

1 **Title: Soil fungal communities and succession following wildfire in Ethiopian dry**  
2 **Afromontane forests, a highly diverse underexplored ecosystem**

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## 54 **Abstract**

55 Ethiopian dry Afromontane forests are complex ecosystems that have important economic and  
56 ecological roles. However, recurrent fire has been a source of disturbance for these forests. We  
57 assessed the effect of fire on soil fungal communities in a remnant dry Afromontane forest in  
58 Wondo Genet, southern Ethiopia, by analysing soil samples collected from unburned stands and  
59 from stands one and ten years after fire using DNA metabarcoding of the ITS2 rDNA. The  
60 analysis indicated that the soil fungal community was most diverse soon after a fire disturbance  
61 and declined over time. Fungal community composition also differed among stands. Our results  
62 also indicated that differences in fungal diversity were stand dependent rather than due to the  
63 chronology of the fire history in this forest system. We found higher numbers of mycorrhizal  
64 species in burned stands, suggesting that these fungal symbionts could compensate for the effects  
65 of nutrient stress caused by fire in these areas. Fungal community composition was also  
66 significantly correlated with organic matter content, potassium and magnesium in soil. This work  
67 could be considered as a case study since the plots were established in a single stand for each  
68 treatment in the dry Afromontane forests of Ethiopia. Thus, we recommend further studies and  
69 conclusions regarding other stands need to be taken with caution.

70 **Keywords:** *Edaphic variable, Ethiopia, Forest fire, fungal functional groups, ion torrent*  
71 *sequencing, tropics*

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## 74 1. Introduction

75 Ethiopia is an ecologically diverse country owing to the varied topographic features and  
76 altitudinal variations (Bongers and Tenngkeit, 2010). The country also experiences a high  
77 variation in macro- and mesoclimatic conditions that have contributed to the formation of  
78 biologically diverse ecosystems. According to Friis et al. (2010), the vegetation of Ethiopia is  
79 classified into 12 types based on the elevation zones in which they occurred. Out of which, the  
80 natural high-elevation forests , that include the Afromontane vegetation, are exclusively found in  
81 the highland regions of Ethiopia between 1500 to 3400 m above sea level (Lemenih and Bekele,  
82 2008).

83 The highlands of Ethiopia occupy more than 44% of the country's land area (Kidanu, 2004;  
84 McCann, 1995). Dry Afromontane forests dominate these highlands and are found mainly in the  
85 central, northern and western parts of the country (Friis et al., 2010). This forest is a complex  
86 ecosystem and is characterized by high humidity, a variable rainfall pattern, and a prolonged dry  
87 season (Friis et al., 2010). The dry Afromontane forest provides important ecosystem services  
88 such as watershed protection and carbon sequestration (Wassie et al., 2005). The dominant tree  
89 species in this forest are *Juniperus procera*, *Podocarpus falcatus*, *Hagenia abyssinica* and *Olea*  
90 *africana*, which are the main source of timber in Ethiopia. In addition, the dry Afromontane  
91 forest harbours various types of non-timber forest products, including wild edible mushrooms  
92 (Dejene et al., 2017b).

93 Anthropogenic factors are negatively affecting the forest resources in Ethiopia (Lemenih  
94 and Bekele, 2008). Fire is a potentially destructive disturbance, affecting the distribution,  
95 diversity and composition of the forest resources (Lemenih and Bekele, 2008; Wassie et al.,  
96 2005). Human-induced fire, mainly for subsistence and economic reasons, is the most important  
97 reason for the depletion and degradation of natural resources in Ethiopia. For instance, the most  
98 destructive forest fires, which occurred in 2000, destroyed over 150,000 ha of forest (Senbeta  
99 and Teketay, 2001). More recently, in 2019, a fire in the northern part of Ethiopia affected 340  
100 ha of forest (New Business Ethiopia, 2019). This trend is more common in the highland areas,  
101 where the dry Afromontane forest is found, and has a direct impact on the biodiversity in the  
102 forest ecosystem (Lemenih and Bekele, 2008). This loss of biodiversity could also occur in the

103 forest soil, which harbours a great diversity of microbial organisms (Fierer and Jackson, 2006),  
104 including fungi. Depending on the severity and frequency, fire could directly or indirectly affect  
105 edaphic variables in the forest ecosystem (Reazin et al., 2016), which in turn could have an  
106 impact on fungal communities dwelling in the soil (Cairney and Bastias, 2007; Dahlberg et al.,  
107 2001; Rincón and Pueyo, 2010).

108 Forest fungi comprise a crucial functional component and contribute to the high ecological  
109 significance of forest ecosystems (Crabtree et al., 2010). Their roles can be described in terms of  
110 nutrient addition and cycling, the carbon pool, soil formation, and the formation of symbiotic  
111 links with plants (Claridge et al., 2009; Fontaine et al., 2007; Van Der Heijden et al., 2008).  
112 Saprotrophic fungi play a key role in the decay of organic matter (Hobbie et al., 1999), whereas  
113 mycorrhizal fungi form symbiotic associations with plants, and can form up to 80% of the fungal  
114 biomass in forest soils in northern temperate regions (Nehls, 2008). The fungal mycelium also  
115 plays an essential role in soil stabilization and helps to increase the water-holding capacity of the  
116 soil (Kennedy and Gewin, 1997). Furthermore, some species of fungi are pathogenic, causing  
117 disease in both above- and belowground components of the forest system (Narayanasamy, 2011).

118 Despite recent advances in determining the diversity and composition of forest fungi in  
119 various biomes, fundamental questions regarding their distribution and function, and the factors  
120 that influence them remain unanswered, particularly in under-sampled biomes (Guo et al., 2013;  
121 Krashevskaya et al., 2015). The majority of soil fungi are unexplored and, the functional  
122 relationship between fungi, soil, and plants remains understudied (Bridge and Spooner, 2011;  
123 Van Der Heijden et al., 2008). Previous investigations have estimated that there are about 5.1  
124 million fungal species worldwide (Taylor et al., 2014). Of these, 2–6% have been described  
125 (O'Brien et al., 2005) and ~1200 new species are described each year (Hibbett and Thorn, 2001),  
126 indicating that there are many more fungal species to be explored, named, and identified. In  
127 addition, further studies are required to increase our understanding of the dynamics of soil fungi  
128 and their community structure (Dhruba et al., 2015) and the impact of various environmental and  
129 anthropogenic factors. Furthermore, to date, most studies of soil fungal communities have  
130 focussed on temperate and Mediterranean forest ecosystems; less consideration has been given to  
131 soil fungal communities in tropical forest ecosystems (Taudière et al., 2017).

132 The effects of fire on soil fungi are determined by the fire severity, changes in soil  
133 properties and post-fire environmental conditions (Bastias et al., 2006; Buscardo et al., 2012,  
134 2010; Neary et al., 1999; Reazin et al., 2016). A change in vegetation following a fire may also  
135 impact on fungi living in a symbiotic or saprophytic relationship with trees. Thus, the subsequent  
136 structure of fungal communities might be influenced by the dynamics of post-fire plant  
137 communities (Cairney and Bastias, 2007). In addition, factors such as the fuel load (Dahlberg et  
138 al., 2001), soil moisture and temperature (Bonet et al., 2010) and other environmental and  
139 weather conditions during the fire event can also influence the effects on soil biota. Furthermore,  
140 differences in the sensitivity of fungal propagules to fire determine the degree to which the  
141 composition of fungal communities changes after the fire (Hernández-Rodríguez et al., 2013).  
142 However, the inconsistency of results from individual studies makes it difficult to provide a  
143 general conclusion regarding the possible dynamics of fire, fungi and ecosystem function.  
144 Consequently, location-based studies are necessary to obtain a better understanding of the effect  
145 of fire on the soil fungal community at a specific location (Taudière et al., 2017).

146 A limited number of studies have investigated fungal communities in the forest systems of  
147 Ethiopia; however, these were mainly focused on above-ground fungal communities (Dejene et  
148 al., 2017b, 2017a). Recently, Castaño et al. (2019) also investigated the soil fungal community  
149 and ecological guilds associated with *Eucalyptus grandis* plantations in Ethiopia. However, the  
150 soil fungal communities associated with the dry Afromontane forests in Ethiopia are undescribed  
151 and the potential effect of fire on soil fungal communities in these ecosystems where forest fire is  
152 a recurrent phenomenon has not yet been analysed. Forest fires are expected to change the  
153 edaphic variables on which the fungi depend for their trophic as well as their community  
154 composition. In addition, it is important to understand how fungal communities respond to the  
155 post-fire environment and to identify which are the most important environmental factors driving  
156 fungal community structure and function to supplement our knowledge of Ethiopian fungal  
157 resources as well as to promote their conservation and development. Thus, the aim of this study  
158 was to provide baseline information on soil fungal communities in the dry Afromontane forests  
159 along a post-fire, secondary succession gradient in Ethiopia.

