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RESEARCH

Dynamics of ectomycorrhizal communities in Sardinian cork oak forests: influence of management system, lithological substrate and season

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Abstract
Ectomycorrhizal fungi represent a key component of forest ecosystems, contributing significantly to tree nutrition, stress tolerance, and overall ecosystem resilience. In the Mediterranean region, cork oak (*Quercus suber* L.) forests, have significant ecological and economic value, and their vitality strongly depends on these belowground mutualistic relationships. Although previous studies have investigated the diversity and function of ectomycorrhizal fungi, several aspects concerning their ecological dynamics remain inadequately understood, particularly in cork oak forests. This study investigates the structural and dynamics of ectomycorrhizal fungal communities in cork oak forests of Sardinia located on granitic, basaltic, and trachytic substrates and subjected to different management practices (natural, grazed, and ploughed). We assess how forest management and seasonal variability interact with lithological conditions to shape community structure and diversity. Three cork oak stands for each lithological substrate (nine in total) were selected in areas where all the three forestry managements were present. Two transects were established in each stand, and soil samples were collected during spring and autumn to assess seasonal variations in the ectomycorrhizal community. In total, 82,345 ectomycorrhizal root tips were morphologically characterized and classified in 167 morphotypes based on morpho-anatomical characteristics. From these, 120 were successfully assigned to distinct Operational Taxonomic Units (OTUs) through internal transcribed spacer (ITS) barcoding. Our results indicate that lithological substrate, management system, and sampling season significantly influence the structure and composition of ectomycorrhizal communities. Notably, ploughing caused a marked reduction in fungal richness, highlighting the sensitivity of these communities to soil disturbance.

Keywords Fungal diversity · Mediterranean ecosystems · Mutualism · *Quercus suber* · Root symbiosis.

Introduction

Cork oak (*Quercus suber* L.) forests represent an important ecosystem of the Mediterranean landscape, with significant ecological and socio-economic value. Covering around 2.5 million hectares across the Mediterranean Basin, these woodlands are widespread in southern European countries such as Portugal, Spain, France, and Italy, as well as in North Africa, including Morocco, Algeria, and Tunisia (Aronson et al. 2009). Besides their economic importance for cork production, which reaches approximately around 300,000 tons annually (Kim et al. 2017), cork oak forests provide fundamental ecosystem services such as soil protection, carbon sequestration, and biodiversity conservation (Bugalho et al. 2011). Their resilience to fire, made possible by the protective properties of cork bark, further reinforces

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their ecological importance in fire-prone Mediterranean ecosystems (Pausas 1997).

The current structure of cork oak forests reflects a long history interaction between human activities and the natural environment. Over the centuries, traditional land uses have deeply influenced both the structure and functionality of these ecosystems (Bugalho et al. 2011). Different management types, ranging from natural stands to agro-silvo-pastoral systems like the "*Montados*" in Portugal and "*Dehesas*" in Spain, have indeed influenced their ecological structure and composition (Ferraz-de-Oliveira et al. 2016). However, climate change and increasing anthropogenic pressures have increased their overall vulnerability (Brasier 1996). The phenomenon of "oak decline", often associated with unsustainable management and environmental stressors, has contributed to significant reductions in cork oak vitality and productivity (Moricca et al. 2016; Corcobado et al. 2015; Seddaiu et al. 2025). Mitigating the impacts of environmental stressors and management practices requires understanding how mutualistic relationships with ectomycorrhizal (ECM) fungi mediate the sustainability and functioning of these ecosystems.

ECM fungi, forming mutualistic associations with fine roots of host trees, including *Q. suber*, play a key role in nutrient cycling by enhancing host uptake of nitrogen, phosphorus, and water in exchange for photosynthates (Smith and Read 2008; Hawkins et al. 2023). Beyond their nutritional role, ECM fungi also enhance the protection of the host plants by modulating phytohormonal signalling, mitigating abiotic stressors (Kebert et al. 2023), and protecting roots against soilborne pathogens through competitive exclusion and antimicrobial metabolite production (Branzanti et al. 1999; Pozo and Azcón-Aguilar 2007; Lancellotti and Franceschini 2013; Gille et al. 2024). Furthermore, ECM fungal networks enable inter-plant communication that mediates nutrient exchange among individual plants and influence plant community dynamics and resilience (Simard et al. 2012; Sharifi and Ryu 2021). Therefore, the composition and structure of ECM fungal community strongly influence forest stability and productivity, making their study essential for understanding the ecological processes underlying forest dynamics (Clemmensen et al. 2015; Peay et al. 2016; Anthony et al. 2022).

The composition and structure of ECM fungal communities are strongly influenced by a complex interplay of abiotic and biotic factors, including host specificity, soil properties, climatic conditions, and forest management practices (Tedersoo et al. 2012; Lladó et al. 2018; Tomao et al. 2020). ECM communities exhibit dynamic responses to fluctuations in soil pH, nutrient availability, and moisture levels, as well as broader environmental gradients (Corrales et al. 2018; Tedersoo and Bahram 2019; Correia et al. 2021). Lithological substrate further contributes to mycorrhizal community dynamics, as variations in mineral composition and resulting soil conditions affect community composition and ecological interactions (Weemstra et al. 2020; Xiao et al. 2024). Forest management represents an additional significant driver shaping ECM fungal communities by altering habitat conditions and resource availability (Azul et al. 2010). Silvicultural interventions such as thinning modify microclimatic parameters and soil organic matter content, while grazing affects litter accumulation and moisture retention, selectively favouring drought-adapted taxa (Azul et al. 2010). Soil disturbances, including ploughing, can alter fungal networks and create transient successional dynamics, often promoting the proliferation of pioneer fungi with rapid colonization strategies (Collier and Bidartondo 2009; Kalucka et al. 2016; Hartmann and Six 2023). Human-driven ecosystem simplifications reshape ECM fungal assemblages, potentially impacting nutrient cycling and tree health.

Seasonal variability is becoming increasingly important due to the rising frequency of climatic anomalies, and it plays a crucial role in regulating the activity and composition of ECM fungal communities. These communities undergo compositional changes in response to temperature and moisture fluctuations, with peak activity generally occurring in spring and autumn, when favourable conditions enhance nutrient exchange and organic matter decomposition (Richard et al. 2011; Voříšková et al. 2014; Corcobado et al. 2015). In contrast, summer droughts limit physiological activity, favouring taxa with greater tolerance to water stress and altering the competitive balance within ECM fungal communities (Cosme 2023; Kebert et al. 2023). Given the increasing environmental pressures and land-use changes affecting Mediterranean ecosystems (Bajocco et al. 2012; Peñuelas et al. 2017), a deeper understanding of the factors shaping ECM fungal communities is essential for assessing the long-term stability and productivity of cork oak forests. While previous studies in the Iberian Peninsula have explored the influence of climate, land use, and soil characteristics on ECM communities (Azul et al. 2010; Reis et al. 2018; Costa et al. 2022), the specific interactions between soil management practices and lithological substrate remain poorly explored in the Mediterranean basin. We hypothesized that ECM fungal community composition and diversity are significantly influenced by the interaction between soil management practices and underlying lithology, leading to distinct community assemblages across different environmental contexts. To test this hypothesis, we investigated the structure and seasonal dynamics of ECM fungal communities in Sardinian cork oak forests using a combined morpho-anatomical and molecular approach, focusing on three key variables: lithological substrate

(granitic, basaltic, trachytic), management practices (natural, grazed, and ploughed), and sampling season (spring and autumn).

