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Soil fungi associated with *Halimium lasianthum* in Mediterranean ecosystems, including *Boletus edulis*, are largely unaffected by wildfire prevention treatments in the long-term

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Abstract

Mediterranean ecosystems are frequently invaded by pyrophytic scrubs that colonize areas traditionally used by livestock. This considerably increases the risk of wildfires in these ecosystems and the consequent loss of their ecological and economic value. Mushrooms can play important economic as well as ecological roles in these ecosystems. To investigate the long-term effect of two different forest fire prevention treatments on the soil fungal community, we analyzed these communities 9 years after prescribed burning or mechanical shredding had been carried out in scrubland dominated by *Halimium lasianthum*, *Pterospartum tridentatum*, and *Erica australis*. We also analyzed environmental variables that may influence soil fungal communities. Remarkably, the highly prized, edible, ectomycorrhizal mushroom *Boletus edulis*, which is associated with these Mediterranean systems, was not affected by the fire prevention treatments. Furthermore, neither of the fire prevention treatments had a negative effect on the abundance or richness of ectomycorrhizal fungi. Soil fertility significantly affected the distribution of fungi according to their functional groups, and pH was the most influential variable in terms of the distribution of edible species. Prior to this study, *B. edulis* had rarely been recorded in this forest system. Our findings indicate that forest management practices to prevent forest fires are compatible with enhancing the production of edible fungi and, hence, could be an interesting way of reducing the risk of wildfires while increasing the incomes of local populations.

1. Introduction

Areas occupied by Mediterranean scrublands are expanding due to land-use changes as a result of the abandonment of agriculture in these areas and changes in management objectives. These ecosystems are not a focus of interest for forest managers due to their lack of timber production, and other potential resources such a mushroom production are underappreciated and underexploited (Oria-De-Rueda et al., 2008). Furthermore, because these scrubland ecosystems are often unmanaged, they are frequently affected by wildfires (Martín-Pinto et al., 2006; Mediavilla et al., 2014), which reduce vegetation cover, stimulate changes in plant composition (Franco-Manchón et al., 2019), and damage fungal communities (Franco-Manchón et al., 2019; Martín-Pinto et al., 2006). These events favor the development of pyrophytic vegetation (Oria-De-Rueda et al., 2008), which is a common part of Mediterranean ecosystems and includes members of the *Cistaceae* family and members of the *Pinus* genus.

Climate change appears to be changing wildfire behavior and, hence, more severe fires are expected to occur in the near future (Vega et al., 2009). In this context, forest management is a key tool that can be used to stop wildfire damage, as well as improving rural development, conservation strategies, and the quality of life of the local population. Traditionally, cattle have grazed in Mediterranean scrublands, (Fernández et al., 2015), which has been a natural, efficient, and productive way of preventing forest fires by reducing the amount of forest fuel available. Nowadays, fewer cattle are grazed in these areas, which has allowed fuel to accumulate. Therefore, other fuel-reduction methods have had to be implemented to prevent potential damage to natural and artificial resources, by modifying the quantity and continuity of fuels, which reduces the risk of high-intensity and severe wildfires (Fernández et al., 2015). An additional benefit of properly managing this ecosystem is that mushroom production can be increased while at the same time reducing wildfire risks (Hernández-Rodríguez et al., 2015b). Prescribed burning and mechanical shredding are the usual practices undertaken to reduce forest fuels (Fernández et al., 2015; Hernández-Rodríguez, et al., 2015b; Oria-De-Rueda et al., 2008). On the one hand, prescribed burning is considered a low-cost option. Although there is some risk that the fire may get out of control (Fernández et al., 2015), the damage is still less than that caused by wildfires (Oria-De-Rueda et al., 2008). On the other hand, mechanical shredding is a safer technique, but is more expensive than prescribed burning and is difficult to undertake on steep slopes and stony terrains (Fernández et al., 2015).

Previous studies undertaken in the study area have compared the effect of prescribed burning and mechanical shredding treatments with different objectives. These studies observed no differences in plant mortality or in the growth of resprouted shoots between treatments (Fernández et al., 2013b), or in vegetation cover and recovery, or even in species richness, diversity, and evenness (Fernández et al., 2015), which agrees with the findings of previous studies of similar ecosystems (Fernández & Vega, 2014). In addition, prescribed burning has been reported to reduce the soil organic layer and to stimulate seed germination (Fernández et al., 2013a). However, prescribed burning has been reported to have less of an effect on the physicochemical properties of mineral soil than on the organic layer (Fontúrbel et al., 2016), although the effects were undetectable after 3 years.

Given the interest in fungi as an important resource in similar *Cistaceae* Mediterranean ecosystems (Hernández-Rodríguez et al., 2015a; Martín-Pinto et al., 2006; Oria-De-Rueda et al., 2008) and their capacity to contribute to rural development (Bonet et al., 2014; Oria-De-Rueda et al., 2008; Román & Boa, 2004), the goal of our study was to analyze soil fungal communities in scrubland 9 years after prescribed burning or mechanical shredding were carried out to determine the long-term effects of these fire prevention treatments. We hypothesized that: ecosystems partially dominated by *Halimium lasianthum* could be associated with edible and marketable species such as *Boletus edulis*, which could be affected by the fire prevention treatment (hypothesis 1); root-associated symbiotic fungi would be more frequent due to a decrease in soil fertility because of treatment effects (hypothesis 2); and soils would be more fertile immediately after being burnt, with a gradual decrease in soil fertility over time, and that the community turnover would partially be explained by these changes in edaphic conditions (hypothesis 3).

