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Assessment of macrofungal diversity in a Silver Fir plantation in Sardinia (Italy) using a standardized sampling procedure

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Abstract

The knowledge of macrofungal diversity associated with Silver Fir forests in Italy is quite scarce. Only a little information is available about macrofungal communities from some Ligurian and Tuscan Silver Fir sites. This study aims to assess the macrofungal diversity of a Silver Fir plantation in Sardinia by the application of a standardized sampling procedure. A total of 606 sporomata were collected and 52 Basidiomycota were identified. The high value of the Shannon Index indicated a considerable level of macrofungal diversity in this plantation. The results were also compared with the diversity indices obtained by a previous 3-years long sampling methodology in the same site. The comparison of the macrofungal diversity values of the Sardinian site with those of the Ligurian Silver Fir forest revealed interesting similarities among natural versus planted coniferous forests.

Keywords: macrofungal diversity; *Abies alba*; conifer plantations; standardized sampling procedure; Shannon Index

Riassunto

Si riportano in questo lavoro i risultati inerenti l'utilizzo di un protocollo standardizzato per la valutazione della diveristà macrofungina. Tale metodologia, sviluppata per lo studio della biodiversità di organismi come i macrofunghi o gli insetti, offre numerosi vantaggi in ambito ecologico, quali: ridurre lo sforzo di campionamento e ottenere dati comparabili con quelli di altre ricerche simili.
Durante il primo workshop nazionale sulle ectomicorrize si è proceduto all'applicazione di questo metodo con la finalità di avere un quadro della micodiversità del sito prescelto: un impianto artificiale di Abete bianco nel nordovest della Sardegna. Basandosi sull'osservazione quali- e quantitativa degli sporomi presenti, sono stati complessivamente rilevati 606 basidiomi, riconducibili a 52 taxa (Basidiomycota). I valori degli indici di biodiversità calcolati, in particolare l'indice di Shannon (H'), mostrano che l'area è

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caratterizzata da un considerevole valore di micodiversità. Inoltre, confrontando i dati ottenuti con quelli di un'indagine precedente, svolta nello stesso sito per un periodo complessivo di tre anni, è interessante notare simili valori di diversità. Ciò induce ad ipotizzare che il metodo di monitoraggio proposto ha consentito di osservare una significativa percentuale di specie rispetto al totale rilevato applicando un approccio di campionamento che ha richiesto un maggiore impegno di risorse.
Si è proceduto inoltre al confronto dei valori ottenuti nel sito sardo con quelli osservati in un'abetina naturale in Liguria, monitorata seguendo lo stesso protocollo. I valori simili di ricchezza di specie e H' index ci permettono di affermare che riforestazioni di abete bianco ospitano un valore di micodiversità comparabile a quello di aree naturali. Studi futuri potranno confermare tali risultati e validare questa metodologia per lo studio della diversità macrofungina in ambiente mediterraneo.

Parole chiave: micodiversità; *Abies alba*; riforestazione; metodo di campionamento standardizzato; indice di Shannon

