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Soil mycobiota under managed and unmanaged forests of *Nothofagus pumilio* in Tierra del Fuego, Argentina

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Abstract

Background: Management practices can modify the productivity of forests and the associated microbial diversity of soil. The soil mycobiota is considered a key factor in the ecological functions of forests. Forests of *Nothofagus pumilio* (Poepp. & Endl.) Krasser (Nothofagaceae) are the main source of timber and one of the most important economic resources in the province of Tierra del Fuego (Argentina). However, there is no information on the impact of forest management interventions for the soil mycobiota, which can be reliable biological indicators of disturbance.

Methods: Fungi were isolated from samples of soil collected under several *Nothofagus pumilio* forests subjected to different types of management and periods of time since the intervention. Types of management were represented by harvested forest with a shelter wood cutting, stockpile area and control forest without intervention and the periods of time since intervention were 1, 5–10 and 50 years. Species richness, evenness and Shannon's diversity index of the mycobiota in each condition of management were calculated. Additionally, the effect of seasonality was analysed.

Results: The soil mycobiota was represented by 70 taxa. Richness and/or Shannon's diversity index of the mycobiota between undisturbed forest and stockpile area were higher in May (autumn) than in September or November. There were no differences in mycobiota diversity between dates in the harvested forest.

Conclusions: Our results indicate that the forest intervention *per se* did not negatively affect the soil culturable mycobiota composition of *N. pumilio* forests in Tierra del Fuego (Argentina).

Keywords: Biodiversity, forest management impact, soil fungi, sustainable forest management

UNCORRECTED PROOFS

Introduction

Fungi are an important and highly diverse component of soil microbial communities (Tedersoo et al. 2014). In forest ecosystems, they perform essential ecological functions including decomposition of organic matter and nutrient cycling and are involved in biotic interactions such as mycorrhizal symbioses. Understanding whether forest-management practices affect the diversity of fungi and/or influence their spatial patterns is one of the central issues in soil microbial ecology (Green and Bohannan 2006). The whole soil microbiota is involved in the formation and stabilisation of organic matter but fungi play a greater role than bacteria in the metabolism and growth of trees. They are also involved

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in most processes that occur in the forest soil, such as the ones related to soil formation, nutrient availability and recycling of organic matter (Elíades et al. 2015). However, anthropic activities such as management practices can affect the forest productivity and the level of timber harvesting that the forest can sustain as well as the number and quality of habitats and of the associated biodiversity (Martínez Pastur et al. 2002).

Fifty-five percent of the area in the province of Tierra del Fuego (Argentina) is currently covered by forests of *Nothofagus pumilio* (Poepp. & Endl.) Krasser (Nothofagaceae), and, within the harvested forest, 2-3% are covered by stockpile ("canchón") areas (Martínez Pastur et al. 2007). *Nothofagus pumilio* is the most important source of timber in southern Patagonia, and the main economic resource since the 19th century. These forests have been managed using several silvicultural systems ranging from light selective harvests to clear-cuts (Gea et al. 2004). Selective cuttings with retained shelter wood or variable-retention cuttings have been the most common systems used over the last decade (Martínez Pastur et al. 2000, 2009). The period of time since intervention also affects the degree of tree cover in the forest.

Scorsetti et al. (2012) described the pathogenic and enzyme activities of the entomopathogenic fungus *Tolypocladium cylindrosporum* (Ascomycota: Hypocreales) from *N. pumilio* forests in Tierra del Fuego, Argentina. More recently, Elíades et al. (2015) reported preliminary data on the growth and enzymatic abilities of the soil fungus *Humicolopsis cephalosporioides* at different incubation temperatures collected under *N. pumilio* forests. However, studies comparing the soil fungal composition and diversity among forests dominated by the same tree species but under different management practices and periods of time since intervention are scarce. The decay rate in these forests is low and there is no information available on the role of these fungi in ecosystem stability so analysing the responses of the diversity of soil fungi to anthropological interventions is a high priority for understanding how forest management practices affect the ecology of *N. pumilio* forests and their components. Therefore, forest management practices can be considered key factors in the ecology of the Patagonian region. The type, intensity and frequency of management may affect soil microorganisms which are reliable biological indicators of disturbance because they react easily to environmental changes such as the soil chemical and physical changes related to timber harvesting (Jurgensen et al. 1997). Hartmann et al. (2014) reported less resistance and resilience of fungi in forest soils compared to bacteria, thus we hypothesised that management of *N. pumilio* forests in Tierra del Fuego causes a decrease in the species richness and diversity of fungi even following intervention. Therefore, the aim of this study was to assess and compare the seasonal structure of soil fungal communities under *N. pumilio* forests subjected to different management practices and periods of time since the intervention in Tierra del Fuego, Argentina.

Methods

Study area

Study sites are located in the forest-steppe ecotone of the central part of Tierra del Fuego Island, Argentina (54° 27'S, 67°27'W; Fig. 1). The forests correspond to the sub-Antarctic forest type (37°–60° South latitude) and are composed of *Nothofagus pumilio, N. antarctica* (Forster f.) Oersted and *N. betuloides* (Mirb.) Oersted as dominant trees sparsely mixed with *Drymis winteri* Forster & Forster f., *Maytenus magellanica* (Lam.) Hooker f. and *Embothrium coccineum* Forster & Forster f. (Moore 1983; Tuhkanem et al. 1990). The landscape occupied by forests has mostly acid brown soils of glacial origin with loess and alluvial materials in the foothills (Frederiksen 1988; Soil Survey Staff 1960).

