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Ectomycorrhizal communities above and below ground and truffle productivity in a *Tuber aestivum* orchard

Elena Salerni, Maria D'Aguanno, Pamela Leonardi and Claudia Perini*

BIOCONNET. BIOdiversity and CONservation NETwork. Department of Life Sciences. University of Siena. via Mattioli 4. 53100 Siena, Italy

Abstract

Aim of study: The diversity of ectomycorrhizal fungal communities (EM) above (EMFb) and below (EMMt) ground associated with *Quercus cerris* L., *Q. pubescens* Willd., and *Pinus nigra* J.F.Arnold was analyzed.

Area of study: A 20 year-old orchard that produces *Tuber aestivum* truffles, located a few kilometers from Chiusi della Verna (latitude 43° 41' 53"; longitude 11° 56' 9") in Tuscany (central Italy) was observed.

Material and methods: This investigation combined analyses of EMFb, EMMt, *T. aestivum* productivity, different host trees, and statistical data on community ecology.

Main results: The EM communities showed high species richness and differed slightly in relation to both the host tree and their location above or below ground, providing frequent findings of *Tricholoma* and *Tomentella*, respectively. Positive correlations were found between the number of truffles and host trees, and between the weight and number of truffles and EMFb.

Research highlights: Mycorrhizal fungi and truffle production are not in competition.

Key words: Fungal communities; fruiting bodies; morphotypes; *Tuber aestivum;* competition; Italy.

Introduction

Tuber aestivum Vittad. is the highly prized fruiting body of a hypogeous ascomycete (Chevalier and Frochot, 1989) that forms ectomycorrhizae in order to promote plant-assimilate uptake for fungal growth and to enhance the water and nutrition uptake of the host plant (Pennisi, 2004; Smith and Read, 2008). Besides its biological relevance for the functioning of ecosystems, the truffle is an important economic factor in many southern European regions (Chevalier and Frochot 1989). It is reported from Spain across Eastern Europe and China, and from Gotland (Sweden), as far as North Africa (Song *et al*., 2005), and is considered the most widespread truffle species in Europe (Gryndler *et al*., 2011; Hall *et al*., 2007).

Records of the human consumption of truffles date back to ancient Greece (Hall *et al.*, 2001). Truffle production in Europe has declined precipitously over the last century, culminating in the cultivation of various truffle species. The first cultivated *T. aestivum* truffles were collected from an orchard of inoculated greenhouse seedlings in the late 1970s (Chevalier and Grente, 1979). Many successful orchards were established in the following years, and currently half of the truffles sold in Europe are harvested in such areas (Hall *et al.*, 2003). Unfortunately, not all orchards are successful and not all trees within a productive orchard generate truffles (Pruett *et al.,* 2008). The causes of orchard failure are poorly understood. The fruiting of ectomycorrhizal fungi is known to be influenced by a broad range of factors, including climate, temperature, soil moisture and disturbance (Vogt *et al.*, 1992). However, the precise requirements for truffle production remain unclear; moreover, conditions are likely to vary between different species (Kıes and Liu, 2000). The role that interspecific competition plays in determining EM community

* Corresponding author: claudia.perini@unisi.it
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Abbreviations used: EMFb [all ectomycorrhizal fruiting bodies (epigeous and hypogeous)]; EMMt (ectomycorrhizal morphotypes on root tips).

composition is also, as yet, not fully understood, although several studies have demonstrated the existence of interspecific competition in ectomycorrhizal community development (Zambonelli *et al.*, 2000; Lilleskov and Bruns, 2003; Koide *et al.*, 2004; Kennedy and Bruns, 2005; Baciarelli-Falini *et al.*, 2006; Kennedy *et al.*, 2007). In this study we analyzed the diversity of ectomycorrhizal fungal communities above and below ground associated with *Quercus cerris* L., *Q. pubescens* Willd., and *Pinus nigra* J.F.Arnold. We aimed to achieve the following:

(a) characterize the ectomycorrhizal communities through observations of the fruiting bodies (EMFb) and morphotypes on root tips (EMMt); (b) verify and quantify the presence and distribution of *T. aestivum* truffles; (c) analyze whether the fungal communities differed in relation to the host trees; (d) compare the fungal communities in relation to truffle production, in both number and weight; (e) identify any interactions between the communities above and below ground.

