Macrofungal diversity in *Pinus nigra* plantations in Northwest Italy (Liguria)

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Liguria (NW Italy) is a region characterised by a great richness in habitats, flora, and mycobiota. In the first half of 20th century, many areas of Liguria were frequently reforested with conifers, mainly pines. This type of habitat is still uninvestigated from a mycological perspective. As a consequence, our work aims at assessing mycodiversity under *Pinus nigra* plantations and at checking possible correlations between fungal diversity/abundance and ecological parameters. Eleven permanent plots were surveyed in two serpentine areas in Liguria (Northwestern Italy) through three years. Sporomata were collected and identified in all seasons of fungal growth. Shannon's diversity index and Jaccard's similarity index were calculated for each plot, and then the indices were correlated with the main ecological parameters. 94 macrofungal species were found. For each plot, Shannon's index shows a low level of macrofungal diversity. Significant correlations exist between the mycodiversity and the pH, the altitude, and the grass cover percentage. Whereas, only sporomata and biomass values are significantly correlated with the ordination performed. The low mycodiversity found is probably due to both the presence of *Pinus nigra* and serpentine soil. Ectomycorrhizal trophic group is the richest in species. Hypogeous species are very few and sporomata are not abundant. Finally, the surveys do not show typical serpentinophilous fungi.

Keywords: fungi, mycodiversity, European Black pine, serpentine soil, Shannon's index.

Liguria is a coastal north-western Italian region with a peculiar geomorphology and climate that allows the coexistence of different habitats such as woods, grasslands, wetlands, and beech forests. The habitat richness affects flora and mycobiota which consist in a total of 3131 plant species and 1994 macrofungal species (Zotti & Orsino 2001, Conti *et al.* 2005, Zotti *et al.* 2008, Venturella *et al.* 2011). Despite the fact that the Ligurian territory cor-

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responds only to 2 % of Italy, the species collected and classified in this region represent 40 % of macrofungi (Onofri *et al.* 2005) and 46 % of wild plants recorded (Conti *et al.* 2005). Geologically, Ligurian soils derived from ultramafics cover 40 % of the total surface (Marsili 2010), frequently planted with pines, especially *Pinus pinaster* Aiton, *Pinus nigra* J. F. Arnold (Black pine), and, sometimes, *P. sylvestris* L. (Scots pine). Black pine and Scots pine plantations on ultramafic outcrops often spread from thermophilous and sub-mesophilous woods to cool montane plains (Aliugi *et al.* 2007). However, in spite of the wide territory covered by coniferous plantations, little information on their biodiversity is available. More recently, developments in forestry policy highlighted the need to increase the knowledge on the structure, composition, and diversity of these commercial forests (Ferris *et al.* 2000, Humphrey *et al.* 2000, Fernández-Toirán *et al.* 2006).

Although forests usually host the species richest habitats, especially of macrofungi (74 % of threatened species are found in forests, Dahlberg *et al.* 2010), they are not usually considered in conservation strategies, land-use planning and forest management, in spite of the functional roles (i.e. biogeochemical cycles, recycling of organic matter, nutrients uptake) played in the whole forest ecosystems (Mueller *et al.*2004). Moreover, thanks to their ecological plasticity, fungi have a great adaptive capability to live where environments conditions become really extreme and crucial for most of the life forms (Selbmann *et al.* 2013). Studies carried out in boreal ecosystems, in fact, highlight the potential role of macrofungal community as a quality indicator of the whole forests (Snäll & Jonsson 2001, Juutinen & Mönkkönen 2004).

As concerns mycobiota of pine plantations, few studies were carried out. For instance, Ferris *et al.* (2000) studied the composition and diversity of fungal communities in planted forests from Southern England, demonstrating lowland conifer plantations as suitable habitats for a number of macrofungal species. Buée *et al.* (2011) investigated how the richness and diversity of natural fungal communities can be influenced by reforestation (especially by coniferous species), and they observed that reduction/decrease of fungal diversity in different plantations, compared with native stands, can be due to several aspects, such as: the absence of late-stage fungi, the mono-specificity of plantations, and the absence of species specific ectomycorrhizal fungi. Finally, Fernández-Toirán *et al.* (2006), in order to reveal succession patterns of macrofungal communities in several *Pinus pinaster* forests, investigated the sporomata production during six years, with the result that fungal diversity can be increased with the presence of a diverse array of forest stand ages in the landscape.

The partial lack of information on fungal community in forests planted with conifers (especially, in Mediterranean areas) propelled us to study some conifer woods, specifically European Black pine (*Pinus nigra*) plantations. The forests taken into account are located in north-western Liguria. The main goals of this paper are: i) to evaluate the mycodiversity associated with young *Pinus nigra* plantations, and ii) to find possible relationships between fungal diversity and ecological parameters.

Materials and methods

Study area

The study was carried out in 11 permanent plots located in two different areas of Liguria (NW Italy) originally characterized by oak woods dominated by *Quercus petraea* (Mattuschka) Liebl. This vegetation type belong to *Physospermo cornubiensi-Quercetum petraeae* (Oberdorfer & Hofmann 1967) association. These woods were exploited by local population and partially destroyed by human impact. In both areas the presence of the Black pine communities originates from the reforestation carried out in the late 19th and mid-20th century in order to protect the slopes from erosion. The former area (labelled S) is in the Beigua Regional Natural Park 7 km far from the sea, it has a more Mediterranean climate (Vagge 1999) and includes six plots (S1-S6). The latter area (labelled G) is in the north-eastern to S area, 20 km far from the sea, with a more continental climate (Vagge 1999) and it includes five plots (G1-G5). More details can be found in Tab. 1.

Tab. 1. Main characteristics of the plots studied.