2. Material and methods

2.1. Study area

The study site is located in the Edreiras mountains (42° 8’ 02” N – 7° 26’ 17” W, 1330 m a.s.l.), in Ourense province (NW Spain). The main characteristics of the site are a low mean slope (10%), Mediterranean climate conditions (rainfall average 1100 mm year–1; mean annual temperature 10°C) and *Alumi-umbric Regosols* (FAO, 1998) developed on schists. The experimental area was mainly covered by heather, the main species being *Erica australis* L., together with *Pterospartum tridentatum* (L.) Wilk. and *Halimium lasianthum* (Lam.) Spach. Cattle and roe deer are very frequent in this area and are two important agents of ecosystem changes. In the past, prescribed burning was commonly motivated by pastoral considerations. Previous studies that have investigated shrub recovery (Fernández et al., 2015) and resprouting (Fernández et al., 2013a), seedling emergence (Fernández et al., 2013c), and microbial responses (Fontúrbel et al., 2016) to fuel reduction treatments were carried out in the same study area, therefore, further information about this area is available.

2.2. Experimental design, soil sampling and molecular work

Plots were established in the same local area in order to reduce the influence that differences in altitude (Geml et al., 2014), aspect (Chu et al., 2016), slope (Geml, 2019), and climatic conditions (Castaño et al., 2018b; Collado et al., 2019) may have on different fungal communities. The experiment involved the analysis of two different fire-prevention treatments to obtain different plot conditions, burned by prescribed burning and cleared by mechanical shredding, which was carried out in 2010, as well as control plots in which vegetation naturally grew since 2003 when the last wildfire affected the study area. A total of four experimental blocks were established. Within each block we sampled three plots for each studied treatment (burned, cleared, and control plots), to reduce the influence of variations in vegetation and stoniness. A total of 36 plots were surveyed. In each plot, fifteen soil core samples were collected in May and June 2019 and then mixed to form one soil pooled sample per plot to preserve the heterogeneity of the terrain, obtaining 36 pooled soil samples in total. Samples were collected after removing superficial stones and roots, saving them in a plastic bag and labeled. They were transported to the lab and processed before 24 hours. Samples were air-dried and sieved through a 1-mm mesh sieve. Each sample was subjected to genomic DNA analysis, which was performed using 100 g of soil, and chemical analysis, which was performed using two 20-g samples of each soil sample, (these two samples were stored in different freezers, in case of machine failures), to determine soil pH by water-based suspension at 1:2.5; dry matter (%) following UNE-ISO 11465 rule; total phosphorus (P) by Olsen methodology; and total carbon (C) and total nitrogen (N) contents (%) by Dumas methodology (Table 1).

Table 1. Mean physicochemical properties for each treatment of soil samples collected 9 years after scrublands were subjected to prescribed burning or mechanical shredding

Soil properties	Treatment		
	Burned	Cleared	Control
pH	4.49a	4.43b	4.46ab
P (mg/kg)	9.32a	12.08b	9.98ab
N (%)	0.65ab	0.68a	0.62b
C (%)	10.47ab	11.30a	9.94b
Dry matter (%)	95.18a	94.27b	95.97a

Different lowercase letters indicate significantly different mean values (*P* < 0.05).

Above-ground vegetation was surveyed by establishing a 50-m long vegetation transect in each plot. Plant cover and height were measured using a line interception methodology, as described by Kent (2011). Plants were identified down to species level when possible.

PCR was used to amplify the ITS2 region (ca. 250 bp) of the nuclear ribosomal DNA repeat by using primers fITS7 (Ihrmark et al., 2012) and ITS4 (White et al., 1990), as described in Geml et al. (2014). Sequencing was performed using an Illumina MiSeq platform (BaseClear BV, Leiden, the Netherlands).

2.3. Bioinformatic analysis

After identifying both DNA sequence senses, we used cutadapt (Martin, 2011) to trim off poor-quality ends in both directions (3' and 5') using a quality criteria value of q = 15. The next step was to join both sequences of each sample using USEARCH v.10.0.240 (Edgar, 2010) and cutadapt, with a minimum sequence length of 200 bp. Primers (ITS4 reverse and fITS7 forward) were trimmed and sequences with expected errors of > 1 were removed. Then, sequences were combined into a single sample and read count numbers were recorded to generate an OTU (operational taxonomic unit) map showing the number of times that an OTU was detected in each sample. We assigned sequences to taxonomic groups based on their similarity to the curated UNITE database (version v.8.0 released on November 18th, 2018), which contains identified and unidentified sequences assigned to species hypothesis (SH) groups defined based on dynamic sequence similarity thresholds (Kõljalg et al., 2013). Excluding OTUs with < 70% similarity or with < 200 bp pairwise alignment length to a fungal sequence, a total of 2225 OTUs were obtained, representing a total of 292,098 quality-filtered sequences, which included 1,139,216 singletons. Taxonomic studies generally only consider OTUs with >95% identity; however, given that we were analyzing fungal communities from an ecological point of view, all the OTUs detected were considered to be of interest in this study.

After assigning OTUs to functional groups using FUNGuild (Nguyen et al., 2016) and correcting ambiguous functional groups, we assigned some additional categories to the ectomycorrhizal (ECM) fungi, following criteria used by Geml (2019), and data published by Agerer (2006), Tedersoo & Smith (2013), and the DEEMY database (http://deemy.de). Edible fungi detected in the samples were assessed using different specialized books, publications, webpages, and fungal guides to determine the commercial importance of the fungal communities in the study area.