Material and methods

Description of the truffle orchard

The study was conducted in a 20 year-old orchard that produces *Tuber aestivum* truffles, located a few kilometers from Chiusi della Verna (latitude 43° 41' 53''; longitude 11° 56' 9'') in Tuscany (central Italy). The site has an area of 0.7 ha, an average altitude of 1000 m a.s.l., and a slight slope (5-7%). The climate is fresh to moderate, with a mean annual rainfall of 900-1000 mm and mean annual temperature of 9.5°C. The soil is constituted of limestones and marly limestones belonging to the Alberese formation. Rock outcrops are absent, while stones of small dimensions (2-75 mm) formed by unaltered calcareous elements are frequent on the surface; the pH ranges from 7.4 to 8.3 (Salerni *et al.*, 2010).

The truffle orchard was established in 1989 by planting 58 *Quercus cerris*, 85 *Q. pubescens* and 85 *Pinus nigra*, all originally inoculated with *Tuber aestivum*. Seedlings were placed with a distance of 5 m between the rows and 4 m within the rows. In 2006 various silvicultural treatments (mulching, adding organic matter and granular gravel) were performed with the aim of improving the habitat for *T. aestivum* fruiting.

Data collection and morphological analysis

In line with studies conducted in the Netherlands (for the methodology see Arnolds 1981; for the trophic groups see Arnolds *et al.*, 1995), epigeous and hypogeous ectomycorrhizal fruiting bodies larger than 1mm (EMFb) were observed from September 2008 to December 2011. The abundance and frequency was considered in the whole orchard and in relation to the 3 types of host tree. Identification was performed with the usual morphological techniques and employing general analytic keys and monographs (Salerni *et al.*, 2010).

The exsiccata are conserved at the *Herbarium Universitatis Senensis* (Siena). The nomenclature of fungal species refers to the CABI Bioscience Database of Fungal Names available on the internet: www.indexfungorum.org/Names/Names.asp, which was updated at the end of August 2012.

Observations on the hypogeous fruiting bodies, were performed in the same period with the help of truffle hunters and trained dogs, to monitor the presence of *T. aestivum* by quantifying its numbers and weight.

To analyze the EM morphotypes present on root tips (EMMt), 84 soil cores of 30x6 cm were collected between September 2008 and December 2011. After removing the litter and organic horizon, cores were taken from the exact points in which EMFb were found. In order to have soil samples for every fungal species and every host tree, samples were taken for the same fungal species when fructifying under different trees. Each sample was individually soaked overnight in tap water and sieved to separate the root fragments and EMMt from the soil; they were then stored at 4°C and processed within the following 10 days. Morphotyping was performed using a stereomicroscope and a light microscope, with reference to the anatomo-morphological characteristics described by Agerer (1987-2008; 1995; 2006) and the on-line EctoMycorrhizal Community DataBase (www.emyco.uniss.it).

Statistical analysis

To estimate the diversity of the communities, Shannon Wiener ($H' = 0 \rightarrow \infty$) and Pielou's ($E = 0 \rightarrow 1$) indices (Magurran, 2004) were calculated using the *Vegan* software package, version 1.17-9 (Oksanen *et al.*, 2011), within the R system for statistical computing (version 2.12.2) (R Development Core Team, 2011).

Spearman's rank correlation coefficient was calculated to determine correlations between the number and weight of the *T. aestivum* fruiting bodies and host trees and the qualitative (number of species) and quantitative (number of fruiting bodies and of morphotypes) data for EMFb and EMMt. The P-value was set at the 5% significance level. Statistical analyses were performed using the STATISTICA 5.0 (StatSoft. Inc., Tulsa, Ok, USA) package of programmes.

