Temporal dynamics of soil fungal communities after partial and total clear-cutting in a managed *Pinus sylvestris* stand.

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Abstract

Forest management aimed to maximize timber production might impact soil fungi, especially those symbiotically associated to tree roots. In this study, we analyse the temporal dynamics of soil fungi along five sampling years after tree removal in a managed *Pinus sylvestris* stand in northern Spain, where timber production is combined with regular mushroom harvesting. Two management methods were tested: total and partial clear-cutting leaving retention trees for seedling regeneration. Undisturbed, uncut plots were also included in the experiment as a control treatment. The whole fungal community (phylotypes and ecological guilds) were analysed by high-throughput Illumina MiSeq sequencing of fungal ITS1 amplicons. We hypothesized that 1) ectomycorrhizal fungal communities will decrease after both clear-cutting treatments with a concurrent increase in the abundance of saprotrophs, 2) the abundance and diversity of the ectomycorrhizal guild will be more preserved in partially clear-cut than in total clear-cut plots, and 3) the overall fungal diversity will decrease in the cut plots leading to major losses of ectomycorrhizal species. Our results show that soil fungal composition changed across the five years after clear-cutting by decreasing ectomycorrhizal fungi and increasing saprotrophs. However, these changes did not significantly affect fungal diversity and there were taxa-specific responses to tree harvest treatments. *Boletus edulis*, the most abundant ectomycorrhizal species fruiting in the study area and a valuable local non-forest resource, was negatively affected by either clear-cutting treatments. Soil fungal community composition in partially clear-cut areas was not different from that of total clear-cut areas. Our results indicate a strong effect of tree harvest on the relative abundance of ectomycorrhizal fungi along the first years after clear-cutting. However, levels of fungal diversity were comparable to the undisturbed forest, thus suggesting a potential further recovery of ectomycorrhizal fungi through the colonization of the regenerated seedlings.

Keywords: Clear-cutting; Ectomycorrhizal edible fungi; Forest regeneration; Fungal diversity; High throughput Illumina MiSeq sequencing; Forest multifunctionality; *Pinus sylvestris*

1. Introduction

Forest management aimed to maximize timber production involves modifications of abiotic and biotic conditions, both above- and below-ground, that significantly affects the diversity of soil fungi with recognised functional importance (Paillet et al. 2010; Goldmann et al. 2015; Lewandowski et al. 2015). Soil fungal communities are essential drivers of many ecosystem processes such as soil organic matter decomposition, nutrient release, and water acquisition (Smith and Read 2008). Thus, fungal community changes will have important consequences for carbon sequestration, nutrient cycling and water acquisition by plants (Clemmensen et al. 2015). Ectomycorrhizal fungi are particularly affected by tree harvest (Jones et al. 2003; Norvell and Exeter 2004; Durall et al. 2006) since they depend on the carbon provided by the host trees (Harvey et al. 1980; Pilz and Molina 2002; Jones et al. 2003; Luoma et al. 2004). Conversely, fungal saprotrophs are involved in the decomposition of plant-derived litter and may be favoured by the flush of litter and dead fine roots derived from clear-cutting (Kyaschenko et al. 2017). Previous studies carried out to determine the effect of forestry practices on ectomycorrhizal fungi showed that the composition of the ectomycorrhizal communities several years after clear-cutting may be different from that of undisturbed stands (Byrd et al. 2000; Durall et al. 2006; Hartmann et al. 2012; Tomao et al. 2017).

Non-timber forest products, such as edible mushrooms, have not typically been included in forest management plannings where timber production is the main objective. However, in Mediterranean forests, wild edible mushrooms can reach a significant level of production which may exceed 4-10 times the value of timber production, depending on the prediction model (Palahí et al. 2009; Aldea et al. 2012). Consequently, the current trend of the forest management plannings is to make non-wood forest products and their related ecosystem services (carbon

77 sequestration, soil protection and water production) compatible to timber products (Küçüker
78 and Baskent 2017). Removal of photosynthetic host trees, which are the main energy sources
79 for sporocarp production, may cause the decrease of ectomycorrhizal fungi in the short term
80 (Amaranthus et al. 1994). The effects of tree cutting at several intensities showed a sharp
81 decrease in *Boletus edulis* Bull. soil mycelium biomass in *Pinus sylvestris* L. stands in Spain, and
82 no recovery was observed three years after tree cutting (Parladé et al. 2017). Other studies
83 showed that moderate tree thinning produce a temporal increase of sporocarp fruiting of
84 certain species as *Lactarius* spp. (Bonet el al. 2012; Tomao et al. 2017).
85 Changes in mycorrhizal fungal diversity in response to climate parameters and forest
86 management have been mainly evaluated through sporocarp assessments (Kropp and Albee
87 1996; Luoma et al. 2004; Martínez de Aragón et al. 2007; Bonet et al. 2012; Martínez-Peña et al.
88 2012b) or mycorrhizal identification and counting (Jones et al. 2003, 2010; Barker et al. 2013).
89 These studies require a high level of expertise to identify fungal species and root morphotypes,
90 some of them with cryptic features, and may recover only a small proportion of the fungi present
91 in the sampled soil. In addition, the occurrence of fruiting bodies and the ectomycorrhizal
92 community inhabiting the soil are poorly correlated (Gardes and Bruns 1996; Dahlberg 2002).
93 Novel high-throughput DNA sequencing methods outperformed earlier approaches to identify
94 and analyse fungal communities, despite these novel techniques are not absent of
95 methodological biases and limitations from taxonomical identification to community profiling
96 (Lindahl et al. 2013). Recent studies using different sequencing platforms showed that the
97 ectomycorrhizal community was more influenced by environmental changes induced by harvest
98 than by the continuity of trees in a *P. sylvestris* stand (Varenius et al 2017). Kyaschenko et al.
99 (2017) studied the effects of clear-cutting on soil fungal communities in a chronosequence of
100 managed *P. sylvestris* and found a negative effect of tree harvest on the abundance and diversity
101 of ectomycorrhizal fungi and a proliferation of saprotrophs after clear-cutting. However, the
102 ectomycorrhizal fungal community was re-established during stand development, thus

maintaining functional diversity and the recycling of organic nutrient pools. Castaño et al. (2018a) evaluated the effects of forest thinning on soil fungal communities and found fungal community changes driven by inter-annual variation of environmental factors, rather than by the forestry practices. The potential exoenzymatic activities of ectomycorrhizal communities change after tree clear-cutting (Kohout et al. 2018) but potential functional complementarity and redundancy may still support growth of the regenerated seedlings (Jones et al. 2010; Walker et al. 2016).