Materials and methods

Study area

A preliminary cartographic analysis using the world reference database for soil resources (FAO 1998), was conducted based on lithological and management criteria to identify the main areas where *Q. suber* forests occur in Sardinia. The comparison of these datasets revealed that the three main cork oak forest management types - natural, grazed, and ploughed - occur on granitic, alluvial, trachytic, basaltic, and metamorphic substrates (Table 1).

Field surveys were subsequently carried out across different areas to identify sites with all three management types within the same lithological context. This condition was met only in areas with trachytic, basaltic, and granitic substrates, which were selected for the study. All selected sites consist of large, continuous cork oak forests, typically extending over several hundred hectares, ensuring that the sampled areas represent extensive forest stands. The selected sites are described below:

Study area 1- Monte Minerva, Villanova Monteleone. The lithological component consists of Cenozoic acidic effusive rocks, leading to the formation of soils classified as Lithic Xerorthents (FAO 1998). These are moderately deep soils with a texture ranging from sandy loam to clayey sand, a subangular polyhedral structure, good permeability, neutral pH, high skeletal content, and notable stoniness (Aru et al. 1991), which reduce the fraction of fine soil particles available for nutrient and water retention, while the texture and neutral pH suggest moderate nutrient availability. The tree layer is dominated by *Q. suber* and *Q. pubescens*, while the shrub layer includes *Arbutus unedo*, *Myrtus communis*, *Phillyrea angustifolia*, and *Erica arborea*.

Study area 2 - *Su Monte*, Santu Lussurgiu. The lithology is characterized by Upper Pliocene basic effusive rocks (basalts). The predominant soils belong to the Rock Outcrop and Lithic Xerorthents classifications (FAO 1998). These soils are shallow, permeable, with a loam-clay texture, a subangular polyhedral structure, and neutral pH. They exhibit excessive skeletal content, high rockiness, and significant stoniness, with low organic matter content (Aru et al. 1991). These soils are characterized by limited nutrient availability, particularly carbon and nitrogen, and by reduced water and nutrient retention capacity due to their high skeletal and stony fraction and the presence of silt and clay (Aru et al. 1991). The tree layer consists of *Q. suber* and *Q. pubescens*, with scattered individuals of *Q. ilex*, while the shrub layer is mainly composed of *Cytisus villosus*. The understory also includes subshrubs such as *Rubus ulmifolius* and climbing species like *Smilax aspera* and *Hedera helix*.

Study area 3 - *Sa Mudditta*, Oschiri. The lithological substrate consists of Paleozoic intrusive rocks (granites, granodiorites, etc.). The dominant soils are classified as Rock Outcrop and Lithic Xerorthents (FAO 1998). These soils are

Table 1 Description of Sardinian Cork oak sites investigated in this study

Sites	Monte Minerva (Natural)	Monte Minerva (Grazed)	Monte Minerva (Ploughed)	Su Monte (Natural)	Su Monte (Grazed)	Su Monte (Ploughed)	Sa Modhita (Natural)	Sa Modhita (Grazed)	Sa Modhita (Ploughed)
Coordinates	40°24'57.04"N 8°32'40.26"E	40°27'1.64"N 8°31'28.23"E	40°26'58.31"N 8°31'29.99"E	40°6'53.45"N 8°37'27.41"E	40°6'46.48"N 8°37'8.00"E	40°6'38.31"N 8°37'59.24"E	40°46'28.02"N 9°5'55.28"E	40°46'52.44"N 9°5'57.66"E	40°46'45.26"N 9°6'3.24"E
Altitude (m a.s.l.)	320	320	330	610	600	531	210	230	225
Exposure	South-East	South-East	South-East	South	South	South	East	East	East
Morphology	Gentle slope	Gentle slope	Gentle slope	Rolling hills	Rolling hills	Rolling hills	Gentle slope	Gentle slope	Gentle slope
Substrate	Trachytic	Trachytic	Trachytic	Basaltic	Basaltic	Basaltic	Granitic	Granitic	Granitic
Management	Natural	Grazed	Ploughed	Natural	Grazed	Ploughed	Natural	Grazed	Ploughed
Forest Type	*Q. suber* *Q. pubescens*	*Q. suber* *Q. pubescens*	*Q. suber* *Q. pubescens*	*Q. suber* *Q. pubescens*	*Q. suber* *Q. pubescens*	*Q. suber* *Q. pubescens*	*Q. suber* *Q. ilex*	*Q. suber* *Q. ilex*	*Q. suber* *Q. ilex*
Forest Size (ha)	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
Mean Annual Temperature (°C)	15.2	15.2	15.2	13.5	13.5	13.5	16.0	16.0	16.0
Annual Rainfall (mm)	650	650	650	800	800	800	600	600	600

shallow, permeable, with a sandy loam to loamy sand texture, a subangular polyhedral structure, and acidic pH. They are characterized by an excess of skeletal material, high rockiness, and substantial stoniness (Aru et al. 1991). Such soils exhibit moderate cation exchange capacity, reflecting their sandy texture and acidity, which may influence nutrient retention and availability. The tree layer is primarily composed of *Q. suber* with occasional *Q. ilex* individuals. The shrub layer includes species such as *A. unedo*, *E. arborea*, *Cistus monspeliensis*, *C. incanus*, *Calicotome villosa*, *Lavandula stoechas*, *P. angustifolia*, *P. latifolia*, *Euphorbia* sp., *Prunus spinosa*, *Pistacia lentiscus*, *Viburnum tinus*, and *Crataegus monogyna*.

The selected study areas are also geographically well separated: sampling sites of the same substrate type are located 300–4,200 m apart, while sites on different substrates are approximately 100 km apart, minimizing potential spatial autocorrelation among ECM communities (Fig. 1).

