

Resistance of the soil fungal communities to medium-intensity fire prevention treatments in a Mediterranean scrubland

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Abstract

Cistus ladanifer scrublands are widely distributed in the Mediterranean basin and represent an early stage of secondary succession following major disturbances (e.g., fire). This vegetation type often establishes on disturbed and poor soils, thereby improving soil stability in stress-prone environments. Fire prevention treatments in these scrublands are often recommended to decrease the risk of wildfires, but the effect of these treatments on associated soil fungi is not known. We studied the effect of distinct fire prevention treatments on soil fungal communities associated with *C. ladanifer* scrublands soils. We used Illumina MiSeq sequencing of the ITS1 region on soil samples taken after distinct fire prevention treatments that were performed in 27 plots belonging to a long-term experiment. Recent fire prevention treatments did not affect overall fungal community composition

nor fungal diversity; however, when analyzing the community according to the functional guilds, the relative abundance of ectomycorrhizal species was significantly lower in burned and 100% cleared plots, compared with control and 50% cleared plots. In contrast, site history affected fungal community composition and richness to a greater extent than the fire prevention treatments. Our results show a higher susceptibility of ectomycorrhizal species to recent high-intensity fire prevention treatments, whereas fire prevention treatments of medium intensity may reduce the risk of wildfire and maintain the soil fungal community.

Keywords: Post-fire succession; Fungi; Biodiversity; Scrubland; Metabarcoding

1 Introduction

Forest management practices (e.g. tree harvesting, prescribed fire) impact above-ground fungal communities, affecting the occurrence, productivity, and production of mushrooms (Savoie and Largeteau, 2011; Tomao et al., 2020). Moreover, forest management activities can also affect below-ground soil fungal composition and biomass (Parladé et al., 2019) and other soil functions such as the release of enzymatic components necessary for the decomposition of the organic matter (Kohout et al., 2018). Below-ground fungal communities are linked to a number of important ecosystem functions such as soil nitrogen and carbon cycling (Clemmensen et al., 2015) and plant production (León-Sánchez et al., 2018). Understanding how management treatments affect the soil fungal communities is important to develop efficient management practices to improve these vegetation types while promoting fruiting body production and preserving the plant-associated microbiome.

Cistus spp. scrublands usually colonize abandoned agricultural lands or recently burned forests and represent an early stage of secondary succession following wildfires. In addition, these scrublands are also frequently affected by wildfires (Martín-Pinto et al., 2006), which can be prevented using fire prevention treatments such as clearing or controlled burning. *Cistus* spp. shows a high plasticity to abiotic stressors and is associated with a surprisingly high diversity of soil fungi (Hernández-Rodríguez et al., 2015; Martín-Pinto et al., 2006), including both ectomycorrhizal and arbuscular mycorrhizal fungi (Comandini et al., 2006). Hence, the use of molecular methods such as high-throughput DNA sequencing can improve our knowledge regarding how these communities are shaped by environmental drivers (Geml et al., 2014; Lindahl et al., 2013). Using these molecular tools, recent studies have shown that high intensity forest management activities, such as clearcutting, shift the soil microbiome and reduce mycorrhizal abundance and diversity (Parladé et al., 2019; Sterkenburg et al., 2019; Kohout et al., 2018). However, limited effects on the soil fungal community have been observed under lower intensity treatments such as thinning (Castaño et al., 2018) or when retention trees are left uncut (Sterkenburg et al., 2018). Several works have focused on the effect of such disturbances in forests systems, but similar studies are lacking for scrublands, despite the importance of this vegetation type to stabilize and protect soils in stress- and fire-prone environments.

Several guilds of fungi coexist and inhabit the forest soils, including ectomycorrhizal, saprotrophs and pathogens (Baldrian, 2016). Ectomycorrhizal species form symbiotic associations with most trees and shrubs under natural conditions, providing their plant hosts with nutrients in return for photosynthetically fixed carbon (Smith and Read, 2008). In contrast, saprotrophs are free-living fungi mainly involved in the first steps of litter decomposition (Lindahl et al., 2007). Both inter- and intraspecific variation in plant hosts determine both mycorrhizal and saprotrophic communities, with potential implications for the carbon and nitrogen cycles (Pérez-Izquierdo et al., 2019). In addition, any disturbance on above-ground host plants may potentially affect

associated symbionts due to a potential reduction in carbon allocation belowground (Högberg et al., 2001). Past disturbance events (e.g. historical legacies) in which communities were impacted may remain even after several years (Alday et al., 2013), thus determining current fungal communities if the community shows a low degree of resilience against disturbances. Changes in litter type and quality can also affect saprotrophs (Štursová et al., 2020), which are usually found in the uppermost soil layers in forest ecosystems (Lindahl et al., 2007). Thus, any fire prevention treatment is expected to impact both ectomycorrhizal and saprotrophic communities. Understanding the disturbance effects on soil fungal communities associated with *C. ladanifer* could provide useful information for managers to decide on the most suitable management treatments for the conservation of these areas.

In this study, we used a long-term experiment where *C. ladanifer* is the dominant scrub species, to test the effect of distinct fire prevention treatments replicated in three distinct sites. Previous studies performed in the same experiment observed negative effects of forest management treatments (e.g. fire or clearing) on the production of fruiting bodies (Hernández-Rodríguez et al., 2015; Martín-Pinto et al., 2006), but similar studies are lacking for the belowground communities. Thus, our aims were i) to study the effects of different fire prevention treatments (namely burned, 100% cleared and 50% cleared) on the soil fungal community composition, on the composition of the fungal guilds and on the total fungal alpha diversity. In addition, since the experiment was replicated in three sites with distinct site histories, we aimed to ii) test whether site differences affect the soil fungal communities more than the fire prevention treatments. We expected that, since ectomycorrhizal species rely on their hosts to obtain carbon, any disturbance occurring on these plant communities, such as fire or clearing treatments, will negatively affect plant symbionts (e.g. ectomycorrhizal species). However, we expect that high-intensity treatments (i.e. burning and 100% clearings) will exert a much stronger influence on these taxa than middle intensity treatments (50% clearings). In addition, we also expect an effect of the fire prevention treatments on saprotrophs, potentially caused either by changes in substrate quality after fire or by organic matter accumulation after clearing.

