

## Anthropological impacts determine the soil fungal distribution of Mediterranean oak stands

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### ABSTRACT

*Quercus pyrenaica*-dominated forests are very widely distributed in Mediterranean ecosystems. Traditional forest use, such as coppicing to obtain firewood or livestock grazing under silvopastoral systems, and the current social abandonment of the rural environment have given rise to forest structures of different ages and at different stages of development. Thus, on the one hand, there are large areas of *Q. pyrenaica* coppice systems that produce a large amount of biomass that have a very high risk of driving forest fires. On the other hand, *dehesas*, which have very low tree density and are composed of very old trees that are susceptible to different types of environmental stress and have serious regeneration problems and a weak phytosanitary status. In addition, previous studies have suggested that the production of economically valuable edible mushrooms is negatively impacted by silvicultural management. To determine the effects of land management on these ecosystems, we analyzed the soil fungal communities associated with coppice stands (i.e., high-density coppice), high forest stands (i.e., low-density coppice that received silvicultural management 15 years ago to reduce the risk of wildfire), and old stands (i.e., *dehesas*) to assess their potential ecological roles in their conservation and the diversity of edible mushrooms. We also analyzed the edaphic variables associated with these systems (carbon, pH and the carbon/nitrogen ratio) to understand the dynamics of these fungal communities. We observed two distinguishable communities: pathogen-, parasite-, and endophyte-dominated *dehesas* and saprotroph- and ectomycorrhizal (ECM)-dominated coppice stands, with a mixed composition in high forest stands. ECM fungi correlated with stand age and structure, showing higher richness levels in high forest stands, particularly ECM fungi with short hyphal exploration type. Finally, the influence of stand age and structure due to land management significantly affected the variety of some edible genera, such as *Boletus*, *Tuber* or *Terfezia*.

### 1. Introduction

Forests dominated by *Quercus pyrenaica* in Mediterranean areas have historically been used as a source of firewood or for grazing livestock (Moreno-Fernández et al., 2021). As a result, these forests structure have developed in different ways in terms of their structure, depending on the type of use (Praeg et al., 2020; Tárrega et al., 2009). In those areas that have been used as a source of firewood, this practice has given rise to large areas of forest stands that are coppice systems dominated by even-age trees. As a result, an intervention is now necessary to reduce the density of these stands and to transform their structure. This is partly due to the danger that the coppice structure represents in the face of very frequent forest fires in these Mediterranean ecosystems (Tárrega et al.,

2009; Tomao et al., 2020). These systems are characterized by a high load of available fuel and the occurrence of very severe forest fires. This promotes the development of scrubland and the generation of aging forest stands that are highly sensitive to environmental abiotic and biotic stress (Hernández-Rodríguez et al., 2015a; Santos-Silva et al., 2011; Sanz-Benito et al., 2022). *Q. pyrenaica* systems that have been subjected to livestock grazing activity are also prevalent in this area, resulting in the creation of extensive *dehesas*, a multifunctional, agrosilvopastoral system. *Dehesas* are characterized by very low tree densities and by the very intensive use of their pastures for grazing, mainly by cattle and sheep (García Jiménez et al., 2020). Although there is no danger of severe fires in *dehesas*, the intensive grazing activity in these systems may be leading to a loss of diversity (Ling et al., 2020). A great diversity of

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fungus species is known to be associated with these forests.

These fungi play a fundamental role in forest systems as responsible of the forest health maintenance. From the development of symbiotic mycorrhizal relationships based on the uptake of nutrients to the plant or conferring resistance against pathogens especially at the root level (Bzdyk et al., 2019; Chagnon et al., 2013; Hannula & Träger, 2020; Viveló & Bhatnagar, 2019). Induce aggregation of soil particles, improving soil aeration and porosity (Ryan & Kirkegaard, 2012). Saprotrophic fungi are responsible of the dead matter transformation and, therefore, the recycling of nutrients in the ecosystems (Alem et al., 2020). Some of the fungal species associated with these systems are highly economically valuable, such as *Boletus edulis*, providing additional income to local populations that inhabit these areas of low productivity (Oria-De-Rueda et al., 2008). The effect that ecosystem management, which is mainly focused on fire prevention in the coppice systems, and the use of pastures in the *dehesa* systems, has on the associated soil fungal community therefore needs to be assessed (García Jiménez et al., 2020; Hernández-Rodríguez et al., 2017; Tárrega et al., 2009). Learning about this associated community can help us to know the already existing diversity associated to our forests and how can be changed by different impacts from early disturbed areas to long-time altered systems. Consequently, this study could provide essential insights in order to develop an adequate forest management in these areas where the mycological resources are important.

In this study, we studied the fungal communities in differently altered and managed *Quercus pyrenaica* ecosystem. The obtained results could serve as an example for other similar forests widely distributed in the Mediterranean Region, specially due to the focus over the relationship with fuel reduction, farming and fungal community.

We hypothesized that the fungal diversity associated with these ecosystems could be maintained by the implementation of sustainable management practices (Mediavilla et al., 2019; Praeg et al., 2020; Schoenbaum et al., 2018; Tomao et al., 2020). We expect that these management actions and land uses will have a different effect depending on the taxonomic group and, particularly, functional groups (Hannula & Träger, 2020; Marín & Kohout, 2021). Thus, mycorrhizal fungi will tend to be more abundant in mature forests, which are characterized by less fertile soils and a dense canopy (Boeraeve et al., 2018; Carteron et al., 2021; Castaño et al., 2019; Cheeke et al., 2016). A similar trend is expected for short-distance mycorrhizal fungi. These will dominate in mature forests with a high density of trees, whereas long-distance exploration types would be widely spread over stands with a low tree density (Geml, 2019). However, saprophytic fungi, which are more dependent on environmental conditions than on the host, would be more prevalent in coppice stands and grazing areas given that both are characterized by a high level of organic matter and nutrient inputs due to animal dung and the presence of litter from annual plant species (Ye et al., 2020; Zhang et al., 2018).

We also hypothesize that functional groups, such as animal parasites, will be associated with the greater presence of cattle in the *dehesas*. Moreover, plant pathogenic fungi are also likely to be associated with these old *dehesas* given that they are known to be very old and highly susceptible to biotic and abiotic stresses (Bai et al., 2019; Castaño et al., 2019; Ruiz Gómez et al., 2019). Thus, we also hypothesize that there will be an effect of management on the presence and abundance of some edible species, such as the highly valued *Boletus edulis* (Martín-Pinto et al., 2006; Mediavilla et al., 2017). This type of forest is seriously threatened in all Mediterranean countries (Tárrega et al., 2009), so we hope that this type of study will lead to a better understanding and management of this type of ecosystem.

To test and verify these hypotheses, we analyzed the fungal communities present in the soil in forest stands dominated by *Q. pyrenaica* under three different management regimes: 1) a high-density coppice system that had not received any fire-prevention treatment; 2) a mature forest that had been coppiced in the past and received fire-prevention treatments in the last 15 years to decrease the density of the stand;

and 3) a grazed, open woodland *dehesa* system. Thus, the specific objectives of this study were to evaluate the possible effects of land management on the diversity and community composition of various functional groups of fungi and to assess the effect of land management on the diversity and distribution of edible fungi among the sampled habitats.

## 2. Materials and methods

### 2.1. Study area and sampling design

The study site was located between the Culebra mountain range (NW–SE orientation), Spain and the border of Portugal (Fig. 1), in order to avoid the influence of climate conditions in the mountains.

The study area is mainly composed of Palaeozoic metamorphic rocks (Ordovician and Silurian shales) and plutonic rocks (granite and Armorican quartzite landforms) (IGME, 2019). The area is characterized by a sub-Mediterranean climate (with a dry season of three months in the summer), a mean annual precipitation of 450–700 mm (most of the annual precipitation falls between October and February) and temperatures ranging from 14.5 to 15.8 °C (Hernández-Rodríguez et al., 2013).

Climax forests are mainly represented by *Q. pyrenaica*-dominated woodland (Quercion pyrenaicae; Natura 2000 code 9230) and, to a lesser extent, by *Quercus rotundifolia* woodland (Quercion broteroi; Natura 2000 code 9340). In addition, plantations of maritime pine (*Pinus pinaster*) and chestnut (*Castanea sativa*) are relatively common in the study area.

