

Diversity and Ecological Distribution of Macrofungi in a Site of Community Importance of Umbria (Central Italy)

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Abstract: This study attempts to assess diversity and distribution of macrofungi in six Collestrada woodland types characterized by a prevalent plant species: the *Carpinus betulus*, the *Quercus cerris*, the *Q. frainetto*, the *Q. petraea*, the *Q. ilex*, and the plantations with *Pinus pinea* and *Pinus pinaster*. The mycological survey was carried out from January 2011 to July 2013, throughout the Collestrada woodland types. A total of 305 species belonging to 61 families and 121 genera were identified from the Collestrada forest over a 2 y and half sampling.

The collected species were mainly *Basidiomycota* (97%), including a high relative abundance of *Agaricomycetes* (96.6%). The *Agaricales* order is the most represented. The diversity and structure of the macrofungi assemblages are mainly affected by the host tree species composition. The macrofungal community at *Q. frainetto* woodland, displayed the highest richness and diversity. The lowest richness and diversity was found in *Pinus* spp. plantation.

Among the species collected, 81 fungal species were included in the Red List (or proposals for Red Lists) in several European countries because of their rarity due to natural or anthropogenic factors. For this it may be worth taking the Collestrada forest into consideration for the in situ conservation of these species.

Keywords: Fungal conservation, hornbeam, host preference, native woodland, oak, plantation, species richness.

INTRODUCTION

Fungi hold key roles in nutrient dynamics, soil health, species mutualisms and interactions, and overall ecosystem processes (Angelini *et al.* 2006, 2008, 2009, Pagiotti *et al.* 2011, Picco *et al.* 2011, Angelini *et al.* 2012a, b, Perotto *et al.* 2013, Angelini *et al.* 2014, 2015a, b). However, despite their functional importance, they are often overlooked and left out of conservation initiatives (Varese *et al.* 2011, Zotti *et al.* 2013, Pecoraro *et al.* 2014).

Fungal biodiversity worldwide has been estimated at over 1.5 million (Hawksworth 2001), i.e. about six–seven times the number of known plant species (Mora *et al.* 2011). Yet, only about 5-10% of fungal species have been discovered and described (Lonsdale *et al.* 2008).

At the regional scale climatic conditions, tree species composition have been identified as important variables influencing species diversity and community structure (Gómez-Hernández & Williams-Linera 2011, O’Hanlon & Harrington 2012a, b).

Currently, there is interest in macromycete distribution patterns in relation to forest tree species composition (O’Hanlon & Harrington 2012a, b). The relationship

between tree and fungal communities is reflected in host trees affecting fungal specialization and providing unique habitat availability and different resource quality (Buee *et al.* 2011). Macromycete richness and distribution patterns have been related to the composition and structure of the tree community in temperate (Gabel & Gabel 2007) and tropical habitats (Ferrer & Gilbert 2003), whereas, in the Mediterranean region, fewer ecological studies have related fungal diversity and distribution to plant diversity (Richard *et al.* 2004).

The influence of the replacement of a native forest by mono and multi-species plantations on fungal diversity has been studied in several countries. Some studies have shown that coniferous plantations can display significant fungal diversity (O’Hanlon & Harrington 2012b), however other studies have shown that exotic conifer plantations display a fungal diversity lower than those of native hardwood stands (Ferris *et al.* 2000). In New Zealand, replacing *Nothofagus* forests with *Pinus radiata* reduced the species richness and diversity of indigenous ectomycorrhizal fungi (Walbert *et al.* 2010). In a French temperate forest stand, replacement of the native beech-oak forest with Norway spruce, Douglas-fir, Nordmann fir and Corsican pine also reduced the diversity of epigeous ectomycorrhizal and saprotrophic fungi (Buee *et al.* 2011).