Introduction

Macrofungi play fundamental roles in forest ecosystems and the measurement of their species richness and diversity can potentially allow us to monitor the health of natural systems (EGLI 2011; FEEST 2013; BODDY et al. 2014). It has been widely demonstrated that environmental changes (e.g. climate change) affect the species richness and composition of the macrofungal communities (TÓTH & BARTA 2010; BÜNTENG et al. 2012; BODDY et al. 2014). Hence, the monitoring of fungal dynamics over time, can be used as an indicator for forest perturbations (JAKUCS 1988; ARNOLDS 1991; FELLNER & PESKOVA 1995; AMARANTHUS 1999; TÓTH & BARTA 2010). Moreover, macrofungal diversity appears to be a useful tool for assessing priorities in sites of conservation, since fungi are considered organisms whose biodiversity is closely correlated with the total biodiversity of a site (ZOTTI & ZAPPATORE 2006; FEEST 2006, 2010; ORTEGA & LORITE 2007).
Among different forest types, coniferous plantations are considered important environments because they provide a habitat for diverse macrofungal species (FERRIS et al. 2000). HUMPHREY et al. (2000) for instance, highlight the role of both native and exotic conifer plantations in the conservation of native fungi.
In Italy, very few studies have been targeted to investigate the above ground macrofungal diversity of coniferous habitats. An example is given by the study of ZOTTI et al. (2013a) concerning the macrofungal diversity associated with European Black pine (*Pinus nigra* Aiton) plantations in North-western Italy (Liguria). Some other information is available about natural and artificial Silver Fir (*Abies alba* Mill.) forests in central Italy (Tuscany) by PERINI et al. (1995), LAGANÀ et al. (2000, 2002), PECORARO et al. (2007), and SALERNI & PERINI (2010). Nevertheless, due to the differences in the sampling methodology adopted in these aforementioned studies (e.g. presence or absence of plots, plots size, sampling effort, time and duration of surveys), making comparisons among these Italian conifer sites is quite difficult.
Due to the importance of the macrofungal diversity assessment and the partial lack of knowledge on the Italian coniferous forests, we carried out a mycological study in a Silver Fir site of Sardinia by the using of a standardized sampling method (FEEST 2006). This site was previously surveyed to assess the macrofungal species diversity, by following a traditional 3-years long survey approach (AMBROSIO et al. 2014).
The FEEST (2006) sampling approach has been developed to describe the biodiversity of cryptic taxonomic groups, such as macrofungi and insects (FEEST 2006, 2010; FEEST et al. 2011; FEEST & CARDOSO 2012), computing a set of diversity indices (MAGURRAN 2004).

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FEEST (2006) proposed an approach that can reduce the time and effort to the survey to one year, as long as it is favourable to sporomata (KIRK et al. 2008) production. This aspect is particularly important in mycology since the traditional sampling methods require a lot of effort: from 2 to 3 (ARNOLDS 1981, 1982; VILLENEUVE et al. 1989, 1991a, 1991b), up to 10 years (ORTON 1986). Moreover, the development of simple and quick methodologies, which could reduce the time and costs involved in monitoring actions, is also needed for studying the total biodiversity (SANTI et al. 2010) as well.
The aims of our study were to: i) assess the macrofungal diversity using a standardized sampling approach (1-y long); ii) to verify whether different sampling methods (1-y and 3-y) provide the same macrofungal diversity indices; and finally iii) to compare the level of macrofungal diversity of natural and artificial coniferous forests in Italy.

Materials and Methods

Study area

Sardinia is the second largest island (24090 km^2) in the Mediterranean basin, characterized by a high number of plants (BLASI et al. 2007) and macrofungi (VENTURELLA et al. 2011), as well as a wide variety of vegetation habitats (BACCHETTA et al. 2009). During the19th century several areas were planted with exotic conifers, such as: *Abies alba* Mill., *A. cephalonica* Loudon, *Cedrus atlantica* (Endl.) Carr., and *Pinus nigra* Arnold.
A mono-specific *A. alba* plantation, located in North-west Sardinia, on the Limbara mountain (Madonna della Neve Locality, OT), was selected to study the macrofungal diversity. This kind of habitat is an unique example in Sardinia, since no other Silver Fir site can be found in the whole region.
The site covers an area of about 4500 m^2 and it is characterized by a Mediterranean climate (RIVAS-MARTINEZ 2008), with the mean annual temperature of 14.2°C [from 3°C (min) in February to 20°C (max) in July)]. The mean annual rainfall is 1300 mm (climate-data.org, 2015).
Geologically, the substrate consists of leucogranite rocks with a pH close to 5 (PASSINO 1981).