Coppice stands are the most prevalent type of *Q. pyrenaica* woodland in the study area, frequently developing after fire disturbance or as a result of coppicing to obtain firewood. In these single-species forests, there is frequently a lack of silvicultural management (of the coppiced forest), leading to a high stem density owing to the capacity of oak to regenerate by sprouting. Nonetheless, a significant proportion of this type of coppiced forest develops to form high forest stands of medium-tall trees (closed canopy timber wood) that are lower in density than the coppice, coppiced stands because they have received some silvicultural management in the past 15 years. Coppice stands were dominated by *Q. pyrenaica* with a cover close to 100% although, in some few open sites, punctual *Cistus ladanifer*, *Genista* sp. and *Calluna vulgaris* plants can be found. The canopy cover of the high forest stands is between 85 and 100%. In this case, understory vegetation mostly composed of *Cistus ladanifer* with some presence *Genista* sp. and *Calluna vulgaris* can be also observed. Mature-growth stands, are associated with silvopastoral use, resulting in an open woodland structure (*dehesa* type) of low-density large, old trees and pasture.

Thirty-six sampling sites were established in *Q. pyrenaica* woodland (Quercion pyrenaicae) in the region of Aliste, 50 km north-west of Zamora (longitude 6°15'–6°32' W, latitude 41°43'–41°45'N), in a study area of c. 50 km<sup>2</sup> (Fig. 1), with an east–west longitudinal gradient, and a mean elevation of 800 m (ranging from 590 to 890 m). The 36 sampling sites were distributed in three forested areas in regions surrounding Nuez, San Vitero and Rabanales (12 sampling sites per area).

The forests sampled in this study vary from subhumid (*Genisto falcatae-Quercetum pyrenaicae*) to humid (*Holco mollis-Quercetum pyrenaicae*) Mediterranean oak forests and comprise the three stand structures mentioned above: (1) high-density, coppice, coppiced stands – single-species forests lacking silvicultural management; (2) intermediate-sized, lower-density high forest stands – closed canopy timber wood that has received silvicultural intervention in the past 15 years; and (3) mature-growth stands with an open woodland structure (*dehesa*). From here onward, these three stand types are referred to as 'coppice', 'high forest', and 'dehesas' stands, respectively.

### 2.2. Sampling and molecular work

The fieldwork was conducted in March 2019. Woodland was selected

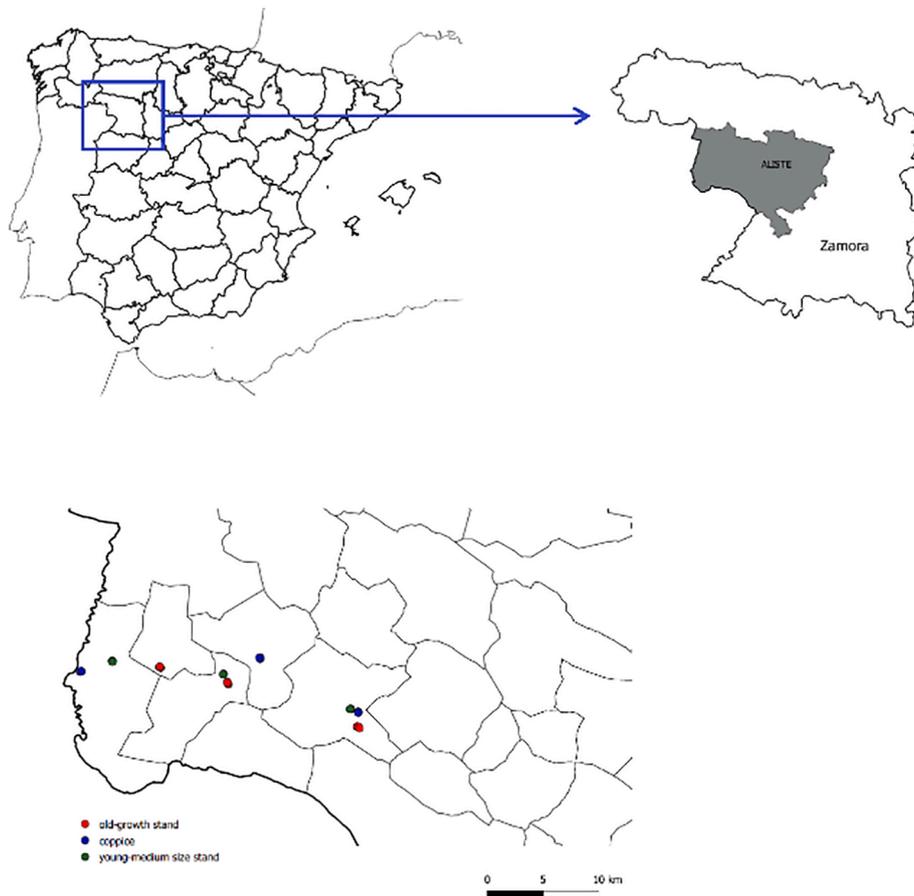


Fig. 1. A map of Spain showing the location of the study sites in Aliste in Zamora province (inset). Red: old growth stands (dehesas); blue: high forests stands; green: coppice stands. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

over a longitudinal gradient and over the three stand-structures stated. At each site (ca. 2500 m<sup>2</sup>), 25 soil cores (200 cm<sup>3</sup> each) of topsoil were taken from underneath the litter layer. We randomly selected five trees located at least 8 m apart. Loose debris was removed from opposite sides of each tree trunk at a distance of approximately 2 m from each trunk before taking five soil cores at least 2 m apart to minimize the probability of sampling the same genet repeatedly. These 25 cores were pooled (coarse roots and stones were removed) resulting in a composite soil sample for each site. A subset of each composite soil sample was dried at room temperature (20–25 °C) and sieved (mesh size 1 mm). Approximately 20 g of each composite sample was kept frozen in zip-lock plastic bags until DNA extraction. The rest of the sample was used for soil chemical analyses to measure pH (water-based), % dry matter, phosphorus (P), and the total carbon (C) and nitrogen (N) content following Sparks et al. (1996).

The ITS2 region (ca. 250 bp) of the nuclear ribosomal DNA repeat was PCR-amplified using primers fITS7 (Ihrmark et al., 2012) and ITS4 (White et al., 1990) and were appended with Illumina adaptors and sample-specific tags using the following amplification program: one cycle of 95 °C for 5 min; then 37 cycles of 95 °C for 20 s, 56 °C for 30 s and 72 °C for 1.5 min; ending with one cycle of 72 °C for 7 min. The amplicon library was sequenced by BaseClear B.V. company (Leiden, The Netherlands) using an Illumina MiSeq platform

### 2.3. Quality control and bioinformatic work

Raw sequence reads were obtained from the Illumina output, which comprised demultiplexed sample reads. The primers were removed, and poor-quality ends were trimmed off based on a quality cut-off threshold of 15, using Cutadapt, version 2.6 (Martin, 2011). Afterwards, sequences

were filtered using USEARCH v.11.0 (Edgar, 2010) using the following settings: all sequences were truncated to 200 bp and sequences with an expected error of > 1 were discarded. For each sample, the remaining sequences were collapsed into unique sequence types with USEARCH, while preserving their read counts. The quality-filtered sequences from all samples were grouped into OTUs at 97% sequence similarity and putative chimeric sequences (2888 chimeras) were removed using USEARCH. After discarding singleton sequence types, the resulting 88,736 high-quality sequences were grouped into 2198 operational taxonomic units (OTUs) with USEARCH at 97% sequence similarity using the UPARSE algorithm.

We assigned sequences to taxonomic groups based on pairwise similarity searches against the curated UNITE fungal ITS sequence database (UNITE Community, 2019), which contains identified fungal sequences with assignments to Species Hypothesis (SH) groups delimited based on dynamic sequence similarity thresholds (Köljalg et al., 2013). Assignment was performed using the PlutoF web workbench (<https://plutof.ut.ee>) (Abarenkov et al., 2010). OTUs with > 90% similarity to a fungal SH with known ecological function were assigned to functional groups according to Pölme et al. (2020). In order to further examine patterns of functional categories within ectomycorrhizal (ECM) fungi, we classified ECM fungal OTUs into two aggregate extramatrical mycelial exploration-type categories: contact/short-distance/medium-distance smooth with hydrophilic hyphae (C/SD/MDS) and medium-distance mat/medium-distance fringe/long-distance with hydrophobic hyphae (MDM/MDF/LD) following Agerer (2006) and Tedersoo & Smith (2013), and the DEEMY database (<http://deemy.de>). Besides morphological differences, these categories also shed light on the different nutrient-acquisition strategies used by ECM fungal OTUs (Hobbie & Agerer, 2010). Finally, fungal OTUs were classified as either

edible or non-edible based on appropriate bibliographical references (mainly Spanish studies of edible mushrooms in the study area; e.g., Hernández-Rodríguez, et al., 2015b).

### 2.4. Statistical analysis

We normalized the OTU table by rarefying the number of high-quality fungal sequences to the smallest library size (34,274 reads) to reduce differences in sequencing depth. The resulting matrix of 2091 OTUs was used for all subsequent statistical analyses. We also compared the total fungal richness and abundance of taxonomic and functional groups detected in the three stand structures using Linear Mixed Effects models (LME,  $p \leq 0.05$ ) that were developed using the package Nlme, (Pinheiro et al., 2016) and by performing Tukey's HSD test.