In this region, the climate is subpolar with short, cool summers and long, snowy winters, influenced by Antarctic ice masses and cold oceanic currents. Mean daily air temperatures above 0°C are found only during three months a year, and the growing season is restricted to approximately five months. Rainfall, including snowfall, reaches up to 600 mm per year. Annual average wind speed outside the forests is 8 km h^{-1}, reaching up to 100 km h^{-1} during storms (Barrera et al. 2000; Martínez Pastur et al. 2009).

Study sites comprised monospecific *N. pumilio* forests and were determined using satellite images from different years and the database of the Natural Resources forest area of the Province. Sites were selected based on their high similarity in soil type, slope, elevation, and land-use history (Martínez Pastur et al. 1997). The harvested forest stands were selected in the central zone of the island, where it is possible to find old and recent cuttings corresponding to the proposed treatments.

Two treatments (types of forest management and period of time since the intervention) were considered. Three types of forest management were selected: (i) harvested forest (HF) with shelter wood cutting; (ii) stockpile area (SA), an area in the forest used during times of harvest to further process stems or trees extracted from the forest, store them, and then load out the logs. This is a designated area that is usually cleared of obstacles such as trees and stumps, and can vary in size depending on the processing, storage and loading-out requirements. This area is ca. 60 x 25 m^2 and represents 2-3% of the harvested forest (Martínez Pastur et al. 2007); (iii) control forest (CF), i.e. without intervention. Harvesting took place during the summer, and assessments were done 1 year, between 5 and 10 years, and 50 years after intervention. Nine stands of forest types (i) and (ii) were selected with three stands for each time period after intervention. In addition, nine unharvested old-growth forests (type iii) without signs of intervention were selected near each harvested forest. These old-growth forests consist of trees with similar diameter at breast height and dominant stand height (28 m total height, 528 trees ha^{-1}, 40.6 cm diameter at breast height, 65.0 m^2 ha^{-1} basal area; Lencinas et al. 2011), corresponding to sites of quality II according to

Martínez Pastur et al. (1997). The three HF sites 1 year after intervention selected were: Ewan River (EW 1), Los Cerros Ranch (LC 1) and Lenga Patagonia Ranch (LP 1). Ushuaia Ranch a (EUa 5–10), Ushuaia Ranch b (EUb 5–10) and Ewan River (EW 5–10) were the three HF sites 5–10 years after intervention, and Ushuaia Ranch (EU 50), Lenga Patagonia Ranch (LP 50) and Reserva Corazón de la Isla (RCI 50) were the three HF sites 50 years after intervention, as shown in Figure 1. The size of each selected site was between 30–60 hectares.

Soil sampling

A transect with five points every 10 m was established within each site. At each point of the transect, three composite samples of soil (each consisting of four subsamples) were collected from the mineral horizon (0–10 cm) using a hole borer according to Dick et al. (1996). Sampling was carried out in November 2009, May and September 2010, which corresponded to late spring, autumn and early spring, respectively. After collection, samples (between 500 g and 1 kg) were stored in plastic bags at 4°C until processing and transported to the laboratory. A fraction of 5 g from each sample was used for fungal isolation while another fraction was oven-dried (105°C) overnight to determine the moisture content.

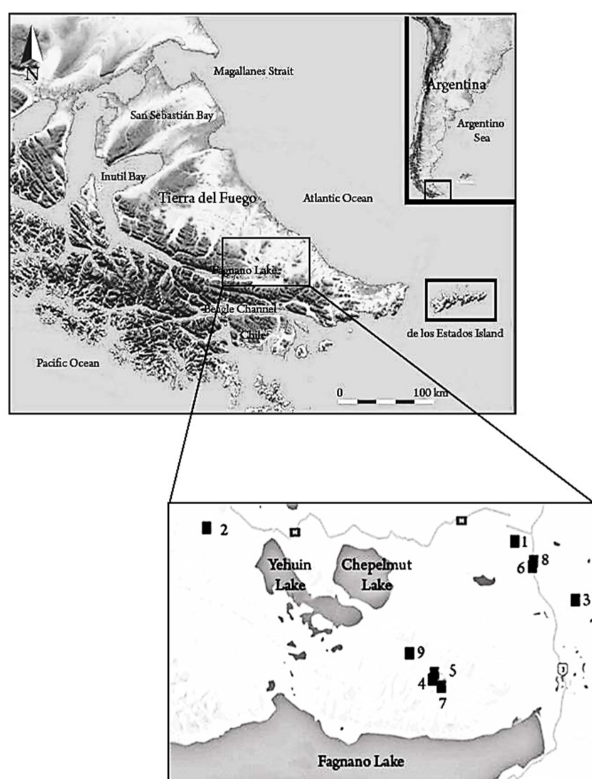

FIGURE 1: Location of sample sites in Tierra del Fuego (54° 27'S, 67° 27'W).

