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

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**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.

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**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 (Kies and Liu, 2000). The role that interspecific competition plays in determining EM community
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 → ∞) and Pielou’s (E = 0 → 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 fungal species were also the most abundant when the area with *Q. pubescens* was dominated by *T. aestivum* (3.052), while *Tuber aestivum*, found 49 times, was the most frequent. In the area with *Q. pubescens* (2.512). The last two species were also among the most abundant were *Cortinarius* and *Laccaria laccata* (3.048), and *Tricholoma terreum* (3.032).

Among the 32 EMMt exclusive to *Quercus cerris* the most abundant were *Inocybe* sp. 8 and *Tricholoma* sp.; 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 Ectomycorrhizal 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     |        |        |        |        |        |        |
| **Boletus Satanus** Lenz                                | 0.009 | 1     | 0.034 | 1     |        |        |        |        |
| **Chroogomphus rattus** (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 rigidulusculus** 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** Arnoldis                     | 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 fuscida** (Berk.) Sacc.                      | 0.039 | 3     | 0.086 | 1     | 0.012 | 1     | 0.035 | 1     |
| **Inocybe fuscicula** var. fuscicula 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. illicina Gillet**             | 0.145 | 6     | 0.388 | 6     |        |        |        |        |
| **Inocybe glabripes** Ricken                           | 0.351 | 14    | 0.941 | 14    |        |        |        |        |
| **Inocybe hirtelloides** Stangl & J. Veselak?          | 0.013 | 1     | 0.052 | 1     |        |        |        |        |
| **Inocybe obscurabadia** (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 lacca** (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 & Leclaire       | 0.013 | 1     | 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     |        |        |
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 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* (★).
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. 8                           | 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     |
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; Bacciarelli 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; García-Barreda and Reyna, 2012; Leonardi et al., 2013). However, according to Tóth and Barta (2010) very few studies have simul-

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**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 | 0.286 1 | 3.600 1 |
| Rhizopogon sp. | 0.714 2 | 1.282 1 | 1.771 4 | |
| Russula sp. 1 | 1.167 5 |  |  | |
| 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 |  | |
| 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 |  |  | |
| Tomentella sp. 6 | 2.262 6 | 3.154 5 | 1.914 1 | 5.500 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 | 2.312 9 | 4.103 6 | 1.457 3 | |
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       | 0.136666       |
| Number of EMFb species | 0.144504       | 0.151502       |
| Abundance of EMFb    | 0.215439       | 0.224748       |
| Number of EMMt       | 0.003820       | 0.004411       |
| Abundance of EMMt    | -0.004941      | -0.002699      |

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.

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. melano- sporum* 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 (Köljalg 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 García-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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