We used the *vegan* R package (Oksanen et al., 2020) to obtain the Hellinger-transformed OTU table and a secondary matrix containing environmental variables, which were standardized using the scale function in R. We used the *envfit* R function to assess relationships between the environmental variables and the fungal community structure

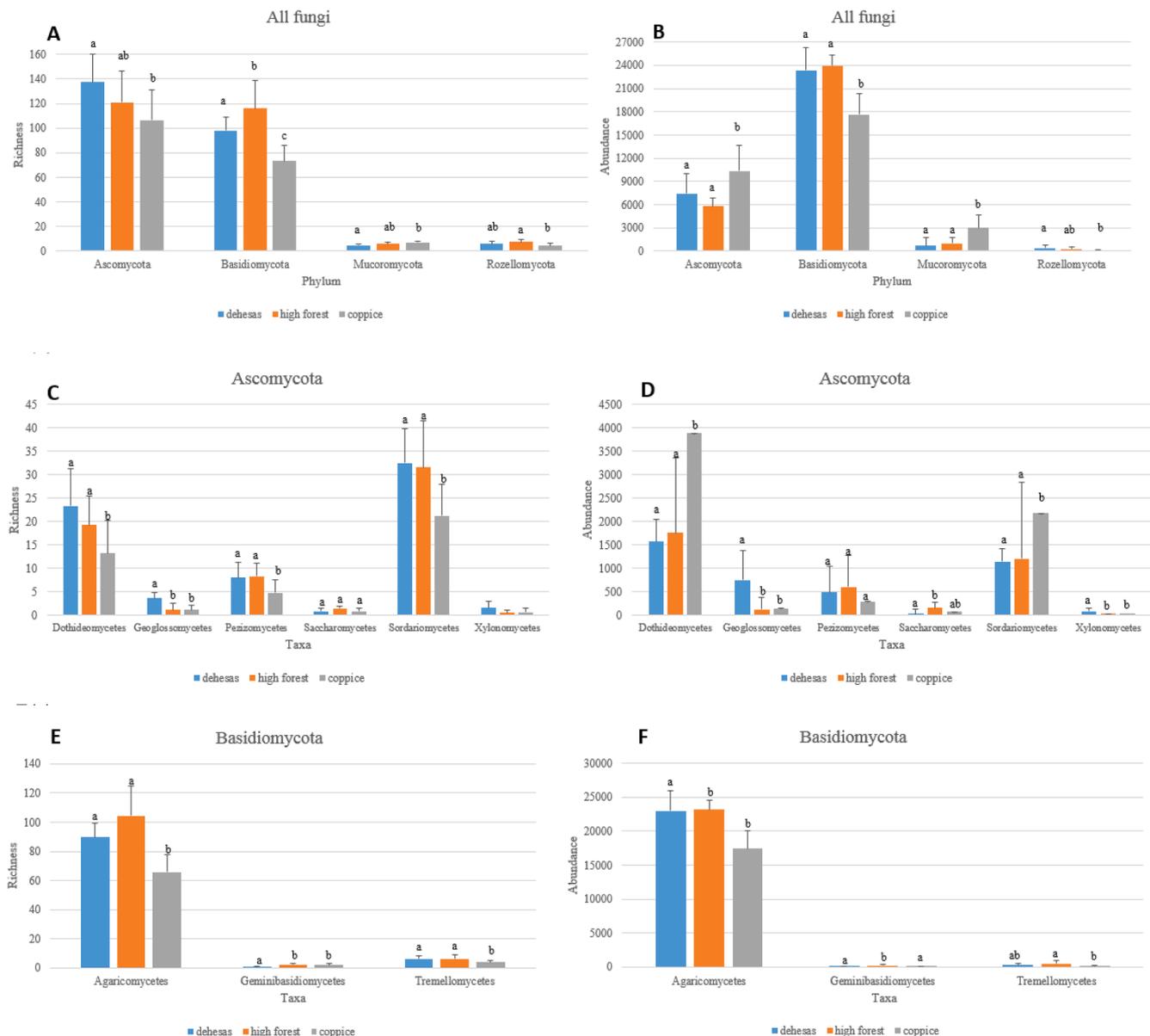
and to fit them onto the correspondence analysis plots. We implemented all these statistical analyses in the R software environment (R Development Core Team, 2015).

The ordination of community data was carried out using CANOCO version 5.0 to perform a Principal component Analysis (PCA), a Redundancy Analysis (RDA) and the Canonical Correspondence Analysis (CCA) (Smilauer & Lepš, 2014). Explanatory ecological variables were selected using a forward selection method and the significance of the environmental variables was tested by performing a Monte Carlo permutations test (999 permutations).

## 3. Results

### 3.1. Patterns of fungal richness and abundance

In total, the quality-filtered and rarefied dataset comprised 2091 OTUs. We obtained an average of  $41,062 \pm 9513$  reads at each site. Total fungal richness was relatively similar for all forest types, with mean per-plot OTU richness levels of  $257 \pm 96$ ; however, the OTU richness of



**Fig. 2.** Comparison of richness and abundance of all fungi (A and B respectively), Ascomycota (C, D respectively) and Basidiomycota (E, F respectively) for the three sampled forest stand structures. Means were compared using LME and Tukey's HSD tests, with different letters denoting significant differences.

coppice forest stands was lower than that of the other stands. In addition, there were significant differences among the taxonomic groups and functional groups with respect to their proportional richness and abundance in samples from the three forest types.

The following phyla showed significant differences in terms of richness among the three types of forest stand: Ascomycota, Basidiomycota, Calcarisporiellomycota, Chytridiomycota, Mucoromycota and Rozellomycota. The richness of these phyla was significantly lower ( $p < 0.05$ ) in coppice stands than in the other stand types whereas the richness of Calcarisporiellomycota was significantly lower in high forest stands ( $p = 0.05$ ) and the richness of Mucoromycota was significantly lower in the dehesas than in the other stand types. The proportional richness of Basidiomycota was highest in high forest stands, whereas the richness of Ascomycota was highest in dehesa stands (Fig. 2A). However, proportional abundance analyses revealed different patterns, with Basidiomycota significantly more abundant than Ascomycota even in coppice stands. Basidiomycota were most abundant in high forest stands. The highest proportional abundance of Ascomycota was detected in coppice stands. Rozellomycota showed it highest proportional abundance in dehesas (Fig. 2B).

The proportional richness and abundance of the Ascomycota, Basidiomycota, Mucoromycota, and Rozellomycota in the three different stand structures were compared (Fig. 2). The proportional richness of Ascomycota tended to be higher in dehesas and lower in coppice stands. Within the Ascomycota, the Dothideomycetes, Geoglossomycetes, Pezizomycetes and Sordariomycetes showed the most significant differences in terms of richness among the different stand types (Table 1).

Dothideomycetes and Sordariomycetes were the richest classes of Ascomycota. Their lowest levels of richness and that of Pezizomycetes ( $p < 0.02$ ) were detected in coppice forest stands, whereas the lowest levels of Geoglossomycete richness were detected in coppice and high forest stands ( $p < 0.009$ ). By contrast, the highest levels of Geoglossomycete richness, and that of Dothideomycetes ( $p < 0.07$ ) and Sordariomycetes ( $p < 0.02$ ) were found in dehesas, although richness levels were not significantly different to that of high forest stands. The highest richness levels of Pezizomycetes were found in high forest stands ( $p < 0.02$ ), although the richness was not significantly different to that of dehesas (Fig. 2C).

**Table 1**

*F* and *p* values for LME comparisons of Ascomycota, Basidiomycota taxa and functional guilds groups among *Quercus* stands of different ages and structure.