2 Materials and methods

2.1 Study site

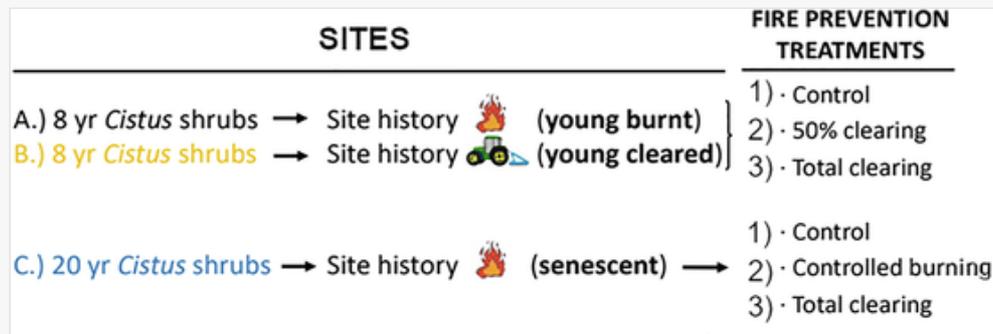
The study area is located in Zamora province in North-western Spain (0730462–0731929 Longitude-UTM, 4619644–4621757 Latitude-UTM 29 T Grid), and is a Mediterranean ecosystem dominated by *C. ladanifer* located 750–780 m above sea level. Paleozoic and Ordovician metamorphic rocks constitute soils in this area and Silurian shales are predominant. Soils are classified as Inceptisol suborder Xerept (Soil Survey Staff, 2010), pH 5.0–5.5, with low calcium and phosphorous contents. More information about the edaphic conditions in this site can be found in Mediavilla et al. (2019). The area is characterized by a sub-Mediterranean climate with a dry season of three months in the summer and a mean annual rainfall ranging from 450 and 700 mm. Mean temperatures range from 14.5 to 15.8 °C.

2.2 Fire prevention treatments

The experimental design of this study was the same than the design used for previous studies focusing on fruiting bodies (Hernández-Rodríguez et al., 2015). Namely, plots were established in three distinct *C. ladanifer* sites with different ages and site histories: A) an eight-year-old stand regenerating from a wildfire in 2002, B) an eight-year-old stand regenerating from a total clearing in 2002, and C) a 20-year-old stand regenerating from a wildfire in 1990 (old-growth stand). In each site, we applied distinct fire prevention treatments. In the eight-years-old sites (A) and (B), the treatments were as follows: 1) control, 2) 50% cleared, 3) 100% cleared. In the

20-year-old site (C), the treatments were as follows: (1) control, (2) burned, (3) 100% cleared (Fig. 1). Treatments were applied following technical recommendations in accordance with the age of the stands and vegetation characteristics, in order to ensure the persistence of these vegetation types and reduce the risk of wildfires, except for the old-growth stands. Each treatment was replicated in three plots from each site, resulting in twenty-seven plots (3 sites × 3 treatments × 3 replicates per site). These sampling plots consisted of transects of 2 m × 50 m, established in accordance with previous studies (Luoma et al., 1991; Smith et al., 2002).

Fig 1



Experimental design of this study. Fire prevention treatments were replicated in three plots in each of the three sites.

2.3 Sampling and molecular work

In each plot, five soil cores were taken using a cylindrical (2 cm radius, 20 cm deep, 250 cm³) soil borer (Taylor, 2002; De la Varga et al., 2012). Cores were extracted along the plots' centerline 5 m from each other to account for the spatial variability and minimize the probability of sampling the same genet repeatedly. Soils were sampled in April 2014, four years after the implementation of the recent fire prevention treatments. Samples were frozen immediately on the sampling date upon return to the laboratory and kept at −20 °C until DNA was extracted.

Soil samples were dried at room temperature with continuous air circulation and then sieved with a mesh size of 1 mm. The five cores of each plot were pooled resulting in a composite soil sample for each plot. DNA extraction was performed from 0.25 g of soil per sample with the PowerSoil™ DNA Isolation Kit (MoBio laboratories Inc., Carlsbad, CA, USA) according to manufacturer's instructions.

The internal transcribed spacer 1 (ITS1) region was amplified using the forward primer ITS1F modified with the 5' Illumina forward adapter as described in Smith and Peay (2014). The reverse primer (ITS2) was based on Caporaso et al. (2012) and Bellemain et al. (2010), using primers modified with the 3' Illumina reverse adapter and individual barcodes for each sample.

Triplicate PCR reactions for each sample were performed in 20 µl reaction volumes containing: 1 × reaction buffer, 800 µM dNTP, 3.5 mM MgCl₂, 0.4 mM forward and reverse primer each, and 2 U Platinum Taq polymerase enzyme (Invitrogen Inc., Carlsbad, CA). PCR conditions were as follows: an initial denaturation step at 94 °C for 3 min; then 35 cycles of 94 °C for 45 s, 50 °C for 1 min and 72 °C for 1.5 min; with a final extension of 10 min at 72 °C. A negative control consisting of sterile water instead of DNA was included in each PCR replicate and underwent the PCR under the same experimental conditions. Negative controls were shown to be amplicon free on an agarose gel.

Amplicons were cleaned with Zymo Clean and Concentrate Kit™ to remove short fragments (Zymo Research, Orange, CA). PCR products were quantified using a Qubit 2.0 Fluorometer with the HS Assay Kit (Invitrogen).

Samples were pooled in equimolecular amounts and sequenced using an Illumina MiSeq 2 × 250 bp sequencer at the Center for Genome Research and Biocomputing of the Oregon State University, U.S.A.

2.4 Bioinformatic analysis

Raw sequence reads were obtained from the Illumina MiSeq output that comprise demultiplexed sample reads. Forward and reverse reads were joined using the `make.contigs` command in MOTHUR v. 1.35 (Schloss et al., 2009), simultaneously trimming off primer sequences. Subsequently, sequences were filtered using MOTHUR based on the following settings: no ambiguous bases (`maxambig = 0`), homopolymers no longer than 10 nucleotides (`maxhomop = 10`), and length range from 150 bp to 400 bp (`minlength = 150`; `maxlength = 400`), resulting in 3,074,348 quality-filtered sequences with an average read length of 229.6 ± 30.4 (mean \pm SD). Sequences were collapsed into unique sequence types, while preserving their original read counts and global singletons (1,189,793) and putative chimeric sequences (6,673) were removed with USEARCH v.8.0 (Edgar, 2010). The curated UNITE dataset of fungal ITS sequences (Abarenkov et al., 2010) was used as reference dataset. The remaining 1,877,876 sequences were grouped into 2,674 operational taxonomic units (OTUs) at 97% sequence similarity using USEARCH. We assigned OTUs to taxonomic groups based on pairwise similarity searches against the curated UNITE fungal ITS sequence database containing identified fungal sequences with assignments to Species Hypothesis groups (Kõljalg et al., 2013). After excluding OTUs with < 70% similarity or < 150 bp pairwise alignment length to a fungal sequence, 1,929 fungal OTUs were retained. Finally, we performed a functional identification of the taxa using FUNGuild (Nguyen et al., 2016) using a cut-off for assigning the OTUs to functional guilds of 97%. Sequencing raw data, together with post clustered fungal community data and the environmental data is stored in Mendeley dataset, DOI: <https://doi.org/10.17632/gd22y3664f.1>.