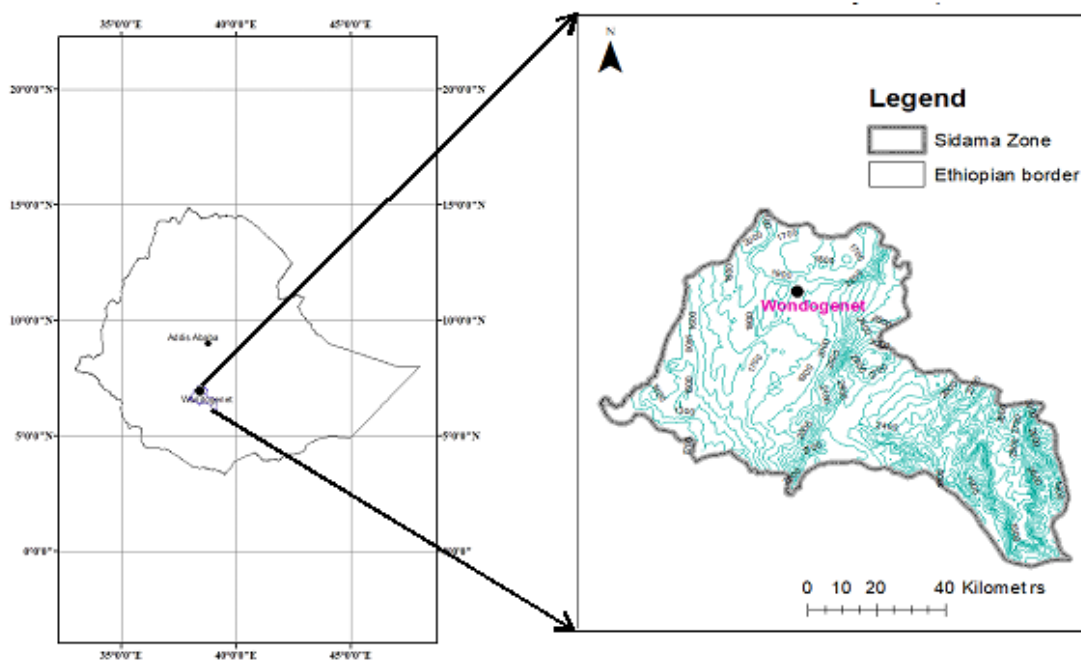
160 We hypothesized that the richness and composition of the entire soil fungal communities  
161 would change substantially during a post-fire forest succession and would differ from those in

162 unburned forest (Hypothesis 1). Specifically, we expected that soils would be more fertile  
163 immediately after fire with a gradual decrease in soil fertility over time and that the community  
164 turnover would partially be explained by these changes in edaphic conditions (Hypothesis 2). As  
165 a consequence, we expected ruderal, generalist saprotrophic fungi, mainly utilizing simple and  
166 easily absorbable forms of nutrients, to be more abundant and species-rich shortly after fire than  
167 in unburned forests (Hypothesis 3). By contrast, root-associated symbiotic fungi were expected  
168 to be more diverse in older burned forests and unburned forests (Hypothesis 4).

## 169 **2. Materials and Methods**

### 170 **2.1. The study area description**

171 The study was conducted in Wondo Genet natural forest area, which is located in southern  
172 Ethiopia, approximately 265 km from Addis Ababa (Figure 1) (between 7°06' N–7°07' N and  
173 38°37' E–38°42' E) at 1,600 to 2,580 m above sea level (Belaynesh, 2002; Fenta, 2014). Wondo  
174 Genet is characterized by remnant dry Afromontane forest patches (Ango and Bewket, 2007;  
175 Belaynesh, 2002; Fenta, 2014). The climate is characterized by the Weyna-Dega agro-climatic  
176 zone, with a bimodal rainfall pattern: the main rainy season is in the summer and a lesser rainy  
177 season is in spring. The mean annual rainfall and mean annual temperature of the study area are  
178 1210 mm and 20°C, respectively (Belay, 2016; Fenta, 2014). The soil is an Andisol with a sandy  
179 loam texture (Eriksson and Stern, 1987) and an average pH value of 5.7 (Eshetu and Högberg,  
180 2000). The study area covers about 797 ha of natural forests lands (Ango and Bewket, 2007;  
181 Belaynesh, 2002; Fenta, 2014) that are characterized by remnant Dry Afromontane forest  
182 patches, home to important fauna and flora. The dominant tree species in the study forest are  
183 *Juniperus procera*, *Albizia gummifera*, *Afrocarpus falcatus*, *Bersama abyssinica*, *Prunus*  
184 *africana*, *Podocarpus falcatus*, *Cordia africana*, *Croton macrostachys* and *Olea africana* (Ango  
185 and Bewket, 2007; Zerga and Berta, 2016). These trees are reported to be associated with  
186 arbuscular mycorrhizal fungi (Wubet et al., 2003). Human-induced fire is a recurrent  
187 phenomenon, occurring yearly in small patches of this natural forest (Bekele et al., 2013; Dejene  
188 et al., 2017b).



189

190 **Figure 1:** Location of the study area, Wondo Genet, Ethiopia.

191 Sample plots were established in the forest in 2015 in stands with similar environmental  
 192 conditions such as climate, altitude and soil. Information about the forest fire history of these  
 193 stands was obtained from the Department of Forest Management at the Wondo Genet College of  
 194 Forestry. The control stand of unburned natural forest (UB) was representative of the original  
 195 natural forest and had not been affected by fire for at least 40 years. Burned stands selected for  
 196 the study were similar in terms of fire severity, i.e., the canopy and understory had burned and  
 197 the soil organic layer had been consumed (Rincón and Pueyo, 2010). In these burned areas, two  
 198 forest stands were selected based on their fire history: (1) one-year-old burned forest (B1); and  
 199 (2) ten-year-old burned forest (B10). Within each of these forest stands, three transects were  
 200 established about 250 m apart from each other. Each transect covered an area of 100 m<sup>2</sup>, with a  
 201 rectangular shape (2 m x 50 m). Because the plots were established in a single stand for each  
 202 treatment, this work could be considered as a case study and conclusions regarding other stands  
 203 need to be taken with caution.

204 **2.2. Soil sampling for molecular work**

205 A total of nine (2 m × 50 m) transects, three per each studied stands (UB, B1 and B10),  
206 were established perpendicular to the slope following Luoma et al. (1991) and Smith et al.  
207 (2002). Five soil cores were extracted 5 m apart along the centreline of each transect using a  
208 cylindrical (2 cm radius, 20 cm long, 250 cm<sup>3</sup>) soil borer (De la Varga et al., 2012; Taylor, 2002)  
209 to sample spatial variability and to minimize the probability of sampling the same genet  
210 repeatedly. From these cores, well-decomposed organic layers and mineral soils were sampled.  
211 The litter layer (intact and partially decomposed leaves) was discarded because the fungal  
212 community composition in litter tends to diverge from that in soil (Voříšková et al., 2014). The  
213 five cores from each transect were pooled to obtain a composite soil sample for each transect for  
214 the final DNA extraction. Soil cores were dried, sieved through a 1 mm<sup>2</sup> mesh and ground to a  
215 fine powder using a mortar and pestle. A subsample of each pooled sample was stored at –20°C  
216 for molecular analysis and the rest of the sample was used for determining selected physical and  
217 chemical properties of the soil (Table 1).

### 218 **2.3. Molecular analysis**

219 DNA was extracted from 0.25 g of soil per sample using a PowerSoil™ DNA Isolation Kit  
220 (MoBio Laboratories Inc., Carlsbad, CA, USA). PCR reactions were performed in triplicate for  
221 each sample to minimize PCR biases. PCR reactions were performed in 20 µl reaction volumes  
222 containing 11.22 µl of MQ water, 1.60 µl of DNA template, 2.00 µl of 10× buffer, 1.40 µl of  
223 MgCl<sub>2</sub> (50 mM), 1.60 µl of dNTPs (10 mM), 0.50 µl of BSA (2%), 0.80 µl of reverse and  
224 forward primers (10 µM) and 0.08 µl of Platinum Taq DNA polymerase (Invitrogen, Carlsbad,  
225 CA, USA). We used the following PCR conditions: an initial denaturation step at 94°C for 3  
226 min; then 35 cycles of 94°C for 45 s, 50°C for 1 min and 72°C for 1.5 min; and a final cycle of  
227 72°C for 10 min. The ITS2 rDNA region was amplified using the forward primer fITS7 (Ihrmark  
228 et al., 2012) and the barcoded reverse primer ITS4 (White et al., 1990). The ITS4 primer was  
229 labelled with sample-specific Multiplex Identification DNA-tags. A negative control consisting  
230 of MQ water instead of DNA was included in each PCR run. The absence of bands on gels  
231 indicated that negative controls were amplicon free. Ion Torrent sequencing was carried out at  
232 the Naturalis Biodiversity Center. We used the Ion 318™ Chip to allow for the highest possible  
233 sequencing coverage.



#### 234 **2.4. Soil sampling and edaphic variables analysis**

235 To relate soil fungal composition to explanatory edaphic variables, additional soil samples  
236 were collected from each transects. Composite soil samples, from the centre and from the four  
237 corners of each plot, were extracted after clearing plant matter and debris from the soil surface.  
238 Soil was extracted to a depth of 20 cm with the aid of an auger and spade. After mixing the  
239 samples thoroughly, approximately 500 g of soil was placed in a plastic bag for transport back to  
240 the laboratory for analysis. After air drying the soil in shade, the chemical and physical  
241 properties of the soil were determined using DTPA extraction,  $\text{KH}_2\text{PO}_4$  extraction, Olsen,  
242 Kjeldahl digestion, Walkley–Black, ammonium acetate and instrumental methods. The analysis  
243 was conducted by Water Works Design and Supervision Enterprise, a laboratory test service for  
244 soil fertility at Addis Ababa, Ethiopia. A soil: water (1:2.5) suspension and the supernatant of the  
245 same suspension were measured using a pH meter and an electrical conductivity meter,  
246 respectively, to determine the soil pH (Reeuwijk, 2002). Organic carbon (C) content was  
247 determined using wet digestion (Walkley and Black, 1934). Total nitrogen (N) content was  
248 determined using the Kjeldahl digestion procedure (Kim, 1996). Available phosphorus (P) was  
249 determined using sodium bicarbonate (0.5M  $\text{NaHCO}_3$ ) as an extraction solution (Olsen and  
250 Sommer, 1982). Available sodium (Na), potassium (K), calcium (Ca) and magnesium (Mg) were  
251 extracted using ammonium acetate. Soil particle size was analysed with a hydrometer  
252 (Bouyoucos, 1951), using sodium hexametaphosphate (Calgon solution) as the dispersing agent.  
253 Once the proportion of sand, silt and clay separates were calculated, the soil was assigned  
254 textural class name using ASTM software. We also used the following formula to convert  
255 organic carbon to organic matter. Organic matter (%) = Total organic carbon (%) x 1.72. The  
256 selected soil properties of the studied plots are provided in Table 1.