Plot	Age of trees (years)	Altitude (m)	Exposition	GPS coordinates WGS-84	Mean annual rainfall (mm)	Mean annual temperatures (mean Min- mean Max°C)
S1	65	920	W	N 44°25.405' E 008°31.802'	1300	6–14
S2	65	911	W	N 44°25.405' E 008°31.802'	1300	6–14
S3	65	976	SE-E	N 44°25.383' E 008°32.254'	1300	6–14
S4	65	1025	S-SE	N 44°25.484' E 008°32.345'	1300	6–14
S5	65	1210	SE	N 44°25.871' E 008°34.399'	1300	6–14
S6	65	1137	W-SW	N 44°25.729' E 008°32.809'	1300	6–14
G1	55	763	S-SW	N 44°32.284' E 008°49.957'	1200	7–16
G2	55	781	S	N 44°32.280 E 008°49.900	1200	7–16
G3	55	837	W	N 44°30.702' E 008°48.016'	1200	7–16
G4	55	838	W-NW	N 44°31.692' E 008°48.804'	1200	7–16
G5	55	831	W	N 44°31.626' E 008°49.812	1200	7–16

The areas are part of a wide ophiolitic zone with an Alpine metamorphic imprint. This zone represents a fragment of the original Jurassic oceanic ba-

sin, and therefore the bedrock consists of serpentinites (Chiesa *et al.* 1975, Vanossi *et al.* 1984, Capponi & Crispini 2002, Spagnolo *et al.* 2007).

Survey and data analysis

We selected randomly 11 permanent non-continuous plots among Ligurian pine plantations, with a size of about 1000 m^2 (approximately of 32 m × 32 m). The plots were surveyed from June 2007 to March 2010, three times a month during autumn season and at two times a month during spring season. Each plot was georeferenced in WGS-84 Global Position System (GPS) and coordinates expressed as decimal degrees. Also altitude, inclination, rockiness, stoniness, floristic composition, and percent coverage of vegetation type (grass-, shrub- and tree-layer) were recorded (Tabs. 1, 2, 3). Nomenclature of plant species listed refers to Conti *et al.* (2005).

Tab. 2. Data of soil in each plot.

Plot	pH	Inclination (°)	Rockiness* %	Stoniness* %	Soil type
S1	5.01 ± 0.31	14	20	2	Humic Dystric Regosol
S2	6.74 ± 0.54	15	2	7	Humic Dystric Regosol
S3	5.00 ± 0.21	14	2	10	Humic Dystric Regosol
S4	4.60 ± 0.15	30	10	3	Humic Dystric Regosol
S5	5.40 ± 0.17	10	5	0	Humic Dystric Regosol
S6	5.84 ± 0.13	15	20	2	Humic Dystric Regosol
G1	7.0 ± 0.42	23	2	2	Humic Hyperdystric Regosol
G2	4.25 ± 0.16	24	2	0	Humic Hyperdystric Regosol
G3	5.86 ± 0.38	13	0	2	Humic Hyperdystric Regosol
G4	4.20 ± 0.12	12	0	0	Humic Hyperdystric Regosol
G5	6.33 ± 0.21	7	0	0	Humic Hyperdystric Regosol

* Rockiness (amount of emerging parent material) and stoniness (amount of stone on the ground) coverage were estimated according to the scale of Braun-Blanquet (1946).

Tab. 3. Vegetation percentage coverage in each plot.

Plot	Pine coverage	Other tree coverage	Shrub coverage	Grass coverage
S1	60	0	5	85
S2	35	0	1	70
S3	80	0	11	20
S4	50	20	20	40
S5	50	0	0	85
S6	50	20	15	65
G1	60	0	25	75
G2	60	0	25	75
G3	75	0	7	95
G4	70	0	10	70
G5	50	0	5	95

To assess the soil characteristics three soil replicate samples were taken from each plot (S1-6; G1-5). Soil sampling was carried out in autumn season,

during the maximum sporomata growing. The performed analyses follow the Italian official methods (MiPAF 2000): soil samples were air-dried, particle size distribution analysis was carried out by wet-sieving for the fraction >50 µm and the composition of the fine fraction (<50 µm) was determined by pipette procedure after dispersion of the sample with sodium hexametaphosphate $(NaPO_3)_6$. The soil profile (one for each plot) consisted of small dug pits large enough to allow examination and description of the different horizons. Soil classification was carried out according to the FAO methods and terminology (FAO 2006 a, b).

The pH was measured with the potentiometric method: soil samples were dried at 50 °C for 24 hours, sieved at 2 mm, mixed in a 1:2.5 soil-water suspension, agitated 15 minutes, left to settle 30 minutes and measured with a glass electrode pH meter (10 replicates for each plot).

Mycological surveys were focused both on epigeous and hypogeous macrofungi. The samplings of hypogeous fungi were performed with the help from professional harvesters and trained dogs. Sporomata were counted and identified on the basis of macroscopic and microscopic characteristics. The observations of microscopic characters were based on material mounted in different media: distilled water, lactic acid plus acid fuchsine, 5 % potassium hydroxide, and Melzer's reagent. In the case of spores and other microscopic characters (e.g. basidia, cystidia) at least 30 measurements were made for each structure. For taxa identification specific mycological literature was consulted. The systematics used follows Hibbet *et al.* (2007), Kirk *et al.* (2008), and Vizzini (2004). Nomenclature and authors abbreviations are used in accordance with CABI, CBS and IMA (see www.indexfungorum.org, www.cbs.knaw.nl, www.mycobank.org).

The different fungal species were classified into the following four trophic groups according to literature (Boccardo *et al.*2008, Rinaldi *et al.* 2008, Tedersoo *et al.* 2010) and to the niches of the collecting site: ectomycorrhizal (EM), saprotrophic in soil, humus or in litter (SH), saprotrophic on dead wood (SW) and parasitic (P).