Tree health assessment and sampling

In each of the nine selected cork oak forests, representative of different lithological substrates (trachytic, basaltic, and granitic) and management regimes (natural, grazed, and ploughed), two transects measuring 30 × 7.5 m were established. In each transect, the abundance of *Quercus* species was recorded, and the health status of individual trees was assessed to ensure data homogeneity for subsequent analyses. Tree health evaluation was conducted according to the protocols described by Franceschini et al. (2002) and Vannini et al. (2010), based on crown transparency and the presence of visible decline symptoms such as necrosis, exudates, cankers, wood decay, and epicormic shoots on trunks and branches. Each tree was assigned to a decline class on an empirical scale ranging from 0 for healthy tree to 4 for dead tree (Supplementary material 1). These classifications were subsequently used to calculate a site-specific Decline Index (DI) using the formula: $DI = \sum(C \times F)/N$, where C is the decline class (0–4), F is the frequency of trees within the same class, and N is the total number of assessed trees. Based on DI values, decline severity thresholds were defined as follows: None ($DI < 0.2$), Mild ($0.21 \leq DI \leq 0.9$), Moderate ($0.91 \leq DI \leq 1.7$), Medium ($1.71 \leq DI \leq 2.6$), and High ($DI > 2.61$).To investigate the ectomycorrhizal community associated with these forests, seasonal soil sampling was carried out in each transect, with one sampling conducted in autumn (10 samples from the first transect) and the other in the subsequent spring (10 samples from the second transect). From each transect, 10 soil samples were collected using a cylindrical soil corer (10 cm diameter × 10 cm depth), following a standardized design (Supplementary material 2). Each sample was immediately sealed in a plastic bag containing moistened filter paper to preserve fungal viability and labeled with a code comprising two letters representing lithology and management type, followed by a numeric identifier: 1–10 for autumn samples and 30–40 for spring samples. After collection, the soil samples were transported to the laboratory for further analyses.

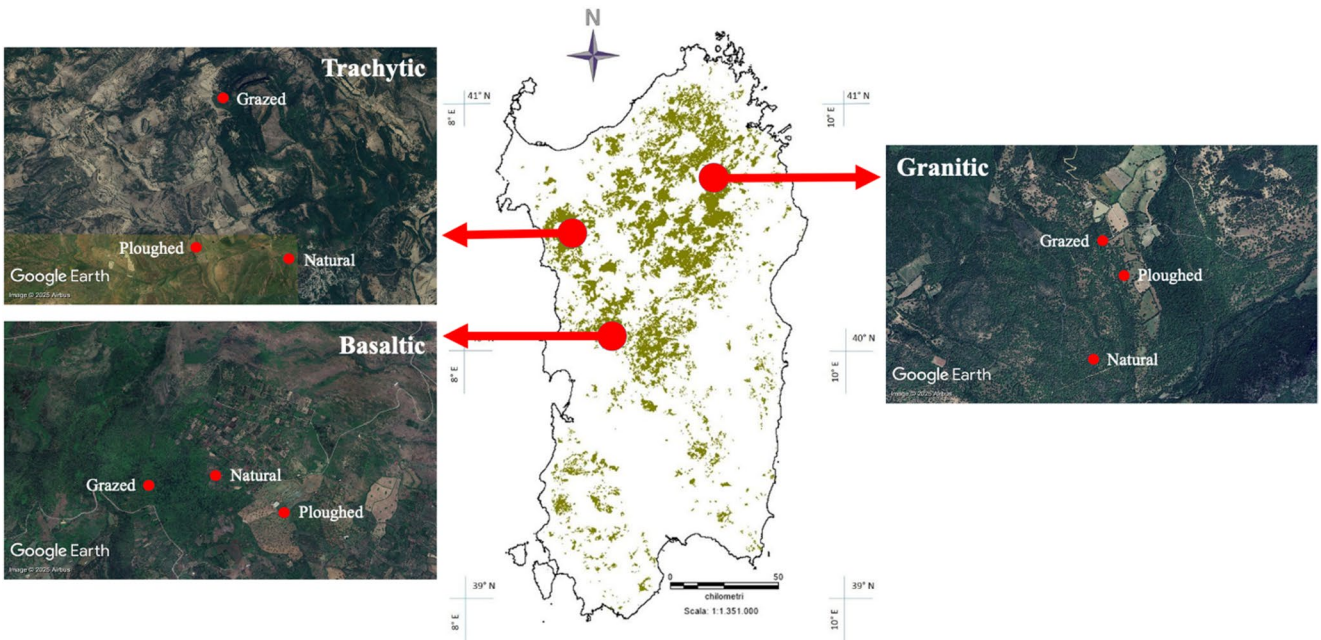

Fig. 1 Sampling sites. Red circles indicate the location of study areas (map) and transects established in the different managing sites (pictures). Green areas in the map indicate the distribution of cork oak in Sardinia

Processing of root samples

Soil samples were stored at 4 °C for up to two months before processing. In the laboratory, each sample was initially cleaned to remove coarse skeletal material, wood debris, and large roots. Subsequently, each sample was divided into five subsamples. Before analysis, each subsample was submerged overnight in tap water to loosen the soil structure. The samples were then placed in a 2 mm mesh sieve and rinsed with low-pressure running water to completely remove free soil particles.

All fine roots were carefully washed and separated into vital ectomycorrhizal (ECM) root tips, old non-turgescent tips, and nonmycorrhizal roots using an Olympus SZX10® dissecting stereomicroscope (Olympus Corp., Tokyo, Japan) with 10–63× magnification and an Olympus Highlight 3100 daylight filter. Special care was taken to preserve the external structures of the ectomycorrhizas. All ECM root tips present in each subsample were counted and subsequently analysed for morphological and anatomical characterization.

Vital ECM root tips were sorted into different ectomycorrhizal morphotypes based on their morphological and anatomical traits using a dissecting microscope and an Olympus BX53® microscope with 100–1000× magnification. Morphotypes were described following the methodology outlined by Agerer (1987). Following Agerer (1987), characters used to classify ECM root tips were: colour, shape, texture, ramification type, cystidia occurrence, mantle structure, and the presence of emanating hyphae and rhizomorphs. Morphotypes displaying slight variations were carefully differentiated to ensure accurate classification. When possible, fungal partners were identified at species level or higher taxonomic ranks by comparing the observed descriptions with published references in Agerer (2008) and Emyco database (Lancellotti et al. 2012).

Finally, for each sample, several tips of selected morphotypes were individually placed in 1.5 ml tubes and stored at −20 °C pending molecular barcoding and OTU definition.

Molecular analysis

DNA was extracted using the CTAB method following Doyle and Doyle (1987). Isolated DNA was employed as the template for polymerase chain reaction of the rRNA ITS-region using the following primer pairs: ITS1 and ITS0F-T (Taylor and McCormick 2008) as forward primers, ITS4B (Gardes and Bruns 1993) specific for Basidiomycota, and ITS4 (White et al. 1990) as reverse primers.