2.5 Statistical analyses

All the analyses were performed using R software environment (version 2.15.3; R Development Core Team, 2013). We used the `'vegdist'` function implemented in the `'vegan'` package (Oksanen et al., 2015), to calculate Bray-Curtis dissimilarity of the Hellinger transformed community matrix. Afterwards, matrices were used to evaluate the homogeneity of multivariate dispersion using the `'betadisper'` function. Thus, in this analysis, we tested differences in beta diversity across the treatments and the distinct sites to study whether a specific treatment or origin resulted in more homogeneous communities. An overall analysis of the fungal community composition was carried out using non-metric multidimensional scaling (NMDS) and permutational multivariate analysis of variance based on distance matrices (Bray–Curtis), using the `'metaMDS'` and `'adonis'` functions in the `'vegan'` package (Oksanen et al., 2015). We performed three separated analysis using i) presence/absence data, ii) raw abundance data, and iii) Hellinger-transformed abundance data. In these analyses, site and fire prevention treatments were used as explanatory variables, and the effects were tested using 500 iterations. We assessed whether a particular OTU could be identified as an indicator species of soil-associated fungal communities for some specific treatment or origin using Indicator Species Analysis implemented in the package `"indicpecies"` (De Cáceres & Legendre, 2009), using the relative abundance of each OTU. We used the function `"multipatt"` together with the parameter `IndVal.g` to manage the unequal group sizes. Changes in the relative abundance of functional guilds across treatments were assessed by Linear Mixed Effects models (LME, Pinheiro et al., 2016), where site was defined as random and fire prevention treatment was defined as fixed factor. Richness (N0) was calculated from asymptotic estimates of detected OTUs against read numbers, implemented in the `"iNEXT"` R package (Hsieh et al., 2016), and curves were obtained from the derived value obtained for each number of reads. Whether richness values were distinct across treatments and origin was tested again by LME, following

previous schemes. When significant effects were found, we performed Tukey post hoc specific contrasts for treatments and origin.

3 Results

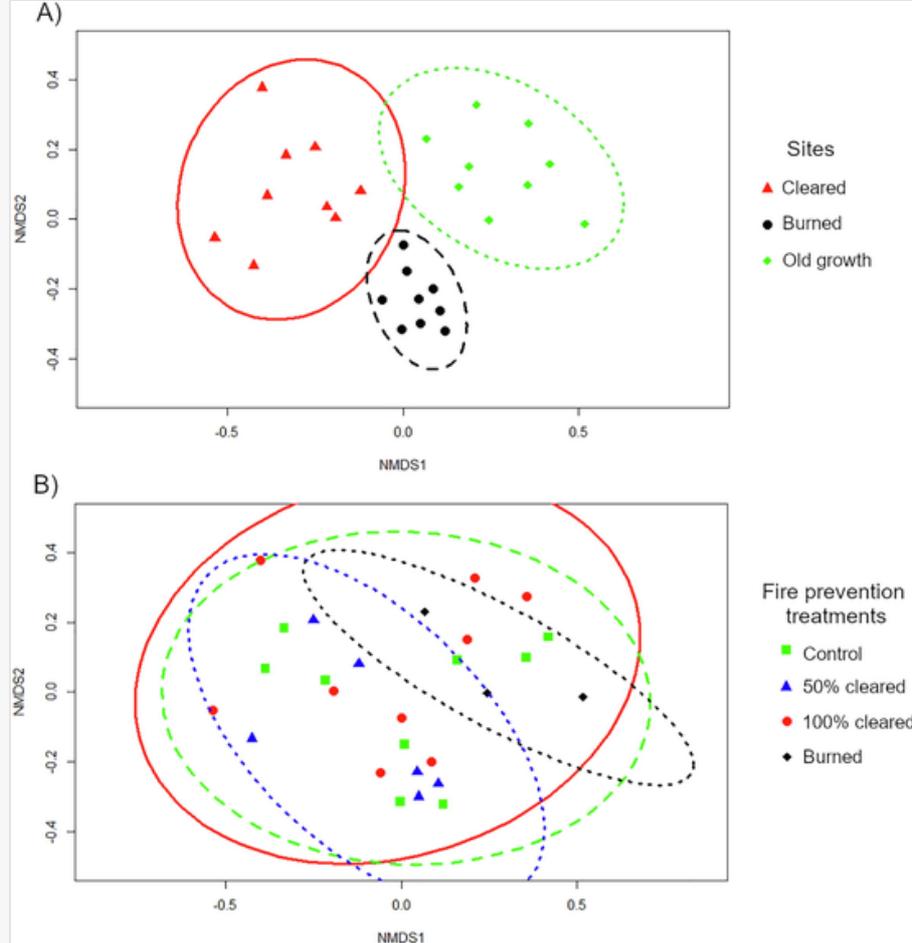
3.1 Sequencing output and fungal community composition

After discarding singletons, sequence clustering resulted in 1688 OTUs of the total 621 517 sequencing reads, with an average of $23,071 \pm 2\ 768$ reads in each sample. The most dominant guilds were, by order of proportional abundance: saprotrophs (31%), ectomycorrhizal fungi (9.3%), ericoid mycorrhizal fungi (2.9%), and plant pathogens (2.0%), whereas other groups represented $<1\%$ (including arbuscular mycorrhizal fungi and fungal parasites, among others). Fungi with unknown guilds represented around 40% of the total abundances.