Successively, all the examined material was deposited at GDOR (Herbarium of the Museo Civico di Storia Naturale Giacomo Doria, Mycologia section, Genova, Italy). Data concerning the collected fungi were inserted in a specific database (Advanced Liguria Check-list of Ectomycorrhizal and other fungi, A.L.C.E.) for statistical analyses.

Biodiversity *H'* was assessed for each trophic group, for each single plot and for the whole studied area by means of Shannon's biodiversity index (Shannon 1948, Magurran 2004, Mueller *et al.* 2004). It can be proven that the maximum value H_{MAX} of Shannon's index is given by:

$$H_{MAX}=log_2 M$$

where M is the total number of species observed. This upper bound of entropy can be useful to compare the diversity of different forests. In fact, if only partial data regarding the fungi collected in some forests are available,

we can compare the H_{MAX} of these forests with the actual diversity of our woods: if the H_{MAX} is less than the actual mycodiversity, then the actual diversity of the forest taken into account cannot exceed the mycodiversity of our surveyed wood. In this sense, the H_{MAX} represents the "Maximal Potential Biodiversity" (MPB).

The evenness (E) of Shannon's index (Magurran 2004, Mueller *et al.* 2004) was calculated as:

$$E=H'/ H_{MAX}$$

Jaccard's similarity coefficient J (1908) was used for comparing the similarity of the plots from a mycological point of view.

Pearson's correlation was computed to interpret relationships among ecological/environmental variables and mycodiversity.

Macrofungal biomass was estimated according to the methodology of Tóth & Feest (2007). We used the number of sporomata per species recorded in each plot and the data on cap diameter from literature (Boccardo *et al.* 2008) to calculate cap area index.

Tab. 4. Data about Shannon's index and evenness for all the plots. The first column shows the plot considered, the second and third columns specify Shannon's index evaluated on all the species (G) and Shannon's index evaluated only on the ectomycorrhizal species (M), respectively. The last column reports the evenness related to the total mycodiversity in all plots. G3 plot in bold shows Shannon's index highest value (3.335).

Plot	Shannon's Index (G)	Shannon's Index (M)	Evenness
S1	2.33	1.22	0.70
S2	2.56	2.15	0.67
S3	1.98	2.23	0.57
S4	2.18	1.16	0.57
S5	2.74	1.89	0.65
S6	1.84	1.66	0.48
G1	2.82	1.05	0.74
G2	2.37	2.37	0.59
G3	3.34	2.66	0.69
G4	2.55	1.40	0.65
G5	2.88	2.01	0.62

Finally, the multivariate analysis of PCA (Principal Component Analysis) was performed in order to understand which variables (vectors) provide to plots variability. Vector fitting (by using function "envfit") was executed to interpret environmental variables onto ordination. The arrow points to the direction of most rapid change in the environmental variables. Often this is called the direction of the gradient. The length of the arrow is proportional to the correlation between ordination and environmental variables. Often this is called the strength of the gradient. All statistical analyses were executed by using Vegan package in R system (2012) for statistical computing (version 2.15.2).

Tab. 5. Jaccard similarity coefficients of macrofungal species between plots (S1-6, G1-5).

	S1	S2	S3	S4	S5	S6	G1	G2	G3	G4	G5
S1	1	0.33	0.11	0.26	0.21	0.20	0.33	0.24	0.22	0.25	0.21
S2		1	0.14	0.27	0.18	0.20	0.22	0.21	0.17	0.11	0.22
S3			1	0.25	0.11	0.19	0.14	0.12	0.15	0.04	0.06
S4				1	0.27	0.22	0.33	0.21	0.20	0.21	0.26
S5					1	0.14	0.18	0.18	0.27	0.21	0.29
S6						1	0.22	0.18	0.14	0.12	0.18
G1							1	0.21	0.24	0.32	0.30
G2								1	0.26	0.14	0.19
G3									1	0.23	0.29
G4										1	0.25
G5											1

Results

Habitat conditions

Both the areas are characterised by serpentine substrates and serpentine soils. The only difference between G an S soils consisted in organic matter content, higher in S (106.9 ± 32.1 g 100 g^{-1}) and lower in G (54.5 ± 24.9 100g^{-1}), and assimilable P content, 10 times lower in S (0.002 ± 0.003 mg kg^{-1}) than in G (0.02 ± 0.01 mg kg^{-1}). The soil of S area can be considered Humic Dystric Regosol and the soil of G area a Humic Hyperdystric Regosol.

As regards vegetation, the dominant tree species is *Pinus nigra*. In the tree layer other conifers occur, such as *Pinus sylvestris* and, at lower altitude, *P. pinaster*, together with individuals of *Fagus sylvatica* L., *Quercus pubescens* Willd., and *Castanea sativa* Mill. In the shrub and grass layers *Sorbus aria* (L.) Crantz, *Ilex aquifolium* L., and some acidophilous species like *Vaccinium myrtillus* L. and *Luzula nivea* (L.) DC. were observed.

The richness of the herb layer changes according to the age and coverage of the Black pine and the soil depth (Tab. 3).

The pH values of S (5.43 ± 0.77) and G areas (5.53 ± 1.26) are acidic with a total average ± standard deviation of 5.48 ± 0.96 (see Tab. 2 for details).

Mycobiota

As concerns the mycobiota, 6926 sporomata were counted and 94 macrofungal species were identified (91 Basidiomycota and 3 Ascomycota) distributed among the trophic groups as follows: 47 EM, 31 SH, 13 SW and 3 P (Tab. 6). The most frequent species, recorded all three years, were: *Suillus bovinus*, *Russula sardonia*, *Hygrophoropsis aurantiaca*, *Strobilurus tenacellus*, *Lactarius deliciosus*, *Russula caerulea*, and *Gymnopilus penetrans*. These species are generally common in coniferous forests. The most occurring species belong to EM and SW trophic groups, such as *Suillus bovinus* and *Hygrophoropsis aurantiaca* (in 10 plots). Details on identified species are listed in Tab. 6, together, with authors' names and trophic groups. The species are organised according to their presence/absence in the plots.