(1) 1 year (Ewan River); (2) 1 year (Los Cerros Ranch); (3) 1 year (Lenga Patagonia Ranch); (4) 5–10 years (Ushuaia Ranch a); (5) 5–10 years (Ushuaia Ranch b); (6) 5–10 years (Ewan River); (7) 50 years (Ushuaia Ranch); (8) 50 years (Lenga Patagonia Ranch); (9) 50 years (Reserva Corazón de la Isla).

Fungal isolation and identification

Each soil sample was processed by the soil washing method according to Parkinson and Williams (1961). A total of 100 soil particles from each sample was used, placed on plates containing cornmeal agar medium supplemented with 0.05% streptomycin sulfate and 0.025% chloramphenicol at rate of five particles per plate (a total of 20 plates by sample) and incubated at 25°C for 10 days (Elíades et al. 2008). Daily observations of the plates were performed under the microscope and a representative of each taxon registered on the particles at each sampling date was isolated. Stock cultures were kept at 4°C on 2% (w v^{-1}) agar-malt extract (MEA) slants, lyophilised and deposited in the culture collection of "Instituto Spegazzini", UNLP, La Plata, Argentina (LPSC). For morphological identification, MEA slide cultures of each isolate were prepared and mounted with lactophenol cotton blue to observe the structures differentiated by hyaline fungi. Original taxonomic papers based on cultural and morphological features and compendia (Ellis 1971; Carmichael et al. 1980; Domsch et al. 1993; Cabello and Arambarri 2002) were used to identify sporulating fungi.

Statistical analyses

The community structure of soil fungi was analysed by (i) frequency (%): number of particles bearing a specific fungus / total number of particles analysed × 100 (Godeas 1983); (ii) species richness (S); (iii) Evenness (E); and (iv) Shannon's diversity index (H'). $H' = \sum p_i \ln p$, where p_i is the relative abundance of the i species compared to the abundance of all identified species in a sample (Magurran 1988; Cabello & Arambarri 2002). A two-way ANOVA was performed to analyse the effects of forest management type and period of time since intervention (between-subject effects) and of season (i.e., sampling dates, as the within-subject effect) on S, E and H'. Prior to analysis, normality and homoscedasticity of the data were tested in order to confirm that they fulfilled the assumptions required for ANOVA.

Principal Component Analysis (PCA; Digby & Kempton 1987) was performed with the frequency data of all species using the Multivariate Statistical Package MVSP 3.1. (Kovach 1999). Wilks' Lambda test was applied to verify if the sample units in the PCA analysis were mainly separated in the two axes by the sampling time, years after intervention or forest-management type.

Results

Total soil mycobiota

The total mycobiota recovered from all 80 soil samples collected was represented by 70 taxa whose higher frequency at each site and sampling time is shown in Tables 1–3. A representative isolate obtained from the particles at each sampling date was obtained. Of the

TABLE 1. Higher percentage frequencies of soil fungi isolated in three sampling times (November 2009, May 2010 and September 2010), which are combined totals from all 27 sites studied.

Fungal species	Nov. 2009	May 2010	Sep. 2010	Fungal species	Nov. 2009	May 2010	Sep. 2010	Fungal species	Nov. 2009	May 2010	Sep. 2010
Absidia coerulea			36	*Fusarium sulphureous*		1.3		*Penicillium frequentans*	20.4	38.5	100
Absidia cylindrospora	16	30.2	58	*Geomyces pannorum*	6.2			*Penicillium nigricans*	28.3	40.7	
Absidia ramosa	7.4			*Humicola fuscoatra*			60.3	*Penicillium odoratum*	6.6	1	
Absidia spinosa	1	29		*Humicola grisea*	9.3	14.6	18.4	*Penicillium purpurascens*	6.6	19	20.4
Acremonium cereales	11.8			*Humicolopsis cephalosporioides*	77.2	58.8	84.6	*Penicillium restrictum*		1	
Acremonium sp.	23.5	3.4		Yeast sp.1	23.5	3.7	9.1	*Penicillium rubrum*		5.8	
Alternaria alternata	1		5.9	Yeast sp. 2		2.4	9.1	*Penicillium* sp.	6.6		1.9
Arthrinium phaeospermun		0.8		Yeast sp. 3			6.1	*Penicillium thomii*	6.6	1.2	3.6
Aspergillus niger	6.6	1.3	2	*Melanospora fallax*		7.4		*Phoma herbarum*			1.56
Aspergillus terreus	2.2	2	9.1	*Metarrhizium anisopliae*	1.08			*Piptocephalis* sp.		1.9	
Aspergillus ustus		3.7		*Mortierella humilis*	30	3.6	30	*Purpureocillium lilacinum*	1.75	4.9	
Basidiomycota mycelium			8.6	*Mortierella hialina*	1.1	24	22	*Rhizopus stolonifer*		3.1	
Beauveria bassiana	19.1			*Mortierella parvispora*			5.8	*Rhodotorula* sp.	16.2		
Beauveria brongniartii	33.3	40.9	1.9	*Mortierella ramosa*	5			*Trichoderma hamatum*		3.5	19.2
Cladosporium herbarum		3.1		*Mortierella* sp.1		5.1	3.7	*Trichoderma harzianum*		1	
Cladosporium cladosporioides	5.9	3.1	70.6	*Mortierella* sp.2			2	*Trichoderma koningii*	3.1	7.8	50.9
Clonostachys rosea		2		*Mortierella vinacea*	47.8	19.5	72.7	*Trichoderma polysporum*	83.3	64.8	65.3
Cordana pauciseptata			4.1	*Mucor genevensis*		35.5	12	*Ulocladium botrytis*			2
Cylindrocarpon sp.			3.8	*Mucor hiemalis*	22.5	18.8	55.8				
Cylindrocarpon didymun			6	*Mucor mucedo*		10.3	4				
Cylindrocarpon fueguianum	15.2			*Mucor subtilissimus*	55.6	22.5	34.7				
Cylindrocarpon tenue	1			*Nigrospora sphaerica*		0.9	5.9				
Dactylium dendroides	66.6			*Paecilomyces carneous*		1.9					
Dematiaceous sterile mycelium	5.4	14.3		*Paecilomyces* sp.	1	55.6					
Fusarium oxysporum	4.4			*Penicillium canescens*	1.9		10				