Studies on ectomycorrhizal community succession after a disturbance such as clear-cutting or fire are still scarce (De Román and De Miguel 2005; Palfner et al. 2005; Twieg et al. 2007; Goicoechea et al. 2009; Taudière et al. 2017). Natural re-establishment of ectomycorrhizal fungi after clear-cutting can be achieved by means of mycelium, sclerotia (vegetative resistance structures formed by a few ectomycorrhizal species) and spores (Brundrett 1991). Mycelium and hyphae from the mantle of old, dead or dying mycorrhizas can act as inoculum for the regenerated seedlings (Bâ et al. 1991). In addition, sclerotia can also be an inoculum source (Ingleby et al. 1990), as well as spores of epigeous sporocarps from surrounding forests dispersed by water, animals, and wind (Peay et al. 2012), or hypogeous sporocarps dispersed by small mammals and arthropods (Miller et al. 1994). Effective inocula of fungi forming a resistant propagule community can persist in the soil, thus contributing to the maintenance of species richness in the ectomycorrhizal community (Taylor and Bruns 1999). It has also been found that numbers of apparently active ectomycorrhizal root tips remain for at least one year after logging, with signs of decay in density appearing after the second year (Harvey et al. 1980; Hagerman et al. 1999).

Most of the experimental studies carried out on the dynamics of fungal communities after forest management have been based on immediate or short-term (2-3 years) responses, and larger data series are needed to extract stronger conclusions on fungal regeneration. In this study, we analyse the temporal dynamics of soil fungi along five sampling years after total and partial clear-

cutting in a managed *Pinus sylvestris* stand. We hypothesize that 1) ectomycorrhizal fungal communities will decrease after both clear-cutting treatments with a concurrent increase in the abundance of saprotrophs, 2) the abundance and diversity of the ectomycorrhizal guild will be more preserved in partially clear-cut than in total clear-cut plots, and 3) the overall fungal diversity will decrease in the clear-cut plots leading to major losses of ectomycorrhizal species.

2. Material & Methods

2.1 Study site

The study was conducted in a managed monospecific Scots pine (*Pinus sylvestris*) forest known as 'Pinar Grande' in the Central Spanish plateau, province of Soria. This stand is located in the Sistema Ibérico mountain range, covering an area of 12500 ha with an altitude between 1100 and 1500 m, with dominating West and East orientations. The accompanying vegetation includes shrubs as *Erica vagans*, *E. tetralix* and herbs as *Agrostis* sp., *Brachypodium* sp., *Cynosurus cristatus*, *Lotus* sp., and *Nardus stricta*. Soils are Regosols, Luvisols, Cambisols and Umbrisols (FAO, 1998) with a markedly acid pH (4- 5), sandy to sandy-loam texture, limited water holding capacity, and low fertility levels. Average annual rainfall is 865 mm, 69 mm falling in July and August, and 132 mm in September and October. Average annual temperature is 8.8°C being July the warmest month with an average of 17.4°C, and January the coldest with an average of 1.9°C. The frost period begins in November and ends in April, with frequent frosts in late spring and early autumn. The climatic variables along the experiment (mean annual T and mean accumulated P) were obtained from the automatic weather station 'La Cuerda del Pozo', code 2-011, located in the Soria province, next to the experimental site (02º-42'-W, 41º-53'-N, altitude 1150 m), and are given in Supplementary Table 1. Forest management consists of alternate and periodic clear-cutting in mosaics with a rotation period of 130 years. In 1995, eighteen fenced permanent plots of 150 m^2 each, assigned with five age classes, were established in the forest site as a part of a long-term experiment to evaluate the yearly production of fungal sporocarps (Martínez-Peña 2009; Martínez-Peña et al. 2012a).

155 *2.2 Experimental design*

156 Three areas of 1 ha within the study site, each containing trees aged 101, 112, and 133-year-old,
157 were totally clear-cut in December 2012. Three additional areas sharing similar ecological
158 conditions, with trees aged 94, 138, and 113-year-old each, were partially clear-cut in the same
159 year, leaving parent trees for seed dispersal. In these areas, the number of trees per ha was
160 reduced from 437, 537, and 400, to 125, 137, and 125, respectively (1-2 trees left per 150 m^2
161 plot). Three additional 150 m^2 control plots (uncut) located next to the cut areas with trees aged
162 between 55 and 134-year-old were also included in the design.

163 Soil sampling was performed annually in autumn along five years, from November 2012 (one
164 month before the clear-cutting treatments) to November 2016, in three 150 m^2 plots included
165 within each cutting area (total and partially cut). Five 250 cm^3 soil samples were obtained
166 annually, along five years, with a metallic soil borer (2 cm radius, 20 cm deep) from each of the
167 nine experimental 150 m^2 plots (total clear-cut, partial clear-cut, and uncut). Soil samples were
168 taken randomly within each plot leaving a minimum distance of 30 cm to the nearest
169 tree/stump. A total of 225 soil samples were taken along the experiment.

170 *2.3 Soil processing and DNA extraction*

171 Soil samples were air-dried at room temperature, sieved through a 2 mm mesh, and maintained
172 at -20ºC until further processing. DNA extraction was performed using the PowerSoil™ DNA
173 Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) from 0,25 g of sieved soil following the
174 manufacturer's instructions. The five DNA extracts from each plot at each sampling date were
175 pooled to have a unique DNA sample per plot.

176 *2.4 Soil fungal community analysis*

177 Each of the 45 DNA pooled samples (9 plots x 5 years) was subjected to high-throughput Illumina
178 MiSeq sequencing (Illumina Inc., San Diego, CA, USA). Nuclear ribosomal ITS1 DNA markers from
179 each sample were amplified using the fungal-specific primers ITS1F (Gardes and Bruns 1993),
180 and ITS2 (White et al. 1990) attached to the Illumina overhang adapter sequences (Illumina

2013). The average length of reads assigned to the ITS1F/ITS2 primers prior to quality checking and trimming was 314 bp, excluding primers and overhang sequences (Op De Beeck et al. 2014). A first-stage PCR was performed using a GeneAmp PCR System 9700 thermocycler (Life Technologies, Carlsbad, CA, USA). PCR was conducted on 10 ng of template DNA employing an initial denaturation of 3 min at 95°C, followed by 25 cycles of 95°C for 30s, 55°C for 30s, 72°C for 30s, and a final step of 72°C for 5 min. The amplicons obtained from each sample were subjected to electrophoresis to detect successful amplification.

Illumina dual Indices (barcodes) with 8 nucleotide sequences were added to individual samples in a second-stage PCR using the Nextera XT Index Kit and the following PCR conditions: 95ºC for 3 min, 8 cycles of 95ºC for 30s, 55ºC for 30s, 72ºC for 30s, and a final extension step of 72ºC for 5 min. The amplicons were then cleaned up using AMPure XP beads, validated with a Bioanalyzer DNA (Agilent Technologies, Santa Clara, CA) with a DNA 1000 chip, and submitted to fluorometric quantification. An equimolar pool (library) with unique indices per sample was then prepared. The amplicon library was sequenced with an Illumina MiSeq system using Reagent Kits v2 at the Genetics and Bioinformatics Service, Autonomous University of Barcelona, Spain.