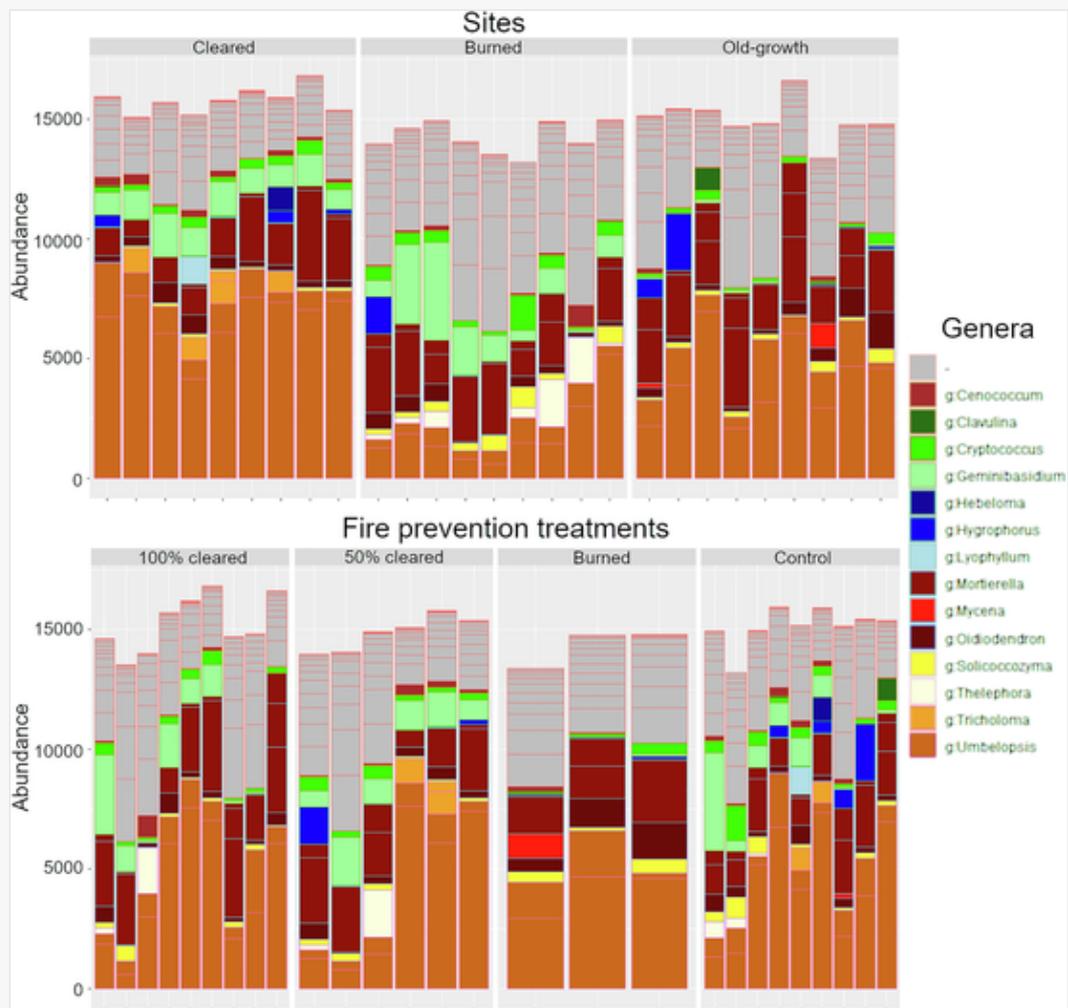
3.2 Effect of treatments on the soil fungal community composition

NMDS ordination plots using Hellinger-transformed data of the whole community revealed a strong structuring of fungal communities according to the site (whether the site was previously burned, old-growth or cleared, [Fig. 2a](#)), but not according to the more recent treatments (50% cleared, 100% cleared, burned, [Fig. 2b](#)). Thus, sampling sites representing the same origin grouped together ([Fig. 2b](#)), showing a potential lack of treatment effect. These results were confirmed by permutational multivariate analysis of variance using Bray Curtis distance, with significant effects found for the different sites ($F_{2,24} = 4.8$, $P < 0.001$, $R^2 = 0.29$) but not for the fire prevention treatments ($F_{2,24} = 1.1$, $P = 0.277$, $R^2 = 0.12$). The same results were observed independently on whether presence/absence data or non-transformed community data was used. Barr plots showed clear differences depending on the sample site, with some taxa dominating samples from specific sites, while absent in other sites ([Fig. 3](#)).

Fig. 2



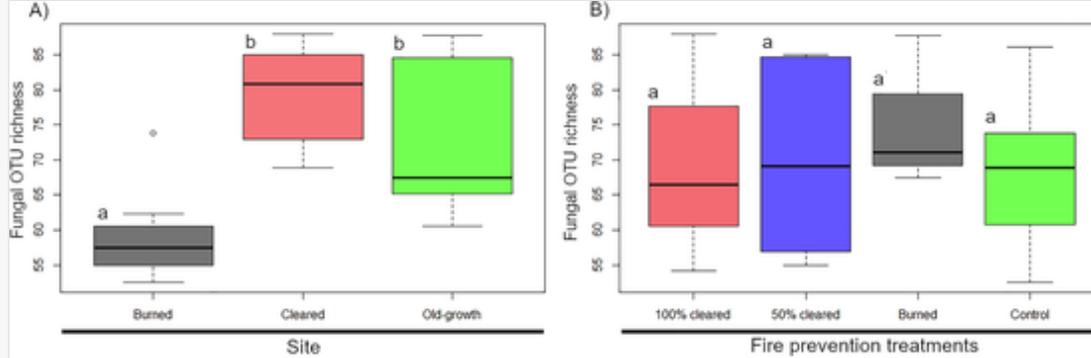
Compositional variation of the soil fungal community with respect to site and fire prevention treatment, based on nonmetric multidimensional scaling. Ellipses represent standard deviations. In (A), ellipses of the distinct sites (cleared, old-growth and burned) show little or no overlap, indicating the strong “site” effect on fungal community composition, whereas in (B) ellipses of the distinct fire prevention treatments effect (100% cleared, 50% cleared, burned, control) overlap to a great extent, indicating no significant effect of treatment. In (a) red colours indicate cleared plots, black colours indicate burned plots, and green colours indicate old-growth plots. In (b) red colours indicate 100% cleared plots, black colours indicate burned plots, green colour indicate control plots and blue colours indicate 50% cleared plots. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 3

Barr plots showing the soil fungal community profile of the most abundant taxa identified at genera level and sorted according to the site and the fire prevention treatments. Abundance represent the number of reads. Taxa identified as “-“ refers to taxa that could not be assigned to genus level.

Similarly, there was a significant site effect on soil fungal compositional multivariate variance (Beta diversity, $F = 5.5$, $P = 0.010$), with communities in the burned plots having lower compositional multivariate variance than old-growth and cleared sites (Fig. S1). However, no effect of the fire prevention treatments was found on compositional multivariate variance ($F_{2,24} = 2.0$, $P = 0.141$). The same trend was observed for the richness values, with a significant site effect on richness ($F = 12.8$, $P < 0.001$, Fig. 4a), but not significant effect of the fire prevention treatments on fungal richness ($F = 0.2$, $P = 0.872$, Fig. 4b). Thus, only distinct and lower richness values were observed in sites that were burned, as compared to sites that were cleared (Specific contrasts; $P < 0.001$, Fig. 4a) or compared to old growth sites (Specific contrasts, $P = 0.001$, Fig. 4a).