The forest of Collestrada (PG) is situated in the region of Umbria (central Italy), covering an area of approximately 68 ha (250-306 m a.s.l.). It was declared a Site of Community Importance (SCI) (IT5210077) in 2008. The climate, as

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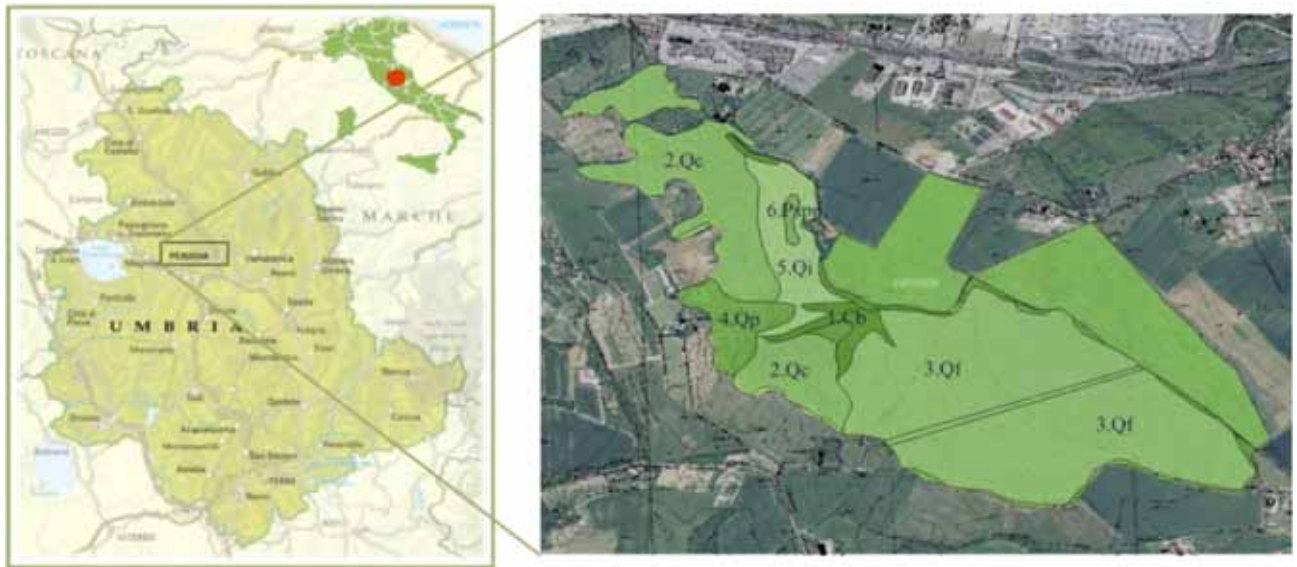


Fig. (1). Map showing the six studied Collestrada woodland types: 1.Cb, *Carpinus betulus*; 2.Qc, *Quercus cerris*; 3.Qf, *Quercus frainetto*; 4.Qp, *Quercus petraea*; 5.Qi, *Quercus ilex*; 6.Pspp., *Pinus* spp.

revealed by the phytoclimatic analysis of the Umbria region, is similar to that of the town of Perugia. According to bioclimatic indices, this can be defined as Temperate Macroclimate, Upper Hilly Thermotype, Lower Humid Umbrotype, with dry summers and maximum precipitation in spring and autumn (Angelini *et al.* 2014).

Recent studies have shown that the biodiversity of several groups of organisms in the Collestrada forest, including that of birds, invertebrates and vascular plants, is surprisingly rich (Orsomando *et al.* 2004, Poponessi *et al.* 2014), but there have been few and brief systematic studies of diversity or ecology of macrofungi (Angelini *et al.* 2012a, 2015a). This paper is the first attempt to assess the diversity and distribution of macrofungi in natural and plantation Collestrada woodlands over a thirty-one month period.

The aims of this study are to describe the macrofungal communities of Collestrada forest and compare the macrofungal species richness and community assemblages in all six Collestrada woodland types characterized by a prevalent plant species.

METHODS

In the forest of Collestrada, the mycological investigation was carried out from January 2011 to July 2013, throughout the most widespread local plant communities: (1) *C. betulus* L. woodland (43.08061111°N 12.46213889°E), (2) *Quercus cerris* L. woodland (43.0795°N 12.462°E), (3) *Quercus frainetto* Ten. woodland (43.0799722°N 12.46569444°E), (4) *Quercus petraea* (Matt.) Liebl. woodland (43.0808889°N 12.4613056°E), (5) *Quercus ilex* L. woodland (43.0812222°N 12.4610278°E), (6) plantation with stone pine (*P. pinea* L.) and maritime pine (*Pinus pinaster* Aiton.) (43.08325°N 12.46091667°E) (Fig. 1).