Nov., November; Sept., September.

TABLE 2.Higher percentage frequencies of soil fungi isolated in three times since intervention averaged over the three sampling dates (November 2009, May 2010 and September 2010).

Fungal specieS	**Time since intervention (years)**			**Fungal species**	**Time since intervention (years)**			**Fungal species**	**Time since intervention (years)**		
	1	**5-10**	**50**		**1**	**5-10**	**50**		**1**	**5-10**	**50**
Absidia coerulea	36			*Fusarium sulphureous*	1	1.3	1.2	*Penicillium frequentans*	40	20	100
Absidia cylindrospora	58	25.3	42	*Geomyces pannorum*			6.2	*Penicillium nigricans*	34.3	30.9	40.7
Absidia ramose	7.4	2.8	2.1	*Humicola fuscoatra*	43.8	3.6	60.3	*Penicillium odoratum*		6.6	1
Absidia spinose	29	19	27.1	*Humicola grisea*	14.6	18.4	9.3	*Penicillium purpurascens*	20.4	19	
Acremonium cereals	11.8			*Humicolopsis cephalosporioides*	54.9	66	84.6	*Penicillium restrictum*	1	0.9	
Acremonium sp.	23.5	3.4	5.2	Yeast sp. 1	23.5	10.9	1.03	*Penicillium rubrum*	5.8	1.3	0.8
Alternaria alternata	1	5.9		Yeast sp. 2	2.4	9.1		*Penicillium* sp.	1.9	6.6	
Arthrinium phaeospermun	0.8			Yeast sp. 3	6.1			*Penicillium thomii*	1.2	6.6	2
Aspergillus niger	2	6.6		*Melanospora fallax*	7.4			*Phoma herbarum*	1.56		
Aspergillus terreus	2	9.1	0.9	*Metarrhizium anisopliae*		1.08		*Piptocephalis* sp.	1.9		
Aspergillus ustus	3.7			*Mortierella humilis*	7.5	26.4	30	*Purpureocillium lilacinum*	1.1	0.9	4.9
Basidiomycota mycelium		8.6		*Mortierella hialina*	24	7.6	22	*Rhizopus stolonifer*	3.1	1.1	
Beauveria bassiana	19.1	6.6	5.1	*Mortierella parvispora*		5.8		*Rhodotorula* sp.	16.2	2.2	3.5
Beauveria brongniartii	27	10.9	40.9	*Mortierella ramosa*	5			*Trichoderma hamatum*	3.5	19.2	3
Cladosporium herbarum			3.1	*Mortierella* sp.1	5.1			*Trichoderma harzianum*	1		
Cladosporium cladosporioides	5.9	70.6	3.1	*Mortierella* sp.2		2		*Trichoderma koningii*	30.6	50.9	3.4
Clonostachys rosea	2			*Mortierella vinacea*	19.5	72.7	22.5	*Trichoderma polysporum*	83.3	77.3	68.5
Cordana pauciseptata	4.1			*Mucor genevensis*	6.4	35.5	7.8	*Ulocladium botrytis*			2
Cylindrocarpon sp.		3.8		*Mucor hiemalis*	16.5	55.8	14				
Cylindrocarpon didymun	6			*Mucor mucedo*	10.3	4	8.2				
Cylindrocarpon fueguianum		15.2	2.1	*Mucor subtilissimus*	34.7	14	55.6				
Cylindrocarpon tenue	1		1	*Nigrospora sphaerica*		5.9					
Dactylium dendroides		0.9	66.6	*Paecilomyces carneous*	1.9						
Dematiaceous sterile mycelium	5.4	2.2	14.3	*Paecilomyces* sp.	55.6	16.8	8.5				
Fusarium oxysporum		4.4	3.1	*Penicillium canescens*		7.3	10				

TABLE 3. Higher percentage frequencies of soil fungi isolated in three types of forest management averaged over the three sampling dates (November 2009, May 2010 and September 2010).