Fig. 4



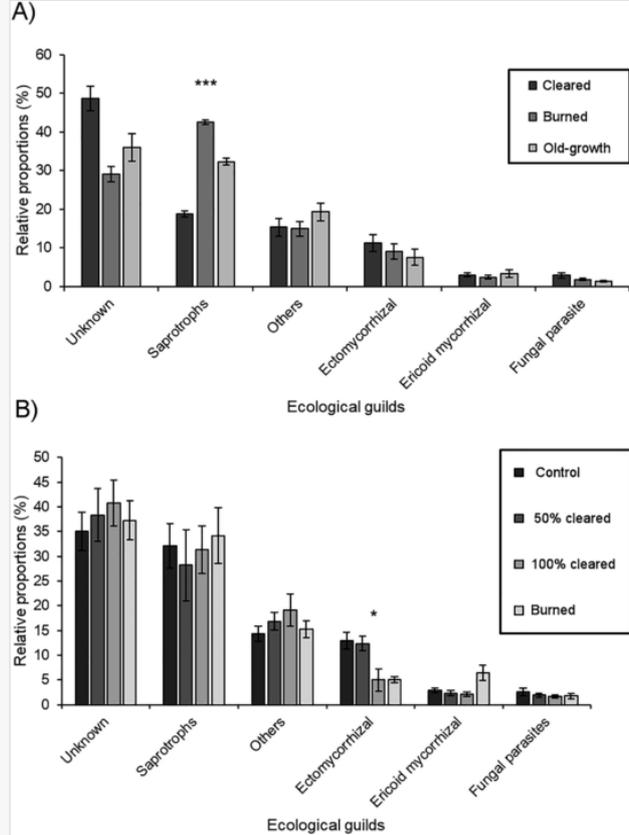
Interpolated richness of soil fungal communities in different a) sites and b) fire prevention treatments. Wording (a, b) indicate significantly different values ($P < 0.05$).

Species indicator analysis stressed the importance of the site on the fungal community, with 219 OTUs significantly associated to specific sites, of which 49 were associated to burned sites, 103 to cleared sites and 67 to old-growth sites. In contrast, the same analysis revealed only 6 OTUs associated to specific fire prevention treatments; of which 4 were associated to 50% cleared plots and 2 to control plots.

3.3 Effect of treatments on the fungal guilds

Analysis of the soil fungal community according to their functional guilds revealed again a significant site effect on the soil fungal community ($F_{2,24} = 5.4$, $P < 0.001$) while only a marginal effect of the fire prevention treatments was found ($F_{2,24} = 1.7$, $P = 0.081$). Saprotrophs were the ecological guild of fungi that were mainly responding to the observed site effects ($F = 23.3$, $P < 0.001$), with a higher relative abundance of this guild in burned plots, but lower relative abundance in cleared plots (Fig. 5a). In contrast, the only ecological guild significantly affected by the fire prevention treatments were the ectomycorrhizal guild ($F = 4.8$, $P = 0.010$, Fig. 5b), with higher relative abundances of ectomycorrhizal fungi in control and 50% cleared plots, as compared to 100% cleared and burned plots (Fig. 5b). Thus, overall, burned and 100% cleared treatments negatively affected the relative abundances of ectomycorrhizal species. For example, the relative abundance of the ectomycorrhizal fungi in 100% cleared and burned plots was less than half of that found in control and 50% cleared plots (Fig. 5b).

Fig. 5



Relative abundances of the most abundant soil fungal trophic guilds (guilds identified using FunGUILD in Nguyen et al., 2016) sorted according to a) sites and b) fire prevention treatments. Asterisks indicate significant differences (significance levels: “***” $P < 0.001$, “**” $P < 0.01$, “*” $P < 0.05$, “.” $P < 0.1$, n.s. non-significant).

4 Discussion

Our results show that site identity exerted a much stronger effect on soil fungal communities in our *Cistus ladanifer* scrublands than the fire prevention treatments. Unexpectedly, we did not see an overall effect of the fire prevention treatments on richness or composition of the total fungal community, except for the significant lower relative abundance of ectomycorrhizal fungi in burned and 100% cleared plots, compared to the control and 50% cleared plots. Previous studies described the fungal species fruiting in the same experiment considered here and studied the effect of fire prevention treatments on the fruiting bodies (Hernández-Rodríguez et al., 2015). In contrast to Hernández-Rodríguez et al., (2015), we found weak effects of the fire prevention treatments on the belowground fungal community, suggesting that fire prevention treatments may be buffered to belowground communities.

Cistaceae has a Holarctic distribution and is an important vegetation type shaping and determining ecosystem dynamics in xeric Mediterranean areas (Ellul et al., 2002). *Cistus* spp. associate with a high diversity of fungi, including arbuscular mycorrhizal and ectomycorrhizal fungi (Comandini et al., 2006). This coexistence has many practical advantages, such as the rapid exchange of water and nutrients through mycorrhizal hyphal networks (Brundrett, 2004, 2002). Therefore, these soil fungal communities likely play crucial roles in

enhancing resistance of *Cistus* to drought and other disturbances in Mediterranean ecosystems. Although we did not find significant treatment effects on the total soil fungal community, the observed lower relative abundance of ectomycorrhizal species in burned and totally cleared plots suggests that severe disturbances that greatly reduce host biomass have a negative effect on ectomycorrhizal fungal abundance, likely due to decreased C allocation belowground. The significant decrease in the relative abundance of ectomycorrhizal species after severe fire prevention treatments parallels with previous studies in which the mycorrhizal community was negatively affected by fire (Day et al., 2019), land use changes (Castaño et al., 2019), insect attacks (Veselá et al., 2019) or forest clear-cuttings (Parladé et al., 2019). However, the lack of effects on the ectomycorrhizal community in 50% cleared plots suggest that medium or low intensity treatments, likely retaining enough host plant biomass and functional roots, may sustain the mycorrhizal community, as previously observed in forest systems (Castaño et al., 2018; Sterkenburg et al., 2019). In fact, a recent study shows that thinning in *Quercus* forests may increase the resistance of the soil microbial communities to drought (Bastida et al., 2019). In addition, our results suggest that the 50% cleared treatment may be the best option to maintain the ectomycorrhizal community in these systems, while reducing the risk of wildfires. In contrast, total clearing or clearcutting may negatively affect the soil fungal community, especially plant symbionts (Parladé et al., 2019). The time elapsed from the execution of the treatments to the soil sampling was four years, suggesting that the ectomycorrhizal community in burned and totally cleared plots may not fully recover even 4 years after treatment.

We found profound site differences in terms of fungal community composition and richness. The lowest richness and the lowest number of indicator species corresponded to the previously burned sites, which suggest that burning may select for a relatively small set of specific and highly abundant pyrophilic taxa (Martín-Pinto et al., 2006; Mediavilla et al., 2014). Recent studies also found a decrease in richness and changes in community composition shortly after wildfires (Day et al., 2019). Surprisingly, the site with the senescent (Old-growth) scrubland did not have higher diversity than cleared plots, but a clear shift in fungal community composition was observed, as compared to the other sites. This is in accordance with the distinct compositional turnover of fungal communities along a post-fire secondary succession observed in other biomes (e.g., Geml et al., 2010), which suggests a restructuring of the fungal community rather than an accumulation of taxa across secondary succession. Similarly, Kyaschenko et al. (2017) related the shifts in fungal composition along a chronosequence to changes in soil chemistry, with an increase in taxa more specialized in mining N from organic matter during the later stages of the succession. The distinct communities observed in senescent plots highlight the importance of preserving old-growth stands harbouring distinct fungal communities.