The number of woodlands visited and the number of surveys per woodland were higher during the fruiting season,

which was from April to May until late July and from September to November for the 2 y and half period. Throughout the fruiting season each woodland type was usually visited once every one or two weeks. During the out of season months, a subset of six woodlands was surveyed once per month. The surveys were limited to macromycetes that were visible to the naked eye (greater than 1 mm in size) (Arnolds 1981).

Collections were identified with reference to standard texts and dried specimens were deposited at the DCBB (Department of Chemistry, Biology and Biotechnology, University of Perugia, Italy) herbarium. Nomenclature follows Index Fungorum (<http://www.indexfungorum.org>) and Dictionary of the Fungi (Kirk *et al.* 2008).

The macrofungal species recorded were split into functional groups based on their primary mode of nutrition: Am associated with bryophytes-possibly weak parasitic, Em ectomycorrhizal, Em (Sh) ectomycorrhizal or sometimes lignicolous saprotroph, Em?(St) maybe ectomycorrhizal or sometimes terrestrial saprotroph, Pn necrotrophic parasite, Pn (Sh) necrotrophic parasite or sometimes lignicolous saprotroph, Pn (Sh?) necrotrophic parasite or maybe lignicolous saprotroph, Sc coprophilous, Sh lignicolous saprotroph, Sh (Em?) lignicolous saprotroph or maybe ectomycorrhizal, Sh (Pn) lignicolous saprotroph or sometimes necrotrophic parasite, Sh (Sk) lignicolous saprotroph or sometimes herbaceous saprotroph, Sh (St) lignicolous saprotroph or sometimes terrestrial saprotroph, Sk herbaceous saprotroph, Sk (Pn) herbaceous saprotroph or sometimes necrotrophic parasite, Sk (Pn?) herbaceous saprotroph or maybe necrotrophic parasite, Sk (Sh) herbaceous saprotroph or sometimes lignicolous saprotroph, St terrestrial saprotroph, St (Am) terrestrial saprotroph or maybe associated with bryophytes-possible weakly parasitic, St (Em?) terrestrial saprotroph or maybe ectomycorrhizal, St (Sh) terrestrial saprotroph or sometimes lignicolous saprotroph, St (Sc) terrestrial saprotroph or sometimes

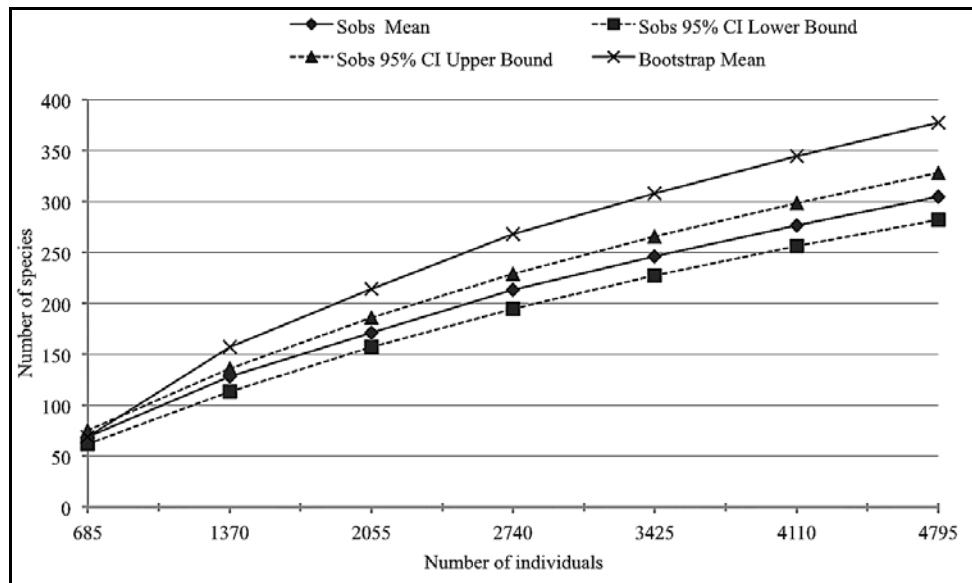


Fig. (2). Species accumulation curves and bootstrap estimate of total richness for random samples of collected macrofungi in the Collestrada forest (EstimateS 8.2).