Fungal specieS	CF	SA	HF	Fungal species	CF	SA	HF	Fungal species	CF	SA	HF
Absidia coerulea	36	8	6	*Fusarium sulphureous*	1			*Penicillium frequentans*	18.4	28	100
Absidia cylindrospora	54	58	48	*Geomyces pannorum*	6.2			*Penicillium nigricans*	30.9	40.7	30
Absidia ramosa	2.8	1.1	7.4	*Humicola fuscoatra*	60.3	41.6	45.5	*Penicillium odoratum*	6.6		
Absidia spinosa	29.8	14.6	15.5	*Humicola grisea*	18.4	9.3	14.6	*Penicillium purpurascens*	6.6	20.4	14
Acremonium cereales			11.8	*Humicolopsis cephalosporioides*	50	84.6	58.8	*Penicillium restrictum*		1	0.9
Acremonium sp.	8.1	5.2	23.5	Yeast sp. 1	23.5			*Penicillium rubrum*	5.8	1.3	
Alternaria alternata			5.9	Yeast sp. 2			9.1	*Penicillium* sp.	6.6	1.9	
Arthrinium phaeospermun	0.8			Yeast sp. 3		6.1		*Penicillium thomii*	6.6	3.6	1.9
Aspergillus niger		1.3	6.6	*Melanospora fallax*			7.4	*Phoma herbarum*	1.56		
Aspergillus terreus	9.1	2.2	0.9	*Metarrhizium anisopliae*		1.08		*Piptocephalis* sp.			1.9
Aspergillus ustus			3.7	*Mortierella humilis*	30			*Purpureocillium lilacinum*		4.9	1,1
Basidiomycota mycelium	8.6			*Mortierella hialina*	24	17.8	1.1	*Rhizopus stolonifer*	1.1	3.1	
Beauveria bassiana	6	2.1	19.1	*Mortierella parvispora*	5.8			*Rhodotorula* sp.	16,2		3.5
Beauveria brongniartii	27	40.9		*Mortierella ramosa*	2.8	1.1	7.4	*Trichoderma hamatum*		19.2	3.5
Cladosporium herbarum	3.1		2	*Mortierella* sp.1		3.7		*Trichoderma harzianum*	1		
Cladosporium cladosporioides	3.1		70.6	*Mortierella* sp.2		2		*Trichoderma koningii*	16.4	50.9	30.8
Clonostachys rosea	2		2	*Mortierella vinacea*	72.7	4.1	19.5	*Trichoderma polysporum*	30.8	46	65.3
Cordana pauciseptata	4.1			*Mucor genevensis*	7.8	35.5	14.7	*Ulocladium botrytis*			2
Cylindrocarpon sp.			3.8	*Mucor hiemalis*	38.5	55.8	36.4				
Cylindrocarpon didymun			6	*Mucor mucedo*	4.9	10.3	7				
Cylindrocarpon fueguianum	15.2		5	*Mucor subtilissimus*	55.6	34.7	4.2				
Cylindrocarpon tenue	1			*Nigrospora sphaerica*	0.9		5.9				
Dactylium dendroides	0.9		66.6	*Paecilomyces carneous*		1.9	4				
Dematiaceous sterile mycelium	5.4			*Paecilomyces* sp.	55.6	24.7					
Fusarium oxysporum	4.4	3.1		*Penicillium canescens*			10				

CF, control forest; SA, stockpiled area; HF, harvested forest.

fungi identified, most were in the Ascomycota phylum although some representatives of Mucoromycota were also found.

The S, J and H' values of the soil mycobiota are shown in Appendices 1–3. Sampling time was the only factor that generated significant differences in the values of S and H' (Table 4). The H' is a parameter that includes S so a Fisher's least-significant-difference test was performed using H' data for the samples corresponding to each forest management situation at the three sampling times (Fig. 2). There were no significant differences between dates in the harvested forest (P≤0.01), although in May H' indices were significantly higher than at the other two sampling dates for both undisturbed forests and stockpile areas (Fig. 2).

The PCA performed with the frequencies of all fungal species showed that the first two axes accounted for 50.7% of the total variance explained (Fig. 3). A Wilks' Lambda test was highly significant with sampling time (Wilk's Lambda: 0.003, F: 180.3, P<0.001), which grouped soil samples according to seasonality and not significant between years after intervention (Wilk's Lambda: 0.957, F: 0.21, P>0.001) and types of forest management (Wilk's Lambda: 0.957, F: 0.85, P>0.001). September 2010 samples were mainly represented by *Humicola fuscoatra*, *Penicillium frequentans*, *Trichoderma koningii* and *T. polysporum*, while the November 2009 samples included *Beauveria brongniarti* and *Mortierella vinacea*, and the May 2010 samples included *Aspergillus niger* and *A. terreus*.

Soil mycobiota at undisturbed sites

The 12 most frequently obtained species found in the nine control forests at each of the three sampling dates are shown in Figure 4. Among these ones, *Mortierella vinacea, Mucor subtilissimus, Humicolopsis cephalosporioides, Penicillium frequentans* and *Trichoderma polysporum* occurred most frequently in November 2009 (late-spring). *Absidia cylindrospora, A. spinosa, Paecilomyces* sp., *P. frequentans*, *Penicillium nigricans* and *T. polysporum* characterised the soil samples corresponding to May 2010 (autumn), while *Humicola fuscoatra* and *Humicolopsis cephalosporioides* exhibited a high frequency in September 2010 (early-spring), together with *A. cylindrospora*, *Mucor hiemalis*, and *T. polysporum.* In these undisturbed sites, the species richness was around 4 and 13, the evenness was between 0.40-0.63 and the species diversity was 0.88-2.13.