2.5 Quality filtering and bioinformatic analysis

Illumina reads were provided as demultiplexed FASTQ files. PIPITS automated pipeline (Gweon et al. 2015) was used for fungal community analysis of the sequences generated on the MiSeq platform using the UNITE fungal ITS reference training data set for taxonomic assignment (http://sourceforge.net/projects/rdp-classifier/files/RDP_Classifier_TrainingData), and the UNITE UCHIME reference data set for chimera removal (http://unite.ut.ee/repository.php). In a first step, the paired-end reads were joined using VSEARCH (https://github.com/torognes/vsearch/) , the resulting FASTAQ files were quality filtered using the FASTX-TOOLKIT (http://hannonlab.cshl.edu) , converted into a FASTA format, and merged into a single file. In a second step, ITS regions were extracted and reoriented using ITSX and sequences shorter than 100 bp were removed. In a third step, unique sequences were removed,

207 the remaining sequences were clustered into OTUs at 97% similarity using VSEARCH, chimeras
208 were removed, and the representative OTUs were taxonomically assigned with the RDP
209 Classifier (Wang et al. 2007) against the UNITE fungal ITS reference data set. The results were
210 then translated into two types of OTU abundance tables. In the first table, typically known as
211 'OTU abundance table', an OTU was defined as a cluster of reads with the user-defined threshold
212 (97% sequence identity by default), motivated by the expectation that these correspond
213 approximately to species. In the second table, typically known as 'phylotype abundance table',
214 an OTU was defined as a cluster of sequences binned into the same taxonomic assignments. The
215 online FUNGuild application (www.stbates.org/guilds/entry.php) (Nguyen et al. 2016) was used
216 to assign ecological information to OTUs: Arbuscular Mycorrhizal, Ectomycorrhizal, Endophyte,
217 Ericoid Mycorrhizal, Fungal Parasite, Lichenized, Plant Pathogen, and Saprotroph. Sequence data
218 are archived at NCBI's Sequence Read Archive under accession number PRJNA540904
219 (www.ncbi.nlm.nih.gov/sra)

220 *2.6 Statistical analyses*

221 The fungal community data were subjected to multivariate analyses using CANOCO version 5.11
222 (Biometris, Wageningen Research Foundation, Wageningen, The Netherlands). Relative
223 abundance data of OTUs and phylotypes were log transformed for analyses.

224 Principal components analysis (PCA) was used to obtain graphical representations of fungal
225 community similarity between both clear-cutting treatments and years. Variation partitioning
226 analysis was applied to study how much of the variation was explained by 'clear-cutting
227 treatment' and 'sampling year' as explanatory variables. The effects of cutting treatments on
228 fungal phylotypes and guild composition were separately tested by Redundancy Analysis (RDA)
229 and Monte-Carlo permutations (999 permutations). The effect of cutting on fungal community
230 composition over five years was evaluated using Principal Response Curves (PRC) to study the
231 temporal response to cutting treatments. The primary result of the PRC is a set of response
232 curves, representing temporal trajectories of community composition for each of the

experimental treatments (Smilauer and Leps 2014). Each factor level is presented as a single response curve in the plot where the horizontal axis represents the time and the vertical axis the PRC score values. Here, year was defined as a factor with 5 levels (2012, 2013, 2014, 2015, 2016) whereas cutting was defined as explanatory factor with 3 levels (control, partial clear-cutting and total clear-cutting). The reference level of the factor (uncut, control plots) has zero PRC values and so its curve overlays the horizontal axis. The clear-cutting effect was tested for significance using Monte Carlo simulations (999 permutations). Two independent tests were carried out with i) relative abundance of phylotypes and ii) relative abundance of ecological guilds.

Changes in the relative abundance of the most abundant phylotypes (represented with more than 1000 sequences) in response to cutting treatments and year were analysed using linear mixed effects models (LME) with JMP® 13.1.0 (SAS Institute, Inc.). 'Plot' was defined as random term, whereas 'year' and 'clear-cutting' were defined as fixed terms.

Hill's series of diversity indices: H0, H1, H2 (Hill 1973) were used to compare differences in diversity values between cutting treatments for both, total fungal community and ectomycorrhizal fungal community. H0 corresponded to the phylotypes richness, H1 (representing the abundant phylotypes in a sample) was calculated as the exponential of the Shannon's diversity index, and H2 (representing the very abundant phylotypes in a sample) was the inverse of the Simpson's diversity index. Communities can be considered more diverse if their diversity ranks higher at all three scale parameters. We did not rarefy the fungal community due to the potential information loss. Instead, we included square-root transformed total read counts per sample as an explaining variable to stand for the bias stemming from differential sequencing success in different samples (Bálint et al. 2015). LME models were used to test significant changes in Hill's numbers between cutting treatments and years. 'Plot' was defined as random term whereas 'year' and 'cutting' were defined as fixed terms.

3. Results

From the 45 samples, we obtained a total of 895320 ITS1 fungal sequences to generate 3107 OTUs and 970 phylotypes. A 66% of the OTUs were identified at different taxonomic levels, being the Ascomycotina the most abundant and accounting for 54% of the identified phyla. Basidiomycotina accounted for 29%, followed by Mortierellomycota (9%), and the rest of the phyla: Mucoromycota, Glomeromycota, Rozellomycota and Chytridiomycota which accounted, altogether, for 8%. A total of 529 phylotypes were assigned to ecological guilds. Most of them (67%) were assigned as saprotrophs, whereas 16% were ectomycorrhizal, and the rest of ecological guilds ranged between 2-4%.

The variation in soil fungal communities after unconstrained linear PCA corresponding to different clear-cutting treatments and years is shown in Fig. 1 for phylotypes (a) and ecological guilds (b) and approximates the dissimilarity of their composition. Variation partitioning shown in Supp. Fig. 1 reveals that clear-cutting treatments explained 16.1% of the total variation at the phylotype level (a), and 14.9% at the ecological guild level (b). The sampling year accounted for 3.4 and 16.8%, respectively. The negative values of the shared variation fraction (c sectors in Supp. Fig. 1) indicated that the joint explanatory effects of 'cutting' and 'sampling year' variables are stronger than the sum of their marginal effects.

The variation in fungal phylotypes composition explained by treatments (cutting and year) is summarized in Fig. 2a. RDA analysis showed that explanatory variables (cutting treatments and sampling year) accounted for 23.98% of the total variation (Pseudo F = 2.0; P=0.002). The RDA biplot showed a clear dissimilarity in phylotypes composition between year 2012 (before clear-cutting) and the following years after clear-cutting (2013-2016) which were grouped. Clear-cutting treatments also showed highly dissimilar phylotypes composition to each other. *Boletus edulis*, the most abundant ectomycorrhizal fungus in the area, was associated to control, uncut plots.