2.4. Statistical analysis

After standardizing the environmental variables and transforming the OTU matrix using the Hellinger transformation method, detrended correspondence analyses (DCA) and canonical correspondence analysis (CCA) were used to analyze the fungal communities associated with the different treatments using CANOCO version 5.0 (Smilauer & Lepš, 2014). Non-linear variable analysis was used to determine the individual significance of each fungal community. After that, we used Bonferroni analysis by being more restrictive, and finally, we calculated the statistical significance of variable groups with treatments using a Monte Carlo permutation test (499 permutations). Proportional richness and relative abundance were compared across stands using one-way ANOVA. Data were scaled using R (R Core Team, 2019) when needed to normalize for ANOVA. Tukey's HSD was used to determine significant differences between means (P ≤ 0.05) among stands, and the Pearson test was used to analyze correlations among environmental variables and *B. edulis* relative abundance.

3. Results

3.1. Taxonomic composition of fungal communities

A total of 2225 fungal OTUs were classified into 13 phyla and 329 genera (Figure 1). Although most of the OTUs were identified down to the genus level, it was not possible to identify all OTUs down to the species level because of database limitations. Based on the identified OTUs, the soil fungal community appeared to be dominated by Ascomycota (54%), which were mainly represented by Agaricomycetes (22% of the total OTUs identified) and Basidiomycota (27%), which were mainly represented by Leotiomycetes (21% of the total OTUs identified). In total, 20% of the total OTUs were unclassified: 5% of OTUs were identified only as members of the Fungi kingdom, and 15% of OTUs could not be identified using database searches.

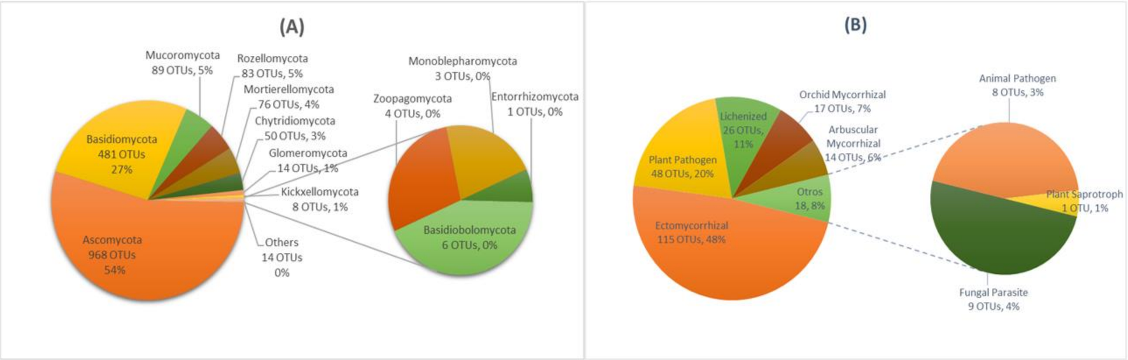

Figure 1. Relative proportions of fungal operational taxonomic units (OTUs): (A) taxonomical classification at the phylum level (name of phylum; number of OTUs; percentage); (B) classification of guilds (guild; number of OTUs; percentage) based on a FUNGuild (**www.funguild.org**) search and manual checking of unrecognized groups.

3.2. Effect of fire prevention treatments on fungal richness and abundance

As already mentioned, Ascomycota was the most abundant group present in soil samples collected from burned, cleared and control plots (Figure 2A); however, their richness was significantly lower in burned and cleared plots than in control plots (P = 0.026 and P = 0.088, respectively). Mortierellomycota richness was significantly lower in burned plots than in cleared plots (P = 0.026), and Zoopagomycota richness was lower in burned plots than in control plots (P = 0.018); however, the rest of the phyla did not significantly differ in richness in different treatment plots. In terms of relative abundance (Figure 2B), Ascomycota levels in cleared plots were significantly lower than those in control plots (P = 0.022); however, although the relative abundance in burned plots tended to be lower than that of control plots, no significant differences were found. The relative abundance levels of the Rozellomycota phylum in the burned and cleared plots were similar to each other but were significantly lower than that in the control plots (P = 0.029 and P = 0.075, respectively). The relative abundance of Zoopagomycota in burned plots was significantly lower than that in control plots, indicating that the prescribed burning treatment had a significant effect on the abundance of those phyla (P = 0.018), whereas the relative abundance of Calcarisporiellomycota in burned plots was higher than that in the control plots (P = 0.053). The relative abundance of other phyla were not significantly different in the different plots, although the relative abundance of Basidiomycota tended to be lower in burned plots than in cleared or control plots.

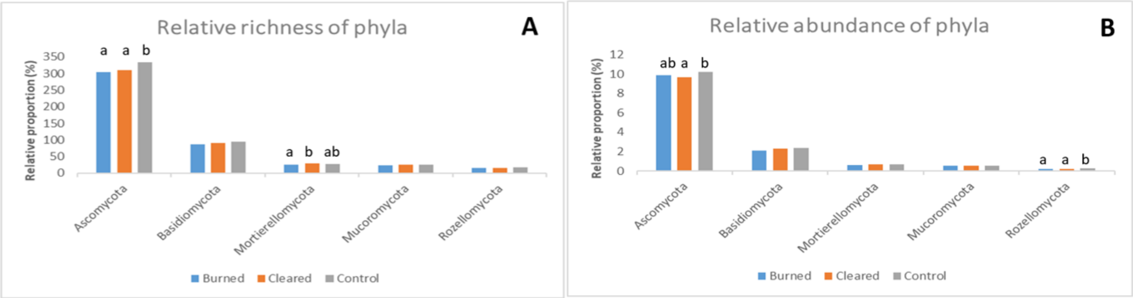

Figure 2. Fungal phyla proportional richness (A) and relative abundance (B) in soil samples collected 9 years after scrublands were subjected to prescribed burning or clearing by mechanical shredding treatments. Different lowercase letters above bars indicate a significant difference between treatments ($P < 0.05$).