Effect of forest-management type and time since intervention

Even though the sampling time was the variable that explained the separation of the units according to their composition, some species were present in recently exploited sites (*Acremonium cerealis*, *Melanospora fallax* and *Mortierella ramosa*) and others in ones intervened 50 years ago (*Cladosporium herbarum, Dactylium dendroides* and *Geomyces pannorum*).

Averaged across all three sampling dates, *H. cephalosporiodes* and *T. polysporum* exhibited the highest frequencies, with the former being more abundant in sites with the shortest periods of time since intervention (1 and 5–10 years) and *T. polysporum* being more abundant in soils at sites after 50 years of intervention. However, there were no differences in S, J and H' in soils of different forest-management type or time since intervention.

Discussion

In our project, we analysed and compared the community structure of soil fungi under forests subjected to different management practices and periods of time since the intervention. However, species richness and diversity of soil mycobiota associated with *N. pumilio* forests estimated here did not confirm the hypothesis that forest management decreases the mycobiota composition. Silviculture practices can be a potential source of stress that influences both the ecophysiology of forests and the associated soil microbiota. It is well-known that forest management practices generate specific microclimate conditions due to changes in the humidity and temperature in the soil and canopy as well as in sunlight availability (Aussenac 2000).

TABLE 4. Results of repeated-measures ANOVA on specific richness (S), equitability (J) and diversity index (H') indicating the effect of sampling time (S), forest management (FM) and years from intervention (YI).

		S		J		H'	
Source of variation	d.f.	F	P value	F	P value	F	P value
FM	2	1.231	0.315	0.014	0.986	0.492	0.620
YI	2	0.625	0.547	0.589	0.565	0.671	0.523
FM x YI	4	0.937	0.465	0.908	0.480	1.952	0.145
S	2	20.258	**0.000**	2.030	0.146	13.185	**0.000**
S x FM	4	0.243	0.912	1.971	0.120	1.179	0.336
S x YI	4	0.832	0.513	0.059	0.993	0.451	0.771
S x FM x YI	8	0.490	0.855	0.491	0.855	0.520	0.833

To date, there is no information about the impact of seasonal changes on the soil microfungi diversity in forests of *N. pumilio* in Tierra del Fuego subjected to different management practices and consequently to different degrees of tree cover. However, this information can be of key relevance for determining the forest productivity status and therefore contribute to the development of new sustainable management strategies (Martínez Pastur et al. 2009).

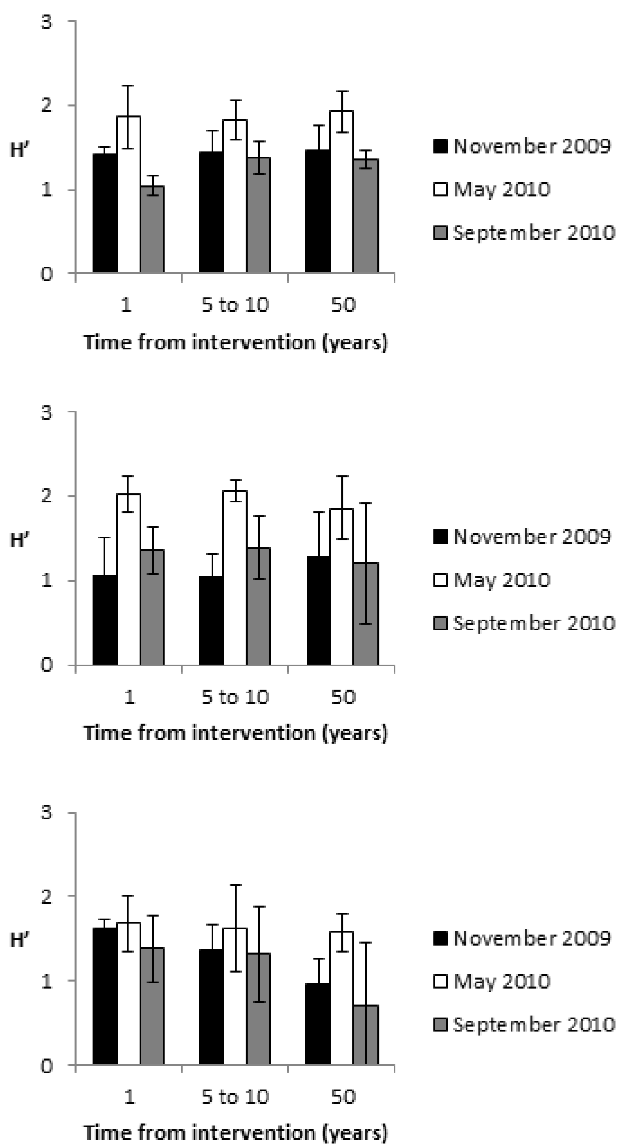

FIGURE 2: H' in control forest (A), stockpile areas (B) and harvested forest (C) at the three periods of time since intervention in November 2009 (Nov), May 2010 (May) and September 2010 (Sept). Values are means that correspond to three forest sites (replicates). The asterisk (*) denotes significant differences between sampling dates for each period of time since the intervention according to ANOVA and Fisher's LSD test ($P \leq 0.05$).