The effects of explanatory variables (clear-cutting treatments and sampling year) on fungal guilds response are summarized in Fig. 2b. Here, RDA analysis showed that explanatory variables

285 accounted for 36.16% of the total variation (Pseudo-F = 3.6; P=0.002). The generated biplot also
286 showed a clear dissimilarity in guild composition between year 2012 (before clear-cutting
287 treatments) and the rest of the years, being 2015 and 2016 the most similar to each other. The
288 three cutting treatments also showed a dissimilarity in guilds composition. Ectomycorrhizal and
289 ericoid fungi were associated to uncut treatments, whereas saprotrophs, lichenized and
290 arbuscular mycorrhizal fungi were associated to partial clear-cut treatments and to the third and
291 fourth years after cutting. Endophytes, plant pathogens and parasitic fungi were mostly found
292 in total clear-cut plots and along the two first years after cutting. A negative correlation was
293 found between ectomycorrhizal fungi and saprotrophs.
294 Principal response curves (PRC) showed a significant effect of clear-cutting treatments on soil
295 fungal phylotypes along time (Fig. 3a) (Pseudo-F=0.4; P=0.044). Soil fungal communities in both,
296 total and partial clear-cuttings, showed a similar trend and parallel responses over time in the
297 ordination plot, with differences that can be attributed to the initial variability already existing
298 in the year 2012, before the clear-cutting treatments. The scores in the additional vertical axis
299 next to PRC (Fig. 3a) showed that the relative appearance of phylotypes as the ectomycorrhizal
300 *Boletus edulis* and the root-associated Archaeorhizomyces fungal class was much lower in the
301 clear-cut plots as compared with the uncut plots.
302 The results of PRC analysis with 'guilds' as response variable are shown in Fig. 3b. Ecological
303 guilds were significantly affected by the clear-cutting treatments over time (Pseudo-F=0.8;
304 P=0.006). PRC curves for both clear-cutting treatments also showed parallel responses and a
305 progressive dissimilarity with the reference plots (control) across years. The scores in the
306 additional vertical axis showed that the ectomycorrhizal and ericoid mycorrhizal guilds were
307 associated to the uncut, control plots whereas fungal plant pathogens and fungal parasites were
308 associated to both clear-cutting treatments.
309 Linear mixed effects models considering 'cutting treatments', 'years', and their interaction as
310 fixed terms, and 'plot' as random term for the phylotypes represented by 1000 or more

311 sequences are summarized in Table 1. Clear-cutting had no effect on most of the species except
312 for *Boletus* and Eurotiales in which cutting treatments decreased the number of sequences. The
313 sampling year had significant effects for *Archaeorhizomyces*, Eurotiales, *Geminibasidium*,
314 *Microdochium*, *Mortierella*, *Oidiodendron*, *Tremellales* and *Umbelopsis*.
315 Hill's diversity values of the total fungal community (total fungal phylotypes) and the
316 ectomycorrhizal fungal community (ectomycorrhizal phylotypes) are represented in Fig. 4 and
317 Supp. Table 2. No significant differences in any of the Hill's diversity parameters between the
318 cutting treatments were found. However, the sampling year affected significantly the
319 parameters N1 and N2 of the total fungal phylotypes, with a sharp and significant decrease of
320 the abundant and very abundant phylotypes across the years after cutting.

321 **4. Discussion**

322 The results presented in this study show that soil fungal dynamics across five years after tree
323 harvest was dependent on the clear-cutting treatments (uncut, partial clear-cutting and total
324 clear-cutting) and the sampling year. Fungal phylotypes and the composition of ecological guilds
325 were different between plots subjected to the two clear-cutting treatments. However, the
326 differences in fungal composition between the sampling years after clear-cutting (2013-2016)
327 were more marked in ecological guilds than in total fungal phylotypes. Direct studies on short-
328 term fungal dynamics after tree clear-cutting are scarce in the literature. Castaño et al. (2018a)
329 found that changes of a fungal community across 4 years after forest thinning in a dry
330 Mediterranean forest were driven by inter-annual variation in precipitation and temperature,
331 and not by the thinning treatment. A former study on the dynamics of the mycelium of the edible
332 ectomycorrhizal fungus *Boletus edulis*, carried out in the same experimental area as in the
333 present study, showed a sharp decrease on this fungal species as soon as 7 months after partial
334 and total clear-cutting treatments, and no recovery was observed 3 years later (Parladé et al.
335 2017). Kohout et al. (2018) assessed the dynamics in fungal community structure during a 2-year

period following clear-cutting and detected profound changes in soil decomposition processes and fungal community composition. On the other hand, Jones et al. (2010) and Barker et al (2013) measured extracellular enzymes in ectomycorrhizal communities 2-3 years after tree harvesting practices and found changes in the structure of the ectomycorrhizal communities before and after the disturbance but functional similarities. Similarly, Kyaschenko et al. (2017) suggested that the maintenance of functional diversity in the ectomycorrhizal fungal community may sustain long-term production by retaining the symbiotic capacity able to recycle the organic nutrients.

Our results confirm the first hypothesis that clear-cutting causes a sharp decrease of the relative abundance of the ectomycorrhizal fungal guild and an increase of saprotrophs and arbuscular mycorrhizal fungi in the short term after the clear-cutting treatments. However, saprotrophs were more abundant in partially clear-cut than in total clear-cut plots, where a higher amount of pathogenic, parasitic, and endophytic fungi was found. Kyaschenko et al. (2017) found a proliferation of saprotrophic fungi in total clear-cut *P. sylvestris* stands which correlated with enzymes involved in holocellulose decomposition. Moreover, root endophytic fungi may have an important role on the early stages after clear-cutting by their contribution to the initial phases of decomposition of host tissues (Kohout et al. 2018). Long-term studies showed that the relative abundance of root-associated communities (i.e. ectomycorrhizal and ericoid mycorrhizal fungi) increased while saprotrophic communities decreased 50 years after logging (Chen et al. 2019), suggesting a progressive recovery of root-associated communities with time. Partially clear-cut plots leaving retention trees may lifeboat ectomycorrhizal fungi and mitigate the negative effects of clear-cutting on biodiversity (Fedrowitz et al. 2014). The efficiency of this practice has been found to be significant only close to the tree (Luoma et al. 2006; Jones et al. 2008). Recent studies in regenerated *P. sylvestris* stands in Sweden showed that retention of seed trees failed to mitigate the impact of harvesting on ectomycorrhizal species composition and diversity (Varenius et al. 2017). However, other studies show that retention trees may