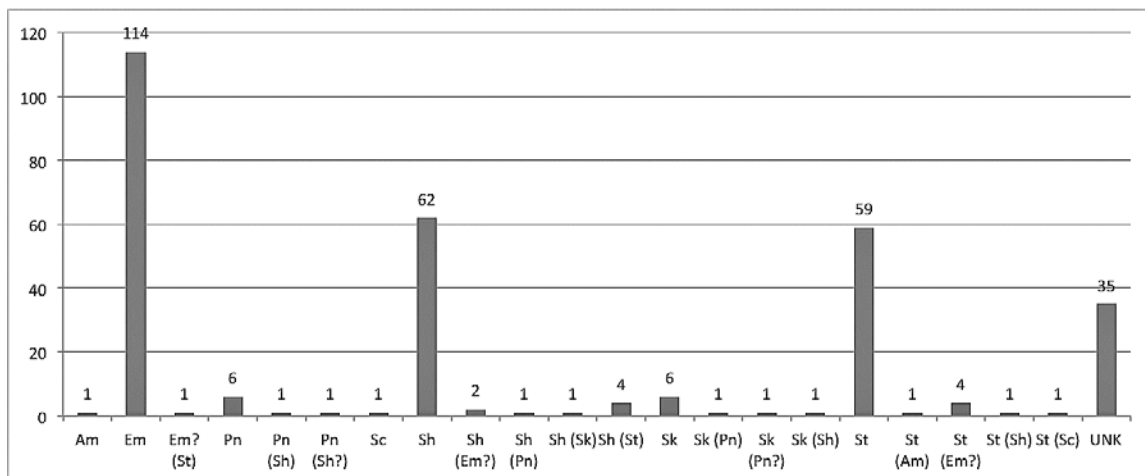


Fig. (3). Number of fungal species from each ecological group recorded in Collestrada forest (PG, Umbria, Italy).

coprophilous, and UNK unknown groups (Arnolds 1995). To determine the dominant macromycete species, abundance and relative abundance were assessed. Abundance is the total number of individuals (basidiomata or ascomata) collected per species, whereas the relative abundance is the proportion of individuals (basidiomata or ascomata) from each species in relation to the total number of basidiomata/ascomata recovered (x100). The distribution of relative species abundance was analysed using a rank–abundance curve. Species accumulation curves, bootstrap estimates of total richness (S), and diversity indexes (Fisher’s α , Shannon J’, Simpson 1/D) were inferred using EstimateS 8.2 (R.K. Colwell, <http://purl.oclc.org/estimateS>). To examine similarity in the communities of macrofungi within and among Collestrada woodland types, only similarity indices based on presence/absence data only (Jaccard’s index) and relative abundance (Morisita-Horn index) (Angelini *et al.* 2012b) were used.

RESULTS

A total of 305 species belonging to 61 families and 121 genera were identified from the woodlands over the 2 y and half (31 months) sampling. The bootstrap analysis estimated a total of 378 species, indicating that our sampling detected 80.7 % of the richness (Fig. 2). The number of fungal species from each ecological group recorded in Collestrada forest are shown in Fig. (3).

In total 4,975 basidiomata and ascomata were harvested. The rank abundance curves for each of the woodland types are similar as they all follow log-normal distribution, although the relative abundance of individual species are different in each of the woodland types (Figs. 4-5). The collected species were mainly *Basidiomycota* (97% of the individuals), including a high relative abundance of *Agaricomycetes* Doweld (99.6%). The *Dacrymycetes* Doweld and *Tremellomycetes* Doweld constituted only 0.06