Interestingly, although highly appreciated species such as *Boletus edulis* and *B. reticulatus* only comprised a small proportion of the total edible fungi detected, their proportional richness and relative abundance did not appear to be affected burned and cleared plots when compared to the control plots (Table 2).

Table 2. Mean proportional richness and relative abundance of different edible fungi detected in soil samples collected 9 years after scrublands were subjected to prescribed burning or clearing by mechanical shredding treatments.

	Proportional richness				Relative abundance			
	Burned	Cleared	Control	F and P values	Burned	Cleared	Control	F and P values
Total edible fungi	6.250	5.833	5.083	F = 0.369 P = 0.694	0.102	0.109	0.090	F = 0.225 P = 0.800
Boletus edulis	0.250	0.167	0.083	F = 0.569 P = 0.572	0.004	0.012	0.004	F = 0.396 P = 0.676
Boletus reticulatus	0.000	0.000	0.083	F = 1 P = 0.379	0.000	0.000	0.001	F = 1 P = 0.379
Boletus grouped	0.500	0.333	0.333	F = 0.164 P = 0.849	0.009	0.023	0.010	F = 0.368 P = 0.695

An analysis of functional groups revealed that the relative richness was significantly lower in the burned and cleared plots than in the control plots in fungal parasites ($P < 0.001$ and $P = 0.005$), lichenized fungi ($P = 0.004$ and $P < 0.001$), and saprotrophs (Figure 3A); relative richness were lower also for plant pathogens in cleared plots, whereas the relative richness of the ECM guild in cleared plots was significantly greater than that in control plots (Figure 3A). No significant differences in the relative richness of other guilds in different plots were observed. The relative abundance distribution of different guilds (Figure 3B) was similar to those observed for richness. The relative abundance levels of fungal parasites ($P < 0.001$), lichenized fungi ($P = 0.003$) and saprotrophs (Figure 3B) were lower in burned plots than in control plots, whereas the relative abundance of ECM fungi was greater in cleared plots than in control plots, as was the case for richness.

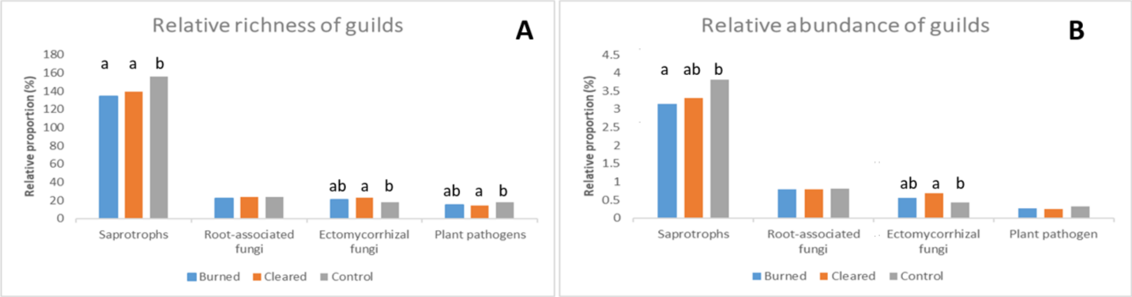

Figure 3. Proportional richness (A) and relative abundance (B) of guilds detected in soil samples collected 9 years after scrublands were subjected to prescribed burning or mechanical shredding treatments. Different lowercase letters above bars indicate a significant difference between treatments (P < 0.05). Different lowercase letters above bars indicate a significant difference between treatments (*P* < 0.05).

Moreover, the relative richness of ECM fungi with short-distance exploratory hyphae was higher in cleared plots than in control plots (P = 0.031); however, the relative richness of ECM fungi with long-distance exploratory hyphae was not significantly different in different plots (Figure 4A). The relative abundance of ECM fungi (Figure 4B), both short- and long-distance exploration types, was significantly higher in cleared plots than in control plots; however, the relative abundance in burned plots was not significantly different from that in the control plots.

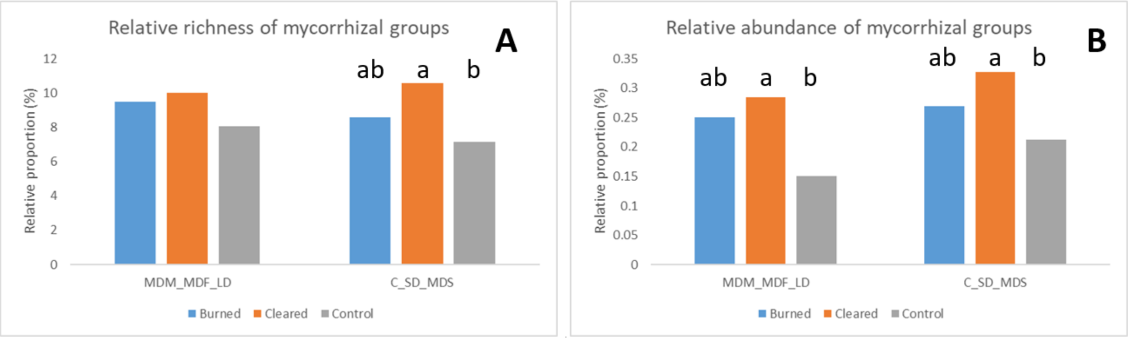

Figure 4. Proportional richness (A) and relative abundance (B) of mycorrhizal groups detected in soil samples collected 9 years after scrublands were subjected to prescribed burning or mechanical shredding treatments. Mycorrhizal exploration types: C_SD_MDS, contact/short-distance/medium-distance smooth with hydrophilic hyphae; MDM_MDF_LD, medium-distance mat/medium-distance fringe/long-distance with hydrophobic hyphae. Different lowercase letters above bars indicate a significant difference between treatments (P < 0.05). Different lowercase letters above bars indicate a significant difference between treatments (P < 0.05).