362 harbour most of the ectomycorrhizal taxa found in conifer forests (Sterkenburg et al. 2019). In
363 the present study, the communities of ectomycorrhizal fungi in both partial and total clear-cut
364 plots were not clearly separated, as shown by the Principal Response Curves (PRC) analysis
365 across the 5-year samplings (Fig. 3), and our second starting hypothesis could not be confirmed.
366 Fungal communities change in response to climatic conditions (Fernandez et al., 2016; Hartmann
367 et al., 2017; Solly et al., 2017). Intra-annual spatio temporal changes of community composition
368 in Mediterranean forests have been correlated significantly with soil moisture and temperature
369 (Castaño et al. 2018b). In addition, inter-annual changes are partly driven by annual variation in
370 precipitation and temperature (Castaño et al. 2018a). The results from PRC showed a decrease
371 in the relative abundance of ectomycorrhizal fungi such as *Boletus edulis* and root-associated
372 Archaeorhizomyces (Pinto-Figueroa et al. 2019) in the clear-cut plots. The increase of relative
373 abundance of saprotrophic phylotypes, such as *Umbelopsis*, in our clear-cut plots, can be caused
374 by the short-term decomposition processes occurring in soil after clearcutting (Kohout et al.
375 2018). The increase of the relative abundance of endophitic fungi (including Pucciniomycotina
376 and root parasites) in the clear-cut plots may indicate their important role at the early stages of
377 root decomposition after clear-cutting (Hilszczańska 2016). Compared to ectomycorrhizal fungi,
378 root endophytes generally feature greater enzymatic capabilities for degradation of the complex
379 organic compounds formed a few months after clearcutting (Schlegel et al. 2016).
380 Hill's diversity values showed no significant changes in fungal diversity between the different
381 clear-cutting treatments. Similar results were found in a forest thinning experiment carried out
382 in Mediterranean forests (Castaño et al. 2018b) and were attributed to the survival of
383 ectomycorrhizal species (the most affected by tree removal) supported by the remaining trees
384 (Varenius et al. 2017). In addition, surviving propagules (spores and sclerotia) may be able to
385 persist long time through unfavourable conditions and disperse into new environments (Nguyen
386 et al. 2012), or colonize regeneration seedlings (Cline et al. 2005). Similarly, fungal diversity and
387 richness of ectomycorrhizal communities in *Pseudotsuga menziesii* forests submitted to clear-

388 cutting, with manual removal of timber and soil retention, were comparable to the undisturbed
389 forest (Barker et al. 2013). However, Hill's diversity parameters N1 and N2 were significantly
390 lower when analysing total fungal phylotypes across years, indicating a decrease of the
391 abundant and very abundant phylotypes in the years following clear-cutting, and suggesting a
392 relative homogenization of fungal abundances following the disturbance. These results do not
393 support the third hypothesis that tree removal affects ectomycorrhizal assemblies. Instead, the
394 conservation of soil propagules seems to be more important that the removal of the tree hosts
395 in the study area, at least during the first years after disturbance when the natural regeneration
396 occurs.
397 Univariate GLM analyses considering the most abundant fungal taxa (represented by more than
398 1000 sequences) showed a significant effect of clear-cutting in the abundance of *Boletus* and
399 Eurotiales, with no significant interaction with the sampling year. In all cases, the effect of clear-
400 cutting was to decrease the abundance of these species in relation to control (uncut) plots and
401 no significant differences were found between the two clear-cutting treatments (partial and
402 total clear-cutting). The results obtained for *Boletus* are especially interesting because the
403 sporocarps of this fungal genus contributes to the highest ectomycorrhizal biomass in the study
404 area (26.6%) (Martínez-Peña 2009) where it is particularly sought as one of the main non-wood
405 forest products. Recent studies on the effects of cutting on mycelium dynamics of *B. edulis* using
406 specific DNA quantification showed similar results as those obtained in the present study, with
407 a significant and rapid decrease of *B. edulis* mycelium biomass starting one month after clear-
408 cutting and maintained at least for three years (Parladé et al. 2017). However, previous results
409 in the area showed that the production is resumed after cutting, reaching up to 16.2 kg of
410 sporocarps/ha 30 years after tree removal (Ortega-Martínez et al. 2011; De la Varga et al. 2013).
411 The quantitative use of high-throughput sequencing data has been much debated since the
412 abundance of genetic markers does not reflect biomass in the samples (Lindahl et al. 2013).
413 Diverging numbers of rDNA repeats in different species, differences in extractability, and a

414 variable number of primer-template mismatches (Piñol et al. 2015) may lead to important
415 quantitative biases. However, in our case we had the opportunity of analysing the same field
416 samples using specific Taqman® real-time PCR for *B. edulis* quantification (Parladé et al. 2017),
417 and high-throughput Illumina sequencing (in this study) and obtained similar results.
418 The order Eurotiales also showed a significant decrease in cut plots. This order comprises both
419 saprotrophic and ectomycorrhizal species and has been found to be abundant in soil litter, but
420 their relative proportion decrease in the soil mycelium decomposition processes following
421 experimental soil disturbances (Brabcová et al. 2016), and forest pest attacks (Veselá et al.
422 2019).
423 Our study demonstrates that clear-cutting significantly affects soil fungal composition in a
424 managed *Pinus sylvestris* forest across five years by decreasing ectomycorrhizal fungi and
425 increasing saprotrophs. However, these changes do not affect fungal diversity and the different
426 species are not affected in the same way. Partial clear-cutting leaving parent trees to facilitate
427 seedling regeneration showed no different ectomycorrhizal communities as compared to clear-
428 cut areas. Although long-term spontaneous regeneration of key ectomycorrhizal fungi occurs,
429 further research involving tracking the ectomycorrhizal status of the regenerated seedlings
430 would improve the integrated management of forests aimed to improve edible mushrooms
431 production.

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Table 1. Clear-cutting treatment and sampling year effects on the most abundant phylotypes (represented by more than 1000 sequences). P values in ***bold*** show a significant effect (p<0,05) after Linear Mixed Model analysis, including clear-cutting treatment and year as fixed terms and plot as random term. Data were log-transformed for the analysis (Y'=log(Y*1000+1)). TotSeq: Total sequences, SAPR: Saprotrophs, ECTO: Ectomycorrhizal, ENDP: Endophytes, ERIC: Ericoids, FPAR: Fungal parasites.