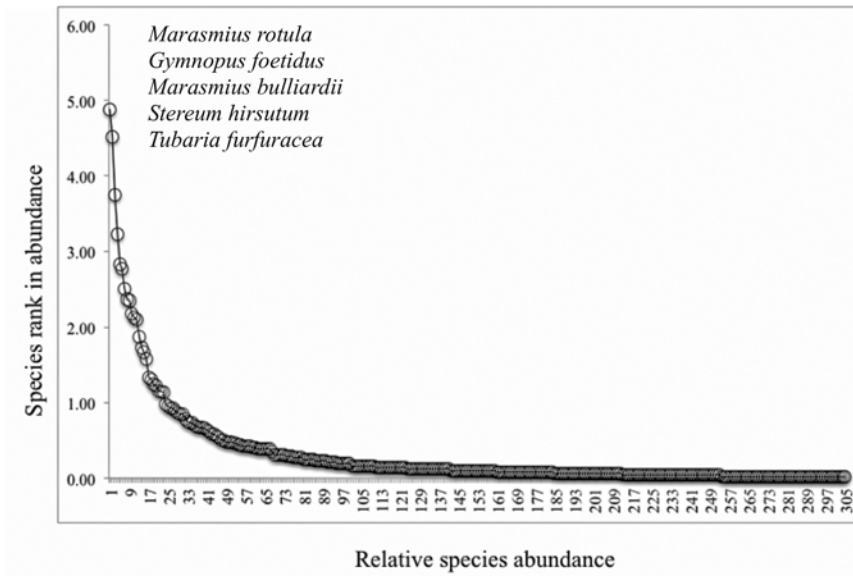


Fig. (4). Dominance–diversity curves for Collestrada species of macrofungi. Most abundante species to least abundante ones are ordered from left to right.