PCR reactions were carried out in a total volume of 50 µl containing 18.7 µl of sterile deionized water, 10 µl of 5× buffer (polymerase buffer solution), 5 µl of 10 mg mL^{-1} bovine serum albumin (BSA), 5 µl of 2 mM dNTP, 5 µl of 10 µM of each primer, 1.5 units of GO-TAQ DNA polymerase (Promega), and 10–30 ng of template DNA. PCR was performed using a "PCR express" thermocycler (Hybaid) with the following program: an initial denaturation step at 94 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 30 s, primer annealing at 60 °C for 30 s, and extension at 72 °C for 1 min, with a final elongation step at 72 °C for 7 min.

PCR products were purified using the Eurogold Gel Extraction Kit (EuroClone S.p.A., Pero, Italy) and sequenced by BMR Genomics sequencing service (https://www.bmr-genomics.it). DNA sequence chromatograms were viewed and edited using BioEdit v. 5.0.6 software. A sequence identity threshold of ≥99% was adopted for validation, allowing taxonomic annotation at the genus or species level. For sequence identities between 98% and 96%, only the genus was assigned, while for sequence identities < 96%, OTUs were classified at the order or family level.

All sequences were deposited in GenBank (http://www.ncbi.nlm.nih.gov/) under accession numbers PV135127 – PV135237; PX723956 – PX723969 and were identified using the GenBank and UNITE databases (https://unite.ut.ee/analysis.php).

Statistical analysis

All ectomycorrhizal root tips isolated from the five subsamples were pooled to represent the ectomycorrhizal community of each soil sample. For each sample, the relative abundance of each detected OTU was assessed, along with three ecological indices: (a) richness (S), representing the number of detected OTUs; (b) evenness (J), following Pielou (1969); and (c) Shannon-Weaver index (H) (Shannon and Weaver 1949).

The effects of substrate, management system, and sampling season on these diversity indices were tested using three-way ANOVA. To determine which fungal taxa were indicative of specific environmental conditions, we conducted an Indicator Species Analysis (ISA) using the IndVal.g method. The analysis was performed on the abundance matrix with 999 permutations, and p-values were corrected for multiple testing using the Benjamini–Hochberg false discovery rate (FDR) (De Cáceres et al. 2010).

ADONIS2 was used to evaluate the influence of substrate type, management system, and sampling season on sample similarity, calculated using the Bray-Curtis dissimilarity index. The ordination of the variables was represented using the Canonical Analysis of Principal Coordinates (CAPSCALE) function. Both ADONIS2 and CAPSCALE analyses were conducted using the BiodiversityR package in R software (R core team 2016; Kindt and Coe 2005).

Results

Preliminary cartographic analysis indicated that 75% of the natural cork oak forests, 40% of the grazed stands, and 60% of the managed cork oak forests within the regional territory are distributed across the three selected substrates (trachytic, basaltic, granitic). The assessment of tree health conditions yielded decline index (DI) values ranging from 0 to 0.89, corresponding to negligible or mild decline thresholds. As a result, the study of ectomycorrhizal communities was conducted under homogeneous conditions, ensuring that the ECM community composition was not influenced by tree health status.

A total of 180 soil samples were collected, of which 167 (93%) contained ectomycorrhizal root tips. Soil sample analysis resulted in the isolation and characterization of 82,345 ectomycorrhizas (ECM), corresponding to 167 morphotypes. From these, 120 were identified at different taxonomic levels based on genetic analysis: 38 at the species level, 62 at the genus level, 15 at the family level, and 5 at higher taxonomic ranks.

The ECM fungal community was dominated by the genera *Tomentella* (22 OTUs), *Inocybe* (18 OTUs), and *Russula* (16 OTUs). Other notable genera included *Lactarius* (6 OTUs), *Cortinarius* (3 OTUs), *Ceratobasidium* (3 OTUs), *Xerocomus* (3 OTUs), *Laccaria* (3 OTUs), *Hebeloma* (2 OTUs), and *Sebacina* (2 OTUs). Less frequent genera, each represented by 1 OTUs, included *Amanita*, *Butyriboletus*, *Cenoccocum*, *Clavulina*, *Hygrophorus*, *Hymenogaster*, *Inosperma*, *Leccinellum*, *Pachyphlodes*, *Peziza*, *Pseudosperma*, *Scleroderma*, *Sistotrema*, *Thelephora*, *Tricholoma*, and *Xerocomellus* (Fig. 2).

Structure and species composition of ectomycorrhizal fungal communities

Ectomycorrhizal colonization varied significantly across forest types and soil substrates. The ploughed cork oak forest on a trachytic substrate exhibited the highest proportion of root samples devoid of ectomycorrhizas, whereas all root samples from natural cork oak forests were colonized. Ectomycorrhizal abundance was greatest in the natural cork oak forest on granite and lowest in the ploughed forest on basalt.

Taxonomic richness (S) varied across sites, ranging from a minimum of 13 OTUs in the ploughed forest on basalt to a maximum of 48 OTUs in the natural forest on granite. The maximum and minimum values of estimated species richness (Chao) were recorded in the basaltic forest, ranging 7.40 in the grazed forest and 2.61 in the ploughed forest (Table 2).

The evenness of ectomycorrhizal species distribution, measured by Pielou's index (J), was relatively homogeneous across sites, ranging from 0.819 in the ploughed cork oak forest on granite to 0.935 in the grazed cork oak forest on basalt. The Shannon–Weaver diversity index (H) further highlighted the influence of forest management and

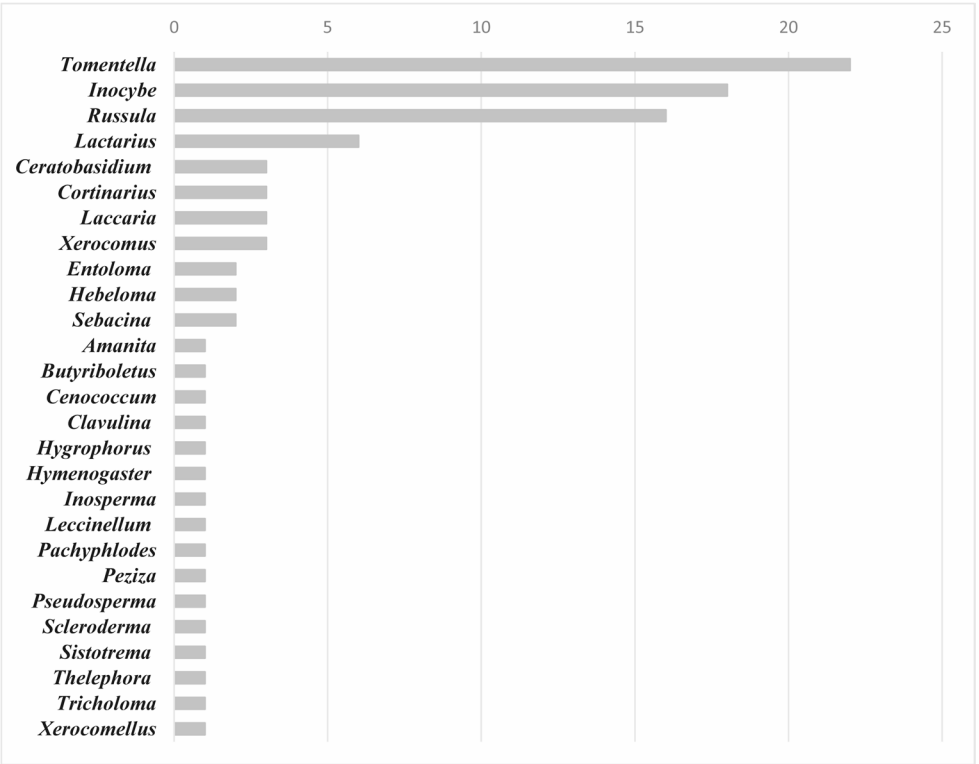

Fig. 2 Total number of detected OTUs subdivided by genera

Table 2 Sample size and main ecological parameters of the analysed ectomycorrhizal communities