#### 257 **2.5. Bioinformatic analysis**

258 Raw sequence reads were obtained from the Ion Torrent output that comprised  
259 demultiplexed sample reads. Primers and poor-quality ends were trimmed off based on a 0.02  
260 error probability limit in Geneious Pro 8.1.8 (BioMatters, Auckland, New Zealand).  
261 Subsequently, sequences were filtered using USEARCH based on the following settings: all  
262 sequences were truncated to 200 bp and sequences with an expected error of  $>1$  were discarded.

263 The remaining sequences were collapsed with USEARCH v.8.0 (Edgar, 2010) into unique  
264 sequence types on a per-sample basis while preserving read counts. Singleton sequence types  
265 were discarded; the resulting 305,520 high-quality sequences were grouped into 3,286  
266 operational taxonomic units (OTUs) with USEARCH at 97% sequence similarity while  
267 simultaneously excluding 181 chimeric sequences. We assigned sequences to taxonomic groups  
268 based on pairwise similarity searches against the curated UNITE+INSD fungal ITS sequence  
269 database containing identified fungal sequences with assignments to species hypothesis groups  
270 (Kõljalg et al., 2013). After excluding OTUs with <70% similarity or with <150 bp pairwise  
271 alignment length to a fungal sequence, the dataset contained 2,898 fungal OTUs, representing a  
272 total of 296,384 high-quality sequences. Functional classification of OTUs at the genus level was  
273 performed using the FUNGuild database.

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276 **Table 1:** Selected soil physic chemical properties of study plots in the dry Afromontane forest of the Wondo Genet area (Ethiopia).

Soil properties	Stand age after burn <sup>a</sup>		
	Unburned	One year	Ten years
Clay	1.60 (0.01)	1.72 (0.02)	1.27 (0.03)
Silt	1.30 (0.1)	1.40 (0.10)	1.38 (0.12)
Sand	1.76 (0.08)	1.38 (0.12)	1.79 (0.04)
pH-H <sub>2</sub> O (1:2.5)	6.51 (0.30)	6.46 (0.1)	6.81(0.30)
Na (meq 100 g <sup>-1</sup> of soil)	0.83 (0.07)	1.00 (0.4)	0.99 (0.10)
K (meq 100 g <sup>-1</sup> of soil)	0.55 (0.12)	0.62 (0.35)	0.80 (0.08)
Ca (meq 100 g <sup>-1</sup> of soil)	28.43 (13.67)	20.85 (5.18)	24.15 (4.98)
Mg (meq 100 g <sup>-1</sup> of soil)	9.77 (5.18)	7.42 (1.42)	8.05 (1.50)
CEC (meq 100 g <sup>-1</sup> of soil)	52.44 (14.91)	43.97(10.9)	42.66 (5.10)
OM	6.05 (1.77)	2.93 (0.36)	5.08 (1.88)
Nitrogen (%)	0.67 (0.17)	0.40 (0.06)	0.54 (0.11)
C/N	5.25 (0.20)	4.25(0.03)	5.46 (3.89)
P (mg P <sub>2</sub> O <sub>5</sub> /kg soil)	43.33 (12.72)	28.89 (4.36)	32.59 (5.18)

277 <sup>a</sup> Numbers in parentheses are the standard deviation of the mean.

278 Abbreviations: CEC, cation exchange capacity; OM, organic matter.

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## 283 2.6. Statistical analysis

284 We normalized the OTU table for subsequent statistical analyses by rarefying the number  
285 of high-quality fungal sequences to the smallest library size (8,361 reads). Shannon's  $H'$   
286 diversity index,  $H = -\sum p_i (\ln p_i)$  (Shannon and Weaver, 1949), was estimated for each stand,  
287 where  $p_i$  indicates the relative abundance of fungal OTUs (Kent and Coker, 1993). The  
288 Simpson's diversity,  $D = 1 / \sum (p_i^2)$ , where  $p_i$  is the importance probability in element  $i$ ; and the  
289 Evenness,  $J = H' / H'_{\max}$ , where  $H'$  is the number derived from the Shannon diversity index and  
290 the  $H'_{\max}$  is the maximum possible value of  $H'$  were also calculated (Magurran, 1988). In  
291 addition, the richness values of all fungal OTUs ( $S$ ) based on stand type were estimated. All  
292 diversity measures were calculated using the BiodiversityR GUI package in R version 3.5.3 (R  
293 Core Team, 2019). Diversity indices and richness were compared across stands using one-way  
294 ANOVA using R (R Core Team, 2019). Data were scaled using R when needed to normalize for  
295 ANOVA. Tukey HSD was used to determine significant differences between means ( $P \leq 0.05$ )  
296 among stands.

297 To compare the entire fungal OTU community composition across the studied stands, we  
298 used PC-ORD v. 6.0 (McCune and Grace, 2002) to run detrended correspondence analyses  
299 (DCAs) on the presence-absence data matrix. Data were also analysed using PERMANOVA  
300 (Adonis) in R to determine the effect of forest types and edaphic variables on the fungal  
301 community (Anderson, 2001). We performed an analysis of Similarity Percentages (SIMPER) to  
302 identify fungal species that are most responsible for the observed patterns (Clarke, 1993) and to  
303 determine the percentage contribution of fungal taxa to significant dissimilarities among the  
304 three stands (Parravicini et al., 2010). Canonical correspondence analysis (CCA) was also used  
305 to relate the selected edaphic variables (Table 1) with the mycorrhizal fungal community  
306 composition using CANOCO version 5.0 (Smilauer and Lepš, 2014). The statistical significance  
307 of the environmental variables was tested using a Monte Carlo permutation test (999  
308 permutations).

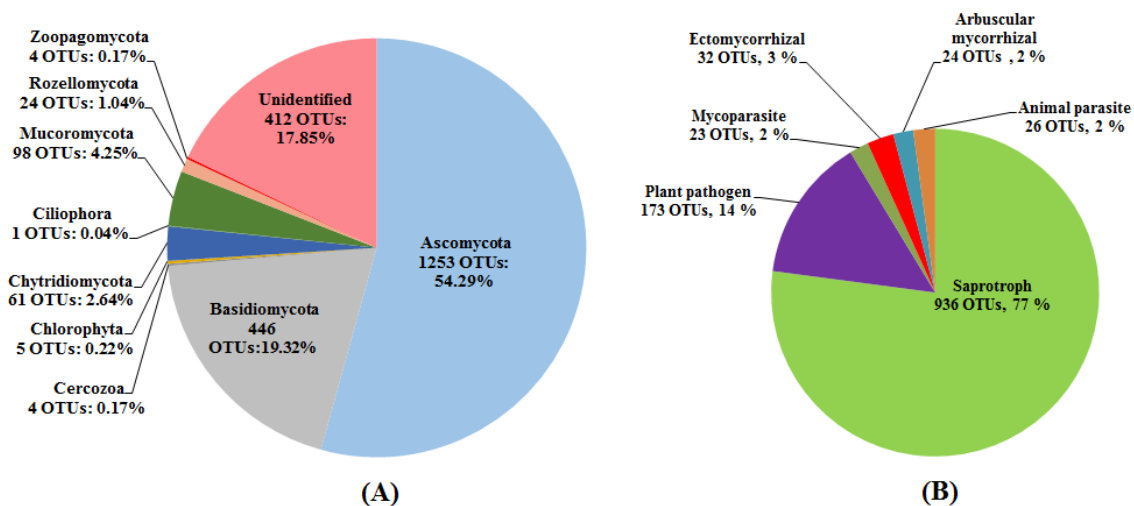
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311 **3. Results**