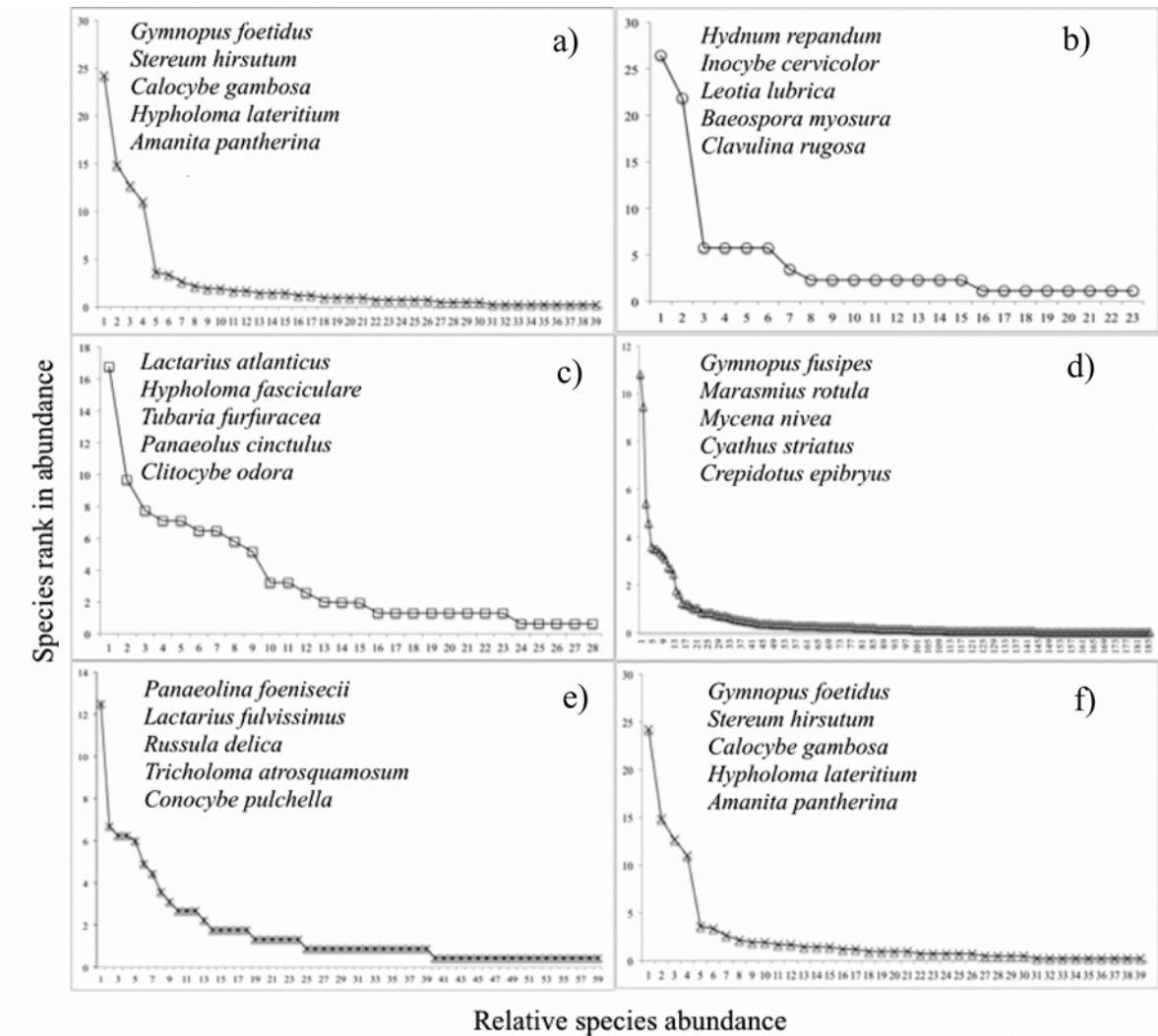


Fig. (5). Dominance–diversity curves for Collestrada woodland types species of macrofungi: **a)** *Carpinus betulus*; **b)** *Pinus* spp.; **c)** *Quercus cerris*; **d)** *Q. frainetto*; **e)** *Q. ilex*; **f)** *Q. petraea*. The most abundant species to least abundant ones are given from left to right.

Table 1. Diversity of macrofungal communities in relation to Collestrada woodland types.

Woodland	<i>Carpinus</i>	<i>Pinus</i>	<i>Quercus</i>	<i>Quercus</i>	<i>Quercus</i>	<i>Quercus</i>	Collestrada
	<i>betulus</i>	spp.	<i>cerris</i>	<i>frainetto</i>	<i>petraea</i>	<i>ilex</i>	forest
Diversity index							
Shannon J'	3.41	2.47	2.91	4.2	2.71	3.6	4.74
Simpson (1/D)	18.41	7.31	13.67	34.97	8.65	23.69	62.98
Fisher's α	21.54	9.48	9.98	48.34	10.43	26.11	72.5
Richness (S)	84	22	28	186	39	59	305

Table 2. Similarity of macrofungi communities in relation to Collestrada woodland type (Cb *Carpinus betulus*; Qc *Quercus cerris*; Qf *Q. frainetto*; Qp *Q. petraea*; Qi *Q. ilex*; Psp *Pinus spp.*), reflecting similarity based on presence/absence data only (Jaccard's index, below diagonal) and relative abundance (Morisita-Horn, above diagonal).

	Cb	Qc	Qf	Qp	Qi	Psp
Cb	-	0.074	0.151	0.33	0.106	0.017
Qc	0.087	-	0.259	0.118	0.021	0.001
Qf	0.174	0.065	-	0.106	0.03	0.04
Qp	0.15	0.047	0.119	-	0.005	0.007
Qi	0.135	0.061	0.104	0.054	-	0.005
Psp	0.029	0.02	0.061	0.052	0.038	-

and 0.28%, respectively, of the collected basidiomycetes. The *Dothideomycetes* O.E. Erikss. & Winka, *Leotiomycetes* O.E. Erikss. & Winka, *Orbilomycetes* O.E. Erikss. & Baral, *Pezizomycetes* O.E. Erikss. & Winka and *Sordariomycetes* O.E. Erikss. & Winka classes belonging to *Ascomycota* were detected in 0.05%, 0.53%, 0.14%, 1.51% and 0.74% of the total basidiomata and ascomata number, respectively. The families that contained the most species were *Russulaceae* Lotsy (27), *Mycenaceae* Overeem (25), *Tricholomataceae* R. Heim ex Pouzar (25), *Marasmiaceae* Roze ex Kühner (18), and *Amanitaceae* R. Heim ex Pouzar (17), which together accounted for about 36.72% of the total surveyed species. *Mycena* (Pers.) Roussel was the most diverse genus with 24 species recorded from the forest plots over the 31-month period, followed by *Amanita* Pers. (17), *Russula* Pers. (17), *Cortinarius* (Pers.) Gray (14), and *Lactarius* Pers. (10).