Cork oak stand	*N.*	*N.* ectomicorrhizas	S	Chao	J	H
bp	18	3527	13	2.61	0.889	0.760
bn	20	7357	26	4.05	0.885	1.131
bg	20	10,410	37	7.40	0.935	1.702
gp	17	4828	23	4.41	0.819	1.066
gn	20	28,108	48	6.70	0.921	1.660
gg	20	10,819	20	2.65	0.919	0.787
tp	15	1015	15	3.73	0.926	1.070
tn	20	11,859	31	4.15	0.934	1.252
tg	17	4422	18	2.82	0.870	0.800

Lithological substrates: b = basaltic, g = granitic, t = trachytic. Management types: p = ploughed, n = natural, g = grazed. N. = number of samples containing ectomycorrhizas. Observed richness (S), richness index (Chao), pielou's evenness index (J), diversity Shannon-Weaver index (H)

substrate type on ectomycorrhizal fungal diversity, with the highest diversity recorded in grazed forests on basalt (H = 1.47) and the lowest in the ploughed forest on basaltic substrate (Table 2).

Ectomycorrhizal communities differed in genus-level composition and diversity among the investigated cork oak forests, while *Cenococcum geophilum* was consistently detected in all communities.

The highest genus-level diversity was recorded in the natural cork oak forest on granite (20 genera), whereas the lowest was observed in the ploughed forest and grazed on trachytic substrate (7 genera). The genus *Tomentella* was a key component of all ectomycorrhizal communities, except in ploughed forests on basaltic substrate. Similarly, *Russula* was highly represented, particularly in grazed and natural forests. *Inocybe* was detected across all cork oak forests but was particularly abundant in the grazed forest on basaltic substrate and in the natural forest on granite. *Cortinarius* was found in all forests on basaltic substrate and in all forests on trachytic substrate except for the grazed forest; on granite, it was restricted to the natural forest. *Xerocomus* was detected in natural and grazed forests on basaltic and trachytic substrates, whereas on granitic substrate it occurred in natural and ploughed forests. *Hebeloma* was recorded exclusively in natural forests on trachytic and granitic substrates, while *Tricholoma* and *Boletus* were confined to ploughed and natural forests on granite.

Combinatory effect of management type, substrate, and sampling season on *Species richness*, *diversity* and evenness

The three-way ANOVA revealed that species richness was influenced by substrate ($F = 5.52$, $p = 0.004$), management ($F = 74.38$, $p < 0.001$), and season ($F = 59.63$, $p < 0.001$), with management emerging as the most important driver of variation (Supplementary material 3). Interactive effects were also highly significant: the interaction between substrate and management ($F = 20.09$, $p < 0.001$) accounted for a large portion of the explained variance. Furthermore, interactions between substrate, management and season ($F = 8.10$, $p < 0.001$), explained a significant portion of the observed variance, suggesting an important effect on the community composition. This confirms that richness was not only shaped by individual factors, but also by their combined influence.

Shannon diversity (H) followed a similar pattern. Management and season were highly significant (respectively: $F = 10.84$, $p < 0.001$; $F = 60.29$, $p < 0.001$), with season again explaining the largest portion of variance. Interactions were equally important, with substrate and management ($F = 16.77$, $p < 0.001$), and the three-way interaction ($F = 4.62$, $p = 0.001$) standing out as particularly effect on the Shannon index. These results confirmed that both abiotic (substrate) and anthropogenic (management) factors modulate ECM community diversity, but management variation remains the dominant driver on the observed Shannon index.

In contrast, evenness (J) was not significantly affected by any of the main factors (most $p > 0.1$). However, significant differences emerged in the three-way interaction ($F = 5.74$, $p < 0.001$), indicating that evenness responded primarily to the combination of factors rather than to individual drivers. Compared to richness and Shannon diversity, effect sizes (F values) were lower, suggesting a more limited sensitivity of evenness to environmental and management gradients.

Indicator species analyses

The Indicator Species Analysis (ISA) revealed a total of 53 significant indicator species ($p < 0.05$), each associated with single habitat combinations, management, and season. Most indicator taxa were detected during the spring season, particularly in grazed site on basaltic substrate (11 OTUs), and natural site on granitic substrate (6 OTUs) (Supplementary material 4). Autumn samples exhibited fewer indicator taxa, with specific associations such as *Hymenogaster* sp.1 in the combination between basaltic–ploughed site and

Sebacinales in trachytic–ploughed site. *Laccaria bicolor* and *Russula violacea* in basaltic–natural sites; *Hygrophorus* sp.1 and *Tomentella* sp.21 in granitic–natural sites. *Inocybe* sp.6 and *Hebeloma parvicystidiatum* in trachytic–natural sites. Other autumn combinations showed between two and three indicator species each. Spring samples showed a higher number of indicator taxa across all substrates and management types. Notable associations included *Inocybe* sp.12, Russulaceae sp.1 and *Inosperma lanatodiscum* in basaltic–ploughed sites; 6 species including several *Tomentella* OTUs in granitic–natural sites; and 11 species in basaltic–grazed sites, including several *Inocybe* and *Thelephora* OTUs. Other spring combinations ranged from one to five indicator species each.

Overall, ISA results showed variation in the number and identity of indicator taxa across rock types, land-use systems, and seasons, consistent with patterns observed in ANOVA and multivariate analyses (Supplementary material 4).

Impact of lithological substrate on ectomycorrhizal community composition

The ADONIS2 analysis revealed that the lithological substrate at the sampling sites significantly influenced sample dissimilarity ($p=0.002$). The graphical representation (Fig. 3) highlights the formation of three distinct clusters, each corresponding to the distribution of samples collected from the three substrate types. These three clusters significantly represent the distribution of the samples across lithological substrates. A hypothesis was tested to determine whether these clusters might be smaller than illustrated; however, permutation tests led to the rejection of this hypothesis, with probabilities of 0.1% for the area encompassing samples from granitic substrate, 0.2% for basaltic substrate, and 0.02% for trachytic substrate.