Tab. 6. List of recorded species and relative abundance value; TG: trophic group (EM = ectomycorrhizal, SH = saprotrophic in soil, humus or in litter, SW = saprotrophic on dead wood, P = parasitic and MP = mycoparasitic); Plots: S1, S2, S3, S4, G1, G2, G3, G4, G5, S5, S6. The species are organised according to their presence/absence in the plots.

Family	Species	TG	S1	S2	S3	S4	S5	S6	G1	G2	G3	G4	G5
Russulaceae	*Russula sardonia* Fr.	M	1	3		6	122	39	2	15	7	52	13
Suillaceae	*Suillus bovinus* (Pers.) Roussel	M	117	47		15	166	4	42	76	137	187	41
Hygrophoropsidaceae	*Hygrophoropsis aurantiaca* (Wulfen) Maire	Sw	5	5	9	117		68	17	159		418	45
Physalacriaceae	*Strobilurus tenacellus* (Pers.) Singer	Sw	3		47	54	22	291	1	2	41		12
Russulaceae	*Lactarius deliciosus* (L.) Gray	M	22	41		1	39		5		15	14	11
Strophariaceae	*Gymnopilus penetrans* (Fr.) Murrill	Sw		17	94	3		2	24	5		21	
Russulaceae	*Russula caerulea* (Pers.) Fr.	M				2	15		2	7	13	23	4
Gomphidiaceae	*Gomphidius roseus* (Fr.) Fr.	M					17	1		4		2	3
Hydnangiaceae	*Laccaria laccata* (Scop.) Cooke	M				5	16			2		5	2
Tremellaceae	*Tremella mesenterica* (Schaeff.) Retz.	MP	1						1		9	5	2
Mycenaceae	*Mycena leptocephala* (Pers.) Gillet	Sh		1			2					1	4
Tricholomataceae	*Tricholomopsis rutilans* (Schaeff.) Singer	Sw	9	1						1			3
Gomphidiaceae	*Chroogomphus rutilus* (Schaeff.) O. K. Mill.	M			2					11			1
Tricholomataceae	*Clitocybe nebularis* (Batsch) P. Kumm.	Sh								4	2		4
Cortinariaceae	*Cortinarius camphoratus* (Fr.) Fr.	M		1		1				5			
Agaricaceae	*Lycoperdon nigrescens* Wahlenb.	Sh					6				8	1	
Agaricaceae	*Lycoperdon perlatum* Pers.	Sh							3			2	1
Mycenaceae	*Mycena epipterygia* (Scop.) Gray	Sh					2					5	1
Rhizopogonaceae	*Rhizopogon luteolus* Fr. & Nordholm	M			1		1						3
Russulaceae	*Russula cessans* A. Pearson	M			1	1	7						
Russulaceae	*Russula sanguinea* (Bull.) Fr.	M	2							2		6	
Russulaceae	*Russula torulosa* Bres.	M									1	6	7
Suillaceae	*Suillus luteus* (L.) Roussel	M	8	12			13						
Boletaceae	*Xerocomus badius* (Fr.) E.-J. Gilbert	M		1	2	1							
Amanitaceae	*Amanita rubescens* Pers.	M				1						6	

Family	Species	TG	S1	S2	S3	S4	S5	S6	G1	G2	G3	G4	G5
Clavicipitaceae	*Cordyceps militaris* (L.) Link	P								1			1
Cantharellaceae	*Craterellus lutescens* (Pers.) Fr.	M		7								22	
Dacrymycetaceae	*Dacrymyces chrysospermus* Berk. & M. A. Curtis	Sw							2		1		
Dacrymycetaceae	*Dacrymyces stillatus* Nees	Sw					1			2			
Tricholomataceae	*Dendrocollybia racemosa* (Pers.) R. H. Petersen & Redhead	Sh							1			3	
Entolomataceae	*Entoloma formosum* (Fr.) Noordel.	M									20	4	
Strophariaceae	*Gymnopilus penetrans* var. *hybridus* (Bull.) P. Roux & Guy Garcia	Sw							15	1			
Marasmiaceae	*Gymnopus androsaceus* (L.) J. L. Mata & R. H. Petersen	Sw					5					42	
Hydnaceae	*Hydnum repandum* L.	M		2						5			
Strophariaceae	*Hypholoma fasciculare* (Huds.) P. Kumm.	Sw	3							19			
Tricholomataceae	*Lepista nuda* (Bull.) Cooke	Sh			5								1
Mycenaceae	*Mycena pura* (Fr.) P. Kumm.	Sh						1				5	
Mycenaceae	*Mycena sanguinolenta* (Alb. & Schwein.) P. Kumm.	Sh									2	14	
Marasmiaceae	*Rhodocollybia butyracea* (Bull.) Lennox	M				1					1		
Russulaceae	*Russula integra* (L.) Fr.	M			5			1					
Russulaceae	*Russula nigricans* Fr.	M		2						1			
Pyronemataceae	*Aleuria aurantia* (Pers.) Fuckel	Sh		4									
Amanitaceae	*Amanita citrina* (Schaeff.) Pers.	M							2				
Amanitaceae	*Amanita* sp. 2 (*Vaginatae* section)	M										1	
Physalacriaceae	*Armillaria* sp. 1	P			1								
Auriscalpiaceae	*Auriscalpium vulgare* Gray	Sw											1
Boletaceae	*Boletus edulis* Bull.	M			1								
Dacrymycetaceae	*Calocera viscosa* (Pers.) Fr.	Sw					1						
Agaricaceae	*Chlorophyllum rachodes* (Vittad.) Vellinga	Sh								6			