Results

Ectomycorrhizal fruiting body (EMFb) communities

In the truffle orchard studied, 48 fungal species were identified (Table 1), of which 44 were epigeous species and 4 hypogeous: 15 genera belonged to the *Basidiomycota* and only 1 to the *Ascomycota*. The genus *Inocybe* had the highest number of species (14), followed by *Cortinarius* with 11*, Hygrophorus, Lactarius* and *Tricholoma* with 3 species each, *Boletus, Hebeloma* and *Tuber* with 2 species, and others with a single species. Regarding the average abundance in the whole area studied, *Tricholoma terreum* produced the highest number of fruiting bodies, with an average of 4.114, followed by *Laccaria laccata* (3.052), while *Tuber aestivum*, found 49 times, was the most frequent. These fungal species were also the most abundant when the three different host trees were observed separately: the most abundant species in the area with *Quercus cerris* was *Laccaria laccata* (11.948), in the *Pinus nigra* area *Tricholoma terreum* (6.882) and, among the few species found in the *Q. pubescens* area, *Tuber aestivum* had the highest productivity and frequency (Table 1). In the *Q. cerris* area the most frequent species, found exclusively here, was *Hebeloma sinapizans*, which was observed in association with 41 of the 58 total trees. The area with 85 *Pinus* trees was strongly preferred by *Chroogomphus rutilus*, with 31 findings. Here one of the most frequent and abundant species was *Suillus granulatus*, known to always be associated with pines. Surprisingly, a few fruiting bodies were also collected in the neighboring oak areas in this study.

The three indices (Table 2) used in this study provided an important tool to summarize the overall diversity of the fruiting body communities. They were characterized by a low diversity and a good evenness of the samples, although these indices varied significantly among the different host trees. In particular, the area planted with *Q. pubescens* showed the lowest values for species richness and Shannon's diversity index (11 and 0.048 respectively).

Rank-abundance curves revealed that the *Quercus pubescens* community showed a different trend in relation to the other two communities, and that only a few species of EMFb dominated in relation to each host species (Fig. 1).

Ectomycorrhizal morphotype (EMMt) communities

The 4.685 colonized root tips present in 84 soil cores were examined and assigned to 74 different EMMt according to their morpho-anatomical features, as described by Agerer (1987-2008; 1995; 2006) and the Ecto-Mycorrhizal Community DataBase (www.emyco.uniss.it) (Table 3).

With regard to their identity, 69 were *Basidiomycota* and only 5 *Ascomycota*. The most common family was *Cortinariaceae* with 14 different ectomycorrhizal morphotypes, 6 of which were identified at genus level (*Cortinarius*) (Table 3), followed by *Inocybaceae* with 12 different EMMt (all of the genus *Inocybe*) and *Thelephoraceae* with 11 (7 of which belonged to the genus *Tomentella*). The genus *Inocybe*, followed by *Tomentella,* also had the highest number of different EMMt. Regarding relative abundance, *Tricholoma* sp. 2 had the largest number of colonized root tips (6.429), followed by *Hebeloma* sp. 2 (3.060), *Inocybe* sp. 8 (3.048), *Cenococcum geophilum* (2.631) and *Tuber aestivum* (2.512). The last two species were also among the most frequent. In the area with *Q. cerris*, *Tricholoma* sp. 2 was again the most abundant (13.769), whereas in the *Pinus nigra* and *Q. pubescens* areas the most abundant species were *Hebeloma* sp. 2, with 4.571 colonized root tips, and *Tomentella* sp. 5, with 5.550, respectively. The most frequent EMMt in the orchard studied as a whole and in the *Pinus* area was *Cenococcum geophilum* (14), while under *Q. cerris* it was *T. aestivum*. The third area showed only single findings. *T. aestivum* was found in only 9 out of the total of 84 soil cores, with a considerable variation in relative abundance between individual samples (from 0.07 to 0.64).

Among the 32 EMMt exclusive to *Quercus cerris* the most abundant were *Inocybe* sp. 8 and *Tricholoma* sp.;

Table 1. Average abundance and frequency of ectomycorrhizal fruiting bodies: in relation to each host tree (*Quercus cerris*, *Pinus nigra, Q. pubescens*) and total