Impact of management system and season on ectomycorrhizal community composition in basaltic substrate samples

The ADONIS2 test revealed a statistically significant effect of the management system on the ectomycorrhizal community composition in samples collected from basaltic substrate ($p=0.001$). The graphical representation (Fig. 4 1a) illustrates well-defined clusters corresponding to the three management systems.

Additionally, the ADONIS2 test showed that the sampling season significantly influenced the ectomycorrhizal community composition ($p=0.002$), based on the two time points analyzed. The ordination graph (Fig. 4 1b) highlights a clear separation between the samples collected in the two periods, although the areas show a common overlapping zone.

Impact of management system and sampling season on ectomycorrhizal community composition in trachytic substrate samples

The ADONIS2 test demonstrated a statistically significant influence of the management system on the ectomycorrhizal community composition in samples collected from trachytic substrate ($p=0.001$). The graphical representation (Fig. 4 2a) highlights three distinct clusters corresponding to the different management systems. A permutation test confirmed the statistical significance of these clusters, with $p=0.001$ for natural forests, $p=0.003$ for grazed forests, and $p=0.001$ for ploughed forests.

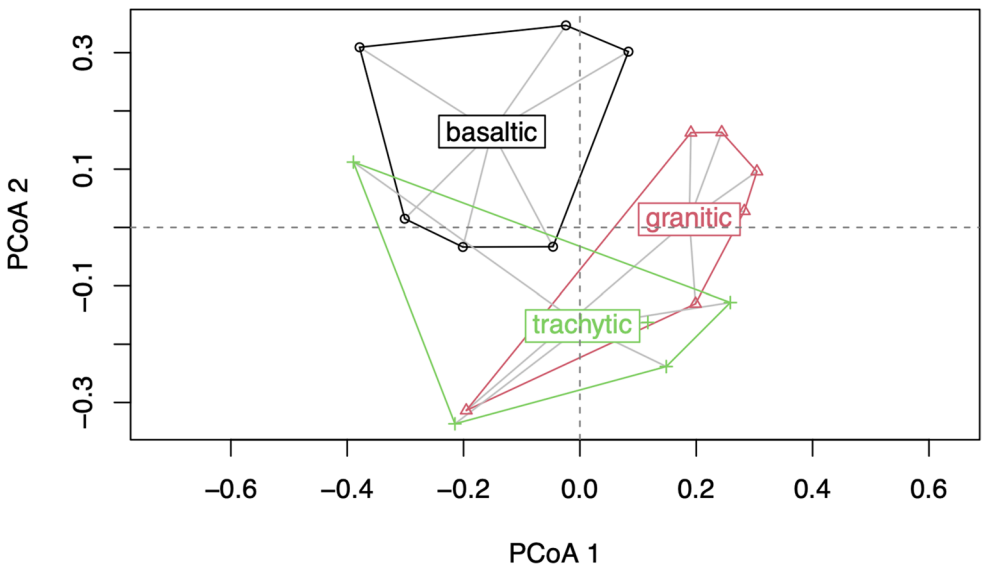

Fig. 3 Ordination of samples obtained through CAPSCALE analysis in relation to the lithological substrates

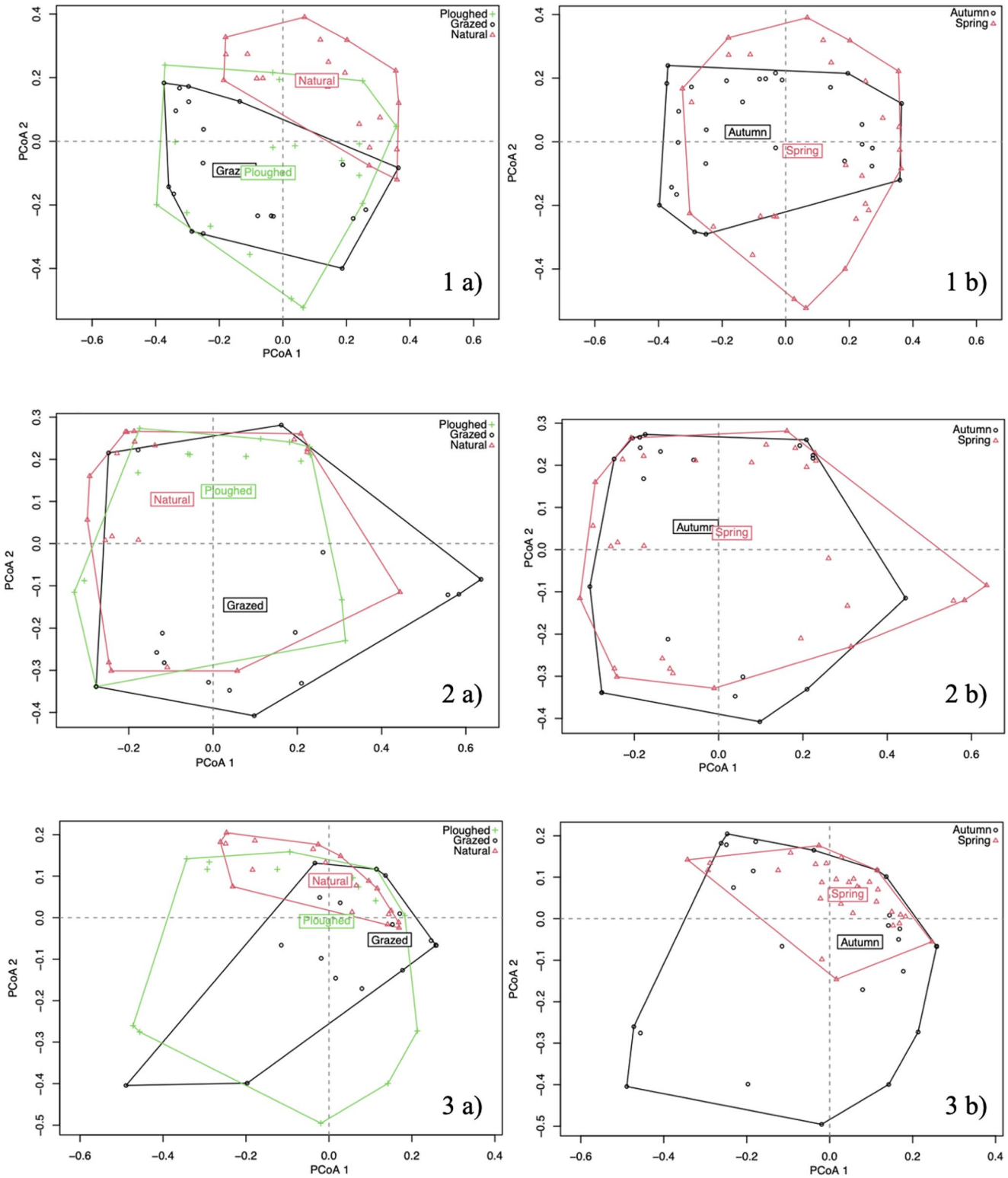

Fig. 4 Ordination of basaltic substrate samples obtained through CAPSCALE analysis in relation to the management system 1**a**) and season 1**b**); Ordination of trachytic substrate samples obtained through CAPSCALE analysis in relation to the management system 2**a**) and season 2**b**); Ordination of granitic substrate samples obtained through CAPSCALE analysis in relation to the management system 3**a**) and season 3**b**)

Additionally, the ADONIS2 test revealed a significant effect of the sampling season on the ectomycorrhizal community composition ($p=0.037$), based on the two time points analyzed, and the ordination plot (Fig. 4 2b) clearly separates the samples collected in the two different seasons.