Family	Species	TG	S1	S2	S3	S4	S5	S6	G1	G2	G3	G4	G5
Clavariaceae	*Clavaria vermicularis* Sw.	Sh					4						
Clavulinaceae	*Clavulina corallides* (L.) J. Schröt.	Sh											1
Tricholomataceae	*Clitocybe phyllophila* (Pers.) P. Kumm.	Sh								3			
Tricholomataceae	*Clitocybe vibecina* (Fr.) Quél.	Sh										1	
Tricholomataceae	*Collybia tuberosa* (Bull.) P. Kumm.	Sh									10		
Cortinariaceae	*Cortinarius purpurascens* Fr.	M						1					
Cortinariaceae	*Cortinarius variicolor* var. *nemorensis* (Fr.) Fr.	M				1							
Cortinariaceae	*Cortinarius spilomeus* (Fr.) Fr.	M									13		
Marasmiaceae	*Crinipellis scabella* (Alb. & Schwein.) Murrill	Sh								6			
Entolomataceae	*Entoloma asprellum* (Fr.) Fayod	M					2						
Strophariaceae	*Hebeloma mesophaeum* (Pers.) Quél.	M									7		
Strophariaceae	*Hebeloma sp.*1 (*Sacchariolens* group)	M		1									
Hygrophoraceae	*Hygrocybe cantharellus* (Schwein.) Murrill	Sh									18		
Hygrophoraceae	*Hygrocybe conica* (Schaeff.) P. Kumm.	Sh								1			
Hygrophoraceae	*Hygrophorus hypothejus* (Fr.) Fr.	M									4		
Inocybaceae	*Inocybe calamistrata* (Fr.) Gillet	M							1				
Inocybaceae	*Inocybe geophylla* (Sowerby) P. Kumm.	M		1									
Inocybaceae	*Inocybe whitei* (Berk. & Broome) Sacc.	M		1									
Hydnangiaceae	*Laccaria amethystina* Cooke	M						3					
Hydnangiaceae	*Laccaria bicolor* (Maire) P. D. Orton	M									6		
Psathyrellaceae	*Lacrymaria lacrymabunda* (Bull.) Pat.	Sh									7		
Russulaceae	*Lactarius deliciosus* var. *quieticolor* (Romagn.) Krieglst.	M											2
Lyophyllaceae	*Lyophyllum putidum* (Fr.) Singer	Sh											6
Agaricaceae	*Macrolepiota excoriata* var. *rubescens* (L. M. Dufour) Bon	Sh									1		
Agaricaceae	*Macrolepiota procera* (Scop.) Singer	Sh								1			

Family	Species	TG	S1	S2	S3	S4	S5	S6	G1	G2	G3	G4	G5
Pyronemataceae	*Melastiza cornubiensis* (Berk. & Broome) J. Moravec	Sh					1						
Mycenaceae	*Mycena aurantiomarginata* (Fr.) Quél.	Sh										3	
Mycenaceae	*Mycena galopus* (Pers.) P. Kumm.	Sh										8	
Mycenaceae	*Mycena haematopus* (Pers.) P. Kumm.	Sh								3			
Strophariaceae	*Flammula pinicola* (Jacobsson) Noordel.	Sh	4										
Pluteaceae	*Pluteus cervinus* (Schaeff.) P. Kumm.	Sw											1
Rhizopogonaceae	*Rhizopogon occidentalis* Zeller & C. W. Dodge	M						1					
Marasmiaceae	*Rhodocollybia maculata* (Alb. & Schwein.) Singer	M								1			
Russulaceae	*Russula acrifolia* Romagn.	M								10			
Russulaceae	*Russula anthracina* Romagn.	M						1					
Russulaceae	*Russula chloroides* (Krombh.) Bres.	M		2									
Russulaceae	*Russula firmula* Jul. Schäff.	M									2		
Russulaceae	*Russula torulosa* f. *alba* Sarnari	M											1
Schizophyllaceae	*Schizophyllum commune* Fr.	Sw		2									
Stereaceae	*Stereum hirsutum* (Willd.) Pers.	Sw											1
Stereaceae	*Stereum sanguinolentum* (Alb. & Schwein.) Fr.	Sw											1
Strophariaceae	*Stropharia semiglobata* (Batsch) Quél.	Sh									5		
Suillaceae	*Suillus granulatus* (L.) Roussel	M								1			
Polyporaceae	*Trametes versicolor* (L.) Lloyd	Sw				1							
Tricholomataceae	*Tricholoma pessundatum* (Fr.) Quél.	M									5		

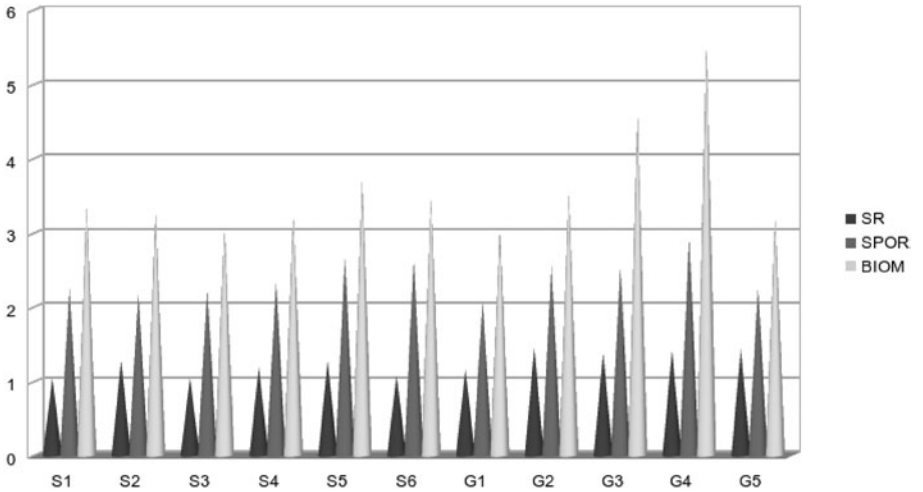

Fig. 1. Species richness (SR), number of sporomata (SPOR) and biomass (BIOM) calculated for each plot (S1-6, G1-5). Values were transformed in Log scale for comparison.