MACROFUNGAL TAXA	Total samples		*Quercus cerris*		*Pinus nigra*		*Quercus pubescens*	
	Abun.	Freq.	Abun	Freq.	Abun.	Freq.	Abun.	Freq.
Boletus fechtneri Velen.	0.004	1					0.012	1
Boletus satanas Lenz	0.009	1	0.034	1				
Chroogomphus rutilus (Schaeff.) O.K. Mill.	0.373	33			0.894	31	0.106	2
Cortinarius anomalus (Pers.) Fr.	0.004	1	0.017	1				
Cortinarius aprinus Melot	0.013	1	0.052	1				
Cortinarius flexipes (Pers.) Fr.	0.009	1	0.034	1				
Cortinarius glaucopus (Schaeff.) Fr.	0.004	1	0.017	1				
Cortinarius hinnuleus Fr.	0.114	2	0.448	2				
Cortinarius paleaceus (Weinm.) Fr.	0.189	7	0.569	6	0.118	1		
Cortinarius rigens (Pers.) Fr.	0.009	1	0.034	1				
Cortinarius rigidiusculus Nezdojm.	0.018	1	0.069	1				
Cortinarius torvus (Fr.) Fr.	0.579	3	2.276	3				
Cortinarius trivialis J.E. Lange	0.057	4	0.224	4				
Cortinarius uraceus Fr	0.026	2	0.103	2				
Hebeloma crustuliniforme (Bull.) Quél.	1.206	15	4.707	14	0.024	1		
Hebeloma sinapizans (Fr.) Sacc.	1.794	41	7.052	41				
Hygrophorus agathosmus (Fr.) Fr.	0.013	1			0.035	1		
Hygrophorus mesotephrum Berk. & Broome	0.013	1			0.035	1		
Hygrophorus persoonii Arnolds	0.004	1			0.012	1		
Hymenogaster olivaceus Vittad.	0.009	1	0.034	1				
Inocybe cincinnata (Fr.) Quél.	0.026	1					0.071	1
Inocybe cincinnata var. major (S. Petersen) Kuyper	0.061	1			0.165	1		
Inocybe flocculosa (Berk.) Sacc.	0.039	3	0.086	1	0.012	1	0.035	1
Inocybe fuscidula var. *fuscidula* Velen.	0.162	9	0.017	1	0.329	7	0.094	1
Inocybe geophylla (Fr.) P. Kumm.	0.447	11	0.017	1	1.188	10		
Inocybe geophylla var. lilacina Gillet	0.145	6			0.388	6		
Inocybe glabripes Ricken	0.351	14			0.941	14		
Inocybe hirtelloides Stangl & J. Veselsk?	0.013	1	0.052	1				
Inocybe obscurobadia (J. Favre) Grund & D.E. Stuntz	0.013	1					0.035	1
Inocybe pseudoreducta Stangl & Glowinski	0.066	1	0.259	1				
Inocybe sindonia (Fr.) P. Karst.	0.013	1					0.035	1
Inocybe sp.1	0.079	1	0.310	1				
Inocybe splendens R. Heim	0.145	7	0.534	6	0.024	1		
Inocybe tenebrosa Quél.	0.351	16	1.379	16				
Laccaria laccata (Scop.) Cooke	3.053	34	11.948	33	0.035	1		
Lactarius deliciosus (L.) Gray	0.184	17			0.494	17		
Lactarius sanguifluus (Paulet) Fr.	0.228	18	0.017	1	0.600	17		
Lactarius semisanguifluus R. Heim & Leclair	0.013	1		3	0.035	1		
Paxillus involutus (Batsch) Fr.	0.469	16	1.845	15				
Rhizopogon roseolus (Corda) Th. Fr.	0.123	8	0.017	1	0.318	7		
Russula torulosa Bres.	0.075	8			0.200	8		
Scleroderma verrucosum (Bull.) Pers.	0.237	15	0.017	1	0.624	14		
Suillus granulatus (L.) Roussel	0.825	24	0.052	1	2.024	19	0.153	3
Tricholoma atrosquamosum Sacc.	0.461	2	1.810	2				
Tricholoma terreum (Schaeff.) P. Kumm.	4.114	45	5.983	16	6.882	28	0.071	1
Tricholoma ustaloides Romagn.	0.044	1			0.118	1		
Tuber aestivum Vittad.	1.018	49	0.655	14	1.812	27	0.471	8
Tuber melanosporum Vittad.	0.004	1					0.012	1

Table 2. Ectomycorrhizal fruiting body and morphotype diversity in the truffle orchard and in each host tree area

