar to other forest environments in the same region, but could have intermediate fungal diversity than forests, which could be attributed to the high plant diversity of these environments. This work revealed a high number of new or non-identified fungi, highlighting the fact that this environment has been little studied, and the importance of doing so.

When the seasonal effect was analyzed, no changes were observed in the diversity of the fungal communities (evaluated by richness and diversity index), whereas modifications were recorded in the taxonomy and ecological guilds of the community, and in certain soil parameters (gravimetric humidity, pH, EC, C, and N); the changes in the proportions of abundant taxa should be considered.

When considering forest management, in general the fungal community (diversity indexes, taxonomy, and ecological guilds) was not affected by this anthropic disturbance, with the exception of species richness. Available P was the only soil chemical characteristic that registered changes associated with forest management.

It should be highlighted that available P and species richness could be proposed as bioindicators. Future studies considering more results from this kind of native program and other aspects such as vegetation, use and system functions of the area, will allow us to assess whether some of these changes are early bioindicators of sustainable use and management.

Future studies with analysis over a longer period of time following management, and a greater number of experimental assays, could also complement the conclusions of this work. Questions remain as to how resilient soil fungi are in the face of disturbances such as forest management, whether they constitute a positive response strategy, and how the duration of the management period influences the system.

CRedit authorship contribution statement

Ayelen Inés Carron: Conceptualization, Methodology, Software, Formal analysis, Data curation, Investigation, Writing - original draft, Writing - review & editing, Visualization, Project administration. **Lucas Alejandro Garibaldi:** Conceptualization, Methodology, Formal analysis, Supervision, Funding acquisition. **Sebastian Marquez:** Methodology. **Sonia Fontenla:** Conceptualization, Methodology, Investigation, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foreco.2020.117955>.

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