In the entire dataset, *Marasmius rotula* (Scop.) Fr. and *Gymnopus foetidus* (Sowerby) J.L. Mata & R.H. Petersen were the most abundant taxa, followed by *Marasmius bulliardii* QuéL., *Stereum hirsutum* (Willd.) Pers. and *Tubaria furfuracea* (Pers.) Gillet (Fig. 4).

Total number of basidiomata and ascomata, species richness and relative abundance of the dominant species differed among woodlands (Table 1, Fig. 5). The species composition differed among the six woodlands (Fig. 4).

One species was found in all woodland types (*Tubaria furfuracea*), 2 were found in five forest types (*Tubaria furfuracea*, *Mycena epipterigia*), 8 were found in four forest types, 35 were found in three forest types, 62 were found in two forest types, and 198 were found in only one forest type.

Diversity of macrofungi ranged from 48.34-9.48 (Fisher's α), 4.2-2.47 (Shannon index), and 34.97-7.31 (Simpson index) depending on woodland type (Table 1). The macrofungal community at *Q. frainetto* woodland, displayed the highest richness and diversity with 186 species among 2,218 carpophores. The smallest number of species (22) was found in *Pinus spp.* plantation.

Similarity indices calculated for macrofungal community of the Collestrada woodland types ranged from 0.02 (*Pinus spp.* vs. *Quercus cerris*) to 0.174 (*Quercus frainetto* vs. *Carpinus betulus*) based on presence-absence data (Jaccard's index), and from 0.001 (*Pinus spp.* vs. *Quercus cerris*) to 0.33 (*Carpinus betulus* vs. *Quercus petraea*) based on relative abundance (Morisita-Horn index) (Table 2).

Among the 305 species collected, 81 fungal species were included in the Red List (or proposals for Red Lists) in several European countries because of their rarity due to natural or anthropogenic factors [i.e. *Boletus permagnificus* Pöder, *B. roseoalbidus* (Alessio & Littini) G. Moreno & Heykoop, *B. ichnusanus* (Alessio, Galli & Littini) Oolbekk.



Fig. (6). *Sarcosphaera coronaria* (Jacq.) J. Schröt. (photo: Giancarlo Bistocchi).

and *Sarcoscypha coccinea* (Gray) Boud.] (<http://www.wsl.ch/eccf/>). Among these, it is noteworthy the presence of *Amanita eliae* Quél. included in the preliminary list of 23 species considered rare and / or threatened to Italy, given by the mycology work group for the Italian Botanical Society (Venturella *et al.* 1997). It is also noteworthy the presence of *Sarcosphaera coronaria* (Jacq.) J. Schröt. (Fig. 6), included in the list of 33 fungal species threatened at the European level included in Annex 1 of the Bern Convention (ECCF, 2001). Finally, also important, is the presence of *B. ichnusanus*. It, along with 12 other 'non policy species', has recently been included in the Red List of the Italian Flora, assessed against the IUCN Criteria and Categories (Rossi *et al.* 2013).

DISCUSSION

This study provides a comprehensive analysis of macrofungal species richness, abundance and diversity in Collestrada forest (Umbria, Italy). Mycoecological studies on *Quercus cerris* L., *Q. frainetto* Ten., *Q. ilex* L. and *Q. petraea* (Matt.) Liebl. woodlands have been carried out in Italian (Venturella 2001, Zotti *et al.* 2008) and in some European forests (O'Hanlon & Harrington 2012a, b, Richard *et al.* 2004, Ortega *et al.* 2010, Fodor 2013). Comparisons of the macrofungal communities of Italian forests (i.e. Collestrada forest) and Spanish Oak forests (Ortega *et al.* 2010, Ortega & Navarro 2006) reveals noticeable similarities