312 **3.1. Sequencing outputs and fungal diversity**

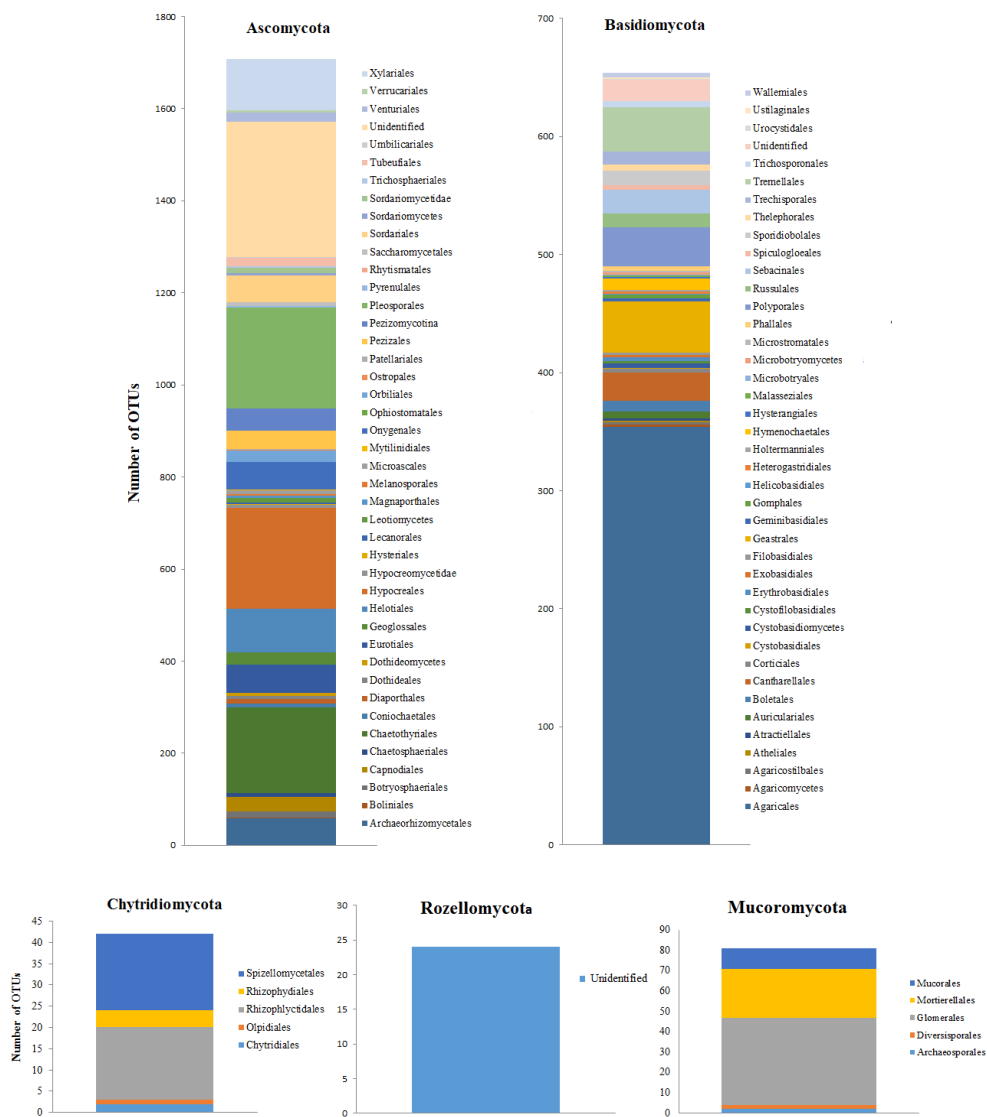
313 A total of 2,898 fungal OTUs of a total of 296,384 sequences were found across all  
314 samples before rarefaction. The taxonomic classification revealed that Ascomycota was the most  
315 diverse fungal phylum, with 1708 OTUs; 52% of the total (Figure 2A). The ranking of  
316 taxonomic orders in Ascomycota, based on the number of representative OTUs, was as follows  
317 Chaetothyriales (128), Pleosporales (106) and Hypocreales (77), followed by many other orders  
318 with less than 50 fungal OTUs (Figure 3A). In Basidiomycota, Agaricales was the most species-  
319 rich order followed by other orders with less than 50 OTUs each. Unidentified fungi were  
320 classified down to kingdom level and represented about 645 OTUs; 20% of the total (Figure 2A).  
321 The number and proportional distribution of fungal OTUs describing all known taxonomic phyla  
322 and orders are shown in Figure 3.



323

324 **Figure 2:** Relative proportions of fungal operational taxonomic units (OTUs): (A) taxonomical  
325 classification at the phylum level (name of phylum; the number of OTUs; percentage); (B)  
326 classification of fungal ecological function at the genus level (ecological function; the number of  
327 OTU; percentage) based on a FUNGuild ([www.funguild.org](http://www.funguild.org)) search.

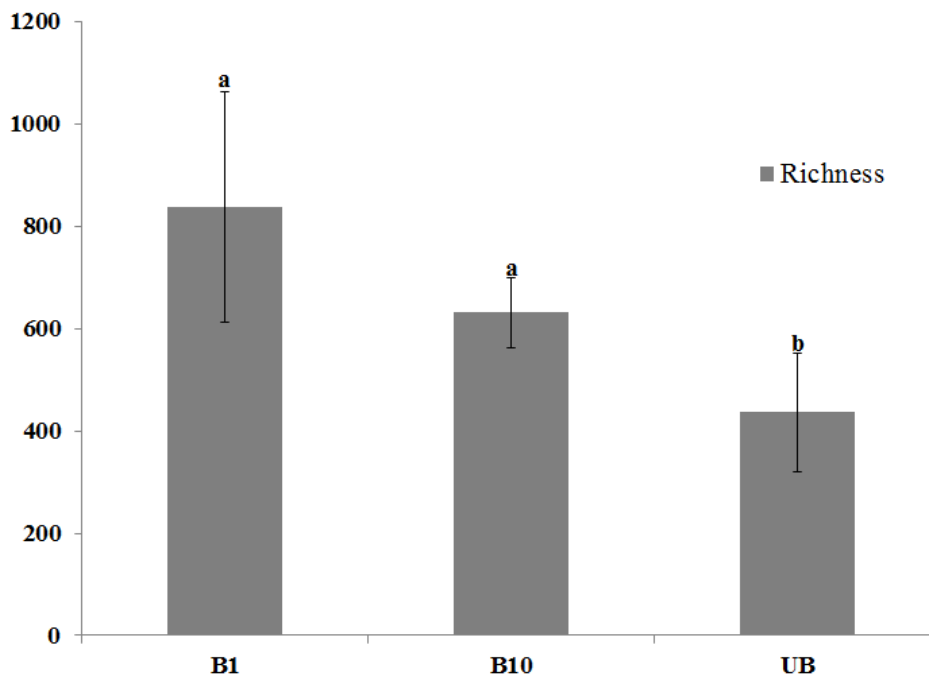
328 About 42% (1217 OTUs) of the fungal OTUs were classified to the genus level. These  
 329 genera were assigned to ecological functional groups, i.e. symbionts (ectomycorrhizal and  
 330 arbuscular mycorrhizal fungi) and non-mutualistic fungi, including saprotrophs, parasites and  
 331 pathogens. The proportion of fungal OTUs at the genus level sharing the same ecological  
 332 function is presented in Figure 2B.



333  
 334 **Figure 3:** Numbers and proportional distribution of fungal operational taxonomic units (OTUs)  
 335 representing all taxonomic phyla and orders found in soil samples collected from the dry  
 336 Afromontane forest of Wondo Genet, Ethiopia.

337 **3.2. Fungal richness and diversity changes after fire**

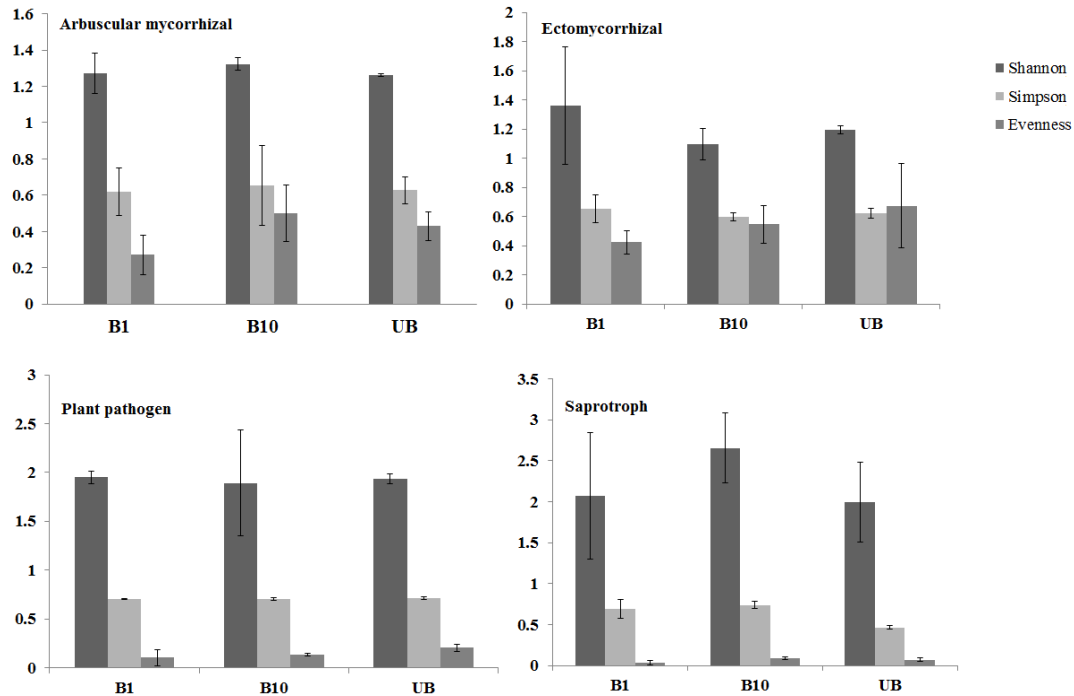
338 The observed total fungal OTU richness was significantly affected by fire ( $P = 0.002$ ;  $F =$   
339 12.48; Figure 4) and was higher in one and ten years after fire, compared with the unburned site.  
340 The highest richness value was observed in one-year-old burned stands, whereas the lowest was  
341 observed in unburned stands, which had an average richness value that was 50% and 18% lower  
342 than that of one- and ten-year old burned stands, respectively.



343 **Figure 4:** Mean total fungal community richness values in a dry Afromontane forest of Wondo  
344 Genet, Ethiopia, following fire. Abbreviations: UB, unburned stand; B1, one-year-old burned  
345 stand; B10, ten-year-old burned stand. Bars denote standard deviation. Different letters above the  
346 bars indicate a significant difference in richness between stand types ( $P < 0.01$ ,  $n = 3$  transects per  
347 stand).  
348

349 Diversity indices for each ecological guild did not differ significantly among treatments  
350 ( $P > 0.05$ ; Figure 5). The observed evenness values also did not differ significantly among  
351 treatments ( $F = 0.18$ ,  $P = 0.72$ ; Figure 5). However, the trend observed for evenness values

352 indicated that ecological guilds are distributed more uniformly in B10 and UB stands than in B1  
 353 stands (Figure 5).