Ascomycota taxa	Richness		Abundance	
	<i>F</i>	<i>p</i> -value	<i>F</i>	<i>p</i> -value
Dothideomycetes	<b>7.793</b>	<b>0.0017</b>	<b>11.940</b>	<b>0.0001</b>
Geoglossomycetes	<b>7.161</b>	<b>0.0026</b>	<b>5.157</b>	<b>0.0112</b>
Pezizomycetes	<b>6.606</b>	<b>0.0039</b>	1.118	0.3390
Sordariomycetes	<b>6.663</b>	<b>0.0037</b>	<b>4.274</b>	<b>0.0223</b>
<b>Basidiomycota taxa</b>				
Agaricomycetes	<b>20.88</b>	<b><math>1.38 \times 10^{-6}</math></b>	<b>21.69</b>	<b><math>9.68 \times 10^{-7}</math></b>
Geminibasidiomycetes	<b>7.352</b>	<b>0.0023</b>	<b>8.184</b>	<b>0.0013</b>
Tremellomycetes	<b>6.117</b>	<b>0.0055</b>	<b>3.257</b>	<b>0.0512</b>
<b>Functional groups</b>				
Animal parasites	3.049	0.061	1.669	0.204
Arbuscular mycorrhizal	1.529	0.232	1.588	0.220
Dung saprotrophs	<b>16</b>	<b><math>1.39 \times 10^{-5}</math></b>	<b>5.837</b>	<b>0.007</b>
Ectomycorrhizal fungi	<b>23.95</b>	<b><math>3.75 \times 10^{-7}</math></b>	1.366	0.269
Foliar endophytes	8.25	0.001	<b>7.601</b>	<b>0.002</b>
Lichens	1.462	0.246	2.722	0.077
Litter saprotrophs	<b>3.3</b>	<b>0.049</b>	0.699	0.504
Mycoparasites	0.596	0.557	0.771	0.471
Plant pathogens	<b>9.673</b>	<b>0.00049</b>	<b>7.382</b>	<b>0.002</b>
Root endophytes	<b>12.7</b>	<b><math>8.1 \times 10^{-5}</math></b>	<b>10.02</b>	<b>0.000</b>
Soil saprotrophs	0.914	0.411	3.069	0.060
Undef-saprotrophs	0.416	0.663	<b>4.902</b>	<b>0.014</b>
Wood saprotrophs	<b>9.022</b>	<b>0.000</b>	2.261	0.120

Significant *F* and *P* values are shown in bold.

In terms of abundance, Dothideomycetes and Sordariomycetes were most abundant in coppice stands ( $p < 0.001$ ) and least abundant in dehesas, whereas Geoglossomycetes were more abundant in dehesas ( $p < 0.03$ ). By contrast, the proportional abundance of Pezizomycetes was not significantly different in the different stand types (Fig. 2D).

Among the Basidiomycota taxa, only the Agaricomycetes, Geminibasidiomycetes and Tremellomycetes differed significantly in terms of their richness among the different stand types (Table 1), with the greatest levels of richness detected in high forest stands. However, neither the richness levels of Agaricomycetes ( $p = 0.05$ ) and Tremellomycetes ( $p = 0.99$ ) in old and high forest stands nor the richness levels of Geminibasidiomycetes in high forest and coppice stands were significantly different ( $p = 0.69$ ) (Fig. 2E). In terms of proportional abundance, these three classes showed the same trend as that for richness, with the highest levels of abundance observed in high forest stands. In the case of Agaricomycetes, abundance was not significantly different to that in dehesas ( $p = 0.98$ ), whereas the abundance of Geminibasidiomycetes in the high forest stands was significantly different to that in the other stands ( $p < 0.02$ ); however, coppice and dehesas were not significantly different ( $p = 0.7$ ). In the case of Tremellomycetes, high forest stands were not significantly different to that of dehesas ( $p = 0.31$ ) (Fig. 2F).

In total, 1216 OTUs (58.18%) were unequivocally assigned to functional groups. Overall, ECM fungi (most of which were Basidiomycota) were the most abundant across the whole dataset, representing 48.4% of the community, followed by saprotrophs (dung, plant and soil saprotrophs, and other saprotrophs), which represented 34.9% of the community. The rest of the groups were far less abundant.

In terms of richness, six out of the thirteen functional groups showed significant differences (Table 1) among the three forest types. The richness levels of ECM fungi were significantly higher in high forest stands than in other stand types ( $p < 0.01$ ), lowest in coppice stands, and not significantly different to that of dehesas ( $p = 0.44$ ). Root endophytes ( $p < 0.01$ ) showed the same pattern of richness as ECM fungi in the three different stand types. Dung saprotrophs were richest in dehesas ( $p < 0.02$ ) whereas coppice stands had the lowest level of richness. Likewise, the richness of foliar endophytes ( $p < 0.01$ ) was lowest in coppice stands, although the richness levels of coppice and high forest stands were not significantly different ( $p = 0.12$ ). Plant pathogens ( $p < 0.01$ ) and wood saprotrophs ( $p < 0.01$ ) showed the same richness trend as foliar endophytes; however, no significant differences were observed between old and high forest stands. LME analysis of animal parasite richness did not reveal any significant differences in richness among the different stand types; however, a comparison of the proportional richness showed a clear difference between old and coppice stands ( $p < 0.05$ ), with coppice stands being the least rich (Fig. 3A).

Regarding abundance, just five of the guilds showed significant differences among the different stand types. Dung saprotrophs ( $p < 0.05$ ), foliar endophytes ( $p < 0.04$ ) and plant pathogens ( $p < 0.03$ ) showed the same trend as that for richness. Root endophytes were significantly more abundant in coppice forest than in high forest stands or dehesas ( $p < 0.01$ ), which were not significantly different ( $p = 0.99$ ). Soil saprotrophs, which were not significantly different in terms of richness, were significantly more abundant in coppice stands than in high forest stands ( $p = 0.04$ ) but the abundance of these stands was not significantly different to that of dehesas. Likewise, unspecified saprotrophs showed a similar trend; however, the abundance of high forest stands was not significantly different to that of old and coppice stands (Fig. 3B).

Only ECM fungi with C/SD/MDS exploration types (e.g., *Russula*, *Inocybeaceae*, and *Tomentella*) showed significant differences in their proportional richness among the three forest types (Table 2), with significantly higher levels in high forest stands ( $p < 0.01$ ) than in coppice and dehesas, which were also significantly different from each other ( $p < 0.05$ ) being richer in dehesas. MDM/MF/LD exploration types did not differ significantly in their richness among the different stand types but showed a decreasing trend with increasing stand age.

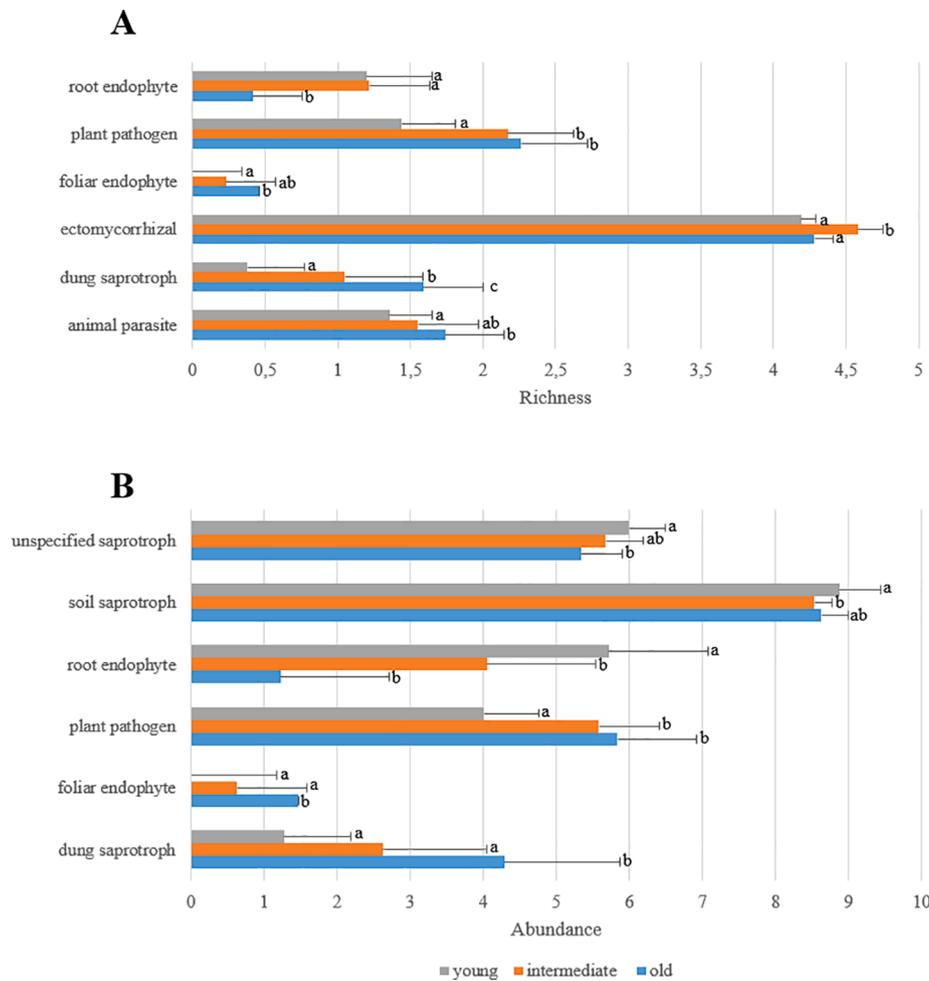


Fig. 3. Comparison of the proportional richness (A) and abundance (B) of functional groups across the three sampled forest types. Means were compared using LME and Tukey’s HSD tests, with different letters denoting significant differences.