In Fig. 1 the values of species richness, sporomata and biomass calculated for each surveyed plots are shown. Highest species richness has been observed in plot G2 (28 species), the lowest in S1 and S3 (11 species). As regards number of sporomata recorded, values range from 118 in plot G1, to 857 in G4, where consequently have been obtained the minimum and maximum biomass values (Tab. 7).

Tab.7. Species richness, sporomata and biomass values calculated for each plot (S1-6, G1-5).

Plot	Species richness	Sporomata	Biomass
S1	11	175	2196,43
S2	19	151	1750,55
S3	11	168	1124,12
S4	15	210	1646,93
S5	19	442	4741,14
S6	12	413	2902,93
G1	14	118	1080,16
G2	28	354	3300,14
G3	24	335	34927,79
G4	26	857	339360,3
G5	27	173	1632,8

Shannon's indices of the plots are summarised in Tab. 4, which reports in the first column the plot considered, in the second column Shannon's index evaluated on all the species (G), in the third column Shannon's index

evaluated taking into account only the ectomycorrhizal species (EM), and in the last column the evenness related to the total mycodiversity in all plots.

Jaccard's index ranges from 0.33 to 0.04 (Tab. 5). Pearson's correlation coefficient (namely R) between Shannon's index and some environmental parameters were summarised in Figs. 2–4. The correlation between Shannon's index and the pH is enough strong (R= 0.40, Fig. 2). However, increasing pH seems to be correlated with increasing mycodiversity, while it decreases with

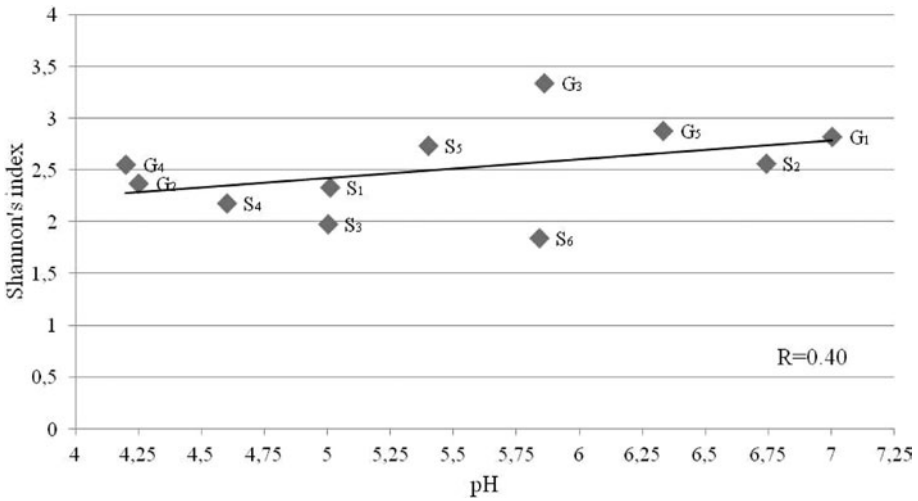

Fig. 2. Pearson's correlation between Shannon's index (all trophic groups together) and soil pH.

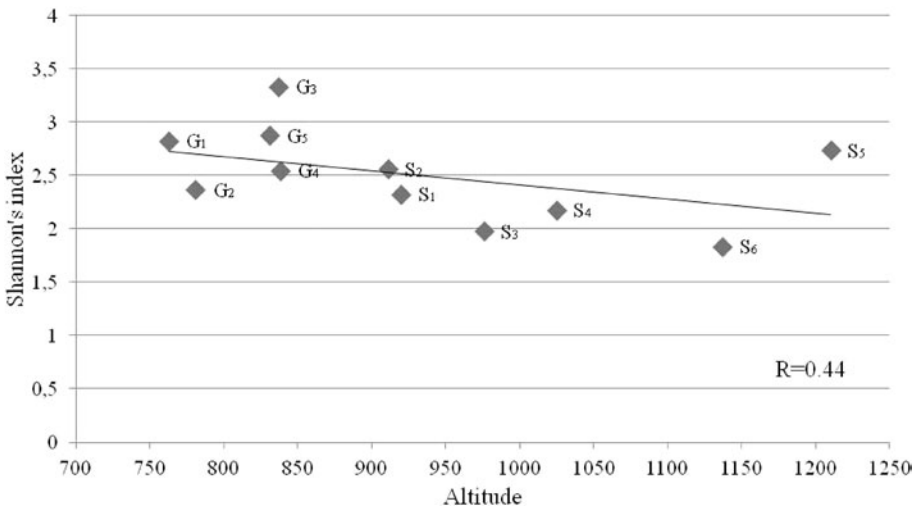

Fig. 3. Pearson's correlation between Shannon's index (all trophic groups together) and altitude.