Sampling procedure and data collection

Accordingly to the FEEST (2006) procedure, during the autumn of 2013, both the qualitative (identification of species) and quantitative (number of sporomata) macrofungal observations were performed in 20 circular plots (4 m radius) selected along line transects (total length of 400 m). The total size of the sample site covers a surface of approximately 1000 m^2.
All the macrofungi (Agarics, Boletes and Gasteromycetes) with a given shape and visible to the naked eye were recorded in each plot. Hypogeous and resupinate lignicolous species (Corticiaceae s.l. and Polyporaceae s.l.) were not taken into account in this study.
The taxonomical identification was performed by analyzing both the macro- and microscopical characters (for more details see AMBROSIO et al. 2014). Systematic classification followed HIBBETT et al. (2007) and KIRK et al. (2008). Nomenclature and author abbreviations were used in accordance with CABI (www.indexfungorum.org), CBS (www.cbs.knaw.nl), and IMA (www.mycobank.org).

Data analyses

The collected dataset was inserted into the specific program FUNGIB (available at www.ecosulis.co.uk/page/fungib-programme) to calculate the following diversity indices:

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- **Species richness** (S) was computed by the number of species recorded. The richness S expresses the alpha diversity and is the simplest measure of biodiversity (MAGURRAN 2004; ZAK & WILLING 2004).

- **Shannon index** (1948) (H') is commonly used to characterize species diversity in a community and it depends on the total number of species and their occurrence. H' was calculated by the formula:

$$H' = -\sum_{i=1}^{M} p_i \ln \; p_i$$

where:
M is the total number of species
p_i is the ratio of individuals found belonging to the *i*-th species.

- **Evenness** (E) (PIELOU 1969, 1975) of the Shannon index was calculated as:

$E = H'/H_{MAX}$

where H_{MAX} is the diversity that can be observed if all species are equally likely.

Evenness is a measure of biodiversity which quantifies how equal the community is numerically.

- **Simpson's index** (D) was computed by the formula:

$$D = \sum_{i=1}^{M} \frac{n_i (n_i - 1)}{N (N - 1)}$$

where n_i is the number of individuals in the *i*-th species and *N* is the total number of individuals. Simpson index is weighted by the abundance of the most common species: when D increases, diversity decreases (SIMPSON 1949).

- **Berger-Parker index** (d) expresses the proportional abundance of the most abundant species (BERGER & PARKER 1970); it was calculated by the formula:

$d = N_{MAX}/N$

where N_{MAX} is the number of individuals in the most abundant species and *N* is the total number of individuals.

- **Density** was used to measure the number of sporomata per total sampled area.

- **Species Conservation Value Index** (SCVI) was used to evaluate the rarity of the recorded species. Each species was labeled with a number (1-5), in order to indicate its distribution in the Italian territory. National references (ONOFRI et al. 2005; BOCCARDO et al. 2008) were used to assign SCVI value to each species. SCVI = 1 indicates the most common species; SCVI = 5 for the rarest.

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- **Non-parametric estimator and rarefaction curve** was used to estimate the species richness and to check whether data collected allowed observation of a notable number of species. CHAO1 is the simplest method to extrapolate the "true" number of species in an assemblage and it is based on the number of singleton and doubleton species (CHAO 1984; MAGURRAN 2004). Rarefaction generates the expected number of species in a small collection of individuals drawn at random from the whole pool of individuals. The curve generally rises very quickly at first and then flattens since a reasonable number of individuals have been considered (GOTELLI & COLWELL 2001, 2011; COLWELL et al. 2012). In this work, sampled-based rarefaction curves were computed and plotted by R system (version 3.1.0, R CORE TEAM 2014). The number of taxa was plotted as a function of the accumulated number of individuals. We used the function "rarefy" in the Vegan package version 2.0-7 (OKSANEN et al. 2013) to plot the curves with the corresponding iterative error around the mean.