in the distributions of many species; however, there are also distinct differences between the two communities, for example some species are much more common in Italian forests [e.g. *Boletus roseoalbidus* (Alessio & Littini) G. Moreno & Heykoop, *Boletus subtomentosus* L., *Clitopilus prunulus* (Scop.) P. Kumm., *Laccaria impolita* Vellinga & G.M. Muell., *Russula torulosa* Bres.] while others are more common in Spanish counterpart forests [e.g. *Boletus spretus* Bertéa, *Leccinum pseudoscabrum* (Kallenb.) Šutara, *Russula amoena* Quél., *Tricholoma stiparophyllum* (S. Lundell) P. Karst., *Entoloma philocistis* Hauskn. & Noordel.]. Larger discrepancies are found between the macrofungal communities of Collestrada Oak woodlands in Italy and Atlantic Oak woodlands in Ireland (O'Hanlon & Harrington 2012b). These communities share few similarities regarding species common in both forests; with just *Stereum hirsutum* (Willd.) Pers. and *Armillaria mellea* (Vahl) P. Kumm. being of similar distribution.

Although there were differences in species richness, the most common species-rich genera in the genera *Mycena* (Pers.) Roussel, *Amanita* Pers., *Russula* Pers., *Lactarius* Pers., *Inocybe* (Fr.) Fr. and *Tricholoma* (Fr.) Staude in our study were also commonly found in the few other studies in Mediterranean oak forests (Richard *et al.* 2004, Ortega & Navarro 2006, Ortega *et al.* 2010). However, these genera are also some of the most species-rich genera worldwide (Kirk *et al.* 2008), so their high species richness may not be a distinguishing factor of Collestrada woodland types.

The study of Collestrada forest is still far from complete, as indicated by both the suggestion that less than 81% of the macromycete species for Collestrada is known and the high diversity predicted by the bootstrap analysis in the present study. Several authors have reported the importance of sampling macrofungi over periods of several years to record the majority of the species present at a site (Gómez-Hernández & Williams-Linera 2011).

Our results indicate differences in macromycete species richness, abundance and diversity patterns between Collestrada woodland types. The existence of distinctive macrofungal communities related to the dominant tree species of the forest has been confirmed in many other studies (Buee *et al.* 2011). The diversity indexes (Fisher's α , Shannon, Simpson) indicated clearly that the macrofungal community of *Q. frainetto* woodland were richer and more diverse than the other woodland types.

Species richness and diversity indexes of *Pinus* spp. plantation were significantly lower than the natural woodlands.

Conifers (*P. pinea* L. and *P. pinaster* Aiton) are not naturally distributed in our area but they are often planted and owing to their capacity for dispersal they are practically present in every wooded area. These sporadic conifers do not have a noticeable influence on the phanerogamic communities of broad-leaved forests but they do affect the mycoflora especially by promoting mycorrhizal fungi (Sousa *et al.* 2011).

Several fungi in the current study have been listed in more than one nutritional category (Table 2) and Arnolds (1995) points out that it is difficult to place some fungi in a nutritional group because of dual roles. Em was the most abundant ecological group, producing around 18.02% of all samples. However, abundance of mycorrhizal mycelium and root tips may be different, as seen in studies examining the below-ground mycorrhizal communities using molecular approaches (Richard *et al.* 2005, Smith *et al.* 2007). The contrasting patterns are suggested to be a consequence of many fungal species being unable to produce basidiomata and ascomata or producing inconspicuous ones. Other explanations include large differences in reproductive and explorative strategies among fungi. In addition, the optimum fruiting conditions for some species may not have occurred in the studied years.

It has been found that 81 fungal species, which appear relatively common in the analysed woodlands of the Collestrada forest, have resulted to be at risk of endangerment or even extinction in various other European countries (<http://www.wsl.ch/eccf/>); for this it may be worth taking the Collestrada forest into consideration for the in situ conservation of these species.

CONCLUSION

This study has shown that Collestrada forest, composed of six different woodland types with particular plant assemblages, provides distinct habitats for diverse macrofungal species, not in the least ectomycorrhizal species. The diversity and distribution of the macrofungi assemblages

are mainly affected by the host tree species composition and by climate.

The information obtained can be used as a baseline to discern future trends associated with climate change, to interpret trends resulting from mushroom harvesting and management, and is therefore important for management, conservation strategies and the conservation policy.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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