	Total	***Quercus cerris***	***Pinus nigra***	***Quercus pubescens***
Number of host trees	228	58	85	85
Number of EMFb	3692	2322	1315	55
EMFb richness	48	32	24	11
EMFb Shannon index	0.420	0.713	0.498	0.048
EMFb Pielou index	0.731	0.741	0.736	0.715
Number of EMMt	4685	2834	1587	264
EMMt richness	74	49	33	17
EMMt Shannon index	0.214	0.394	0.200	0.047
EMMt Pielou index	0.629	0.686	0.676	0.526
Number of *T. aestivum* truffles	232	38	154	40
Total weight of *T. aestivum* truffles	5485	1220	3205	1060

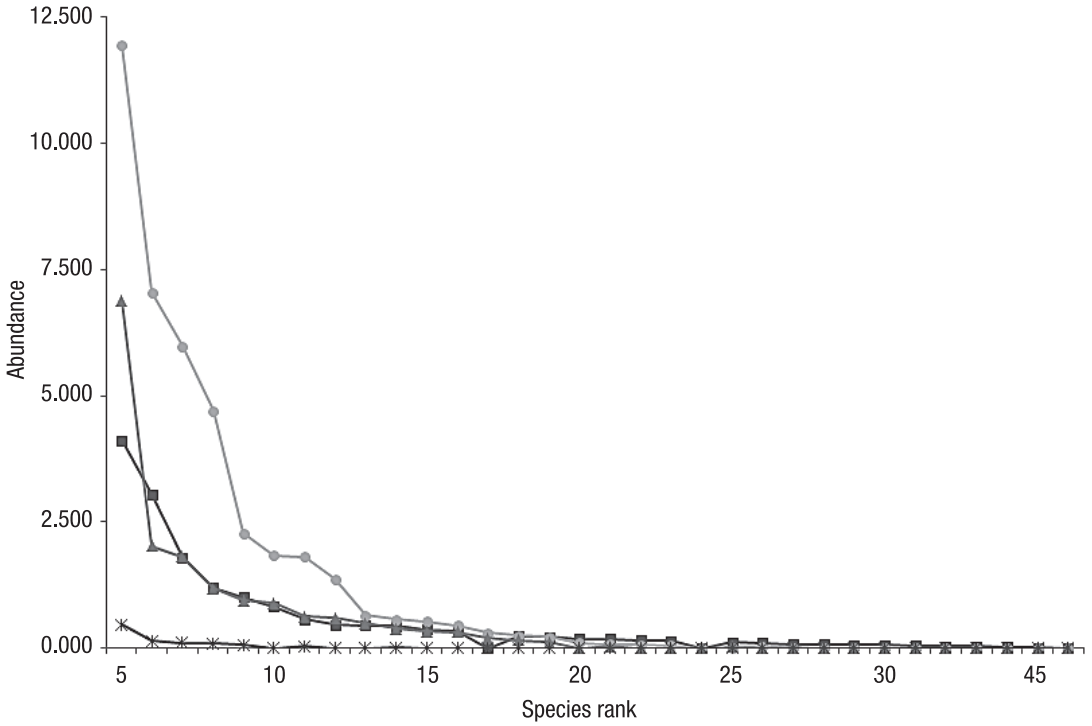

Figure 1. Rank-abundance curves: abundance of EMFb species in relation to total community (■); *Quercus cerris* community (●); *Pinus nigra* (▲) and *Quercus pubescens* (✳).

of the taxa exclusive to pines, *Inocybe* sp. 2, *I. geophylla* and *Lactarius* sp. 3 were the most abundant, and the latter was also the most frequent. *Quercus pubescens* was generally associated with few taxa, but these included 7 exclusive to this area, with very abundant *Tomentella* sp. 5, followed by *Inocybe* sp., *Melanogaster broomeianus* and *Boletus* sp. 1. In contrast, *Cenococcum geophilum, Pezizales* sp. 2, *Tomentella ferruginea* and *Thelephoraceae* sp. 1 were present in all areas. A special mention should be made of *Suillus* sp. 1, which was very abundant and frequent under pines but also observed under oaks, and *Suillus* sp., which was exclusive to pines, albeit not as abundant.