The strong site effect on the soil fungal community composition is consistent with previous findings targeting sporocarp production (Hernández-Rodríguez et al., 2015) and recently soil bacteria (Mediavilla et al., 2019) in the same study area. However, a strong significant effect of these treatments was observed also at the fruiting body community, suggesting that fruiting body production is more sensitive to environmental drivers and climatic conditions than soil mycelia (Alday et al., 2017). The relatively higher resilience of the soil fungal communities to mechanical treatments (Jennings et al., 2012) may be explained by the capability of these vegetation types to keep the radical system alive, despite the reduction of above-ground biomass, but also to the fast germination of the seed bank. In addition, thinning or 50% cleared treatments may boost plant growth and could eventually stimulate associated symbionts. This may have been also the case of the fire treatments, since prescribed fires are usually conducted under low fire intensity conditions, likely with limited effect on soil biota (Oliver et al., 2015). Hence, the lower richness found in historically burned areas stresses the need for conducting these fires under relatively low temperatures (as compared to wildfires) if the fungal community is to be maintained. Finally, the increase in relative abundances of saprotrophs in the burned site contrasts with previous

findings in which relative proportions of amplicons from saprotrophs decrease after fire (Day et al., 2019), but also sporocarps (Mediavilla et al., 2014). Our results and current literature suggest that effects of fire on saprotrophic communities may be context and host dependent.

5 Conclusions

Our work provides new information on fungal communities associated with *Cistus ladanifer* soils and the effect of different fire prevention treatments on fungal taxa. We highlight that fungal communities found in these scrublands are primarily shaped by site differences, whereas recent severe fire prevention treatments significantly decreased the relative abundance of mycorrhizal fungi in burned and totally cleared treatments. This study provides potentially useful information for the conservation of fungal communities in scrublands and highlights the use of management tools aiming to reduce the risk of wildfires; 50% clearing probably best preserves the fungal community while decrease the risk of wildfires.

Uncited references

~~Ekblad et al. (2016), Gardes and Bruns (1996), Horton and Bruns (2001).~~

CRedit authorship contribution statement

Carles Castaño: Analysis, writing and editing. **María Hernández-Rodríguez:** Investigation, Methodology. **József Geml:** Analysis and review. **Joyce Eberhart:** Methodology, Investigation. **Jaime Olazola:** Supervision. **Juan Andrés Oria-de-Rueda:** Supervision. **Pablo Martín-Pinto:** Conceptualization, Methodology, Supervision.

Declaration of Competing Interest

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

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Appendix A Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foreco.2020.118217>.

References



The corrections made in this section will be reviewed and approved by a journal production editor. The newly added/removed references and its citations will be reordered and rearranged by the production team.

- Abarenkov, K., Henrik Nilsson, R., Larsson, K.-H., Alexander, I.J., Eberhardt, U., Erland, S., Høiland, K., Kjølner, R., Larsson, E., Pennanen, T., et al., 2010. The UNITE database for molecular identification of fungi - recent updates and future perspectives. *New Phytol.* 186, 281–285.
- Alday, J.G., Bonet, J.A., Oria-de-Rueda, J.A., Martínez-de-Aragón, J., Aldea, J., Martín-Pinto, P., de-Miguel, S., Hernández-Rodríguez, M., Martínez-Peña, F., 2017. Record breaking mushroom yields in Spain. *Fungal Ecol.* 26, 144–146. <https://doi.org/10.1016/j.funeco.2017.01.004>.
- Alday, J.G., Cox, E.S., Pakeman, R.J., Harris, M.P.K., Leduc, M.G., Marrs, R., 2013. Overcoming resistance and resilience of an invaded community is necessary for effective restoration: a multi-site bracken control study. *J. Appl. Ecol.* 50 (1), 156–167.
- Baldrian, P., 2016. Forest microbiome: diversity, complexity and dynamics. *FEMS Microbiol. Rev.* doi:10.1093/femsre/fuw040.
- Bastida, F., López-Mondéjar, R., Baldrian, P., Andrés-Abellán, M., Jehmlich, N., Torres, I.F., García, C., López-Serrano, F.R., 2019. When drought meets forest management: effects on the soil microbial community of a Holm oak forest ecosystem. *Sci. Total Environ.* 662, 276–286. doi:10.1016/J.SCITOTENV.2019.01.233.
- Bellemain, E., Carlsen, T., Brochmann, C., Coissac, E., Taberlet, P., Kauserud, H., 2010. ITS as an environmental DNA barcode for fungi: an in silico approach reveals potential PCR biases. *BMC Microbiol.* 10, 189.
- Brundrett, M., 2004. Diversity and classification of mycorrhizal associations. *Biol. Rev.* 79, 473–495. doi:10.1017/S1464793103006316.
- Brundrett, M.C., 2002. Coevolution of roots and Mycorrhiza of land plants. *New Phytol.* 154, 275–304.
- Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J.A., Smith, G., Knight, R., 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J.* 6 (8), 1621–1624.
- Castaño, C., Alday, J.G., Lindahl, B.D., Martínez de Aragón, J., de-Miguel, S., Colinas, C., Parladé, J., Pera, J., Bonet, J.A., 2018. Lack of thinning effects over inter-annual changes in soil fungal community and diversity in a Mediterranean pine forest. *For. Ecol. Manage.* 424, 420–427. <https://doi.org/10.1016/j.foreco.2018.05.004>.
- Castaño, C., Dejene, T., Mediavilla, O., Geml, J., Oria-de-Rueda, J.A., Martín-Pinto, P., 2019. Changes in fungal diversity and composition along a chronosequence of *Eucalyptus grandis* plantations in Ethiopia. *Fungal Ecol.* 39, 328–335. doi:10.1016/J.FUNECO.2019.02.003.
- Clemmensen, K.E., Finlay, R.D., Dahlberg, A., Stenlid, J., Wardle, D.A., 2015. Carbon sequestration is related to mycorrhizal fungal community shifts during long-term succession in boreal forests. *New Phytol.* 205, 1525–1536.
- Comandini, O., Contu, M., Rinaldi, A.C., 2006. An overview of *Cistus* ectomycorrhizal fungi. *Mycorrhiza* 16, 381–395. doi:10.1007/s00572-006-0047-8.

Day, N.J., Dunfield, K.E., Johnstone, J.F., Mack, M.C., Turetsky, M.R., Walker, X.J., White, A.L., Baltzer, J.L., 2019. Wildfire severity reduces richness and alters composition of soil fungal communities in boreal forests of western Canada. *Glob. Chang. Biol.* 25 (7), 2310–2324. doi:10.1111/gcb.14641.