Higher S and/or H' were found in undisturbed forests and stockpile areas in autumn (May 2010) compared to those from the other two seasons we analysed. Similarly, differences in the diversity of microfungi associated with seasonality and temperature conditions in cold environments were reported by Coleine et al. (2015) and Rodolfi et al. (2015). Voříšková et al. (2014) also found that the soil fungal community under a temperate oak forest was affected by seasonality, with the highest number of genera found in autumn. The two processes that probably contributed most to those differences were litter decomposition and allocation of photosynthates. Since temperature can also affect nutrient recycling in *N. pumilio* forests and consequently influence soil biological activity (including microfungi and their enzymes), further analysis is necessary to demonstrate whether these differences in the diversity of microfungi are in fact related to the amount and availability of specific nutrients. Preliminary studies on the *in-vitro* conditions of *H. cephalosporioides*, a fungus with a high frequency in forest soils of *N. pumilio* in Tierra del Fuego, revealed that its enzyme activity is affected by the temperature of incubation (Elíades et al. 2015).

All study sites were exposed to the same stressful conditions that prevailed due to the seasonal presence of snow (characteristic of the sub–Antarctic climate) but this did not seem to have affected fungal diversity in the harvested forest with shelter wood. One possible reason could be the existence of a mosaic of vegetation types and floristic composition in disturbed ecosystems that minimised the regional effect of climate. Bradley et al. (2001) compared the chemical and microbial properties of the forest floor between shelter wood, adjacent old-growth and clear-cut plots in the montane coastal western hemlock of British Columbia (Canada). These authors found that forest floor can develop under shelter wood plots with atypical properties of either clear-cut or old-growth plots. The absence of differences in the community of soil microfungi associated with different forest management practices and periods of time since intervention observed in the present study could be due to mechanisms of ecological compensation that mitigated the impact of the intervention. The presence of new substrates (such as wood particles and other organic remains), and of microhabitats equivalent to the original ones as a result of cutting and delimbing of trees, may contribute to the restoration of appropriate conditions for soil fungi to thrive, leading to the maintenance of a similar fungal community. Yet, this also depends on the ability of each fungal species to survive in the modified environment. Soil fungi are an ecological group composed of generalist representatives, as in the case of most *Aspergillus* and *Penicillium*, which are able to survive in both natural and man-made environments due to their ability to use a wide variety of carbon sources for growth (Kowalczyk et al. 2014). Ecological compensation is a mechanism that allows these fungi to survive different kinds of disturbances and therefore these organisms might play an important role in the sustainable development of a region (Wang et al. 2007). They can establisher new

FIGURE 3: PCA of soil mycobiota data at three collection dates (November 2009, circles; May 2010, inverted triangles; September 2010, squares) with site codes above the symbols. The main species vectors are indicated with arrows. Fungal names are abbreviated. Red symbols indicate control samples. The code of each site is formed by: period of time (in years) since intervention (1, 5-10 or 50), followed by type of forest management (control forest, CF; stockpiled area, SA; harvest forest, HF) and season sampling (November 2009, N; May 2010, M; September 2010, S). The abbreviations of the taxa names are: *Abs coe*, *Absidia coerulea*; *Abs cyl*, *Absidia cylindrospora*; *Asp nig*, *Aspergillus niger*; *Asp ter*, *Aspergillus terreus; Bea bro*, *Beauveria brongniartii*; *Hum fus*, *Humicola fuscoatra*; *Hum cep*, *Humicolopsis cephalosporioides*; *Lev*, *Yeast sp.1*; *Mor hum*, *Mortierella humilis*; *Mor par*, *Mortierella parvispora*; *Mor vio*, *Mortierella vinacea*; *Muc hie*, *Mucor hiemalis*; *Muc sub*, *Mucor subtilissimus*; *Pen fre*, *Penicillium frequentans*; *Pen pur*, *Penicillium purpurascens*; *Pen tho*, *Penicillium thomii*; *Pip* sp., *Piptocephalis* sp.; *Tri kon*, *Trichoderma koningii*; *Tri pol*, *Trichoderma polysporum*; *Ulo bot*, *Ulocladium botrytis*.

FIGURE 4: Accumulated frequency of the 12 most abundant fungal species in samples from the three control forests at each of the three sampling dates (November, November 2009; May, May 2010; September, September 2010) and their average values (AVE).

biotic interactions that often result in changes to the environment and activate inducible metabolic pathways that allow their growth under stressful conditions such as when nutrient resources are scarce (Troncozo et al. 2015). This includes phenotypic plasticity according to available organic substrates and synthesis of lytic enzymes involved in soil formation, which have adaptive and ecological significance (Franco et al. 2018). Lin et al. (2016) reported that the soil mycobiota of a Taiwanese *Cryptomeria japonica* forest regained system stability and recovered from tree thinning disturbance in a relatively short period of time. Forest management practices, including harvesting and forest conversion, could affect the soil microbial community in montane forest (Chang et al. 2017). Therefore, additional studies including other recently disturbed sites of *N. pumilio* forests in Tierra del Fuego are needed.