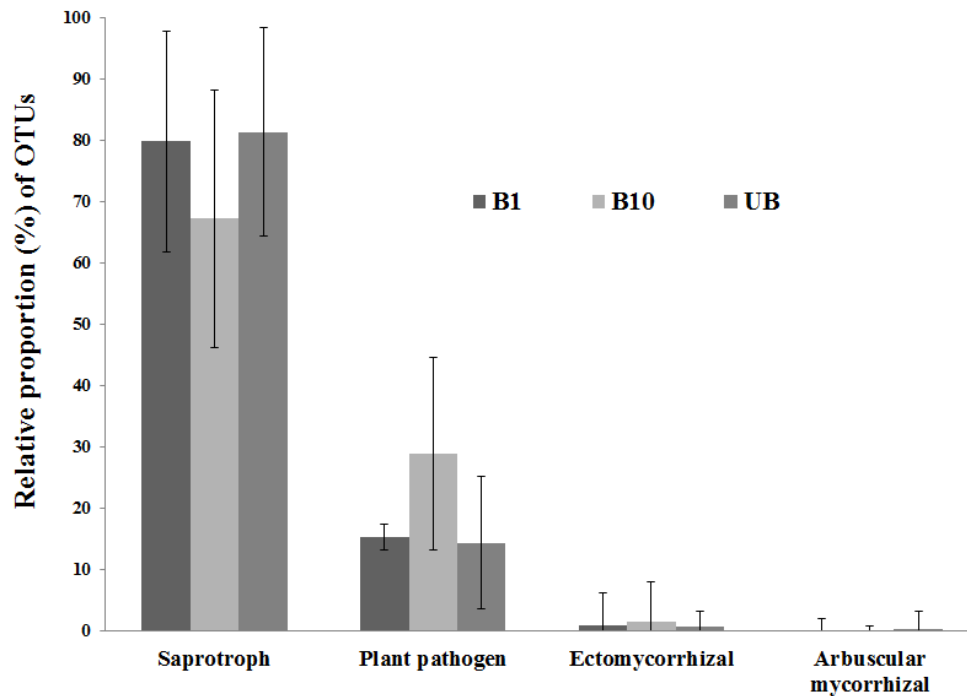


354

355 **Figure 5:** Mean soil fungal community diversity and evenness estimated for functional guilds  
 356 detected in three types of forest stand with different fire histories. Abbreviations: UB, unburned  
 357 stand; B1, one-year-old burned stand; B10, ten-year-old burned stand; Key: Shannon = Shannon  
 358 diversity values; Simpson = Simpson diversity values; Evenness = evenness values. Bars denote  
 359 standard deviation, n = 3 transects per stand.

360 When the relative proportions of each of the ecological guilds in each of the different types  
 361 of forest stand were considered separately, only the proportions of plant pathogens differed  
 362 significantly in the different types of forest stand. The relative proportion of plant pathogens in  
 363 B10 soils was significantly greater than in UB or B1 soils ( $P = 0.001$ ;  $F = 5.04$ ;  $n = 3$  transects).  
 364 The relative proportions of the other ecological guilds were not significantly different ( $P > 0.05$ )  
 365 in burned and unburned forest stands (Figure 6).





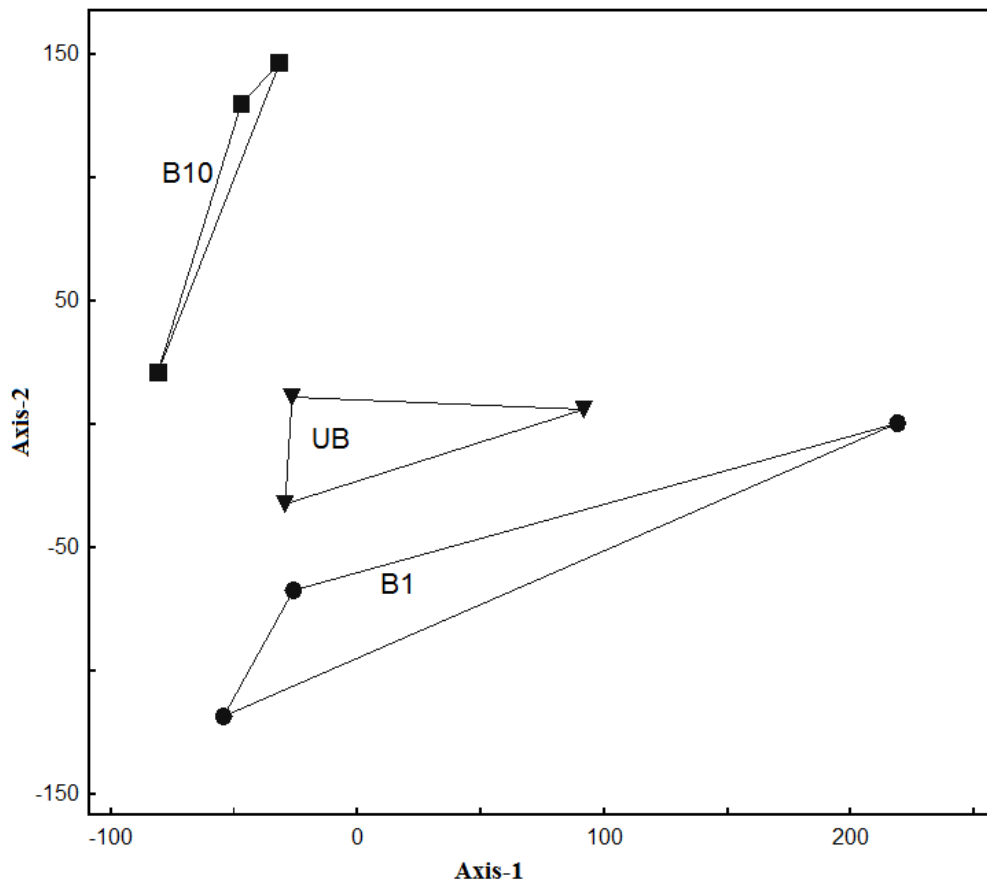
366

367 **Figure 6:** Relative proportions of fungal operational taxonomic units (OTUs) in different  
 368 ecological guilds in unburned stands (UB), one-year-old burned stands (B1) and ten-year-old  
 369 burned stands (B10). Bars denote standard deviation.

### 370 3.3. Soil fungal communities and environmental variables

371 The DCA showed that the variation in fungal community composition can be partially  
 372 explained by the successional stage following fire (Figure 7), indicating distinct fungal  
 373 compositions in each treatment. The PERMANOVA analyses confirmed that the composition of  
 374 the fungal OTUs in the three stand types were significantly different ( $F = 1.54$ ,  $P = 0.02$ ),  
 375 indicating that the fungal communities are differently associated with the three forest stands due  
 376 primarily to soil fertility. With respect to the edaphic variables, Nitrogen (N) ( $R^2 = 0.5685$ ), C/N  
 377 ratio ( $R^2 = 0.6355$ ), and Phosphorus (P) ( $R^2 = 0.6387$ ) correlated most strongly with fungal  
 378 community composition ( $P < 0.05$ ). The SIMPER analysis identified fungal species that typified  
 379 and distinguished between the three treatments (Table S1). The overall between-group  
 380 dissimilarity was 79.59% for UB and B1 treatments, 74.47% for UB and B10 treatments and

381 72.91% for B1 and B10 treatments. *Agaricus campestris* and *Gymnopilus ochraceus* were the  
382 most influential species and, along with other species such as *Hypocreales* sp. and *Onygenales*  
383 sp., made the greatest cumulative contribution towards differences between the three stands,  
384 often accounting for more than 25% of the observed value of dissimilarity (Table S1).  
385 Furthermore, the one-year-old burned treatment (B1) dataset contained a relatively higher  
386 number of OTUs as compared to the ten-year old burned (B10) and unburned (UB) treatments.



387

388 **Figure 7:** Detrended Correspondence Analysis ordination plot for soil fungal communities  
389 detected in the three treatment groups: B1, plots in one-year-old burned stands; B10, ten-year-old  
390 burned stands; UB, unburned stands. Plots with the same symbol are in the same treatment  
391 group.

392 CCA of ectomycorrhizal fungal OTUs based on simple term effects revealed that edaphic  
393 variables such as the Mg, K and OM were significantly correlated with ectomycorrhizal fungal

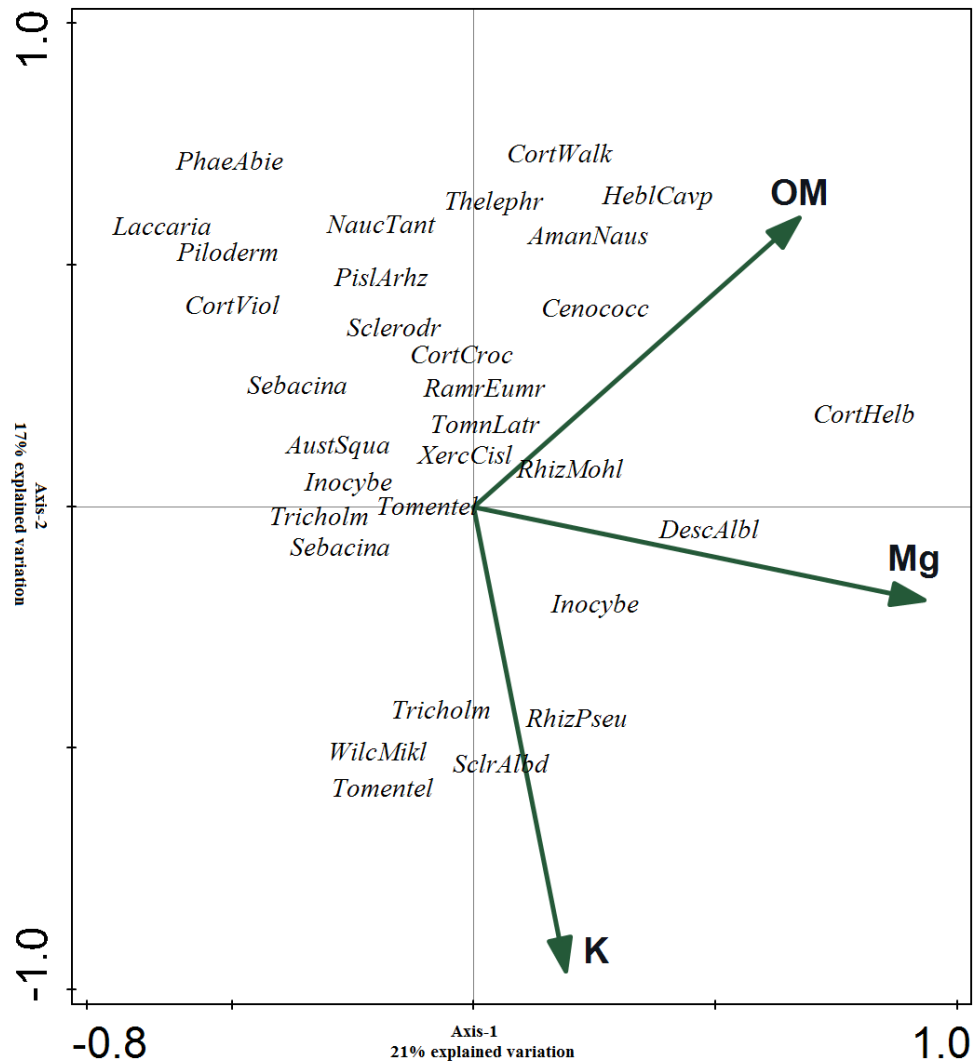
394 community composition in our forest study area (Table 2; Figure 8). The cumulative contribution  
 395 of the explained variation data for the interaction between ectomycorrhizal soil fungal  
 396 composition and soil variables are shown in Figure 8.