Table 2

F and p values for LME comparisons of ECM exploration types among *Quercus* stands of different ages and structure.

ECM exploration types	Richness		Abundance	
	F	p-value	F	p-value
C/SD/MDS	<b>28.040</b>	<b>7.65 × 10<sup>-8</sup></b>	1.343	0.275
MDM/MDF/LD	3.721	0.0506	2.297	0.116
Mixed types	2.275	0.119	2.275	0.119

Significant F and P values are shown in bold.

However, the proportional abundance of the different ECM exploration types did not differ significantly among the different stand types.

Among the edible fungi, some specific genera differed significantly across the different stand types (Table 3). *Boletus*, *Choiromyces*, *Cortinarius*, *Terfezia* and *Xerocomellus* were much richer in coppice stands than in other stands, *Craterellus* and *Hygrophorus* were much richer in dehesas, and *Tuber* was richest in high forest stands (Fig. 4).

### 3.2. Effect of stand age and structure on fungal community composition

Fungal community composition analyses revealed three populations when defined in terms of functional guilds, with coppice stands showing a clear difference in community composition compared with those detected in high forest stands and dehesas, which shared some trophic guilds. Principal Component Analysis (PCA) (Fig. 5A) showed that the first axis explained 51.19% of the variation mainly responsible for the

Table 3

F and p values for LME comparisons of edible fungi among *Quercus* stands of different ages and structure.

Edible genera	Richness	
	F	p-value
<i>Boletus</i>	6.494	<b>0.0042</b>
<i>Choiromyces</i>	3.667	<b>0.0365</b>
<i>Cortinarius</i>	3.667	<b>0.0365</b>
<i>Craterellus</i>	3.371	<b>0.0465</b>
<i>Hygrophorus</i>	15.740	<b>1.58 × 10<sup>-5</sup></b>
<i>Terfezia</i>	4.569	<b>0.0177</b>
<i>Tuber</i>	7.857	<b>0.0016</b>
<i>Xerocomellus</i>	7.732	<b>0.0018</b>

Significant P values are shown in bold.

community composition. The variables related to the first axis were the C/N ratio and dry matter, which clearly defined the coppice stands, whereas pH and the amount of N in the soil defined old and high forest stands. The composition of coppice stands was clearly distinguishable, with the composition dominated primarily by saprotrophs, with a noticeable presence of root endophytes and ECM fungi. By contrast, the composition of dehesas was defined by a mixture of guilds, including parasites and pathogens, some saprotrophs, foliar endophytes and lichens. Finally, high forest stands showed a convergent community composed of guilds present in the other two stand types. The second axis was closely associated with the amount of soil C, which defined the differences between old and high forest stands. Interestingly, root

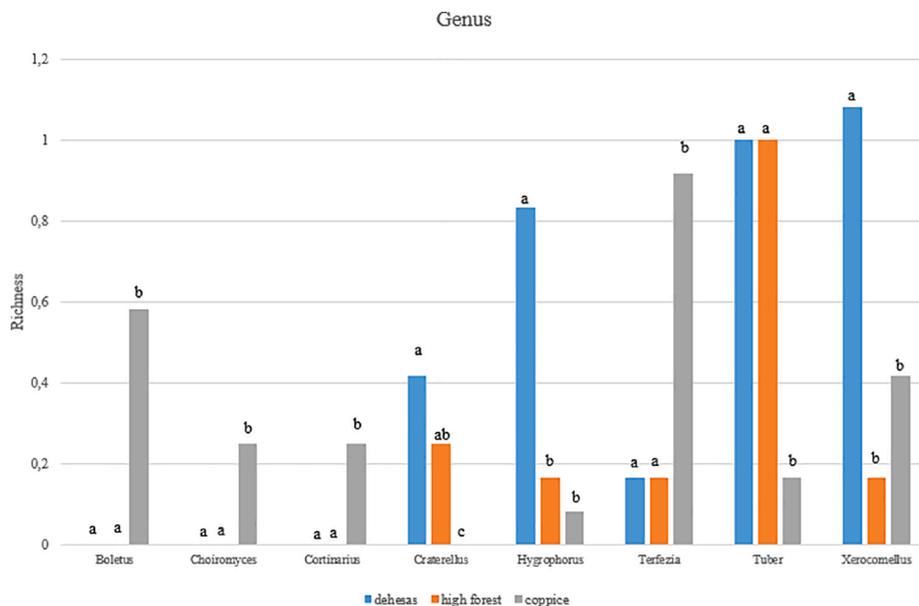


Fig. 4. Proportional richness of edible fungi across the three sampled forest types. Means were compared using LME and Tukey’s HSD tests, with different letters denoting significant differences.

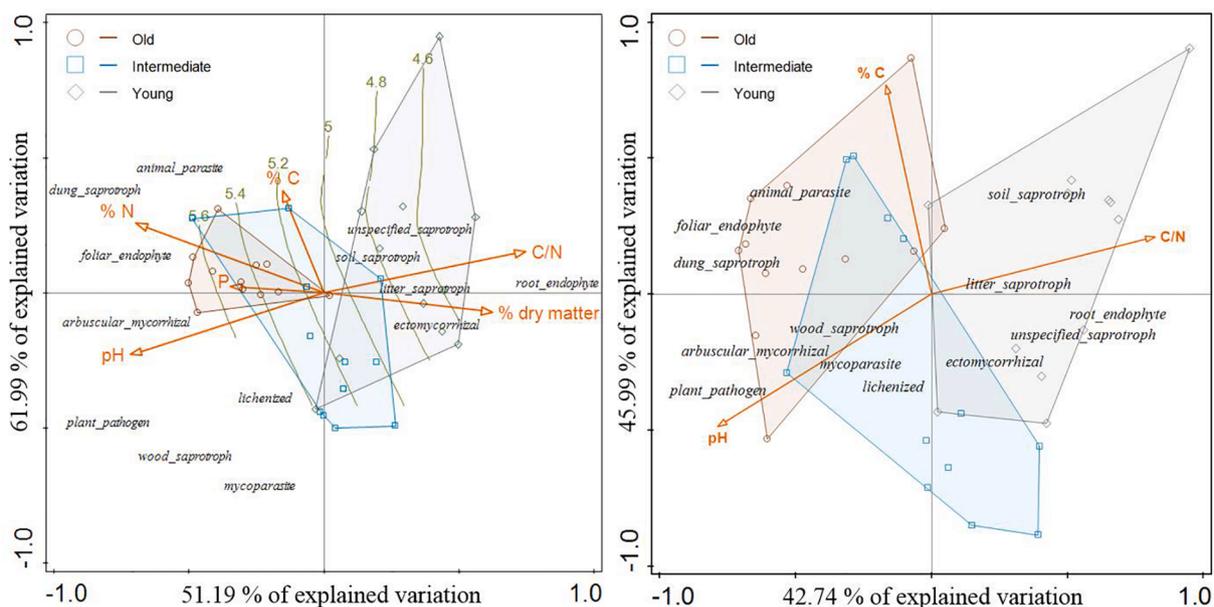


Fig. 5. Principal component analysis (A) and redundancy analysis (B) of fungal community composition in the three oak stand types. Edaphic variables are shown in orange. Isolines indicate the pH gradient following Loess Model  $R^2 = 60$ ; Residual SE = 0.32. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

endophytes were strongly associated with the C/N rate whereas foliar endophytes were associated with soil N. Furthermore, dung saprotrophs were clearly associated with N content, whereas litter saprotrophs were associated with the C/N ratio and obviously influenced by dry matter.

Redundancy Analysis (RDA) (Fig. 5B) was performed to explain the distribution of guilds according to statistically significant variables. The C/N rate explained 57.8% of the distribution of guilds, dominating the ordination of the community. The highest C/N rates were associated with coppice stands where saprotroph guilds were more dominant. The RDA placed ECM fungi in an intermediate position between coppice and high forest stands, revealing that ECM were highly influenced by low amounts of C and were associated with moderate pH values. C and pH explained 24.1% and 12.1%, respectively, of the ordination of the guilds. Plant pathogens and arbuscular mycorrhizal fungi were

associated with the highest pH values, which were associated with dehesas. Other taxa such as dung saprotrophs, foliar endophytes and animal parasites were directly explained by high C and very low C/N rates, which also explained the strong influence of N on these groups, which were closely associated with dehesas. The RDA also indicated that C/N rates and soil C content had opposite effects on the distribution of root/foliar endophytes and on litter/dung saprotrophs. Finally, mycoparasites and lichenized fungi were influenced by high pH and low C/N rates, unlike animal parasites, which were correlated with high soil C and N content levels associated with dehesas used as grazing areas.