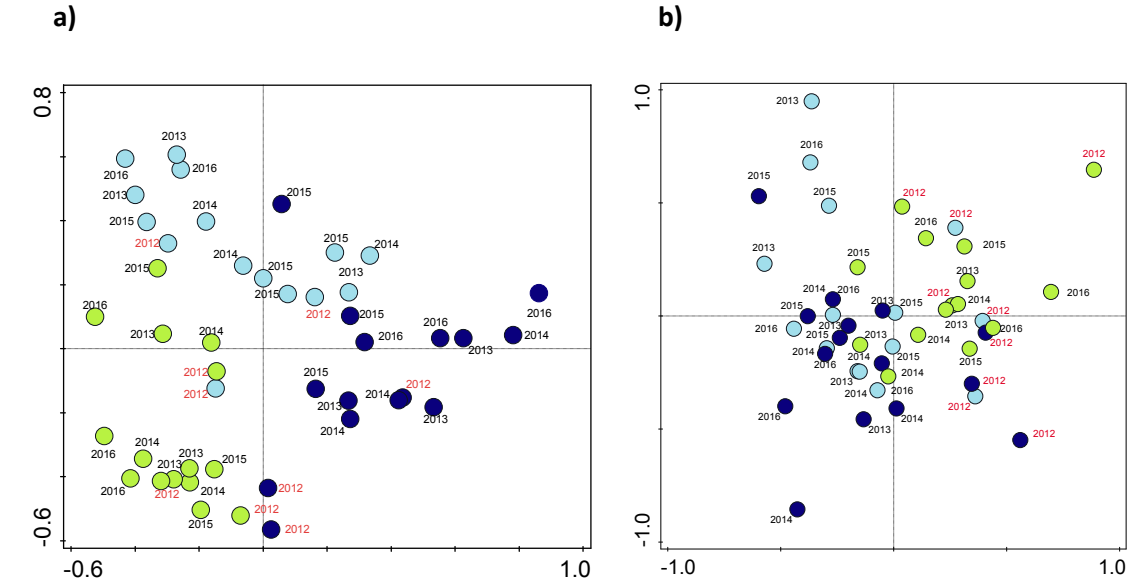
Phylotype	Total Sequences	Guild	p Cutting	p Year	p Interaction	Effect (**)
Archaeorhizomyces	80589	SAPR	0.0792	***0.0092***	***0.036***	D,I
Boletus	1366	ECTO	***0.0026***	0.4484	0.3134	D
Cenococcum	1944	ECTO	0.9653	0.8573	0.3751	
Ceratobasidiaceae	1127	SAPR	0.5365	0.5408	0.5745	
Chaetosphaeriaceae	1797	SAPR	0.506	0.1237	0.1051	
Clavulina	2325	ECTO	0.3181	0.6956	0.9577	
Cortinarius *	1005	ECTO	0.5606	0.1206	0.0574	
Eurotiales	18448	SAPR	***0.0038***	***0.0025***	0.1368	D
Geminibasidium	5540	SAPR	0.1929	***0.0459***	0.1261	D
Hypocreales	15858	SAPR	0.124	0.7582	0.1475	
Luellia	1931	SAPR	0.2399	0.291	0.4599	
Microdochium	1343	ENDP	0.3701	***0.0251***	0.1257	I
Mortierella	420021	SAPR	0.0784	***<.0001***	***0.036***	I,D
Oidiodendron	15453	ERIC	0.0854	***0.0132***	0.2439	D
Pseudeurotium	1446	SAPR	0.4304	0.2779	***0.0286***	
Russula	10138	ECTO	0.5239	***0.0019***	***0.0018***	D
Saccharomycetales	2498	SAPR	0.0817	0.2682	0.1352	
Sistotrema	1736	ECTO	0.5838	0.4661	0.4624	
Trechispora	1263	SAPR	0.5183	0.3735	0.5521	
Tremellales	3136	FPAR	0.0663	***0.0051***	0.0621	I
Umbelopsis	46225	SAPR	0.0567	***0.0495***	0.6046	D

*: Including 6 sequences of Cortinariaceae

**: D: Decrease with cutting or year respect to control

I: Increase with cutting or year respect to control

Fig. 1. Variation in soil fungal community composition on the 45 soil samples after unconstrained linear PCA (Principal Component Analysis) ordination of fungal phylotypes (a) and fungal guilds (b). Response data have been log-transformed for the analysis (Y'=log(Y*1000+1)). The sampling year is indicated next to each point with different colours representing the clear-cutting treatments. Points marked with the year 2012 (in red) represent the plots before clear-cutting and the color indicates the assigned treatment (control, partial clear-cutting and total clear-cutting) applied in December 2012.



: uncut; : partial clear-cutting; : total clear-cutting

Fig. 2. Variation in fungal community composition explained by treatments (clear-cutting and year) after performing constrained redundancy analysis (RDA) ordination of fungal phylotypes (a) and fungal guilds (b). Response data have been log-transformed for the analysis (Y'=log(Y*1000+1)). CONTROL: Uncut plots; PCUT: partially clear-cut plots; TCUT: total clear-cut plots. In graph a) only the 30 best-fitting phylotypes are represented.