The values of the Shannon and Pielou indices (0.214 and 0.629, respectively) indicated that the EMMt communities were also characterized by a low diversity

Table 3. Average abundance and frequency of ectomycorrhizal morphotypes: in relation to each host tree (*Quercus cerris*, *Pinus nigra, Q. pubescens*) and total samples

EMMt	Total samples		*Quercus cerris*		*Pinus nigra*		*Quercus pubescens*	
	Abun.	Freq.	Abun.	Freq.	Abun.	Freq.	Abun.	Freq.
Agaricales sp. 1	0.250	2			0.600	2		
Agaricales sp. 2	0.119	1					1.000	1
Agaricales sp. 3	0.131	1	0.282	1				
Agaricales sp. 4	0.440	2			1.057	2		
Agaricales sp. 5	0.048	1					0.400	1
Agaricales sp. 6	0.238	1	0.513	1				
Boletus sp. 1	0.202	1					1.700	1
Boletus sp. 2	0.238	1	0.513	1				
Cenococcum geophilum	2.631	14	1.641	4	3.514	9	3.400	1
Chroogomphus sp.	0.083	1			0.200	1		
Cortinariaceae sp. 2	0.512	2	0.821	1	0.314	1		
Cortinariaceae sp. 3	0.250	1	0.538	1				
Cortinariaceae sp. 4	0.202	1	0.436	1				
Cortinariaceae sp. 5	0.048	1	0.103	1				
Cortinariaceae sp. 6	0.250	2	0.538	2				
Cortinariaceae sp. 7	0.190	1	0.410	1				
Cortinariaceae sp. 8	0.071	1					0.600	1
Cortinariaceae sp. 9	0.143	2	0.308	2				
Cortinarius sp. 1	0.595	1	1.282	1				
Cortinarius sp. 2	0.119	1	0.256	1				
Cortinarius sp. 3	1.143	2	2.462	2				
Cortinarius sp. 5	0.238	1	0.513	1				
Cortinarius sp. 6	0.036	1	0.077	1				
Cortinarius sp. 7	0.155	2	0.333	2				
Hebeloma sp. 1	1.143	2	2.462	2				
Hebeloma sp. 2	3.060	3	2.487	2	4.571	1		
Hygrophorus sp. 1	0.238	1			0.571	1		
Hygrophorus sp. 2	0.512	1			1.229	1		
Hygrophorus sp. 3	0.083	1			0.200	1		
Hymenogaster sp.	0.595	1	1.282	1				
Inocybe geophylla	0.631	1			1.514	1		
Inocybe sp.	0.298	1					2.500	1
Inocybe sp. 9	0.298	1			0.714	1		
Inocybe sp. 1	1.131	2	2.436	2				
Inocybe sp. 10	0.024	1			0.057	1		
Inocybe sp. 11	0.012	1	0.026	1				
Inocybe sp. 2	1.012	2			2.429	2		
Inocybe sp. 3	0.536	2	1.154	2				
Inocybe sp. 4	0.560	2			1.086	1	0.900	1
Inocybe sp. 5	0.845	3	0.462	2	1.514	1		
Inocybe sp. 6	0.238	1			0.571	1		
Inocybe sp. 8	3.048	1	6.564	1				
Laccaria sp.	0.036	1	0.077	1				
Lactarius sp.	0.595	1	1.282	1				
Lactarius sp. 1	0.083	2			0.200	2		
Lactarius sp. 2	0.131	4			0.286	3	0.100	1
Lactarius sp. 3	0.821	4			1.971	4		
Melanogaster broomeianus	0.238	1					2.000	1
Paxillus involutus	0.060	1	0.128	1				
Pezizales	0.476	1	1.026	1				
Pezizales sp. 2	1.202	3	1.795	1	0.457	1	1.500	1

Table 3 (cont.). Average abundance and frequency of ectomycorrhizal morphotypes: in relation to each host tree (*Quercus cerris*, *Pinus nigra, Q. pubescens*) and total samples