Three more Canonical Correlation Analysis (CCA) were performed to observe how the taxonomical distribution within the principal guilds is developed (Fig. 6). In all of them the pH showed the largest explaining contribution over the community distribution (40.8% for ECM and

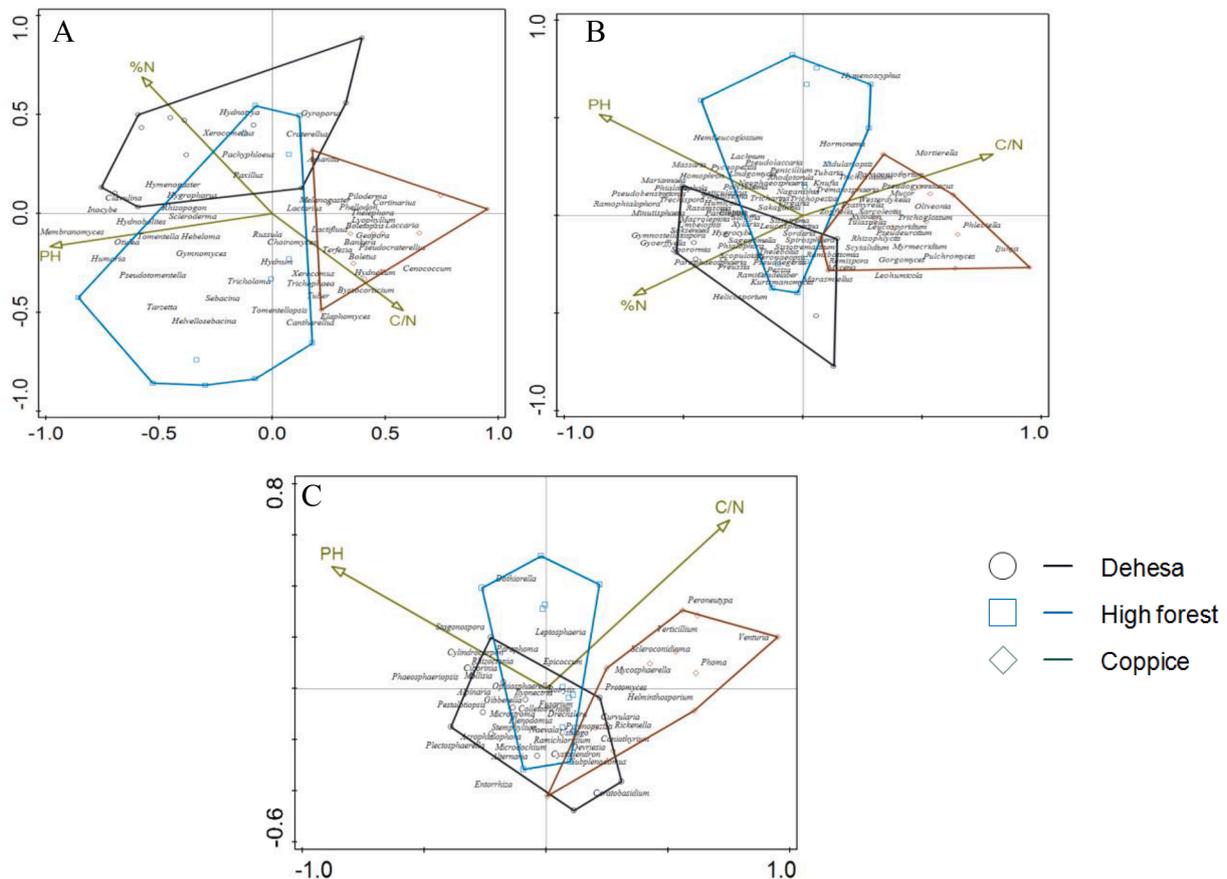


Fig. 6. Canonical Correlation Analysis of the taxonomic composition of ECM (A) Saprotrrophic (B) and Pathogenic (C) fungi oak stand types. Edaphic variables are shown in green. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

saprotrophic fungi and 34.4% for pathogens fungi). In the case of ECM fungi, coppice stands were clearly influenced by the C/N ratio having within group taxa corresponding to *Boletus*, *Cortinarius* (both MDM/MDF/LD) or *Laccaria* (C/SD/MDS). In opposition to the C/N ratio, the N % dominated the influence over the dehesa stands, showing taxa like *Craterellus* (C/SD/MDS), *Xerocomellus* and *Paxillus* (MDM/MDF/LD). Finally, high forest stands showed a mixed influenced of C/N ratio and % N, but the main influence was the pH. It showed a middle position between coppice and dehesa stands, with a presence of taxa such as *Russula*, *Cantharellus*, *Tomentella* and *Inocybe* (all of them are C/SD/MDS).

Saprotrophic fungi showed a clear differentiation among types of stands related to the pH. Coppice and dehesa stands showed a separation marked by the %N and C/N ratio, in the same way that was seen for ECM. Coppice stands showed the most differentiated population of the three kind of stands, containing species such as *Mucor*, *Moterialia*, both soil saprotrophs. Dehesa stands, marked by a high %N and higher pH, contained species such as *Umbelopsis* (e.g., soil saprotrophs), *Macrolepiota*, an edible litter saprotroph, or *Thelebolus* which is a dung saprotroph, while high forest stands, characterized by intermediates levels of each soil factor, contained species such as *Tubaria*, which is a litter saprotroph, *Peziza* which is a soil saprotroph or *Penicillium* which is an unspecified saprotroph.

Finally, for Pathogens a more differentiated community is observed in coppice stands, while intermediate and dehesas present more similarities in comparison with the other guilds. The main difference between coppice stands and the two other types of stands is driven by the pH, as for the other guilds. Coppice stands are characterized by a lower pH, but a higher C/N ratio. Some of the taxa that can be found in this type of stands are *Phoma*, *Venturia* or *Protomyces*. On the other hand, for pathogen fungi the community presented in intermediate and dehesa

stands were not highly driven by either C/N ratio or pH. A higher preference for nitrogen rich soils, especially in the fungal community of dehesas, could be seen as for in the case of species such as *Ramichloridium*, *Alternaria* or *Microdochium*. Some taxa were more attached to intermediate stands such as *Dothiorella* or *Leptosphaeria* while other such as *Entorrhiza* or *Plectosphaerella* were associated just to old dehesas. All the taxa observed in this CCA are plant pathogens, as they were selected in terms of larger abundance. The rest of the specific pathogenic guilds were not selected as they were quite far less abundant.

## 4. Discussion

### 4.1. General data

The data presented here show that stand structure and correlating changes in edaphic factors, have profound influence on the taxonomic and functional composition of soil fungal communities sites investigated in this study. Fungal community differences were higher among than within stand types, which was also confirmed by the numerous indicator taxa associated with the three different forest stand structures, confirming our hypothesis. Certain fungal groups differed in their preference for distinct stand types, which was apparent not only among, but within functional groups, as shown here for ECM fungi. Total fungal richness was highest in high forest stands and statistically lower in coppice stands. Possibly because high forest stands provide a more suitable environment for a greater diversity of taxa than coppice stands due to better preservation of soil moisture and greater control over atmospheric incidence owing to the closure of the forest canopy (Dejene et al., 2017; Zhang et al., 2018; Sanz-Benito et al., 2022). Furthermore, the presence of shrubs, which act as alternative hosts or reservoir

species, enables a wide diversity of fungal species to persist (Tomao et al., 2017). The highest total fungal richness was detected in high forest stands of closed canopy timber, which is in accordance with the intermediate disturbance hypothesis (IDH) proposed by Connell et al. (1978). The IDH proposes that highest species diversity is recovered in environments experiencing disturbance of intermediate intensity after an intermediate timespan (Bendix et al., 2017).