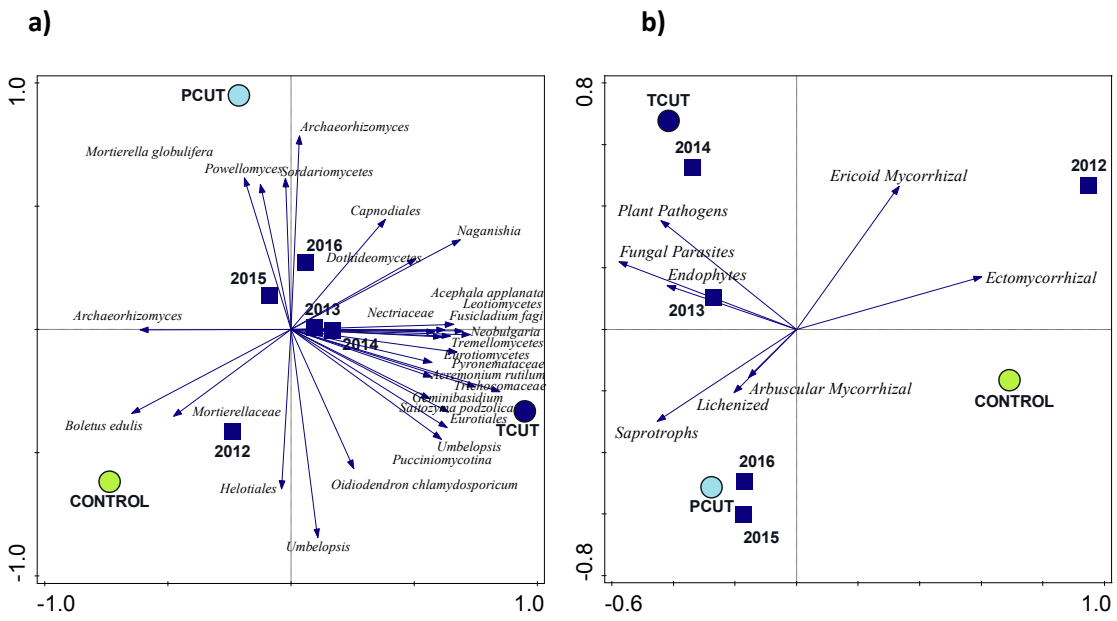
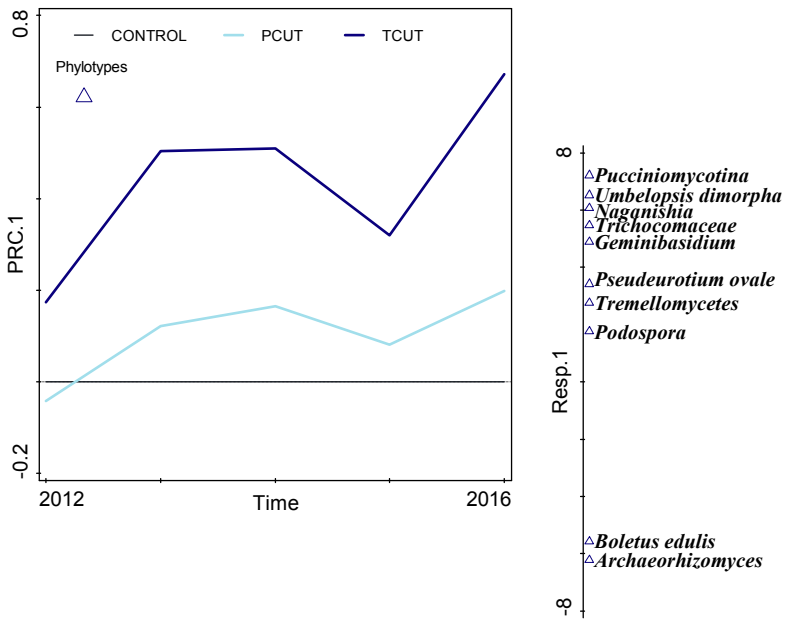
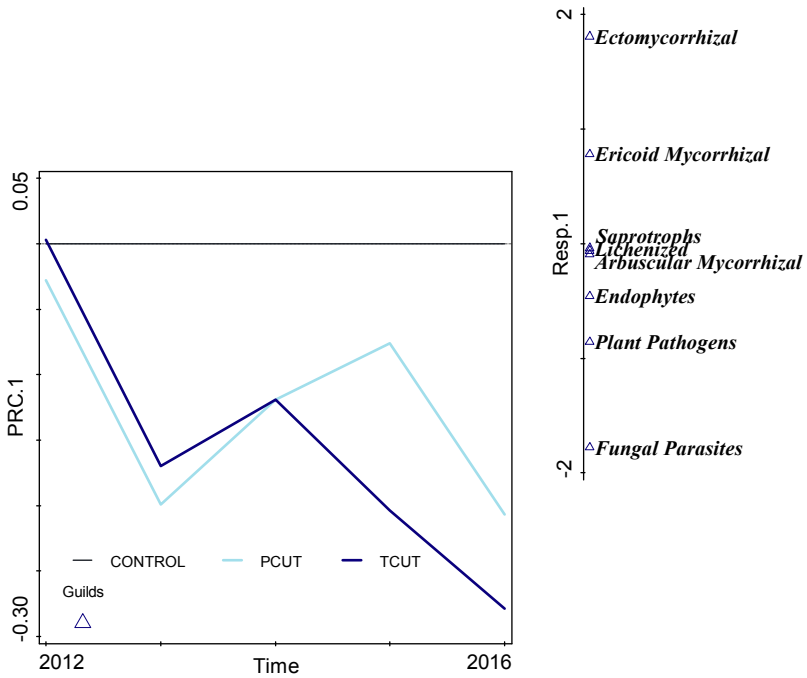


Fig. 3. Principal response Curves (PRC) for the first RDA axis showing the effects of clear-cutting treatments on fungal phylotypes (a) and fungal guilds (b) composition over five years. The reference level of the cutting factor (uncut) is represented by a straight horizontal line overlaying the horizontal axis. The one-dimensional diagram in the right side shows the response variables (phylotypes or guilds) scores on the corresponding RDA axis. PCUT: Partial clear-cutting; TCUT: Total clear-cutting; CONTROL: Uncut plots.

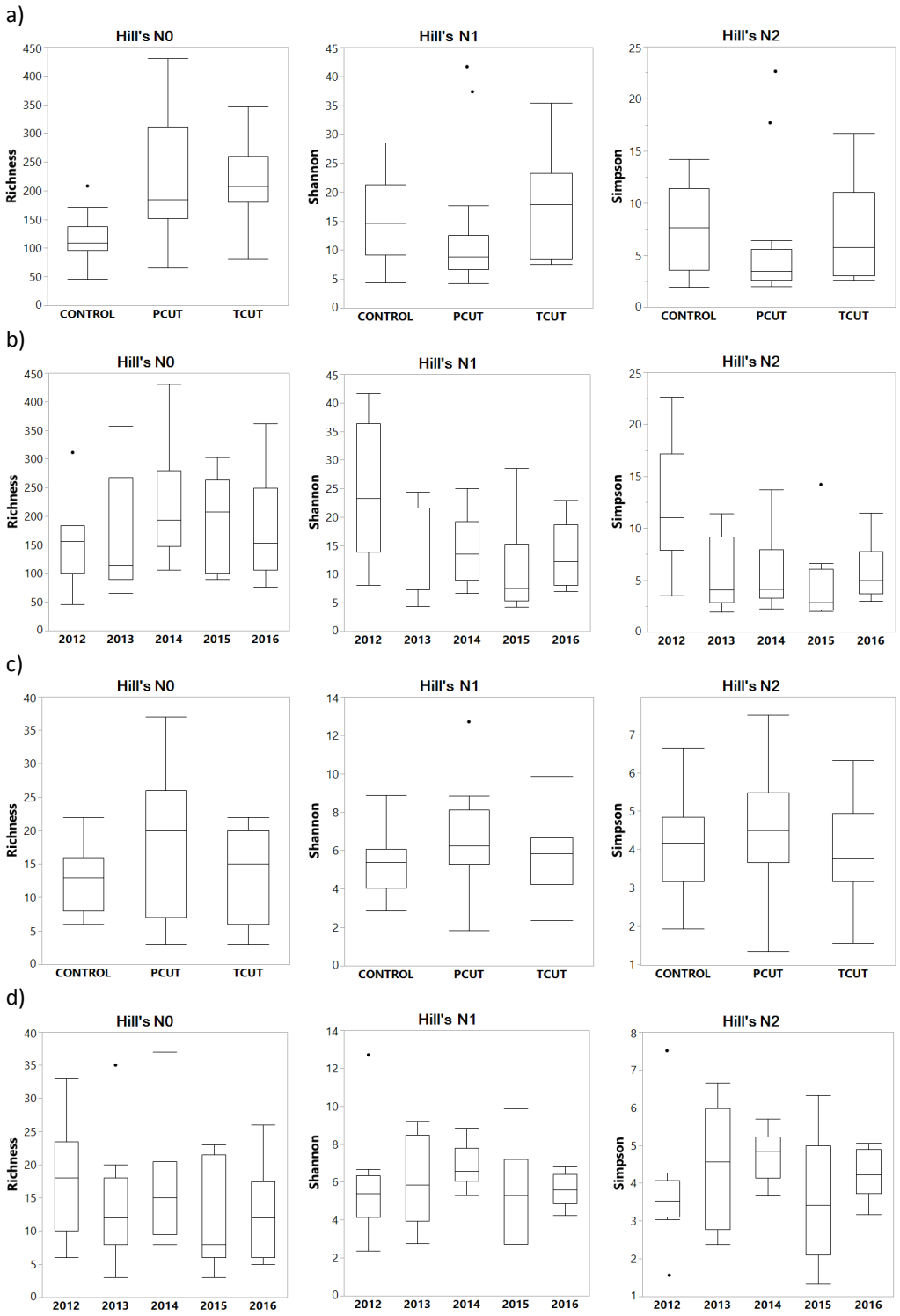
a)



b)