Impact of management system and sampling season on ectomycorrhizal community composition in granitic substrate samples

The ADONIS2 analysis revealed a statistically significant effect of the management system on the ectomycorrhizal community composition in samples collected from granitic substrate ($p=0.001$).The graphical representation (Fig. 4 3a) highlights distinct clusters corresponding to different management systems, with statistically significant separation.

Similarly, ADONIS2 analysis showed that the sampling season significantly influenced ectomycorrhizal community composition ($p=0.001$), based on the two time points analysed. The graphical representation (Fig. 4 3b) clearly distinguishes samples collected in spring from those collected in autumn.

Discussion

Structure and species composition of ectomycorrhizal fungal communities

The structure and diversity of ectomycorrhizal (ECM) fungal communities are shaped by a complex interplay between environmental factors and forest management practices (Courty et al. 2010; Goldmann et al. 2015; Tomao et al. 2020). By examining diverse forest types and soil substrates in two different seasons, this study provides novel insights into the ecological drivers of these communities in cork oak forests. The selected transects cover a substantial portion of cork oak forests in Sardinia, representing a wide range of natural, grazed, and managed stands, and offering a comprehensive assessment of ECM community dynamics across distinct ecological contexts.

We analysed more than 80,000 ectomycorrhizas from 180 soil samples, representing 167 morphotypes, and 120 genetically assigned OTUs. ECM richness and diversity varied according to forest type: natural forests on granite substrate showed the highest values, while ploughed forests on basalt showed the lowest. Observed differences reflect variations in lithological substrate and management intensity. ECM fungal diversity is primarily influenced by host composition and soil properties, while the effects of aboveground disturbances are context-dependent and do not necessarily reduce richness (Goldmann et al. 2015). Even in disturbed or managed forests, diversified ECM communities can persist, shaped by factors such as understory vegetation, climate, root depth, and exudates, as well as land use history, which affects organic matter, network resilience, and fungal responses to nitrogen availability (Correia et al. 2021).

Among the ECM fungi detected in this study, *Cenococcum geophilum* was ubiquitous across investigated forest types, confirming its status as a cosmopolitan, able to persist even in grazed or ploughed sites, probably due to the presence of resistant sclerotia, drought tolerance, and broad host range (Obase et al. 2017).

Genus-level analysis revealed distinct distributional partners, highlighting the ecological relevance of specific ECM fungal groups. ISA results reflected variation in the number and identity of indicator species across substrate, management systems, and seasons, providing quantitative support for the genus-level patterns observed in ECM community composition. These findings are consistent with previous studies reporting that intensive soil disturbance and plant stress can negatively affect ECM communities and reproductive capacity (Glassman et al. 2016; Policelli et al. 2020). For example, soil tillage combined with grazing or scrub removal may reduce fruiting body production and alter community composition, whereas low-impact management practices can support gradual recovery over time (Azul et al. 2009; Aponte et al. 2010). The genus *Russula* was a dominant component of ECM communities in all forest types investigated, as reflected in ISA results showing association with different basalt sites, suggesting that, although this genus is widely adaptable, it may be sensitive to intense soil disturbances. *Tomentella* was strongly represented, especially in natural and grazed forests, and ISA indicated its presence mainly in spring indicator taxa across multiple substrate–management combinations. The differential distribution of *Inocybe* and *Cortinarius* across forest contexts reflects environmental selection influenced by soil nutrients and management. ISA indicated higher number of *Inocybe* OTUs associated with basalt–grazed–spring soils, while *Cortinarius* taxa were indicated in basalt natural–spring and trachyte–ploughed–spring forests except natural sites, and in granitic substrate only in natural site. *Cortinarius*, known for decomposing complex organic matter and mobilising organically bound nitrogen (Sun et al. 2015; Arraiano-Castilho et al. 2021), and *Inocybe* showed distribution patterns consistent with these ISA observations, suggesting an influence of soil properties and management practices on fungal community structure (Suz et al. 2014; Kałucka and Jagodziński 2017; Glassman et al. 2017). Finally, *Xerocomellus* were associated with basaltic soils, probably due to their mineral composition

and organic matter dynamics. In contrast, *Hebeloma* and *Entoloma* showed narrower distributions in ISA indicator species, consistent with higher ecological specialization (Santos-Silva et al. 2016; Kałucka and Jagodziński 2017; Correia et al. 2021).

Effect of lithological substrate on ectomycorrhizal diversity and community composition

The lithological substrate significantly influences ectomycorrhizal (ECM) diversity, particularly species diversity, while its effect on species richness and evenness is comparatively limited. The three-way ANOVA showed that species richness and Shannon diversity were significantly affected by substrate, management, and season, with management emerging as the strongest driver of variation. Significant interactions among factors, especially between substrate and management, underscore the complex, multifactorial nature of ECM diversity. These results suggest that, despite similar overall species accumulation, specific lithological conditions can favour distinct community structures (Adamo et al. 2021; Weemstra et al. 2020). ECM communities in granitic soils exhibited higher diversity, consistent with known effects of soil physicochemical properties on ECM composition (Tedersoo et al. 2012; Liu et al. 2020; Prieto-Rubio et al. 2024). Seasonal effects should be further investigated through repeated monitoring across multiple years to confirm the observed patterns.

Natural cork oak forests on granitic substrates may exhibit greater environmental heterogeneity and a wider range of potential host species, thereby enhancing ECM richness and functional diversity through niche differentiation and resource partitioning (Ishida et al. 2007; Tedersoo et al. 2024). Evenness (J) was not significantly affected by lithology or management alone, but responded to the combination of factors, indicating that relative species abundance is more stable than species number across substrates.