397 The response of the ectomycorrhizal species was unique to the different types of stands. In  
 398 contrast to our expectation, number ectomycorrhizal taxa were associated with recently burned  
 399 stands, which had lower soil fertility levels compared with unburned and 10-year-old burned  
 400 stands (Table 1). Species such as *Tricholoma* sp. and *Inocybe* sp. were among the observed taxa  
 401 associated to these 1-year-old burned stands. In older stands i.e. the 10-year-old burned and  
 402 unburned stands, where the soil fertility was high, species such as *Amanita* sp. and *Laccaria* sp.  
 403 were found together with other species. Other species such as *Entoloma* sp. and *Cortinarius* sp.  
 404 were associated with all stand age groups in the forest study area.

405 **Table 2:** Canonical correspondence analysis showing the significance ( $P < 0.05$ ) of edaphic  
 406 variables based on simple term effects on the mycorrhizal fungal species.

Variable	Simple term effects		
	Explains %	pseudo-F	P
Mg	22.6	1.71	0.032
K	19.7	1.63	0.036
OM	17.0	1.40	0.047

407



408

409 **Figure 8:** Canonical Correspondence Analysis (CCA) of the species level community  
 410 composition of ectomycorrhizal fungi in a dry Afromontane forest in Ethiopia. Abbreviations:  
 411 OM, organic matter; Mg, magnesium; K, potassium. Species names are abbreviated (the full  
 412 names of the ectomycorrhizal species used in the ordination are provided in supplementary Table  
 413 S2).

414

415

## 416 4. Discussion

### 417 4.1. Fungal OTU diversity

418 Afromontane forests in Ethiopia are considered to be a major reservoir of biodiversity  
419 (Lemenih and Bekele, 2008), and may support a high level of microorganism diversity, including  
420 fungi. Our study revealed that the dry Afromontane forest in our study area harbours many more  
421 fungal species than previously reported in studies based on sporocarps sampling at a given period  
422 of time (Dejene et al., 2017b). However, the taxonomic classification was challenging owing to  
423 the lack of matches in the database. Thus, only about 48% and 24.13% of the OTUs detected  
424 could be identified to the genus and species level, respectively, indicating that the majority of  
425 fungal in this region had not been sequenced before this study and that many possibly are  
426 undescribed species. This may also be due to the uniqueness of the dry Afromontane forests in  
427 terms of the diversity of soil fungi as well as the lack of scientific studies that have investigated  
428 the local mycota (Dejene et al., 2017a). Furthermore, about 20% of sequences were not identified  
429 even at the phylum level, highlighting the current lack of data from understudied tropical and  
430 subtropical forest ecosystems (Tedersoo et al., 2014), such as the forest systems in Ethiopia. In  
431 light of this, the present study provides important information that contributes to the Ethiopian  
432 fungal biodiversity knowledge base.

433 The largest group of fungi found in this study belonged to the Ascomycota; previous  
434 studies of different forest ecosystems have also reported a dominance of Ascomycota taxa in the  
435 soil (Dhruba et al., 2015; Geml et al., 2014; Reazin et al., 2016; Smith et al., 2017). The  
436 Ascomycota is the most species-rich phylum of fungi (Araujo and Hughes, 2016), which may  
437 explain its dominance. The Hypocreales, Pleosporales, and Chaetothyriales were the three largest  
438 orders of Ascomycota found in this study. The order Hypocreales is a group whose taxonomy  
439 has been relatively well studied and identification aids are widely available (Chaverri and  
440 Samuels, 2003; Schroers, 2001). The fungi in this order can also be saprotrophic,  
441 entomopathogenic, and mycoparasitic (Rossman et al., 1999). In addition to their ecological and  
442 economic importance, the Hypocreales are also considered to be the most important regulators of  
443 insect and fungal populations and, therefore, are used in agriculture as biocontrol agents  
444 (Carruthers and Hural, 1990; Esser and El-Gholl, 1993; Rossman et al., 1999; Samuels, 1996).  
445 The second largest order of Ascomycota detected in this study was the Pleosporales. This order

446 comprises saprotrophs or fungi that are parasites of vascular plants (Kruys et al., 2006). Some  
447 species from this order are also found on animal dung (Kruys et al., 2006), a small number occur  
448 as lichens (Semenova-Nelsen et al., 2019) and as rock-inhabiting fungi (Ruibal et al., 2009). The  
449 epiphytic or endophytic fungi of the Pleosporales are mainly saprotrophic but also play a key  
450 role in causing plant diseases such as stem canker (Zhang et al., 2009). A considerable number of  
451 fungi belonging to the order Chaetothyriales were also detected in this study. This order includes  
452 fungi that are known to be epiphytes, colonizing the leaves and the bark of trees in tropical forest  
453 ecosystems (Arnold et al., 2000; Batista and Ciferri, 1962).

454 The order Agaricales was the largest order of Basidiomycota detected in this study:  
455 members of this order produce the familiar gilled mushroom (Binder et al., 2005; Hibbett and  
456 Thorn, 2001; Stajich, 2015). Agaricales are widespread in diverse ecosystems (Kirk et al., 2008)  
457 and many form ectomycorrhizae by engaging in mutualistic symbioses with vascular plants  
458 (Alexopoulos et al., 1996). Some Agaricales are known to be termite symbionts, some are  
459 valuable as a source of food for animals, including humans (Kirk et al., 2008), whereas others  
460 have hallucinogenic properties or produce toxins lethal to humans (Nichols, 2003). Most of the  
461 species of Agaricales detected in this study are well known soil saprotrophs, such as those  
462 belonging to the genera *Agaricus*, *Calvatia*, *Coprinellus*, *Gymnopilus*, *Leucoagaricus*,  
463 *Lycoperdon*, *Marasmius*, *Psathyrella* and *Psilocybe*, have been reported previously as fruit  
464 bodies from our study area (Dejene et al., 2017b) providing validation of our molecular  
465 techniques.

#### 466 **4.2. Fungal richness and diversity changes after fire**

467 Our results from the post-fire successional chronosequence showed that soil fungal richness  
468 was related to fire. In this study, we found higher total richness and diversity values in the forest  
469 stands recently affected by fire than unburned stands. This may be attributed to the new  
470 ecological conditions created owing to differences in fire severity, which may incite or support  
471 spore germination of several fungal species in the soil (Heino, 2012) following the fire in the  
472 investigated forests. In addition, the mycelium of fungal species in the rhizosphere may persist  
473 (Cowan et al., 2016; Shen et al., 2016) or the fungal community may be resilient to the effects of  
474 fire to some extent (Cowan et al., 2016; Jennings et al., 2012). Furthermore, the intensity of the

475 fire might not have been high enough to affect the below-ground fungal communities given that  
476 low-intensity fires may have little effect on below-ground fungal communities (Bárcenas-  
477 Moreno et al., 2009; Egidi et al., 2016). Thus, the responses of soil fungi to reoccurring low-  
478 intensity fire also appear to be minimal (Johnson et al., 2013; Oliver et al., 2015) and ephemeral  
479 (Hart et al., 2005). Contrary to our expectations, we found that the amount of time since the fire  
480 did not seem to affect fungal guild diversity. These results agree with the findings of a meta-  
481 analysis of fire effects on soil fungi (Egidi et al., 2016), which highlighted the absence of a  
482 significant change in fungal diversity following fire. This might be because little heat is  
483 transferred to the soil because fuel loads are low (Lunt and Morgan, 2002) or might indicate that  
484 the fungal guild communities in burned and unburned forest stands shared similar gene profiles,  
485 which may promote functional similarities among fungal communities with differing  
486 compositions (Mundra, 2015). On the other hand, the absence of significant difference in fungal  
487 richness and diversity in fire affected areas might be due to the fact that recurrent fires consume  
488 less fuel and produce less heat, which does not penetrate into soil as deeply as during high-  
489 intensity fires (Reazin et al., 2016; Semenova-Nelsen et al., 2019). Accordingly, fungal  
490 community shifts in such recurrent fire ecosystems, like that of the dry Afromontane forest, may  
491 be relatively modest (Choromanska and DeLuca, 2001; Korb et al., 2004) and the change may be  
492 driven by indirect fire-induced changes in soil properties or by the change in the plant  
493 communities (Hart et al., 2005; Oliver et al., 2015; Ponder et al., 2009; Trappe et al., 2009).  
494 Also, fungi in a recurrent forest ecosystem may be adapted to frequent fires. Some fungi  
495 produce heat- and smoke-activated spores (Semenova-Nelsen et al., 2019) and some may benefit  
496 from post fire ash deposits (Dean et al., 2015; Hart et al., 2005) or reduced competition from  
497 other species (Semenova-Nelsen et al., 2019). However, factors other than fire might have a  
498 greater effect on the richness and diversity of soil fungal communities. Therefore, further  
499 research is needed to better understand the dynamics and characteristics of soil fungal  
500 communities.