703 **Fig. 4.** Hill's diversity values of the total fungal phylotypes (a, b) and the ectomycorrhizal
704 phylotypes (c, d) across the clear-cutting treatments and the sampling years. PCUT: Partial clear-
705 cutting; TCUT: Total clear-cutting; CONTROL: Uncut plots.



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Supplementary Table 1. Climatic conditions in the study area (monthly accumulated Precipitation and monthly mean Temperature) during the experiment samplings (years 2012-2016).

Year	Month	Accumulated P (mm)	Mean T (ºC)
2012	1	11.1	1.3
2012	2	15.8	-0.7
2012	3	32.3	5
2012	4	132.7	3.9
2012	5	48	10
2012	6	16.4	15.6
2012	7	40.4	17.1
2012	8	4.4	18.2
2012	9	49.4	13.1
2012	10	72.4	9.2
2012	11	51.7	4.3
2012	12	59.7	1.3
2013	1	66.3	3.1
2013	2	159.3	4.1
2013	3	235.5	5.3
2013	4	89.7	8
2013	5	73.5	9.3
2013	6	48.5	15.1
2013	7	38.9	20.3
2013	8	8.8	19.5
2013	9	52.1	16.4
2013	10	81.5	11.9
2013	11	37.4	6.2
2013	12	112.3	3.2
2014	1	123.7	3.85
2014	2	125.3	3.32
2014	3	54.7	6.83
2014	4	78	11.12
2014	5	38.3	11.48
2014	6	31.1	16.38
2014	7	60.2	17.90
2014	8	9.1	18.99
2014	9	70.2	17.33
2014	10	63.3	14.19
2014	11	159.9	7.29
2014	12	28.4	4.27
2015	1	79	3.34
2015	2	66.9	1.83
2015	3	88.6	7.02
2015	4	35.7	9.92
2015	5	12.8	13.89
2015	6	97.0	17.64
2015	7	15.6	22.20
2015	8	77.7	19.23
2015	9	33.8	14.19
2015	10	40.4	11.20
2015	11	48.2	8.73
2015	12	8	5.21
2016	1	191.2	4.77
2016	2	175.3	4.21
2016	3	75.1	4.29
2016	4	78.8	7.49
2016	5	58.1	12.10
2016	6	26.1	16.84
2016	7	48.8	20.31
2016	8	3.3	20.23
2016	9	8.4	16.72
2016	10	25.6	11.97
2016	11	101.1	5.54
2016	12	14.2	4.65

Supplementary Table 2. Cutting treatment and year effects on belowground fungal diversity (Hill's Numbers N0, N1, and N2) for the a) total fungal community, and b) ectomycorrhizal community.

a) Total fungal community (phylotypes)

Fixed Effect Tests. **N0** (Species Richness)

Source	Nparm	DF	DFDen	F Ratio	Prob > F
Treatment	2	2	6.356	3.5408	0.0926
Year	4	4	23.95	2.0748	0.1157
Year*Treatment	8	8	23.85	0.7334	0.6615

Fixed Effect Tests. **N1** (Exponential of Shannon diversity index)

Source	Nparm	DF	DFDen	F Ratio	Prob > F
Treatment	2	2	6.331	0.3615	0.7101
Year	4	4	23.55	5.4210	0.0031*
Year*Treatment	8	8	23.48	2.3399	0.0521

Fixed Effect Tests. **N2** (Inverse of Simpson diversity index)

Source	Nparm	DF	DFDen	F Ratio	Prob > F
Treatment	2	2	6.02	0.0331	0.9676
Year	4	4	23.17	8.8684	0.0002*
Year*Treatment	8	8	23.12	3.4003	0.0100*

b) Ectomycorrhizal community

Fixed Effect Tests. **N0** (Species Richness)

Source	Nparm	DF	DFDen	F Ratio	Prob > F
Treatment	2	2	5.023	1.3728	0.3345
Year	4	4	23.24	1.9077	0.1429
Year*Treatment	8	8	22.94	0.1656	0.9935

Fixed Effect Tests. **N1** (Exponential of Shannon diversity index)

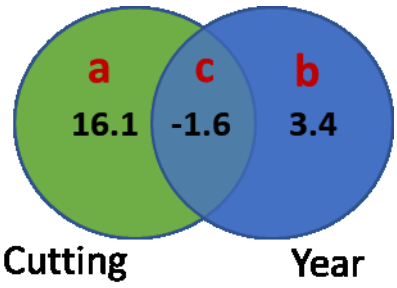
Source	Nparm	DF	DFDen	F Ratio	Prob > F
Treatment	2	2	6.018	0.3678	0.7068
Year	4	4	24.57	1.7712	0.1668
Year*Treatment	8	8	24.25	0.7979	0.6101

Fixed Effect Tests. **N2** (Inverse of Simpson diversity index)

Source	Nparm	DF	DFDen	F Ratio	Prob > F
Treatment	2	2	5.901	0.2102	0.8162
Year	4	4	24.79	1.5024	0.2319
Year*Treatment	8	8	24.43	0.8088	0.6014

Supplementary Fig. 1. Variance partitioning analyses including the clear-cutting treatment and the sampling effects on a) fungal phylotypes response, and b) fungal guilds response. Values show the fraction of variation explained by each parameter, as well as the shared contribution of each of the parameter's combination.

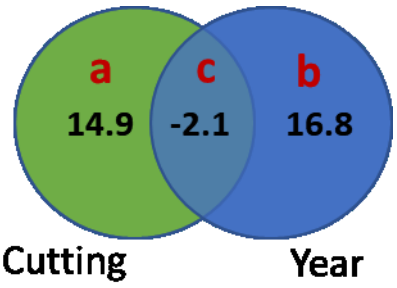
a)



Significance tests

Tested Fraction	F	P
a+b+c	2.6	**0.002**
a+c	4.7	**0.002**
b+c	1.2	0.088

b)



Significance tests:

Tested Fraction	F	P
a+b+c	4.1	**0.002**
a+c	4.2	**0.006**
b+c	2.9	**0.002**