Community composition analysis further supports the role of lithology as a structuring factor in ECM fungi distribution. ADONIS2 and CAPSCALE analyses revealed distinct clustering patterns, with substrate type explaining a relevant portion of the observed variability in fungal assemblages. These results are consistent with previous studies showing that soil mineralogy and texture can influence mycorrhizal symbioses by altering root penetration depth, moisture retention and nutrient cycling dynamics (Cheeke et al. 2017; Meng et al. 2023). The significant aggregation of ECM communities along lithological gradients highlights the role of the substrate as an environmental filter in shaping fungal assemblages in cork oak ecosystems (Glassman et al. 2017; Correia et al. 2021).

Impact of management system and sampling season on ectomycorrhizal community diversity and composition

The results demonstrate that both forest management practices and seasonal variability significantly affect ECM fungal diversity and community composition, with some variation in effect magnitude across substrates. However, the observed seasonal patterns should be considered preliminary, as they are based on only two sampling points.

Across all lithological substrates, ectomycorrhizal (ECM) diversity patterns were primarily driven by management intensity. Species richness and Shannon diversity varied significantly in relation to management and season, with higher values generally associated with less intensive management, whereas evenness exhibited a more limited response. Lower evenness values indicated the dominance of a few ECM taxa, which may be associated with seasonal shifts in resource availability and host root dynamics (Suz et al. 2014).

The impact of land use practices on ECM communities aligns with previous findings in managed *Montados* systems, where tillage and permanent grazing significantly influenced fungal community structure (Azul et al. 2010; Santos-Silva et al. 2016). Disturbance-driven shifts may be mediated by fungal foraging strategies, with long-distance exploration types able to access distant resources, while short-distance or contact types, being less carbon-demanding, can persist under limited resource availability, showing resilience that depends on the ecological context (Tedersoo and Smith 2013; Kałucka and Jagodziński 2016; Jörgensen et al. 2023). Grazing, by controlling shrub encroachment, can influence ECM diversity, as previous studies suggested that shrub management through cutting, without soil disturbance, favours ECM fungi over sites subjected to tillage (Santos-Silva et al. 2016; Rodríguez-Rojo et al. 2022). Seasonal variability also influenced ECM diversity. Species richness varied across seasons regardless of management type, with higher values in spring and lower values in autumn, likely due to limited ECM recovery following summer drought stress (Reis et al. 2018).

Community composition analyses highlighted the influence of both management and seasonal variation on ECM fungal assemblages, particularly on granitic and basaltic substrates. ADONIS2 results showed that management practices explained a significant proportion of ECM community variation, forming distinct clusters for natural, grazed, and ploughed woodlands. Seasonal effects were most pronounced on granite, where spring and autumn samples formed distinct clusters, likely due to shifts in root exudation, soil moisture, and fungal life cycles (Mrak et al. 2021).

The observed differences in ECM diversity and community structure across management types highlight the impact of anthropogenic disturbances on fungal community structure and composition (Tomao et al. 2020). Ploughing disrupts ECM networks by disturbing the soil organic layer, reducing ECM fungal abundance due to lower carbon allocation from autotrophic hosts. Additionally, understory removal shifts fungal composition, increasing saprotrophic fungi in response to root and aboveground litter inputs (Reis et al. 2018; Rodriguez-Ramos et al. 2021).

The effects of grazing appear more complex. Moderate grazing alters organic matter dynamics and soil microclimate conditions depending on intensity, while excessive disturbance can degrade ECM communities through soil compaction and nutrient imbalances (Azul et al. 2010; Reis et al. 2018). Recent studies have highlighted that grazing can substantially modify belowground fungal dynamics and soil nutrient availability. Yang et al. (2020) found that moderate grazing increases soil carbon and nitrogen availability compared to both ungrazed and heavily grazed conditions, potentially altering nutrient balances that influence ectomycorrhizal and arbuscular mycorrhizal community composition. Moreover, grazing intensity modulates mycorrhizal abundance and structure, affecting network complexity and species dominance through grazing-induced changes in soil pH and nutrient status (Zhou et al. 2025). In our study, ectomycorrhizal genera associated with different ecological responses to environmental gradients were observed across substrates and management types. Some genera tended to occur more frequently in grazed sites, including *Cenococcum*, *Cortinarius*, and *Inocybe*, while others were widespread across both natural and ploughed forests. Notably, the genera *Russula* and *Tomentella* were found across different conditions, consistent with evidence that species within these genera can exhibit variable responses to environmental factors such as nitrogen availability, including both nitrophilic and nitrophobic tendencies in different species (Ning et al. 2018; Defrenne et al. 2019; Kuyper and Suz 2023; Jörgensen et al. 2024). These ecological patterns suggest that site-specific management practices can interact with environmental conditions to shape ECM community composition. Consequently, understanding fungal responses requires considering both biotic and abiotic factors influenced by land use.

In regions where cork oak forests are primarily privately owned, management choices depend largely on cork market value. Low economic returns often shift land use toward forage cultivation or grazing, whereas high-quality cork production encourages ecosystem conservation, limiting management-driven simplification. The significant role of shrub cover and understory species composition in shaping fungal communities and multiple functional guilds reinforces the intricate relationships between vegetation structure and mycorrhizal symbionts. These interactions play a crucial role in maintaining belowground diversity and ecosystem functioning (Wang and Qiu 2006). However, the magnitude of management effects is context-dependent, varying according to lithological conditions and seasonal dynamics.

Conclusions

This study investigates the complex interactions between lithology, forest management, and seasonal variability in the formation of ECM fungal communities in cork oak forests. While lithology establishes the basic structure of these communities, management practices and seasonal dynamics may contribute to additional variability, influencing both diversity and composition. In particular, ploughing consistently reduced ECM diversity, especially on basaltic substrates, suggesting that soil disturbance critically compromises the resilience of belowground mutualistic networks. Conversely, trachytic soils appeared more tolerant of such disturbance, supporting richer ECM assemblages even under tillage intervention. These results argue for the importance of substrate-specific management strategies: while trachytic substrates appear more tolerant of limited ploughing, grazing systems on basaltic and granitic substrates are more susceptible to fungal community disturbance. From a conservation perspective, minimizing soil disturbance, especially on ecologically sensitive or less resilient substrates, should be a priority to preserve the functional integrity of ECM communities. Furthermore, the observed seasonal changes in community structure highlight the need for long-term, multi-seasonal monitoring to obtain more robust data on ECM dynamics and to better assess the stability of fungal networks over time, also considering potential impacts of climate change in the Mediterranean region. Given the central role of ECM fungi in nutrient cycling, tree health, and forest ecosystem resilience, incorporating mycorrhizal indicators into forest monitoring networks and forest management planning is essential. Such integration will be crucial to ensure the sustainability of cork oak woodlands under ongoing environmental change and land-use pressure.

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Author contributions SS, AF, EL conceived and designed the study. SS and EL performed the experiments. SS, CM, AF, MI, ES and EL analysed the data. All the authors contributed to manuscript development and revisions.

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Declarations

Competing interests The authors declare no competing interests.

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