EMMt	Total samples		*Quercus cerris*		*Pinus nigra*		*Quercus pubescens*	
	Abun.	Freq.	Abun.	Freq.	Abun.	Freq.	Abun.	Freq.
Pyronemataceae sp. 1	0.262	1	0.564	1				
Rhizopogon sp.	0.714	2	1.282	1	0.286	1		
Russula sp. 1	1.167	5			1.771	4	3.600	1
Sebacina sp. 1	0.095	1	0.205	1				
Sebacina sp. 2	0.655	1	1.410	1				
Sebacina sp. 4	0.464	2	0.410	1	0.657	1		
Suillus sp.	0.357	1			0.857	1		
Suillus sp. 1	1.310	8			3.057	7	0.300	1
Thelephoraceae sp. 1	2.357	6	2.846	3	2.400	2	0.300	1
Thelephoraceae sp. 3	1.571	3	0.949	2	2.714	1		
Thelephoraceae sp. 4	0.786	4	1.641	3			0.200	1
Thelephoraceae sp. 5	2.464	11	1.513	5			0.900	1
Tomentella ferruginea	0.583	5	0.667	3	0.229	1	1.500	1
Tomentella sp. 2	0.250	2	0.513	1	0.029	1		
Tomentella sp. 3	0.226	1	0.487	1				
Tomentella sp. 4	0.381	2	0.821	2				
Tomentella sp. 5	0.655	1					5.500	1
Tomentella sp. 6	2.262	6	3.154	5	1.914	1		
Tomentella sp. 7	0.333	1	0.718	1				
Tricholoma sp.	1.429	1	3.077	1				
Tricholoma sp. 1	2.274	7	2.333	4	2.857	3		
Tricholoma sp. 2	6.429	5	13.769	4	0.086	1		
Tuber aestivum	2.512	9	4.103	6	1.457	3		

and an even distribution of the samples (Table 2). As described above for the EMFb, the fungal community below *Quercus pubescens* showed lower values for richness and Shannon's diversity index (17 and 0.047 respectively).

Tuber aestivum

In total 232 truffles were collected, weighing 5485 grams. The largest number of truffles was counted in the area with *Pinus nigra* (154), while in each of the 2 oak areas less than a quarter of this number and weight was collected (Table 2).

The Kruskal-Wallis test ($\alpha = 0.05$) was performed on the host trees to compare the number of truffles and total weight of *Tuber aestivum* with the species richness and abundance of EMFb and EMMt communities. Significant differences were found in all cases ($p < 0.001$).

Spearman's rank correlation coefficient showed significant correlations ($p < 0.05$) between the number and weight of *T. aestivum* fruiting bodies and the number of EMFb species and their abundance, but not the number of species or abundance of the EMMt community. The host trees showed a statistically significant correlation ($p < 0.05$) with the number of truffles, but not their weight (Table 4).

Discussion

This is the first study to date in which fungal communities above and below ground, analyzed through observations of fruiting bodies (EMFb) and morphotypes (EMMt), have been compared with the production of *T. aestivum*, expressed in both the number and weight of fruiting bodies. Ectomycorrhizal communities on root tips in natural and cultivated truffières have been amply investigated (Donnini and Bencivenga, 1995; Donnini *et al.*, 1999; Murat *et al.*, 2005; Baciarelli Falini *et al.,* 2006; Pruett *et al.*, 2008; Águeda *et al.*, 2010; González-Armada *et al.,* 2010; Iotti *et al.*, 2010; Benucci *et al.*, 2011; Garcia-Barreda and Reyna, 2012; Leonardi *et al.*, 2013). However, according to Tóth and Barta (2010) very few studies have simul-

Table 4. Spearman's correlation coefficient between the weight and number of *T. aestivum* and the host trees (*Quercus cerris*, *Pinus nigra* and *Q. pubescens*), species richness and abundance of EMFb and EMMt

	Truffle weight	Truffle number
Host trees	0.118098 NS	0.136666 $p < 0.05$
Number of EMFb species	0.144504 $p < 0.05$	0.151502 $p < 0.05$
Abundance of EMFb	0.215439 $p < 0.05$	0.224748 $p < 0.05$
Number of EMMt	0.003820 NS	0.004411 NS
Abundance of EMMt	–0.004941 NS	–0.002699 NS

taneously analyzed fungal species both above and below ground in relation to varying environmental factors or with the aim of determining their suitability as indicators of environmental change. In particular, there is limited information available on the ecology of ectomycorrhizae that combines both communities in truffle environments (Donnini *et al.*, 2008; Salerni *et al.*, 2011).