Results

Altogether 606 sporomata were collected and 52 species belong to Basidiomycota were identified. Table 1 lists the species recorded with the respective number of sporomata and SCVI value.
Figures 1 and 2 display the distribution of species richness and abundance values per plots, respectively. The mean richness value was 7.5 ± 2.2. The highest number of species was 12 (in plot 7), the lowest number was 4 (in both plot 14 and 18). The mean number of sporomata was 30 ± 16.2. The highest number of sporomata (viz. 60) was collected in plot 15; the lowest (viz. 5) in plot number 14.
The diversity indices (e.g. S= 52; H'= 3.26; E= 0.82; SCVI= 3.13) and non-parametric estimator (viz. CHAO= 56.05) results are displayed in Table 2. Finally, the accumulation curve is shown in Figure 3. Figures 4-9 show some of the most interesting species.

Discussion

The application of a standardized sampling procedure allowed us to measure the macrofungal diversity of a Silver Fir site in Sardinia. The results showed that this area is characterized by a considerable value of macrofungal diversity. The Shannon value obtained (H'= 3.26) for instance, revealed a high level of macrofungal diversity since, this index usually falls between 1-4 (MAGURRAN 2004). This result agrees with the data observed by a previous mycological investigations performed in the same area (AMBROSIO et al. 2014) and other reports on macrofungal communities in Italy (ONOFRI et al. 2005; PAUTASSO & ZOTTI 2009; VENTURELLA et al. 2011), which highlighted that Sardinia is the most diverse and richest macrofungal Italian region.
In spite of the high macrofungal richness, several areas of this region are still uninvestigated or poorly-known from a mycological point of view. Thanks to the results obtained by the present work and by AMBROSIO et al. (2014), new information and records are now available for Sardinia. No macrofungal records associated to *Abies alba* in Sardinia were in fact, included in the Italian macrofungal checklist (ONOFRI et al. 2005). Moreover, the high SCVI value (= 3.13) confirms the presence of rare and uncommon species as also detailed in AMBROSIO et al. (2014).
The comparison between the results presented in this study with those of AMBROSIO et al. (2014) highlight that Feest's proposed method (FEEST 2006) allowed us to record a significant percentage of species. The number of the observed *taxa* is very close to the estimated CHAO1 value and it corresponds to the 43% of the total species collected over three consecutive sampling years, using a "traditional" macrofungal survey.

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Moreover, it is worth noting that the Shannon Index obtained by the traditional 3-y long survey method (AMBROSIO et al. 2014) is quite similar to the H' value computed, in this study, by the standardized 1-y long survey method (see Tab. 3).

This result is relevant if we consider that Feest's approach requires a lower sampling effort (AMBROSIO 2015) than traditional methods used for the macrofungal survey (i.e. ARNOLDS 1981, 1982).

The application of a common sampling procedure made it possible to compare the Sardinian site with a natural Silver Fir site located in Liguria (NW Italy), where Feest's sampling methodology was applied in order to assess the macrofungal diversity value as well (AMBROSIO et al. 2012; ZOTTI et al. 2013b).