The richness of the general fungal community was significantly lower in burned plots than in control plots (P = 0.027), whereas the richness in cleared plots was not-significantly different to that of the control plots (P = 0.231).

3.3. Ecological factors driving fungal community composition and edible fungi

DCA analysis of DCA analysis of fungal communities did not reveal significant differences between treatment plots when grouped by phyla (Figure 5A). Most of the phyla were homogeneously distributed among the different treatment plots. However, CCA analysis revealed that soil fertility (C and N, because although N is not shown in the CCA, the direction and magnitude of C and N in the DCA indicate their influence on the distribution of fungal phyla) and pH influenced the distribution of some phyla (Figure 5B).

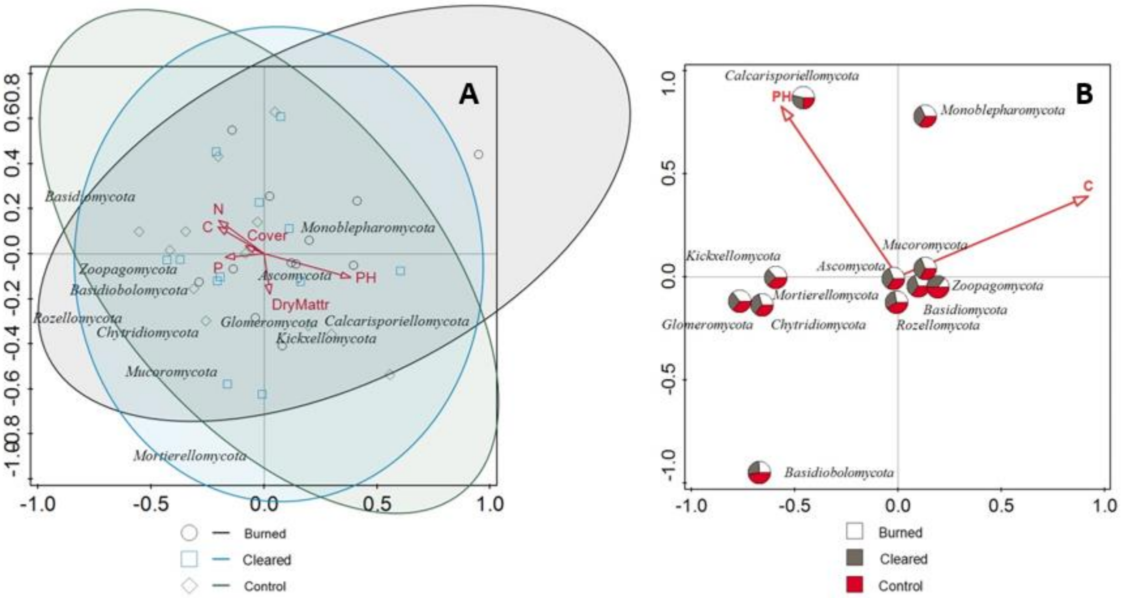

Figure 5. Detrended correspondence analysis (A) and canonical correlation analysis (B) of phyla across three different types of plot (burned, cleared, and control).

The higher relative richness and relative abundance values of Calcarisporiellomycota were associated with soils with a high pH content, with almost 50% of reads detected in burned plots, whereas Basidiobolomycota were more abundant and richer in control plots, which had significantly lower soil fertility than cleared plots (Table 1; Figure 6). However, in both cases, differences between treatments were not statistically significant.

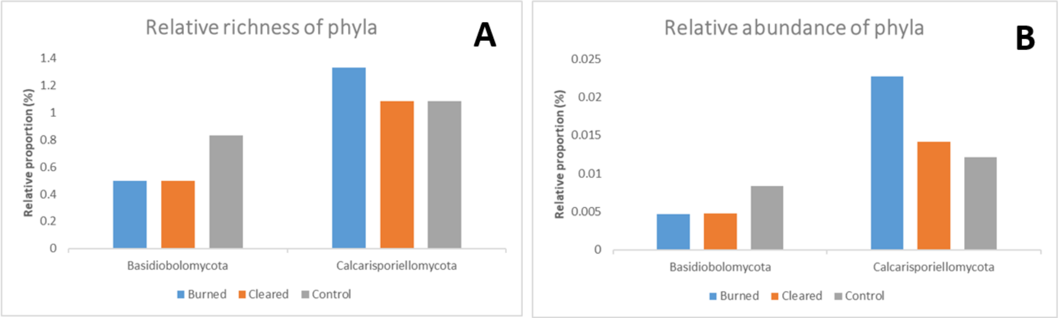

Figure 6. Proportional richness (A) and relative abundance (B) of fungal phyla affected by environmental variables.

Analyses of the functional guilds present in the different plots revealed that the fire prevention treatments had a clear influence on the fungal communities present in the soil. DCA analysis showed that burned and control plots were differently grouped, and cleared plots were placed between them (Figure 7A). CCA analysis revealed that soil fertility had an important influence on fungal communities, revealing a separated distribution tendency of ECM fungi that was associated with both fire-prevention treatments, and that lichenized fungi, saprotrophic, and fungal parasite guilds were associated with control plots (Figure 7B).