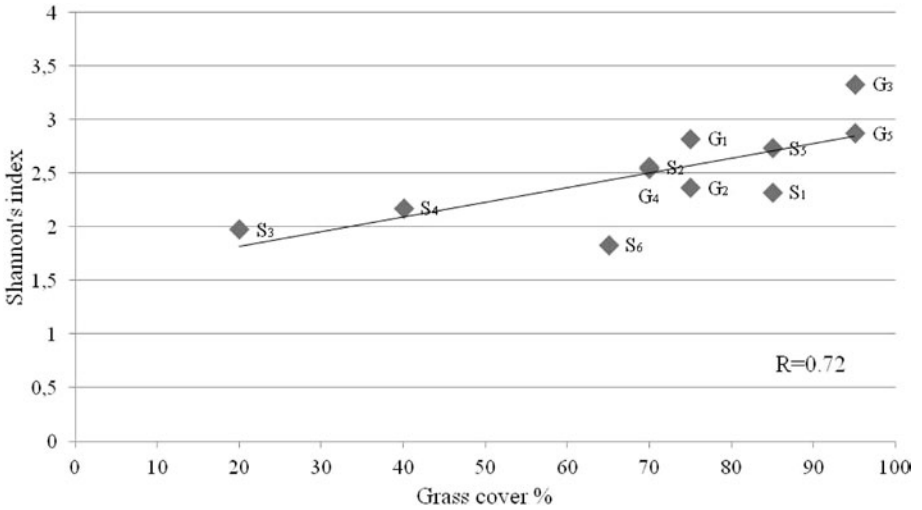

Fig. 4. Pearson's correlation between Shannon's index (all trophic groups together) and grass cover expressed as percentage.

higher altitude (R = 0.44, Fig. 3). The strongest correlation was found between the grass coverage and the overall Shannon's index (R= 0.72, Fig. 4). Other possible correlations, namely between the number of sporomata and the pH, the altitude, grass coverage, soil elements and organic matter, respectively, were checked but proved to be insignificant.

Tab. 8. Results of vector fitting by using "envfit" function. The first column shows the environmental variables; the second and third columns show eighvalues for Axis 1 (PC1) and Axis2 (PC2). r^2 correspond to correlation value. P values, in the last column, is based on 1000 permutations. Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

	PC1	PC2	r2	Pr(>r)
Species richness	0.8704661	0.4922283	0.3698	0.11588
Sporomata	0.9736030	–0.2282480	0.7561	0.03397*
Biomass	0.9999573	–0.0092381	0.8492	0.08891.
Altitude	–0.5679234	–0.8230814	0.2270	0.34865
Rainfall	–0.8720389	-0.4894366	0.2477	0.28971
pH	–0.9923853	0.1231720	0.3847	0.12188
Inclination	0.1075835	–0.9941961	0.0223	0.90410
Pine coverage	0.9401833	0.3406689	0.0624	0.77922
Shrub coverage	0.3980263	–0.9173740	0.1334	0.60739
Grass coverage	0.0678729	0.9976940	0.1377	0.49950
Shannon index	0.0186465	0.9998261	0.4215	0.13387

Result of PCA is shown in Fig. 5. The first four axes account for 97 % of the data variability (PC1 56.4 %; PC2 26.4 %; PC3 11.8; PC4 2.4 %). In Tab. 8 results of vector fitting are summarized. Sporomata and biomass are positively correlated with Axis 1 (PC1) and negatively correlated with Axes 2 (PC2). These two variables probably explain the gradient of ordination. In fact, the vector variables (the arrows in the diagram, Fig. 5) are perpendicular to the contours (the black and grey lines) which reflect the gradient variation. Other environmental variables do not explain significantly the ordination (Tab. 8).

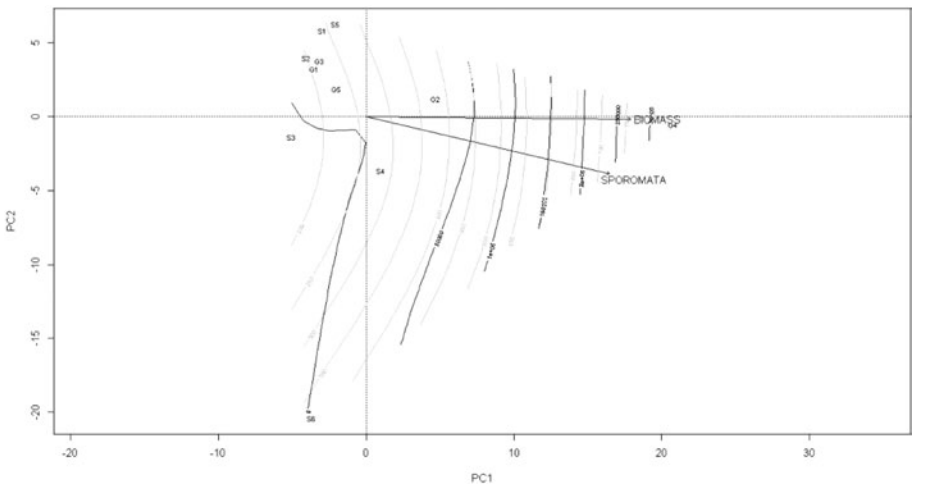

Fig. 5. Principal Component Analysis (PCA) based on plots (S1-6, G1-5) species composition and vector fitting for sporomata and biomass. In grey the contours of sporomata; in black the contours of biomass.

Discussion

Our study is based on sporomata surveys. This method is complex, time expensive and its effectiveness is still a long debated issue. The main problem is that sporomata surveys are often interpreted as representing a sub-sample of the actual underground fungal community (Dahlberg *et al.* 1997). This, in turn, could yield difficulties in data interpretation. However, a recent paper (Tóth & Barta 2010) on the effectiveness of surveys methods for ecological studies of ECM fungi suggests that sporomata investigations permit to obtain valuable and reliable (concerning the identification of fungal species) information on ecosystems provided that surveys are carried out for, at least, two seasons. Therefore, the results here represent a solid basis for discussions and comparisons.

The performed investigations reveal that the most frequent fungal species belong to EM and SW trophic groups. The most EM fungi are symbiotic

partners of coniferous trees (e.g. *Suillus bovinus, Lactarius deliciosus, Chroogomphus rutilus, Suillus luteus, Russula integra)*; similarly most of the SW fungi prefer dead coniferous wood or cones (i.e. *Hygrophoropsis aurantiaca, Auriscalpium vulgaris, Mycena seynii*).