De la Varga, H. De, Águeda, B., Martínez-peña, F., Parladé, J., Pera, J., 2012. Quantification of extraradical soil mycelium and ectomycorrhizas of *Boletus edulis* in a Scots pine forest with variable sporocarp productivity. *Mycorrhiza* 22(1), 59–68. <https://doi.org/10.1007/s00572-011-0382-2>.

De Cáceres, M., Legendre, P., 2009. Associations between species and groups of sites: indices and statistical inference. *Ecology* 90, 3566–3574.

Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26, 2460–2461. doi:10.1093/bioinformatics/btq461.

~~Ekblad, A., Mikusinska, A., Agren, G.I., Menichetti, L., Wallander, H., Vilgalys, R., Bahr, A., Eriksson, U., 2016. Production and turnover of ectomycorrhizal extramatrical mycelial biomass and necromass under elevated CO₂ and nitrogen fertilization. *New Phytol.* 211 (3), 874–885.~~

Ellul, P., Boscaiu, M., Vicente, O., Moreno, V., Rosselló, J.A., 2002. Intra- and interspecific variation in DNA content in *Cistus* (Cistaceae). *Ann. Bot.* 90, 345–351. doi:10.1093/aob/mcf194.

~~Gardes, M., Bruns, T.D., 1996. Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: above- and below-ground views. *Can. J. Bot.* 74, 1572–1583. doi:10.1139/b96-190.~~

Geml, J., Gravendeel, B., Gaag, K.J. Van Der, Neilen, M., Lammers, Y., Raes, N., Semenova, T.A., Knijff, P. De, Noordeloos, M.E., 2014. The contribution of DNA metabarcoding to fungal conservation: Diversity assessment, habitat partitioning and mapping red-listed fungi in protected coastal *Salix repens* communities in the Netherlands. *PLoS ONE* 9(6), e99852. <https://doi.org/10.1371/journal.pone.0099852>.

Geml, J., Laursen, G.A., Herriott, I., McFarland, J.M., Booth, M.G., Lennon, N., Nusbaum, H.C., Taylor, D.L., 2010. Phylogenetic and ecological analyses of soil and sporocarp DNA sequences reveal high diversity and strong habitat partitioning in the boreal ectomycorrhizal genus *Russula* Pers. (Russulales; Basidiomycota). *New Phytol.* 187, 494–507.

Hernández-Rodríguez, M., Oria-de-Rueda, J.A., Pando, V., Martín-Pinto, P., 2015. Impact of fuel reduction treatments on fungal sporocarp production and diversity associated with *Cistus ladanifer* L. ecosystems. *For. Ecol. Manage.* 353, 10–20. doi:10.1016/j.foreco.2015.05.007.

Högberg, P., Nordgren, A., Buchmann, N., Taylor, A.F., Ekblad, A., Högberg, M.N., Nyberg, G., Ottosson-Löfvenius, M., Read, D.J., 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. *Nature* 411, 789–792. doi:10.1038/35081058.

~~Horton, T.R., Bruns, T.D., 2001. The molecular revolution in ectomycorrhizal ecology: peeking into the black box. *Mol. Ecol.* 10, 1855–1871. doi:10.1046/j.0962-1083.2001.01333.x.~~

Hsieh, T., C., Ma, K., H., Chao, A., 2016. iNEXT: an R package for rarefaction and extrapolation of species diversity (Hill numbers). *Methods Ecol Evol* 7, 1451–1456.

Jennings, T.N., Smith, J.E., Cromack, K., Jr., Sulzman, E.W., McKay, D., Caldwell, B.A., Beldin, S.I., 2012. Impact of postfire logging on soil bacterial and fungal communities and soil biogeochemistry in a

mixed-conifer forest in central Oregon. *Plant Soil* 350, 393–411. doi:10.1007/s11104-011-0925-5.

Kohout, P., Charvátová, M., Štursová, M., Mašínová, T., Tomšovský, M., Baldrian, P., 2018. Clearcutting alters decomposition processes and initiates complex restructuring of fungal communities in soil and tree roots. *ISME J.* 12, 692–703. doi:10.1038/s41396-017-0027-3.

Kõljalg, U., Nilsson, R.H., Abarenkov, K., Tedersoo, L., Taylor, A.F.S., Bahram, M., 2013. Towards a unified paradigm for sequence-based identification of fungi. *Mol. Ecol.* 22, 5271–5277. doi:10.1111/mec.12481.

Kyaschenko, J, Clemmensen, K, Karlton, E, Lindahl, B, D, 2017. Below-ground organic matter accumulation along a boreal forest fertility gradient relates to guild interaction within fungal communities. *Ecol Lett* 20, 1546–1555.

León-Sánchez, L., Nicolás, E., Goberna, M., Prieto, I., Maestre, F.T., Querejeta, J.I., 2018. Poor plant performance under simulated climate change is linked to mycorrhizal responses in a semi-arid shrubland. *J. Ecol.* 106, 960–976. doi:10.1111/1365-2745.12888.

Lindahl, B.D., Ihrmark, K., Boberg, J., Trumbore, S.E., Högberg, P., Stenlid, J., Finlay, R.D., 2007. Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. *New Phytol.* 173, 611–620.

Lindahl, B.D., Nilsson, R.H., Tedersoo, L., Abarenkov, K., Carlsen, T., Kjoller, R., Kõljalg, U., Pennanen, T., Rosendahl, S., Stenlid, J., Kauserud, H., 2013. Fungal community analysis by high-throughput sequencing of amplified markers - a user's guide. *New Phytol.* 199, 288–299.

Luoma, D.L., Frenkel, R.E., Trappe, J.M., 1991. Fruiting of hypogeous fungi in Oregon Douglas-Fir forests: Seasonal and habitat variation. *Mycologia* 83, 335–353. doi:10.2307/3759994.

Martín-Pinto, P., Vaquerizo, H., Peñalver, F., Olaizola, J., Oria-de-Rueda, J.A., 2006. Early effects of a wildfire on the diversity and production of fungal communities in Mediterranean vegetation types dominated by *Cistus ladanifer* and *Pinus pinaster* in Spain. *For. Ecol. Manage.* 225, 296–305. doi:10.1016/J.FORECO.2006.01.006.

Mediavilla, O., Geml, J., Olaizola, J., Oria-de-Rueda, J.A., Baldrian, P., Martín-Pinto, P., 2019. Effect of forest fire prevention treatments on bacterial communities associated with productive *Boletus edulis* sites. *Microb. Biotechnol.* 12, 1188–1198. doi:10.1111/1751-7915.13395.