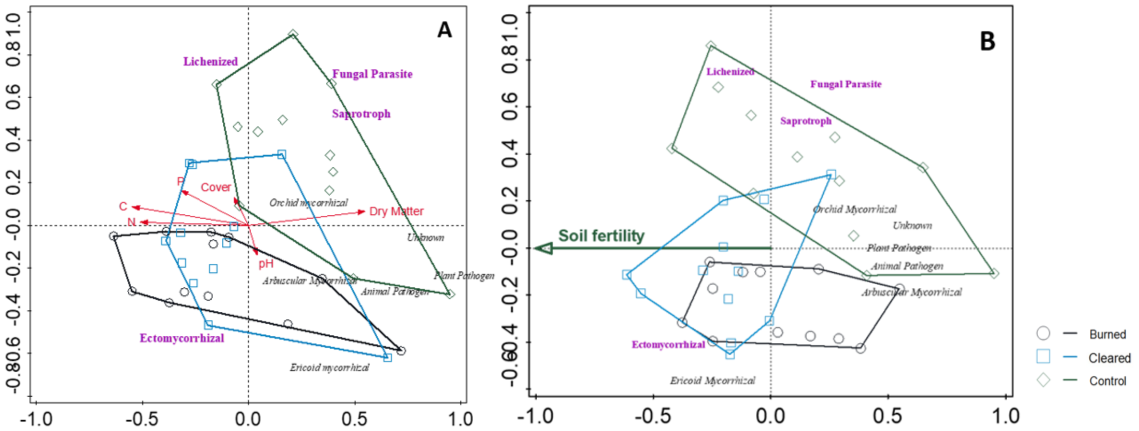

Figure 7. Detrended Correspondence Analysis (DCA) (A) and Canonical Correlation Analysis (CCA) (B) of fungal guilds across three different types of plot (burned, cleared, control)

Although edible OTUs did not appear to be significantly associated with a particular treatment plot, CCA analysis revealed a distribution between treatments that was influenced by the pH gradient associated with axis 1 (Figure 8). Although most of the edible fungi were detected throughout the three treatment plots, the *Boletus* group and *B. edulis* tended to be detected more frequently in cleared plots.

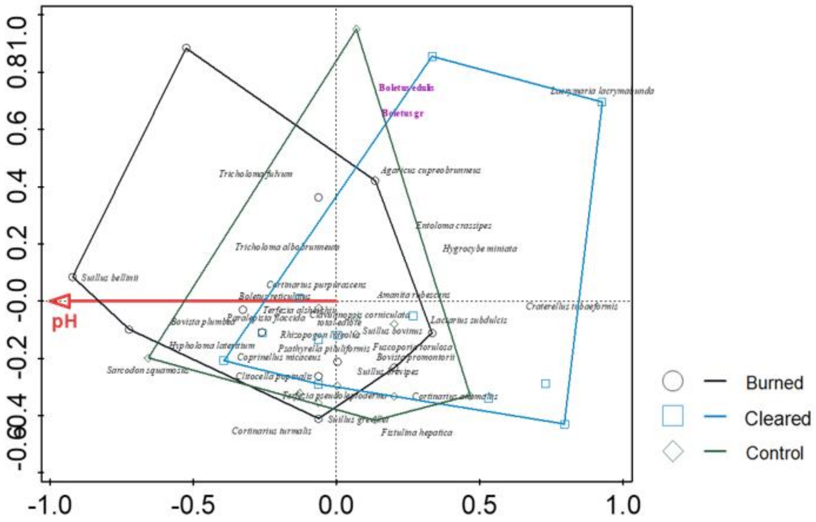

Figure 8. Canonical Correlation Analysis (CCA) of edible fungal communities across three different types of plot (burned, cleared, control)

The pioneer/invasive behavior of *H. lasianthum*, which was one of the main plant species growing in the study area, correlated positively with total vegetation cover (Figure 9). A strong relationship was found between the C and N content of soil, which was expected owing to the close relationship of these elements as fertilizers. A significant negative relationship was established between dry matter and the C, N and P content of soil, and a weak positive relationship was detected between dry matter and pH. Even if we included *B. edulis* in this analysis, the abundance of this species was not significantly correlated with the other studied variables, although their presence was more closely associated with soil fertility and vegetation variables than with pH and dry matter content.

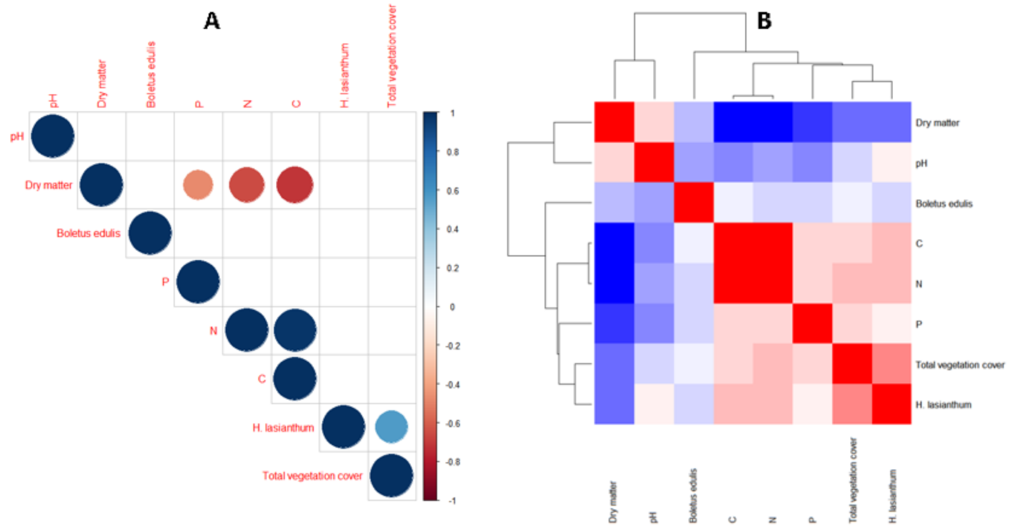

Figure 9. Pearson correlations among soil chemical properties, vegetation cover and *Boletus edulis* abundance (A) and analysis of clustered correlated variables (B). Blue and red colors indicate positive and negative correlations, respectively. Empty cells indicate no significant correlation.