#### 4.2. Patterns of fungal richness and abundance

Previous studies have reported that a larger tree canopy and the presence of shrubs as accompanying species favor ECM sporocarp production, which are mainly Basidiomycota taxa, facilitating the reproduction and presence of ECM in the area (Hernández-Rodríguez et al., 2015b; Tomao et al., 2017). Basidiomycota, including taxa that are able to degrade lignin, appear to be more abundant in later successional stages (Vivelo & Bhatnagar, 2019). Some of which appear to be associated with increases in laccase enzyme and hydrolytic activity (Rosales-Castillo et al., 2017). The high levels of richness and abundance of Agaricomycetes in high forest stands is likely explained by their mycorrhizal associations (Zhang et al., 2018), which are less frequent in places with a small tree canopy and a high soil C content, as is often the case in coppice stands. Tremellomycetes are also more abundant and richer in stands with a closed canopy and low soil C content (Liu et al., 2015). The lower, but no significant, abundance and richness of Agaricomycete and Tremellomycete in dehesas could be affected by the limited hyphal dispersion of clades due to the influence of spatial distance between trees in the “dehesas” (Kingsly & Corlett, 2019). The saprotrophic fungi detected in this study were mainly Ascomycota species. These fungi are more abundant in early stages of ecosystem development. Probably owing to their use of labile soil C and asexual reproduction, which facilitates their dominance at this stage (Vivelo & Bhatnagar, 2019). This was observed in our study for Dothideomycetes and Sordariomycetes, in terms of abundance, probably due to the high level of accessible soil C (Liu et al., 2015). Sordariomycetes are saprotrophic fungi that are usually associated with deposits of animal dung, which could explain why their richness levels were higher in dehesas than in other stands (Zhang et al., 2018). Ascomycota populations are known to decrease with stand age and are inhibited by high nutrient content additions (Ye et al., 2020; Zhang et al., 2018). However, it is probably that competition between Basidiomycota and Ascomycota taxa (Bai et al., 2019; Corrales et al., 2017; Tomao et al., 2017) leads to the displacement of Ascomycota from stages of stand development where ECM are more abundant and soils are usually more acidic. This can be due to their shared capacity for degrading plant residues (Ren et al., 2019). The higher richness of Pezizomycetes in dehesas could be because some species of Pezizomycetes are capable of developing symbiotic relationships with trees. And the addition of organic N could lead to an increase in their abundance (Ning et al., 2020). Geoglossomycetes as well are typical colonizers of grazing areas (Orgiazzi et al., 2012). Rozellomycota taxa were characterized as pathogens and were correlated with high rates of soluble N. Therefore, their association with dehesas can be explained by the abundance of soil N due to the presence of livestock, a reduction in the abundance of ECM, and the pruning of trees by livestock, which debilitates trees, increasing their susceptibility to infections (García Jiménez et al., 2020). Mucoromycota taxa were characterized as saprotrophs, mycopathogens and plant pathogens. Mucoromycota are normally correlated with later decay states and moisture (Vivelo & Bhatnagar, 2019). But they were probably much more abundant in coppice forest stands than in older stands due to competition with Rozellomycota and the plant defensive response induced by associations with ECM fungi (Bai et al., 2019). Contrary to the type of Chytridiomycota functional groups that we expected to find in this study, it seems likely that the Chytridiomycota taxa were mainly composed of saprotrophs. These findings support the silvicultural management of coppiced stands, not only to reduce the risk of wildfire

but also to promote fungal diversity.

Corresponding with the findings for Basidiomycota, the proportional richness of ECM fungi was higher in high forest stands than in coppice stands and old, open forests. This supports previous studies that have shown that as the forest develops more layers, ECM diversity increases, correlating with the understory density (Richard et al., 2004) or *Quercus* abundance. Furthermore, tree isolation negatively affects ECM richness (Saitta et al., 2018). Physico-chemical disturbances of the soil and vegetation turnover affect ECM composition (Odriozola et al., 2020). Increases in soil N, such as that associated with livestock activities in dehesas, reduce ECM enzyme activity. Moreover, it explains why ECM colonization dominates soils in N-limited areas (Cheeke et al., 2016; Corrales et al., 2017; Luptáková & Mihál, 2020). Previous studies have shown that activities such as pruning and thinning reduce the ability of soil to retain water, which affects the ECM community, and is accompanied by host loss (Tomao et al., 2017). There is an explanation for ECM fungi having higher levels of richness than other functional guilds in all stand types. It could be related to the wide diversity of hyphae, exploration types and slow hyphal turnover, leading to high biomass accumulation, as well as their greater longevity, larger sporocarp and higher level of spore production, increasing their dispersal capacity (Bässler et al., 2014). Saprotrophs have been reported to be more abundant in chaparral-coppice stands, diminishing in abundance as the tree canopy increases (Richard et al., 2004). This corresponds with the observed trend for a greater abundance of soil and unspecified saprotrophs in coppice stands than in other stands in this study. Furthermore, competition with ECM fungi and the negative effect of N on nitrophobic ECM species could have displaced saprotrophs to the coppice and dehesas, from high forest stands (Cheeke et al., 2016; Corrales et al., 2017; García Jiménez et al., 2020). This could also be seen as an example of the ‘Gadgil effect’, where saprotrophic species benefit thanks to decreased competition with ECM fungi due to the rising nutrient content of the soil (Gadgil & Gadgil, 1974; Peltoniemi et al., 2021). Higher levels of plant pathogen, foliar endophyte, animal pathogen and dung saprotroph richness were detected in dehesas than in coppice stands. Probably because the stimulation of tree defense responses was weaker because fewer root tips were protected by mycorrhizal associations and, therefore, there was a weaker response to pathogen attack (Ruiz Gómez et al., 2019). In addition, the presence of animal hosts and the presence of suitable substrates such as dung should have influenced. In general, in the Mediterranean ecosystem, root endophytes form a mutualistic or neutral association with oaks (Moricca & Ragazzi, 2008). This can explain their richness and abundance in high forest stands where photosynthetic production levels should be highest. The lower richness and abundance of root endophytes in dehesas is likely due to competition with both ECM and pathogenic fungi. Also, because of the possible negative influence of N accumulation (Mayerhofer et al., 2013). Finally, the increased abundance of soil and unspecified saprotrophs in coppice forest stands can be explained by the already mentioned ‘Gadgil effect’ and the litter input of annual understory (Fernandez & Kennedy, 2016; Goldmann et al., 2015).

Our observations of the richness and abundance of ECM exploration types support previous studies that show that high forest stands with a high rooting density favor short- and medium-distance exploration types (Geml, 2019; Peay et al., 2010). An explanation for differences in ECM exploration types between dehesas and coppice stands is that old dehesa trees are already associated with a fungal community. The major production of laccase enzyme by C/SD/MDS (Hupperts et al., 2017) and the increase in laccase enzyme activity at later successional stages must be related to a larger presence of these exploration types in old and, especially, high forest stands (Rosales-Castillo et al., 2017). Moreover, C/SD/MDS exploration types with hydrophilic hyphae are usually present in soils with labile N, whereas MDF/LD exploration types are more common in soils with recalcitrant N. This is due to their proteolytic activity and their ability to avoid leaching (Geml et al., 2017). Contrary to our expectations, MDF/LD exploration types were less rich in dehesas.

However, this is likely because the soil in these types of stands can be dry due to pastoral pressure. That means making the nonsuberized root tips spots of higher moisture, benefiting a more gathered mycorrhizome (Castaño et al., 2018a). By contrast, non-ECM root-associated fungi showed a higher proportional richness in coppice, coppiced forest than in old, open forests. Mesic microclimate conditions appeared to benefit non-ECM root-associated fungi, endophytes and ericoid mycorrhizal fungi (Geml, 2019).

The greater richness of *Boletus* sp. in coppice forest stands is related to the wide range of hosts that are able to form a symbiotic relationship with *Boletus* sp. (Albuquerque-Martins et al., 2019; Hernández-Rodríguez et al., 2017; Oria-De-Rueda et al., 2008; Salerni & Perini, 2004), which can maintain the supply of photosynthate in a coppice stand. *Cortinarius* spp. are associated with shrubs such as rockroses, which can influence their richness in this stands, as occurs with *Choiromyces* sp. (Comandini et al., 2006). *Terfezia* sp. also has a wide capacity and plasticity of association with different shrubs, especially *Cistus* sp., appearing in primary successional stages, in agreement with our findings (Louro et al., 2021). Many studies have reported that *Craterellus* is associated with dehesas (Hernández-Rodríguez et al., 2013; Martín-Pinto et al., 2006; Odriozola et al., 2020), concurring with our findings. The same trend was also seen for *Hygrophorus* (Luptáková & Mihál, 2020), and its greater presence in dehesas revealed a non-nitrophobic behavior. *Tuber* sp. richness increased with stand age, with up to 18 species recorded in stands aged 20 years or even older (Queralt et al., 2017). This is in accordance with our findings, probably due to the hoarding of photosynthates derived from the plant host until it is able to maintain a wider diversity. *Xerocomellus* sp. was significantly much richer in dehesas, agreeing with the findings reported by Luptáková & Mihál (2020), but less rich in high forest stands, which may reflect its possible displacement by other more competitive species.