501 A previous study reported the absence of ectomycorrhizal fungi in the dry Afromontane  
502 forests of Ethiopia (Dejene et al., 2017a). This finding was not exceptional as the majority of  
503 tropical woody tree species are unable to form associations with ectomycorrhizal fungi  
504 (Brundrett, 2009). However, in this study, we observed different groups of mycorrhizal fungi and

505 they were identified and classified as ectomycorrhizal and arbuscular mycorrhizal (Figure 2).  
506 This association may be due to the diverse vegetation (Friis et al., 2010) and, hence, there may  
507 be more trees present that can act as hosts for mycorrhizal fungi, or may be due to the dispersion  
508 of mycorrhizal inocula from nearby plantation forests, which are dominated by *Eucalyptus* and  
509 *Pinus* species (Castaño et al., 2019; Dejene et al., 2017a; Urcelay et al., 2017). Thus, the findings  
510 presented here may have important implications for the indigenous forest system for the  
511 maintenance of functional guild diversity in Ethiopia given that mycorrhizal fungi have  
512 previously only been reported from exotic tree plantations (Dejene et al., 2017a). However, the  
513 importance of ectomycorrhizae and arbuscular mycorrhizae in indigenous forest systems in  
514 Ethiopia needs empirical data to confirm. In addition, the coexistence of these fungi has many  
515 practical advantages, such as the exchange of water and nutrients through mycorrhizal hyphal  
516 networks (Brundrett, 2002, 2004). Thus, our analysis of the fungal communities in these forest  
517 soils presents an insight into the conservation of functional guilds in the forest system in the  
518 study area.

519 The vegetation changes after a fire may affect the soil microbial community (Hart et al.,  
520 2005). Previous studies have reported that the loss of host plants after fire decreases mycorrhizal  
521 fungal diversity (Pattinson et al., 2006; Smith et al., 2005). In our study, both the richness and  
522 diversity of ectomycorrhizal fungi increased in the recently burned stands, which could indicated  
523 an immediate post-fire mycorrhizal colonization in fire-affected forest stands (Dahlberg, 2002;  
524 Rincón et al., 2015), while the saprotrophic fungi mineralize nutrients and stabilize the soil  
525 moisture after the fire (Dighton et al., 1986). The ectomycorrhizal taxa may also have established  
526 dominance immediately after burning owing to their tolerance of fire effects (Dahlberg, 2002;  
527 Kipfer et al., 2010) or they may have survived in a mycelial state during the fire event (Hewitt et  
528 al., 2013). However, the colonization of mycorrhizal fungi could also be governed by burn  
529 severity and by the depth of burning in the soil profile (Hewitt et al., 2013). Thus, the effect of  
530 fire on mycorrhizae could be reduced when the fire only occurs at the soil surface, and the effect  
531 of the fire reduces with soil depth (Danielson, 1984; Pattinson et al., 2006; Visser, 1995). Thus,  
532 the fire that occurred in our forest study area might not have been strong enough to affect the  
533 mycorrhizal fungi or may have only affected fungi on the soil surface. It may also be influenced  
534 by the host plant's response to fire. However, in Ethiopia the mycorrhizal-associations for most



535 plants are not yet well known. Thus, this should be investigated in future studies, including  
536 ectomycorrhizal root-tip samples, to learn more about the diversity of ectomycorrhizal host tree  
537 species and their associated fungi in dry Afromontane forests.

#### 538 **4.3. Soil fungal communities and environmental variables**

539 The DCA indicated that the fungal communities detected in the three stand types were  
540 different. The SIMPER analysis also distinguished the total dissimilarity between stands and the  
541 relative contribution of each fungal species to the observed dissimilarity. The species making the  
542 highest contributions to the dissimilarity between the one- and ten-year-old burned stands  
543 (10.95%) and the one-year-old burned stands and the unburned stands (10.92%) was *Agaricus*  
544 *campestroides*. This species was highly abundant (N ~14916) in one-year-old burned stands but  
545 much less abundant in ten-year-old burned and unburned stands (N= 2 and N= 386,  
546 respectively). The contribution of the species might be partially responsible for the differences  
547 between stands, suggesting that time after fire is also probably responsible for the variation in the  
548 dominance of some species and their exclusive occurrence in certain stands. This is supported by  
549 previous findings that, for a given stand, certain fungal species tend to be abundant and  
550 characterize its composition (Zhu et al., 2010). Thus, a species with a consistently high  
551 contribution to the dissimilarity is a good discriminating species (Clarke, 1993).

552 Soil microorganisms, including fungi, are influenced by edaphic parameters (Drenovsky et  
553 al., 2004; Lauber et al., 2009, 2008). Our edaphic data from the dry Afromontane forest showed  
554 that more fungal species were detected in the burned forest areas (B1 and B10) where the soil  
555 fertility was relatively low than in unburned areas, which could be related to depositions of ash  
556 after the fire (Hul et al., 2015). Ash depositions could create empty niches that provide  
557 opportunities for the area to be rapidly colonized by fungal species at the early stages of  
558 succession (Fritze et al., 1993). However, the dry Afromontane forest area has suffered erosion  
559 caused by heavy rainfall soon after the fire events. As a result, there is a potential for sediment  
560 transportation from fire-affected areas and, thus, changes in soil fertility levels among stands. For  
561 instance, pH was assumed to be increased in newly burned areas, owing to the production of  
562 oxides and hydroxides (Hul et al., 2015). However, in our study forests, we recorded slightly  
563 high soil pH values in unburned forest stands. We found also a significant influence of the N,

564 C/N ration and P on the entire fungal community in this study. For example, N and P reported  
565 could affect the structure of fungi in the soil, particularly of the mycorrhizal fungi (Zhao et al.,  
566 2018). The higher availability of these elements could decrease plant dependency on mycorrhizal  
567 fungi. This condition could also reduce the carbon allocation to fungi (Liu et al., 2019), which  
568 could increase competition and affect community composition (Wang and Wang, 2008; Zhao et  
569 al., 2018). Our result also confirmed that the fungal richness is low in stands where the soil C/N  
570 ratio is higher.

571 Previous studies have reported that after fire, the abundance of ectomycorrhizal fungi is  
572 reduced owing to the loss of host plants (Hart et al., 2005). However, in our study, the total  
573 fungal OTU richness in fire-affected stands, which had poor soil fertility, was high compared  
574 with that of unburned stands (Fig. 4), although such conditions remain to be interpreted.  
575 However, Castaño et al. (2019), reported high levels of ectomycorrhizal fungi in stands with  
576 poor soil quality. The occurrence of mycorrhizal species in poor quality soils suggests that the  
577 nutrient stress created in the fire-affected area could be compensated for by the increased  
578 dependency of trees on fungal symbionts (Read and Perez-Moreno, 2003). In this regard, the  
579 mycorrhizal ruderal guild in the spore bank would play an important role by quickly colonizing  
580 roots of plants, and will likely aid the survival of trees after the fire (Glassman et al., 2016).  
581 Species of *Wilcoxina*, *Tomentella*, *Tricholoma* and *Laccaria* were among the ectomycorrhizal  
582 species represented in the fire-affected stands, where soil fertility was low. Some of these genera  
583 such as *Laccaria* are considered ruderal species (Ishida et al., 2007) and are known to form an  
584 ectomycorrhizal association with several host tree species (Glassman et al., 2016; Hul et al.,  
585 2015).

#### 586 4.4. Conclusions

587 This pioneer study is the first attempt to describe the soil fungal community in a dry  
588 Afromontane forest system of Ethiopia using next-generation sequencing and to investigate the  
589 effect of fire disturbance on these fungal communities. Data obtained in this study will  
590 significantly contribute to the body of knowledge regarding soil fungal communities in Ethiopia;  
591 however, the taxonomy of these fungi remains challenging and about 20% of the fungal species  
592 detected have not been described even at the phylum level. We conclude that, in general, the

593 fungal diversity in Ethiopian forest systems is as yet largely undescribed and likely includes  
594 many taxa unknown to science. Thus, we advise that additional scientific investigations of this  
595 highly diverse but unexplored forest ecosystem are needed to consolidate the Ethiopian fungal  
596 biodiversity database. Also in this study, the soil fungal communities expected to be changed  
597 substantially along a post-fire forest succession and in comparison to those in unburned forest.  
598 However, we found that fire did not have a significant negative effect on fungal richness and  
599 diversity in the burned stands. Our study also highlighted that soil fungal composition differed  
600 across a chronosequence after fire and was correlated with soil fertility conditions and the  
601 changes would be explained partially by the edaphic conditions. Contrary to our expectation,  
602 root-associated symbiotic fungi like that of the mycorrhizal fungi were not lacking in fire-  
603 affected stands. We assume that mycorrhizal fungi present in the spore bank were able to  
604 colonize the roots of plants that survived the fire. In the fire-affected forests, we also found fungi  
605 that are known to form ectomycorrhizal associations with several host tree species. This key  
606 ecological role could provide support for the importance of fungal conservation in the dry  
607 Afromontane forest systems in Ethiopia. Similarly, vital edaphic variables such as OM and K  
608 also appear to be important in shaping the composition of mycorrhizal soil fungi in different  
609 ways. Thus, the effect of forest management practices such as thinning and harvesting on soil  
610 fertility should be taken into consideration owing to the important relationship between these  
611 ecological parameters and the soil fungal composition in the dry Afromontane forests of  
612 Ethiopia.