4. Discussion

In this study, we analyzed soil fungal communities in scrubland 9 years after prescribed burning or mechanical shredding had been undertaken. Our three main hypotheses appeared to be correct. Hypothesis 1: we found that appreciable edible and marketable species were found in this ecosystem, which had previously been considered unproductive, and fire prevention treatments did not appear to have affected their richness, abundance, or composition. Hypothesis 2: 9 years after the fire prevention treatments were carried out, the richness of ECM fungi had fully recovered and did not appear to have been negatively affected by the fire prevention treatments, consistent with the effect of the fire prevention treatments on C and N. Hypothesis 3: soil fertility was the main driver explaining the fungal community composition, mainly that of the ECM community.

4.1. Effect of fire prevention treatments on fungal richness and abundance

Ascomycota was the most species rich and abundant phylum detected in our study, which agrees with the findings of many studies of different ecosystems (Castaño et al., 2020; Egidi et al., 2019; Geml et al., 2014). Their dominance can be explained by their species richness (Alem et al., 2020a). However, both the richness and abundance of Ascomycota appeared to be reduced by cleared plots, whereas burned plots only appeared to reduce richness. These findings were also observed for the Zoopagomycota phylum. Differences between treatments can be linked to a higher bulk root biomass in control plots than in the fire prevention treatment plots, as Solly et al. (2017) reported in conifer stands, who also found a high dependence between species-specific associations and local host species.

Interestingly, we found some highly appreciated edible species in the study area, such as *B. edulis* and *B. reticulatus*. The fire prevention treatments that had been undertaken 9 years previously had not had a negative effect on the presence of these taxa in terms of richness and abundance or on their potential fructification. Indeed, the composition of edible species was not significantly different in different treatment plots. Studies focused on fruiting bodies have reported a significant reduction in the abundance of fruiting bodies just after fires (Hernández-Rodríguez et al., 2015b; Martín-Pinto et al., 2006; Oria-De-Rueda et al., 2008; Vásquez-Gassibe et al., 2016). However, after a few years, edible fungi appear to be fully recovered and, indeed, production levels can even be higher than those of senescent untreated scrublands (Franco-Manchón et al., 2019; Hernández-Rodríguez et al., 2015b; Mediavilla et al., 2014; Oria-De-Rueda et al., 2008). Our results suggest that following mechanical shredding or prescribed burning, the edible fungal population had recovered well. Even though *B. edulis* is normally linked to late-stage forest stands (Martín-Pinto et al., 2006), when this species forms an association with *Cistus ladanifer* (similar to *H. lasianthum),* fruiting bodies develop soon after (Hernández-Rodríguez et al., 2013). According to Hernández-Rodríguez et al. (2015a), maximum yields of *B. edulis* are found in 14-year-old scrublands of *C. ladanifer*, and high yields are produced between 8 and 20 years after disturbances. Furthermore, considerable yields of *B. edulis* and *B. aereus* are produced in these ecosystems in early (4–6 years) and mature growth (7–17 years) systems that have recovered following a fire (Oria-De-Rueda et al., 2008), which strengthens the view that this fungal group is unaffected by plot type.

The behavior of ECM fungi, both short- and long-distance exploratory types, was different from that of saprotrophs. These fungi were expected to suffer a decrease in their populations in the years following the fire-prevention treatments, followed by a recovery of their populations after some time (Hernández-Rodríguez, 2017) due to the high level of photosynthetic activity and rapid development of young scrublands (Hernández-Rodríguez et al., 2015). The speed of this recovery following a fire is linked to the intensity of the fire (Salo et al., 2019). In our study, 9-years- after the fire prevention treatments were carried out, we found that the communities present in cleared and burned plots had been restored, and that their richness and abundance levels were higher than those of the control. Previous studies that have focused on the production of sporocarps in *C. ladanifer* scrublands have reported that the production levels of mycorrhizal fungi peaked 8 years after the disturbance, followed by a decrease owing to the lower photosynthetic activity levels of older scrubland (Hernández-Rodríguez et al., 2015) and fewer interactions between fungi and hosts (Alem et al., 2020a; Castaño et al., 2018a). This suggests that ECM fruiting body production would have been close to peak levels when we analyzed the soil samples in our study, so a reduction in their populations would be expected in the years following our sampling. Furthermore, the richness of ECMs in the control and burned plots were not significantly different, which is consistent with the finding that the C and N levels in soil samples collected from these plots were not significantly different. Previous studies by Fernández et al. (2013a and c) in the study area showed that prescribed burning reduces the soil organic layer, particularly when high temperatures are reached in this stratum, but has less effect on the physicochemical properties of mineral soil (Fontúrbel et al., 2016), with no noticeable effects three years after treatment. Thus, the rapid colonization of burned plots by ECM could also be linked to lower fire severities and the more limited depth of burning in the soil profile during prescribed burning than with wildfire (Alem et al., 2020a), and the influence of the host plant response to fire (Alem et al., 2020a), which in this study was dominated by pyrophytic hosts (Oria-De-Rueda et al., 2008). As already mentioned, no differences were observed between short-distance and long-distance exploratory types of ECM, with both of these ECM groups showing the same levels of abundance and richness as ECM fungi in general. Geml (2019) has suggested that differences between these two ECM groups are linked to rooting density, with high root density and high abundance levels of short-distance ECM found in mature stands. However, at our study site, the scrubland rooting conditions are different, with each plant closer to its neighbors than those in mature stands, which creates a different root ecosystem structure. Furthermore, host density at the time of sampling all plots was almost 100%, therefore, any differences between plots that may have been present in younger ecosystems were not detected.