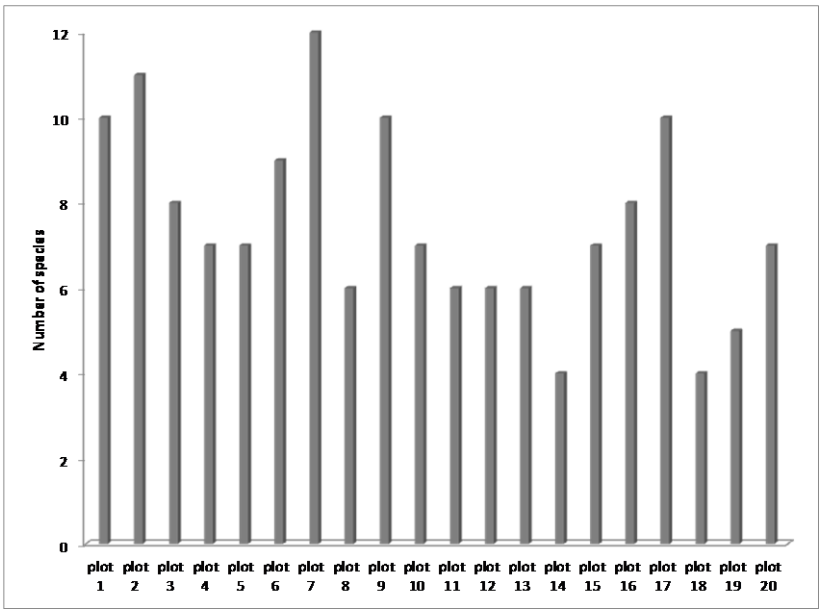

Fig. 1: Distribution of species richness per plot.
Fig. 1: Rappresentazione grafica del numero totale di specie osservate in ogni singolo plot.

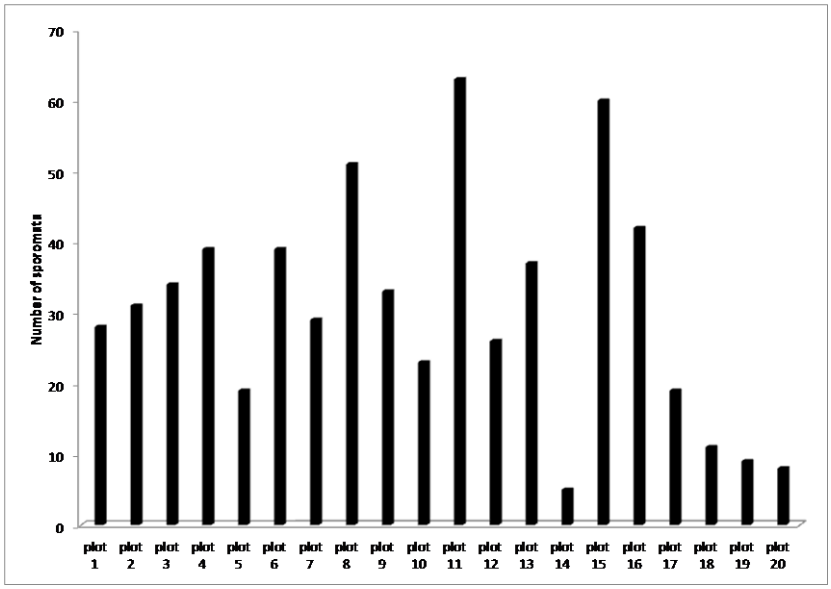

Fig. 2: Distribution of the sporomata abundance per plot.
Fig. 2: Rappresentazione grafica del numero totale di sporomi raccolti in ogni singolo plot.

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Tab. 2: Summary of diversity indices calculated for the macrofungal community.
Tab. 2: Sintesi dei valori degli indici di biodiversità calcolati.

Index	Value
Species Richness (S)	52
Shannon-Wiener Index (H')	3.26
Eveness (E)	0.82
Simpson Index (D)	18.38
Berger-Parker Dominance Index (d)	0.11
Density	0.60 per m^2
Species Conservation Value Index (SCVI)	3.13
CHAO 1	56.05

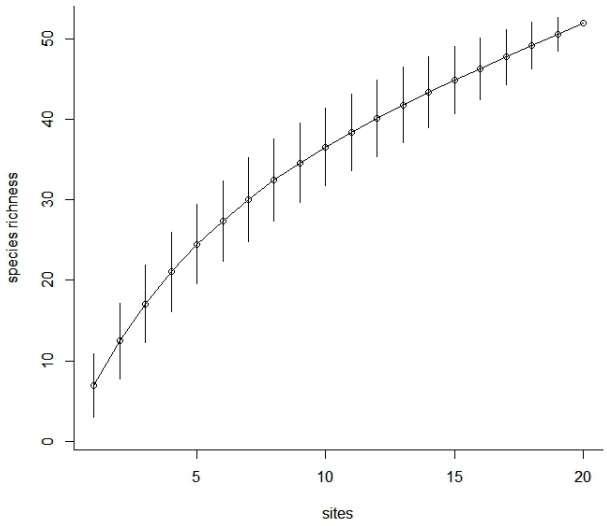

Fig. 3: Sampled-based accumulation curve. Sites refer to plots.
Fig. 3: Rappresentazione grafica della curva di rarefazione costruita sulla ricchezza di specie osservata in ogni plot.