Our result confirmed that fungal communities differed significantly in relation to the host trees. *Quercus pubescens* showed lower levels of richness than *Q. cerris* and *Pinus nigra* for both fruiting bodies (EMFb) and root tips (EMMt). The Shannon index values obtained in this study were rather low, which may be due to the non-natural character of the truffières. In fact, this index is much higher in natural environments: Buée *et al.* (2011) reported a Shannon index of 4.84 for epigeous saprotrophic and ectomycorrhizal fungi studied in a temperate deciduous forest in France, while Oria-de-Rueda *et al.* (2010) found an index of 1.2-1.3 for fungal communities observed in natural *Quercus faginea* and *Q. pyrenaica* forests. The Shannon index for the EMMt communities was closer to agreement with, but still lower than, other studies (Pruett *et al.*, 2008; Benucci *et al.*, 2011; Garcia-Barreda and Reyna, 2012). In contrast Leonardi *et al.* (2013) found relatively high values for the ectomycorrhizal communities present in four natural *Tuber magnatum* truffle grounds. More host plants are generally present in natural truffle grounds. This was confirmed by Belfiori *et al.* (2012), who studied fungal communities in natural and cultivated *T. melanosporum* sites, also providing evidence that fungal communities can diverge under identical environmental conditions.

The genera *Inocybe*, *Cortinarius*, *Hygrophorus, Lactarius* and *Tricholoma* are fairly common ectomycorrhizal fruiting bodies in various plant communities (Oria-de-Rueda *et al.*, 2010; Buée *et al.*, 2011; Hernández-Rodriguez *et al.*, 2013) and in environments in which truffles are produced (Donnini *et al.*, 2008; Salerni *et al.*, 2011). Similarly, *Cortinariaceae* and *Thelephoraceae* are fairly common morphotypes on root tips in various communities (Kõljalg *et al.*, 2000; Glen *et al.*, 2002; Selosse *et al.*, 2002; Urban *et al.*, 2003) and in environments characterized by truffle production (Murat *et al.*, 2005; Baciarelli Falini *et al.*, 2006; Pruett *et al.*, 2008; Iotti *et al.*, 2010; Benucci *et al.*, 2011; Belfiori *et al.*, 2012; Leonardi *et al.*, 2013). Our data confirmed that the genus *Tomentella* is widespread and an important component in orchards with *Tuber* spp. (Murat *et al.*, 2005; Pruett *et al.*, 2008; Águeda *et al.,* 2010; Iotti *et al.*, 2010; Benucci *et al.*, 2011; Belfiori *et al.*, 2012; Leonardi *et al.*, 2013). In agreement with Donnini *et al.* (2008), who reported the frequent fruiting of *Tricholoma* species in truffières, *Tricholoma terreum* was also found to be more abundant than *Tuber aestivum* in the present study. Note that *Tricholoma* sp., together with *Inocybe* sp. 8, were the most abundant EMMt growing exclusively on the roots of *Quercus cerris*. A separate mention is merited by *Suillus* species, which is always associated with pine trees. In the present study *S. granulatus* fruiting bodies and morphotypes were also observed under the nearby oak plantation. This could be related to the root system of *P. nigra*, which often extends well beyond the projection of its foliage.

Positive correlations were found between the number of *T. aestivum* fruiting bodies and the host species. This is in agreement with Garcìa-Montero *et al.* (2007), who found a significant variability in *T. melanosporum* truffle production in five types of woods in Spain. Nevertheless, Benucci *et al.* (2011) suggested that *T. aestivum* production was not affected by differences between the ectomycorrhizal communities associated with hazel and hornbeam in a 24-year-old orchard. This was also confirmed by our data, which showed no correlation between the production of truffles and the EMMt community below ground. Finally, positive correlations were identified between the weight and number of *T. aestivum* fruiting bodies and qualitative and quantitative data for EMFb above

ground. This result confirms the hypothesis supported by Donnini *et al.* (2008) that mycorrhizal fungi and truffle production are not in competition.

It is known that ectomycorrhizal fungi provide mineral nutrition to dominant trees, but differ in their enzymatic activities (Courty *et al.*, 2005). Moreover, they deliver species-specific benefits to their host plants (van der Heijden and Kuyper, 2003), which render their biodiversity of high importance to plant nutrition (Tedersoo *et al.*, 2006). Ectomycorrhizal fungi are highly diverse in most ecosystems, even though the mechanisms behind this diversity remain unclear. Their different preference for soil conditions and host plants seems to play a key role (Bruns, 1995). Koide *et al.* (2004), on the other hand, argue that significant associations between species occur when resources are not a limiting factor. In this case competition would not be prejudicial.

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