It is worth noting that *Entoloma formosum* and *Dacrymyces chrysospermus* were recorded in Liguria for the first time (Zotti & Orsino 2001, Zotti *et al*. 2008).

Some EM species are also species of the broadleaved and mixed woods (e.g. *Laccaria laccata, Xerocomus badius, Hydnum repandum, Amanita rubescens, Russula nigricans, Rhodocollybia butyracea)*: this is due to the presence of young individuals of *Fagus sylvatica, Quercus pubescens* and *Castanea sativa*. According to Chiarucci & Mariotti (1997) *Pinus nigra* plantations on serpentine soils represent a transient phase in a succession to broadleaved woods on these inhospitable substrates. This was confirmed by the presence of both young broadleaved trees and fungi linked to them.

The number of SH fungi is lower than that of fungi belonging to the EM and SW trophic groups: this fact is caused by the presence of pine needles which acidify the substrate of fungi living on litter, as already demonstrated in a previous work on Ligurian serpentine (Chiarucci & Mariotti 1997), thus limiting the number of SH species (Villeneuve *et al*. 1989, Baar 1996, Ferris *et al*. 2000).

As regards the assessed mycodiversity, we can observe that is quite low in comparisons with other kinds of woods in Liguria (e.g., beech woods, holm-oak woods), whose mycodiversity was evaluated in some previous works (Zotti 2004, Zotti & Zappatore 2006). In these areas Shannon's index values ranged from 5.369 to 6.44 in beech woods, and from 2.444 to 4.977 in holm-oak woods. Similarly, also Villeneuve *et al*. (1989) have observed that the richness and diversity of mycobiota are significantly lower in the coniferous forests when compared with the broadleaved forests. Differently, in other countries of Europe (i.e. Britain and Ireland), comparisons between planted coniferous forests and native oak forests have shown no differences in macrofungal species richness and low difference in community composition (Humphrey *et al*. 2000, O'Hanlon & Harrington 2011).

However, comparisons with other studies are difficult as there are neither papers dealing with other Ligurian conifer woods nor mycological investigations involving serpertine soils in Italy. Anyway, a comparison with the results achieved in mycological studies on serpentine soils carried out in other countries is difficult to perform: indeed, other studies on serpentine soils (Moser *et al*. 2005, 2009; Branco 2010; Branco & Ree 2010; Gladish *et al*. 2010) take into account plant communities different from the ones here considered. Most of this works (Branco 2010, Branco & Ree 2010, Moser *et al*. 2005, Moser *et al*. 2009) shows an high species richness probably due to the rich plant community and the kind of soil.

However, for our study it is reasonable to suppose, that the low mycodiversity may be due to both the presence of *Pinus nigra* and the serpentine

soil. As a matter of fact, *Pinus nigra* is a species which does not generally show a high richness in fungal species (Buéè *et al.* 2011, Trocha *et al.* 2012), probably because of the chemical composition of pine litter. This aspect was already highlighted for microfungi (Kara & Asan 2007). Moreover, Gladish *et al.* (2010) report that Black pine woods on serpentine soils are more poor in species than Black pine woods on not serpentine soils.

Furthermore, considering our correlation results (see Figs. 2–4), the mycodiversity seems to be related with some other environmental factors. Specifically, the mycodiversity increases when the pH and the grass coverage increase, on the contrary mycodiversity decreases when the altitude increases. As concerns pH, probably less acidic soils allow the growth of a larger number of species (Christensen 1989). Serpentine soils are generally shallow, so we can suppose that the presence of a high grass coverage detected in some plots may reflect the occurrence of a quite well structured soil, which allows fungi to better grow and develop. Finally, the higher altitude and the consequent lower temperature partially limit the chances of development for thermophilic fungi, common in Mediterranean areas.

Jaccard's indices demonstrate low similarity levels: only four species (*Russula sardonia*, *Suillus bovinus*, *Hygrophoropsis aurantiaca*, *Strobilurus tenacellus*) are present in more than 80 % of the plots. This proves that our *Pinus nigra* plantations on serpentine soils have no specific fungal community. It should be also noted that the EM species *Russula sardonia, Suillus bovinus,* and *Lactarius deliciosus*, if present, are generally very abundant: a number of 50-200 sporomata were counted during a single visit in a plot. Also ordination results (see Fig. 5 and Tab. 8) show limited differences among plots: in fact, only two variables (sporomata and biomass) are significantly correlated.

This data on the abundance does not seem to agree with the serpentine syndrome discussed by Gladish *et al.* (2010): "fewer species of ECM and fewer fruiting bodies". On the contrary, the data on hypogeous fungi confirm what stated by Gladish *et al.* (2010): that is the number of hypogeous sporomata and total number of species are low on serpentine soils. Moreover, as concerns hypogeous fungi, recent studies carried out in different vegetation types of Liguria (both coniferous and broadleaved forests), show a significant number of species (61 species, Zotti *et al.* 2010 a, b), confirming that coniferous plantations on serpentine soils are characterized by few macrofungal species.

Conclusion

Our study gives a contribution to the knowledge of macrofungi in *Pinus nigra* plantations in serpentine areas of NW Italy, where we assessed a low mycodiversity. Ectomycorrhizal fungi dominate for the number of species and abundance. Hypogeous species are very few and sporomata are not abundant, only these traits seem to agree with the "serpentine syndrome".

Our surveys do not highlight fungi strictly related to *Pinus nigra* and serpentine soil.

More data on fungi on serpentine soils are needed to better characterise serpentine mycobiota and its relationship with soil. In future it will be important to study also natural *Pinus nigra* woods.

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