#### 4.3. Fungal community composition

Silvicultural management and stand age are known to drive fungal communities in different types of ecosystems (Tomao et al., 2020). In this sense, taxa composition was clearly affected by forest management, which also influenced the stage of development of the studied stands. Our data showed that saprotrophs dominated fungal communities in coppice stands, which suggests that the higher C content of these soils was being used by OTUs within this guild (Bai et al., 2019). This is in congruence with a greater production of cellulolytic and ligninolytic enzymes by saprotrophic fungi (Frey et al., 2004) that are able to decompose dry matter. PCA and RDA indicated that ECM fungi had an intermediate position between coppice and high forest stands. This is probably due to their wide association with a wide range of shrubs (Albuquerque-Martins et al., 2019). Also, because of the displacement of saprotrophic fungi by ECM to coppice stands where the soil C content is higher and photosynthate production by trees is low (Fernandez & Kennedy, 2016). Finally, this could also be explained by a neighbor effect. The enhancement of ECM fungi in coppice stands could be supported by surrounding stands that are mycorrhizal that have not lost their photosynthetic efficiency because of the lack of a thinning treatment (Moeller et al., 2016).

The pH level is also a well-known diversity driver (Zhang et al., 2018), influencing the distribution in the dehesas, as does soil N content. Arbuscular mycorrhizal (AM) fungi appear to be associated with old forests. This is not unexpected given that AM fungi are usually present in prairies (Zhang et al., 2018), which are present in the dehesa systems. Moreover, AM fungi have been observed to be displaced to areas with a lower C/N ratio and a higher N soil content where ECM do not proliferate (Corrales et al., 2017). Plant pathogens, related to a high pH are characteristic of old stand areas, resulting in debilitated trees due to a lack of ECM fungi and, likely, also due to excessive pruning (García Jiménez et al., 2020). Both in the PCA and in the RDA, a strong divergence was observed between root and foliar endophytes, as was also

observed between litter and dung saprotrophs. This divergence was strongly influenced by N soil content and, in congruency, by C/N rates. Foliar endophytes are early decomposers that live in foliar tissues, becoming decomposers during defoliation (Vivelo & Bhatnagar, 2019). This may explain their dominance in the dehesas because leaves and dung are the main organic material sensitive to decomposition. Another hypothesis is that these foliar endophytes migrate from the soil to the leaves via the inner part of the plant (Gomes et al., 2018), therefore it is likely that these foliar endophytes require high soil N and C content levels prior to the infection of the tree. The differentiation between beneficial and pathogenic endophytic fungi is complicated because their roles can change depending on the state of the host. However, a possible explanation for higher levels of root endophytes has been suggested by the “cry-for-help” hypothesis. This supports the idea that the exudation of substances by tree roots induces the recruitment of protective microbes under stress situations (Costa et al., 2020). It can be seen in the coppice and high forest stands. Alternatively, these root endophytes could be dormant root-decaying decomposers (Otsing et al., 2018), including shrub root decomposers, or contain a high quantity of fine-root decomposer fungi.

In the Canonical Correlation Analyses, different ECM taxa of economic interest were associated with each of the three types of stands. Contrary to what was expected, *B. edulis* was more associated with coppice stands (Tomao et al., 2017). Perhaps this could be because coppice stands represented a suitable environment for *Boletus* development owing to the presence of dry matter as litter (Salerni & Perini, 2004). Moreover, its association with shrubs such as *Cistus ladanifer* could help (Hernández-Rodríguez et al., 2015a). The presence of *Cortinarius* was congruent with the major presence of MDM/MDF/LD exploration types in coppice stands and was correlated with a negative response to N addition (Corrales et al., 2017). *Laccaria* has also been shown having a negative correlation with fertilization in pine stands, which might explain its absence in dehesas (Buée et al., 2011). Dehesa associated species detected in our study have also been reported in recent studies, such as *Craterellus* and *Xerocomellus* (Luptáková et al., 2018; Luptáková & Mihál, 2020; Odriozola et al., 2020). *Paxillus* has been seen associated with young pine stands, but when the stand is composed of *Quercus* it is normally associated with old trees (Keizer & Arnolds, 1994). *Russula* was associated with high forest stands, which relates with the major presence of contact mycorrhizal exploration types in high forest stands. Its association with laccase activity related to lignin degradation and its association can explain its presence in a stand with more developed trees that produce more wood susceptible of being degraded (Hupperts et al., 2017). Furthermore, species such as *Cantharellus*, which is highly affected by thinning treatments (Tomao et al., 2017), *Tomentella* and *Inocybe*, which appears to be a late successional-stage taxon, were mainly present in dehesas correlating with its characteristic contact exploration types (Carrino-Kyker et al., 2016; Godbold et al., 2015). Molds are known to be associated with litter, fresh and decomposed (Brabcová et al., 2018), which explains their association with coppice stands due to the large amount of litter biomass that was observed to be dry matter in these stands in the case of *Mucor* and *Moterialia*. Related to dehesa's saprotrophic fungi, among the species that were found, *Macrolepiota* was expected as a typical mushroom from grasslands like the ones found in this type of stands (Hernández-Rodríguez et al., 2015a), or *Thelebolus* which is a typical coprophilous fungus (Orgiazzi et al., 2012). *Penicillium* is an unspecified saprotroph found more attached to the high forest stands, even that is highly ubiquitous genus Liang et al., 2021) and normally is associated to a higher C content and thinned plots (Bastida et al., 2019; Vašutová et al., 2017). Due to its protease and cellulose decomposing activity (Park et al., 2019) it is possible that high forest stands can offer a wider woody and litter material to decompose than coppice or dehesa stands.

Finally, regarding pathogens, genus such as *Phoma* and *Venturia* are more abundant in earlier stages and had a positive effect with disturbances (Castaño et al., 2018b; Rodríguez-Ramos et al., 2021),

respectively, as it occurred in our study. *Alternaria* presence in dehesas is in agreement with recent research as typical pathogenic species found in pastures and oaks (Santamaria et al., 2018; Gómez et al., 2019; Penagos-Tabares et al., 2021). *Dothiorella* has been seen as wide distributed pathogen linked with the dieback of *Quercus* species (Mahamedi et al., 2020) but also with no health effects over the hosts (Batista et al., 2020). It is possible that it could be displaced by others pathogenic genus more dominant in the others stand structures, as for example *Alternaria* in dehesas, which has been seen to have an antifungal and biocontrol activity over other plant pathogens (Costa et al., 2020).

The strong influence of pH over the taxonomical community distribution among the principal guilds in the studied stands it is remarkable. It has been seen in others studies that pH drives ecosystem factors such as the  $\alpha$ -diversity, depending on the coverage and vegetation (Zhang et al., 2018), or nutrient availability and enzyme activity (Cheeke et al., 2016; Praeg et al., 2020). This means that pH could be used as a sum parameter to predict community distribution in terms of taxonomical structure among the present guilds in a studied ecosystem (Praeg et al., 2020).

All this showed that different practices end up developing differentiated abundance and richness of ecological guilds or fungal life strategies. Therefore, management of these areas should take into account measures to prevent fuel continuity. In this sense, mosaic structure management previously assessed (Castaño et al., 2018b; Moreno-Fernández et al., 2021), including coppice, high stands and dehesas, may help to conserve fungal abundance and richness while preventing the occurrence of large uncontrolled fires. In addition, an adequate management of *Quercus* forests maintaining different management options, can provide production of some appreciated and demanded edible species (Hernández-Rodríguez et al., 2013; Sanz-Benito et al., 2022) while coexisting with livestock production.

## 5. Conclusions

Our study showed that land management has a strong impact on the belowground fungal community at a taxonomic and functional group level. In addition, human actions resulted in a wider fungal diversity due to the different affects and inputs derived from cattle rearing and silvicultural treatments. In summary, an interesting benefit of this diversification could be an increase in the production of diverse economically valuable edible fungi that need different kinds of substrate. The higher richness levels of the C/SD/MDS ECM exploration types in dehesas was likely due to the scarcity of water due to grazing pressure. Saprotrophs were much more abundant in coppice stands, whereas dehesas had a larger presence of pathogens and endophytes. The high forest stands were characterized by the diversity of mycorrhizal fungi, likely due to the higher photosynthetic productivity of these stands. The strongest community drivers were the pH level, the soil C content, and the C/N rates, which are directly linked to the amount of biomass produced by stands that can be degraded, and N deposition, which is driven by livestock activity. The management of these three stand types created three clearly distinguishable communities, inducing a highly diverse mosaic ecosystem that could likely maintain compositional as well as nutritional interactions among them. Further research should be undertaken to determine how these types of stand behave to improve our knowledge and management of areas adjacent to this iconic Iberian landscape.

## CRedit authorship contribution statement

**Pablo Martín-Pinto:** Conceptualization, Methodology, Investigation, Supervision, Writing – review & editing. **Ignacio Sanz-Benito:** Writing – review & editing. **María Santos:** Methodology, Investigation. **Juan Andrés Oria-de-Rueda:** Supervision. **József Geml:** Conceptualization, Methodology, Investigation, 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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