The results summarized in Table 3 show that both natural and artificial Silver Fir sites are characterized by similar values of species richness and diversity. This result agrees with other European studies (FERRIS et al. 2000; HUMPHREY et al. 2000; O'HANLON & HARRINGTON 2012). In Britain and Ireland the comparison of macrofungal diversity (in terms of both Species Richness and the Shannon Index), of conifer plantations and semi-natural woodlands have shown no differences in species diversity and composition.

Tab. 3: Biodiversity indices of coniferous forests of Sardinia and Liguria.
Tab. 3: Indici di biodiversità relativi a foreste di Abete bianco della Sardegna e Liguria.

Location	Site	Vegetation	Species Richness	Shannon Index	References	Number of sampling years
Sardinia	Plantation	Silver Fir	52	3.26	Present study	1
			119	4.35	AMBROSIO et al. 2014	3
Liguria	Natural	Silver Fir	64	4.00	AMBROSIO et al. 2012	1

Conclusions

Our study gives a contribution to the knowledge on macrofungal diversity associated with the Silver Fir plantation in Sardinia, which is characterized by a high value of Shannon Index.
The use of the same standardized sampling approach has allowed the comparison between two coniferous sites in Italy. The obtained results indicate that the artificial Silver Fir site of Sardinia shows a macrofungal diversity value similar to those of the natural Silver Fir forest of Liguria.

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Further studies are needed to improve the knowledge of macrofungal diversity associated with both natural and artificial Silver Fir forests of Italy. Moreover, a wider number of studies are needed to confirm that 3-y and 1-y long sampling approaches give comparable results in terms of biodiversity. If the effectiveness of this method can be shown, it could be of considerable benefit for biodiversity assessment, by the study of difficult taxonomical group of organisms, such as macrofungi.

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Tab. 1: List of the recorded species. For each taxon is given the number of sporomata per each plot. Total number of sporomata per species is indicated in the first of the right hand column, the species SCVI in the final column. The last line reports total number of sporomata per plot.
Tab. 1: Elenco delle specie censite nell'area di studio. Per ogni taxon sono riportati il rispettivo numero di sporomi totale e quello rilevato in ogni plot. Nell'ultima colonna a destra sono riportati i valori SCVI assegnati ad ogni singola specie.

Species name	**Plot 1**	**Plot 2**	**Plot 3**	**Plot 4**	**Plot 5**	**Plot 6**	**Plot 7**	**Plot 8**	**Plot 9**	**Plot 10**	**Plot 11**	**Plot 12**	**Plot 13**	**Plot 14**	**Plot 15**	**Plot 16**	**Plot 17**	**Plot 18**	**Plot 19**	**Plot 20**	**Tot**	**SCVI**
Agaricus porphyrizon P.D. Orton						1				5					1						7	5
Amanita gemmata (Fr.) Bertill.																				2	2	2
Amanita muscaria (L.) Lam.			5		3	3			1			2					1				15	2
Amanita rubescens Pers		1	1							2										1	5	2
Amanita vaginata (Bull.) Lam.				1	1								2								4	4
Baeospora myosura (Fr.) Singe	1						2	4				1									8	3
Chroogomphus rutilus (Schaeff.) O.K. Mill.		2	1							3					1						7	3
Clitocybe phaeophthalma (Pers.) Kuyper						3															3	4
Clitopilus prunulus (Scop.) P. Kumm.		5		10	8	5			2		20		15					3			68	3
Crepidotus variabilis (Pers.) P. Kumm.	2	10									30					10					52	2
Cystoderma carcharias (Pers.) Fayod														2							2	5
Gymnopus dryophilus (Bull.) Murrill							5														5	2
Gymnopus fusipes (Bull.) Gray	2																				2	4
Hygrophoropsis aurantiaca (Wulfen) Maire			5		2	15	3			10			3		10	3	2				53	2
Hygrophorus hypothejus (Fr.) Fr.																2			2		4	3
Hymenopellis radicata (Relhan) R.H. Petersen				20					3			4							2		29	4
Hypholoma fasciculare (Huds.) P. Kumm.	10						5		3						30						48	2