Conclusions

We showed that the diversity of soil mycobiota associated with *N. pumilio* forests was not affected by silviculture practices and time since intervention. Although 70 fungal taxa were recovered from these soils, a change in S and/or H' was only found for undisturbed forests and stockpile areas in autumn compared to those from seasons more favourable to plant growth. Therefore, our results indicate that forest harvesting *per se* does not affect the diversity of soil mycobiota in *N. pumilio* forests in Tierra del Fuego, since there were no changes in any of the structural parameters analysed associated with the harvested forest with sheltering wood cutting.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

LAE conducted most of the planning, experimental design, sample processing, identification, data analysis and the writing of the manuscript. MNC advised on the identification of fungal isolates and was involved in the data analysis. VP conducted the field work. AM helped with fieldwork. NAF helped performing different tasks in laboratory. MCNS was involved in the analysis and discussion of the data and assisted with the writing of the paper. MDB advised on the application of statistical tools and was involved in the statistical analysis of the data. All authors read and approved the final version of the manuscript.

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Appendix 1. Richness (S), Evenness (J) and Shannon Weiner index (H') from control forest (CF), stockpiled area (SA) and harvested forest (HF) of the studied sites in November 2009.

	CF			SA			HF		
Site*	S	J	H'	S	J	H'	S	J	H'
LP 1	6	0.54	1.41	12	0.41	1.47	12	0.48	1.72
LC 1	7	0.47	1.32	6	0.24	0.64	7	0.54	1.51
EW 1	9	0.47	1.51	9	0.35	1.11	6	0.62	1.62
EW 5-10	8	0.31	0.94	10	0.32	1.07	6	0.43	1.11
EUa 5-10	9	0.50	1.61	5	0.34	0.80	8	0.42	1.27
Eub 5-10	4	0.55	1.11	9	0.40	1.29	8	0.56	1.70
LP 50	8	0.52	1.56	9	0.52	1.67	10	0.36	1.19
EU 50	8	0.57	1.71	4	0.34	0.69	2	0.63	0.63
RCI 50	8	0.37	1.13	10	0.44	1.47	7	0.38	1.08

*LP 1, Lenga Patagonia Ranch; LC 1, Los Cerros Ranch; EW 1, Ewan River; EW 5–10, Ewan River; EUa 5–10, Ushuaia Ranch a; EUb 5–10, Ushuaia Ranch b; EU 50, Ushuaia Ranch; LP 50, Lenga Patagonia Ranch; RCI 50, Reserva Corazón de la Isla. For more information to see the section "Methods".

Appendix 2. Richness (S), Evenness (J) and Shannon Weiner index (H') from control forest (CF), stockpiled area (SA) and harvested forest (HF) of the studied sites in May 2010.

	CF			SA			HF		
Site*	S	J	H'	S	J	H'	S	J	H'
LP 1	8	0.47	1.42	13	0.56	2.09	13	0.52	1.94
LC 1	10	0.63	2.11	10	0.53	1.77	11	0.52	1.80
EW 1	12	0.56	2.04	14	0.57	2.17	7	0.46	1.31
EW 5-10	11	0.53	1.84	14	0.57	2.19	5	0.51	1.20
Eua 5-10	-	-	-	14	0.51	1.95	12	0.61	2.19
Eub 5-10	12	0.55	1.98	10	0.60	2.02	8	0.49	1.47
LP 50	10	0.49	1.64	10	0.50	1.68	8	0.59	1.79
EU 50	11	0.57	1.98	13	0.61	2.28	10	0.40	1.35
RCI 50	13	0.57	2.13	7	0.57	1.60	12	0.44	1.59

*LP 1, Lenga Patagonia Ranch; LC 1, Los Cerros Ranch; EW 1, Ewan River; EW 5–10, Ewan River; EUa 5–10, Ushuaia Ranch a; EUb 5–10, Ushuaia Ranch b; EU 50, Ushuaia Ranch; LP 50, Lenga Patagonia Ranch; RCI 50, Reserva Corazón de la Isla. For more information to see the section "Methods".

Appendix 3. Richness (S), Evenness (J) and Shannon Weiner index (H') from control forest (CF), stockpiled area (SA) and harvested forest (HF) of the studied sites in September 2010.

	CF			SA			HF		
Site*	S	J	H'	S	J	H'	S	J	H'
LP 1	5	0.40	0.94	7	0.52	1.47	7	0.43	1.21
LC 1	5	0.50	1.17	7	0.55	1.55	8	0.61	1.83
EW 1	4	0.49	0.99	4	0.52	1.04	5	0.47	1.09
EW 5-10	4	0.44	0.88	7	0.43	1.20	4	0.44	0.88
Eua 5-10	6	0.48	1.24	6	0.43	1.13	2	0.54	1.95
Eub 5-10	8	0.53	1.59	8	0.60	1.80	6	0.43	1.12
LP 50	7	0.45	1.28	6	0.51	1.32	1	0	0
EU 50	6	0.57	1.47	2	0.42	0.42	2	0.68	0.68
RCI 50	5	0.55	1.29	8	0.61	1.84	6	0.56	1.46

*LP 1, Lenga Patagonia Ranch; LC 1, Los Cerros Ranch; EW 1, Ewan River; EW 5–10, Ewan River; EUa 5–10, Ushuaia Ranch a; EUb 5–10, Ushuaia Ranch b; EU 50, Ushuaia Ranch; LP 50, Lenga Patagonia Ranch; RCI 50, Reserva Corazón de la Isla. For more information to see the section "Methods".