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1033 **Supplementary material -1**

1034 **Table S1:** Summary of similarity percentage (SIMPER) results showing contrasts between the  
 1035 cumulative total contribution (50% cut-off) and the contribution (%) of the most influential  
 1036 fungal operational taxonomic units (OTUs) to the dissimilarity between the three types of stand  
 1037 in the study area, Wondo Genet, Ethiopia.

Species	Individual contribution to the dissimilarity	Cumulative contribution to the dissimilarity
<b>One- and 10-year-old burned stands</b>		
<i>Agaricus campestroides</i>	10.95	10.95
<i>Gymnopilus ochraceus</i>	6.69	17.65
<i>Hypocreales</i> sp.	4.57	22.22
<i>Onygenales</i> sp.	3.02	28.71
<i>Mortierella</i> sp.	2.67	31.38
<i>Mortierella</i> sp.	2.11	33.49
<i>Postia</i> sp.	1.82	35.31
<i>Penicillium pimateouiense</i>	1.36	36.68
<i>Alternaria</i> sp.	1.34	38.02
<i>Geastrum</i> sp.	1.24	39.26
<i>Onygenaceae</i> sp.	1.04	40.3
<i>Fusarium</i> sp.	0.87	44.02
<i>Dactylonectria macrodidyma</i>	0.85	44.87
<i>Metarhizium marquandii</i>	0.70	46.31
<i>Helotiales</i> sp.	0.68	46.99
<i>Capnodiales</i> sp.	0.68	47.68
<i>Cylindrocarpon</i> sp.	0.68	48.36
<i>Lecanicillium fungicola</i>	0.67	49.04
<i>Chaetothyriales</i> sp.	0.63	49.68
<b>One-year-old burned stands and unburned stands</b>		
<i>Agaricus campestroides</i>	10.92	10.92
<i>Gymnopilus ochraceus</i>	9.52	20.45
<i>Hypocreales</i> sp.	4.83	25.29
<i>Onygenales</i> sp.	3.03	28.31
<i>Mortierella</i> sp.	2.84	34.17
<i>Mortierella</i> sp.	2.04	36.21
<i>Alternaria</i> sp.	1.87	38.08

Species	Individual contribution to the dissimilarity	Cumulative contribution to the dissimilarity
<i>Penicillium pimateouiense</i>	1.57	39.65
<i>Postia</i> sp.	1.48	41.13
<i>Geastrum</i> sp.	1.25	43.70
<i>Vanrija humicola</i>	1.19	44.89
<i>Dactylonectria macrodidyma</i>	1.05	47.01
<i>Fusarium</i> sp.	0.97	49.01
Ascomycota sp.	0.77	50.58
<b>10-year-old burned stands and unburned stands</b>		
<i>Gymnopilus ochraceus</i>	13.23	13.23
<i>Dothideomycetes</i> sp.	2.73	15.97
<i>Vanrija humicola</i>	2.43	18.4
<i>Postia</i> sp.	2.19	23.00
<i>Alternaria</i> sp.	1.98	24.98
<i>Hypocreales</i> sp.	1.82	26.80
<i>Onygenaceae</i> sp.	1.50	28.30
<i>Psathyrellaceae</i> sp.	1.29	29.59
<i>Agaricales</i> sp.	1.19	30.79
<i>Onygenales</i> sp.	1.15	33.11
<i>Dactylonectria macrodidyma</i>	1.13	34.25
<i>Pseudaleuria</i> sp.	1.12	35.38
<i>Chaetothyriales</i> sp.	1.09	36.48
<i>Fusarium buharicum</i>	1.09	37.57
<i>Coprinopsis igarashii</i>	0.91	39.44
<i>Archaeorhizomyces</i> sp.	0.84	40.29
<i>Dothideomycetes</i> sp.	0.80	41.09
<i>Penicillium pimateouiense</i>	0.75	41.84
<i>Mortierella</i> sp.	0.69	42.53
<i>Agaricus campestris</i>	0.60	43.14
<i>Xylariales</i> sp.	0.59	43.73
<i>Geoglossum difforme</i>	0.56	44.29
<i>Fusarium</i> sp.	0.56	44.86
<i>Onygenales</i> sp.	0.55	45.41
<i>Fusarium brasiliense</i>	0.53	46.48
<i>Onygenales</i> sp.	0.51	47.00
<i>Herpotrichiellaceae</i> sp.	0.51	47.51
<i>Eurotiales</i> sp.	0.48	47.99
<i>Onygenaceae</i> sp.	0.46	48.45
<i>Dothideomycetes</i> sp.	0.46	48.91
<i>Chaetomium homopilatum</i>	0.45	49.36
<i>Pyronemataceae</i> sp.	0.44	49.81
<i>Eurotiales</i> sp.	0.44	50.25

## Supplementary material -2

**Table S2:** List of the most abundant mycorrhizal species used in the canonical correspondence analysis. Abbreviations: UB, unburned stands; Y1B, one-year-old burned stands; Y10B, ten-year-old burned stands; OTUs, operational taxonomic units.

OTUs	Mycorrhizal species	Species abbreviation	UB-1	UB-2	UB-3	Y1B-1	Y1B-2	Y1B-3	Y10B-1	Y10B-2	Y10B-3
OTU_3067	<i>Cenococcum</i>	<i>Cenococc</i>	6	0	0	0	2	0	0	0	0
OTU_70	<i>Wilcoxina mikolae</i>	<i>WilcMikl</i>	0	15	0	0	196	0	0	0	0
OTU_103	<i>Tomentella</i>	<i>Tomentel</i>	0	0	0	0	2	0	0	0	0
OTU_124	<i>Rhizopogon pseudoroseolus</i>	<i>RhizPseu</i>	0	0	2	0	13	0	0	0	0
OTU_1307	<i>Scleroderma</i>	<i>Sclerodr</i>	0	0	0	2	0	0	0	0	0
OTU_1596	<i>Rhizopogon mohelnensis</i>	<i>RhizMohl</i>	3	0	0	0	3	0	0	0	0
OTU_1690	<i>Phaeoclavulina abietina</i>	<i>PhaeAbie</i>	0	0	0	0	0	13	0	0	0
OTU_2511	<i>Laccaria</i>	<i>Laccaria</i>	0	0	0	0	0	0	0	0	0
OTU_2558	<i>Sebacina</i>	<i>Sebacina</i>	0	0	0	0	0	3	0	0	8
OTU_2591	<i>Austropaxillus squarrosus</i>	<i>AustSqua</i>	0	0	0	2	0	0	0	0	2
OTU_2678	<i>Cortinarius walkeri</i>	<i>CortWalk</i>	2	0	0	0	0	0	0	0	0
OTU_2680	<i>Cortinarius croceocoeruleus</i>	<i>CortCroc</i>	0	0	0	2	0	0	0	0	0
OTU_2967	<i>Tricholoma</i>	<i>Tricoholm</i>	0	0	0	0	0	0	0	0	4
OTU_2985	<i>Naucoria tantilla</i>	<i>NaucTant</i>	0	7	0	0	0	0	0	0	0
OTU_313	<i>Tomentella lateritia</i>	<i>TomnLatr</i>	11	8	0	40	11	4	10	3	186
OTU_3443	<i>Tomentella</i>	<i>Tomentel</i>	0	0	0	4	2	0	2	0	13
OTU_3455	<i>Sebacina</i>	<i>Sebacina</i>	0	0	0	0	0	0	0	0	3
OTU_3502	<i>Xerocomellus cisalpinus</i>	<i>XercCisl</i>	0	0	0	0	0	0	0	0	3
OTU_3945	<i>Piloderma</i>	<i>Piloderm</i>	0	0	0	0	0	3	0	0	0
OTU_4	<i>Scleroderma albidum</i>	<i>SclrAlbd</i>	0	0	0	0	5	0	0	0	0
OTU_40	<i>Descomyces albellus</i>	<i>DescAlbl</i>	0	0	0	0	0	0	0	2	0
OTU_4244	<i>Pisolithus arhizus</i>	<i>PislArhz</i>	0	2	0	0	0	0	0	0	0
OTU_4697	<i>Inocybe</i>	<i>Inocybe</i>	0	0	0	0	0	0	0	0	2
OTU_5053	<i>Laccaria</i>	<i>Laccaria</i>	0	0	0	0	0	2	0	0	0

OTUs	Mycorrhizal species	Species abbreviation	UB-1	UB-2	UB-3	Y1B-1	Y1B-2	Y1B-3	Y10B-1	Y10B-2	Y10B-3
OTU_5162	<i>Amanita nauseosa</i>	<i>AmanNaus</i>	2	0	0	0	0	0	0	0	0
OTU_5181	<i>Hebeloma cavipes</i>	<i>HebelCav</i>	2	0	0	0	0	0	0	0	0
OTU_5185	<i>Thelephora</i>	<i>Thelephr</i>	2	0	0	0	0	0	0	0	0
OTU_566	<i>Cortinarius helobius</i>	<i>CortHelb</i>	0	0	77	0	2	0	11	0	0
OTU_646	<i>Cortinarius violaceipes</i>	<i>CortViol</i>	0	0	0	19	3	44	0	0	7
OTU_654	<i>Ramaria eumorpha</i>	<i>RamrEumr</i>	0	0	0	35	3	0	2	0	0
OTU_842	<i>Inocybe</i>	<i>Inocyne</i>	0	0	0	9	23	0	0	35	0
OTU_936	<i>Tricholoma</i>	<i>Tricholm</i>	0	0	0	0	30	0	0	0	0