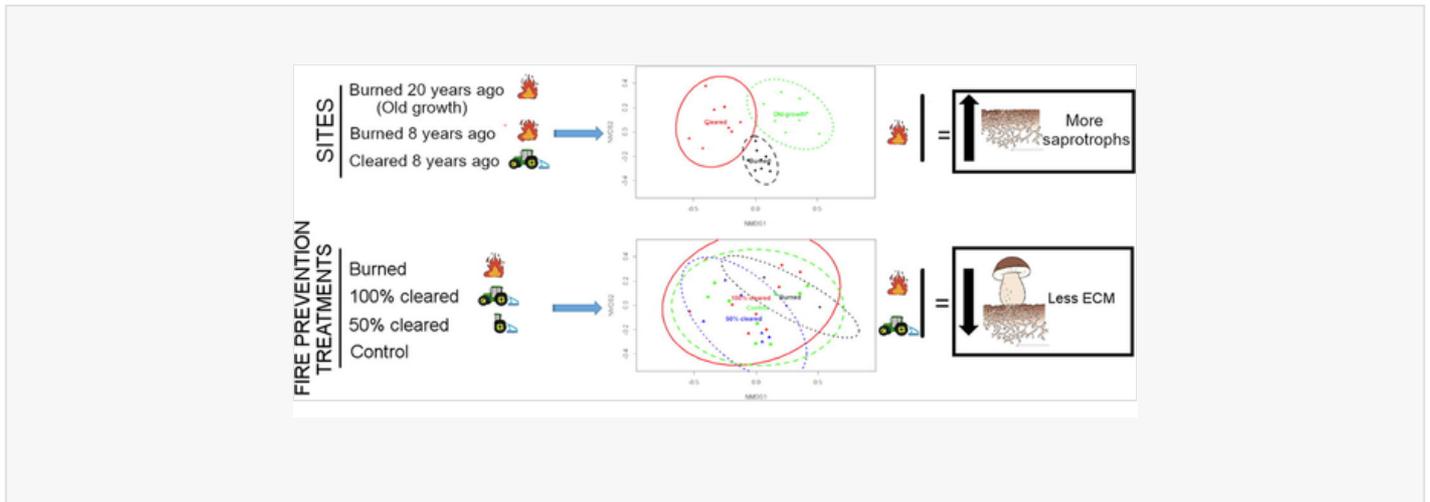
Mediavilla, O., Oria-de-Rueda, J.A., Martín-Pinto, P., 2014. Changes in sporocarp production and vegetation following wildfire in a Mediterranean Forest Ecosystem dominated by *Pinus nigra* in Northern Spain. *For. Ecol. Manage.* 331, 85–92. doi:10.1016/j.foreco.2014.07.033.

Nguyen, N., H., Song, Z., Bates, S., T., Branco, S., Tedersoo, L., Menke, J., Schilling, J., S., Kennedy, P., G., 2016. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecol* 20, 241–248.

Oksanen, J., Blanchet, F., G., Kindt, R., Legendre, P., Minchin, P., R., O'Hara, B., Wagner, H., 2015. VEGAN: Community ecology package. R package version 2.2-1.

- Oliver, A.K., Callaham, M.A., Jumpponen, A., 2015. Soil fungal communities respond compositionally to recurring frequent prescribed burning in a managed southeastern US forest ecosystem. *For. Ecol. Manage.* 345, 1–9. doi:10.1016/j.foreco.2015.02.020.
- Parladé, J., Queralt, M., Pera, J., Bonet, J.A., Castaño, C., Martínez-Peña, F., Piñol, J., Senar, M.A., De Miguel, A.M., 2019. Temporal dynamics of soil fungal communities after partial and total clear-cutting in a managed *Pinus sylvestris* stand. *For. Ecol. Manage.* 449, 117456. doi:10.1016/j.foreco.2019.117456.
- Pérez-Izquierdo, L., Zabal-Aguirre, M., González-Martínez, S., Bueé, M., Verdú, M., Rincón, A., Goberna, M., 2019. Plant intraspecific variation modulates nutrient cycling through its below ground rhizospheric microbiome. *J Ecol.* 107, 1594–1605.
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., R Core Team, 2016. *Nlme: Linear and Nonlinear Mixed Effects Models*. R Package Version 3.1-128. <http://CRAN.R-project.org/package=nlme>.
- Savoie, J.M., Largeteau, M.L., 2011. Production of edible mushrooms in forests: trends in development of a mycosilviculture. *Appl. Microbiol. Biotechnol.* 89, 971–979.
- Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* 75, 7537–7541.
- Štursová, M., Šnajdr, J., Koukol, O., Tláškal, V., Cajthaml, T., Baldrian, P., 2020. Long-term decomposition of litter in the montane forest and the definition of fungal traits in the successional space. *Fungal Ecol.* 100913. doi:10.1016/j.funeco.2020.100913.
- Smith, D.P., Peay, K.G., 2014. Sequence depth, not PCR replication, improves ecological inference from next generation DNA sequencing. *Plos One* 9 (2). doi:10.1371/journal.pone.0090234.
- Smith, S.E., Read, D.J. 2008. *Mycorrhizal Symbiosis*, third ed. Academic Press, London, UK.
- Smith, J.E., Molina, R., Huso, M.M.P., Luoma, D.L., McKay, D., Castellano, M.A., Lebel, T., Valachovic, Y., 2002. Species richness, abundance, and composition of hypogeous and epigeous ectomycorrhizal fungal sporocarps in young, rotation-age, and old-growth stands of Douglas-fir (*Pseudotsuga menziesii*) in the Cascade Range of Oregon, USA. *Can. J. Bot.* 80, 186–204. doi:10.1139/B02-003.
- Sterkenburg, E., Clemmensen, K.E., Lindahl, B.D., Dahlberg, A., 2019. The significance of retention trees for survival of ectomycorrhizal fungi in clear-cut Scots pine forests. *J. Appl. Ecol.* 56, 1367–1378. doi:10.1111/1365-2664.13363.
- Taylor, A.F.S., 2002. Fungal diversity in ectomycorrhizal communities: sampling effort and species detection. *Plant Soil* 244, 19–28. doi:10.1023/A:1020279815472.
- Tomao, A., Antonio Bonet, J., Castaño, C., de-Miguel, S., 2020. How does forest management affect fungal diversity and community composition? Current knowledge and future perspectives for the conservation of forest fungi. *For. Ecol. Manage.* 457, 117678. <https://doi.org/10.1016/J.FORECO.2019.117678>.

Graphical abstract



Highlights

- We studied the effect of fire prevention treatments on soil fungal communities inhabiting scrublands.
- Soil fungal community was profiled using high-throughput sequencing of fungal markers.
- Site differences exerted stronger effect than fire prevention treatments on soil fungal communities.
- Controlled fire and 100% clearing decreased the relative proportions of ectomycorrhizal species.
- 50% cleared treatments may decrease the risk of fire while maintaining the fungal communities.

Appendix A Supplementary material

The following are the Supplementary data to this article:

[Multimedia Component 1](#)

Supplementary data 1

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