We detected lower levels of saprotroph richness and abundance in the fire prevention plots than in the control plots. This finding was expected because previous studies of sporocarps have shown an increase in the saprotroph population just after a disturbance while the vegetation and symbiont fungi are recovering, followed by a reduction in saprotroph abundance over time. A decrease in the saprotroph population has previously been observed in *C. ladanifer* scrublands at a molecular (Castaño et al., 2020) and sporocarp (Hernández-Rodríguez et al., 2015b) level, as well as in *Pinus nigra* stands (Mediavilla et al., 2014), where the development of vegetation and the restoration of competitor communities were linked to reductions in saprotrophic fungi. Moreover, the same effect was found by Chen et al. (2019) following disturbance caused by logging. Like the saprotrophs, fungal parasites and lichenized fungi also tended to be more abundant in control plots than in the fire prevention plots. The common factor in all these groups is a relationship with other organisms (i.e., other fungi, algae, or plants), which is likely to be affected by the fire-prevention treatments, which could explain our findings.

Overall, we observed that fungal richness in burned plots was lower than that in cleared or control plots, whereas no differences were found between cleared and control plots. These findings match those reported by Castaño et al. (2020) and Martín-Pinto et al. (2006) for *Cistaceae* ecosystems. With regards to the effects of the prescribed burning treatment on soil fungal communities, Martín-Pinto et al. (2006) noticed losses in fungal richness linked to a reduction in the vegetal richness after fire as well as damage to the viability and dormancy of fungal spores, adding to the direct damage of the fire. In this study, we found an increase in pH on burned plots comparing to the other ones, which was mainly associated with the reduction in fungal richness, as many authors have found before (Day et al., 2019). Moreover, Tedersoo et al. (2020) noticed that pH was the main driver of fungal diversity, structure, and richness, reinforcing the relationship between pH and fungal diversity. Furthermore, burned plots had lower P contents than cleared plots, which supports the findings of Alem et al. (2020b), who found that P explained 19% of the ECM variability in *Pinus patula* plantations.

4.2. Ecological factors driving fungal community composition and edible fungi production

The composition of the soil fungal communities in plots that had been burned or cleared 9 years previously was not significantly different from that of the control plots, indicating that these communities had fully recovered from the fire prevention treatments. This could be related to the vegetation dynamic in the study area, which is mostly dominated by *H. lasianthum*, a rapid colonizer and pyrophytic species belonging to the *Cistaceae* family (Oria-De-Rueda et al., 2008). Previous studies carried out in the same area reported no differences in plant mortality and resprouted shoots between treatments (Fernández et al., 2013b); no differences in vegetation cover and recovery, or even in species richness, diversity, and evenness (Fernández et al., 2015), in concordance with previous studies (Fernández & Vega, 2014); and even the stimulation of seed germination by fire (Fernández et al., 2013a). Furthermore, the dominance of *H. lasianthum* was confirmed in our study by the relationship between vegetation cover and the presence of *H. lasianthum*. The rapid establishment of *H. lasianthum* after a disturbance, due to the persistent seed bank in the soil (Oria-De-Rueda et al., 2008) and the rapid recovery of the pretreatment ecological conditions, led to the development of a fungal community that was not significantly different from that of the untreated plots.

The composition of phyla was mainly affected by pH and soil fertility (C and N content), although only Calcarisporiellomycota and Basidiobolomycota were significantly affected. Although pH can affect fungal diversity (Tedersoo et al., 2020), the small but significant difference in pH in the burned plots compared with the cleared plots does not seem to have affected the general distribution of fungal communities although it may have been sufficient to have an effect on the distribution of the Calcarisporiellomycota phyla. With regard to soil fertility, significantly lower C and N values were obtained in control plots than in cleared plots. However, these differences in soil fertility were small, and the low fertility of the study area *per se* is likely to have had a greater influence on fungal community composition than the small differences in C and N between treatments.

The community composition of ECM fungi was driven by soil fertility and was mainly correlated with higher C and N levels. ECM fungi were more abundant in cleared plots, which had significantly higher C and N values than control plots, which is contrary to the findings of previous studies (Alem et al., 2020A; Castaño et al., 2020). However, this may be because differences in C and N levels between treatments were small and the disturbance created by the clearance treatment 9 years earlier had a stronger influence on ECM fungal community development than C and N.

Conclusions

Mediterranean ecosystems have traditionally been considered unproductive in terms of their ecological and economic values; however, we detected a high diversity of fungal species, including some edible and highly appreciated species such a *B. edulis*. Due to the high combustibility of these ecosystems, they are strongly affected by recurrent fires. In this context, specific management is needed that focuses on the prevention of forest fires or on reducing their severity in these systems. This strategy could maintain or even increase the ecological and economic values of these ecosystems by promoting the production or collection of edible fungal species. Our results showed that mechanical shredding and prescribed burning are compatible with these objectives because they did not have an adverse effect on the diversity of the fungal community or on the presence of edible species. Several ecological factors drive these results, including the rapid recovery of the pretreatment vegetation, enabling similar fungal communities to develop after fire prevention treatments have been undertaken. Furthermore, soil parameters such as C, N, and P, had no significant effect on ECM richness following prescribed burning, including edible species.

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