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Tab. 1 (Continues)

Infundibulicybe geotropa (Bull.) Harmaja	5																				5	4
Inocybe calamistrata (Fr.) Gillet				5																	5	5
Inocybe geophylla (Bull.) P. Kumm.			3								2					10	3	2			20	2
Inocybe rimosa (Bull.) P. Kumm.		1																			1	3
Inocybe splendens R. Heim											5										5	5
Laccaria laccata (Scop.) Cooke		2	2				3		8			1			6			5			27	2
Lactarius aurantiacus (Pers.) Gray																				2	2	3
Lactarius deliciosus (L.) Gray			15					2				3				10					30	2
Lactarius quieticolor Romagn.				2			5		4							2			2		15	5
Lactarius rufus (Scop.) Fr.													8								8	3
Lepista flaccida (Sowerby) Pat.		2				2		20					6							1	31	2
Lepista nuda (Bull.) Cooke		4			1												2				7	2
Leratiomyces squamosus (Pers.) Bridge & Spooner													1							1	5	
Lycoperdon perlatum Pers.								10								3	7				20	2
Mycena epipterygia (Scop.) Gray							1														1	3
Mycena filopes (Bull.) P. Kumm.					2																2	3
Mycena inclinata (Fr.) Quél.									2												2	4
Mycena metata (Secr. ex Fr.) P. Kumm.			2																		2	3
Mycena pura (Pers.) P. Kumm.	1																				1	2
Rhodocollybia butyracea (Bull.) Lennox		2					1				3				10						16	3
Russula caerulea Fr.																			1		1	3
Russula chloroides (Krombh.) Bres.		1				2															3	4
Russula maculata Quél.																	1				1	4
Russula nigricans Fr.	5																				5	2

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Tab.1 (Continues)

Russula risigallina (Batsch) Sacc.									2												2	2
Russula sanguinea Fr.																			2		2	4
Russula sardonia Fr.		1				8	3	15	5	3			3		2		2				42	2
Russula xerampelina (Schaeff.) Fr.																				1	1	2
Stropharia caerulea Kreisel																		1			1	4
Suillus granulatus (L.) Roussel	2															2	1				5	2
Tricholoma bresadolanum Clémençon							1				3			2						1	7	5
Tricholoma imbricatum (Fr.) P. Kumm.				1																	1	4
Tricholoma pessundatum (Fr.) Quél.									3												3	2
Tricholoma ustale (Fr.) P. Kumm.					2																2	4
Tubaria furfuracea (Pers.) Gillet												15									15	4
Total	**28**	**31**	**34**	**39**	**19**	**39**	**29**	**51**	**33**	**23**	**63**	**26**	**37**	**5**	**60**	**42**	**19**	**11**	**9**	**8**	**606**	**3.13**

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Fig. 4: *Amanita rubescens*. Photo by R. Brotzu.

Fig. 5: *Amanita vaginata*. Photo by R. Brotzu

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Fig. 6: *Crepidotus variabilis*. Photo by R. Brotzu

Fig. 7: *Inocybe geophylla*. Photo by A. Brigo.

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Fig. 8: *Lactarius deliciosus*. Photo by R. Brotzu.

Fig. 9: *Lycoperdon perlatum*. Photo by R. Brotzu

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