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REVIEW

Ectomycorrhizal fungus diversity and community structure with natural and cultivated truffle hosts: applying lessons learned to future truffle culture

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Abstract Since the first truffle plantations were established in France, Italy and other parts in the world, many studies have been carried out to improve their productivity and sustainability. Success of plantations is clearly related to the mycorrhizal status of the host trees over the years, from inoculated seedlings to truffle-producing trees. The experience gained in monitoring the ectomycorrhizal fungus status in cultivated truffle grounds has allowed us to develop an extensive catalogue of the ectomycorrhizal fungi present in truffle plantations. Herein, we summarize fungal community data from 85 references that represent different truffle studies in natural habitats and plantations. Approximately 25 % of the ectomycorrhizae reported in the 85 references are common to most of the studies. In general, more fungal species are detected in productive plantations than in the non-productive ones. Truffle plantations display a diverse ectomycorrhizal fungal community, in which species of the genus *Tuber* are well represented. *Tuber rufum* and some members of Boletales are typically restricted to productive truffle plots. On the other hand, *Hebeloma*, *Laccaria* and *Russula* species are mostly associated with unproductive plots. Ectomycorrhizae belonging to Thelephoraceae are frequently found in mature truffle orchards but do not seem to affect sporocarp production. Several biotic and abiotic factors affect the ectomycorrhizal fungus communities associated with truffle orchards. Among them are plantation age, host species and its growth, the surrounding environment (particularly the presence of other ectomycorrhizal hosts), and plantation management. Understanding the ectomycorrhizal fungal communities inhabiting different plantations may give us clues about the dynamics of the targeted truffles and the possibility of identifying mycorrhizal fungal species that are good indicators of successful truffle plantations.

Keywords Ectomycorrhizal fungus diversity · *Tuber* plantations · Black truffles · Truffle cultivation · Thelephoraceae · Ectomycorrhizal exploration types

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Introduction

The discovery and description of mycorrhizae by A.B. Frank in 1885 was achieved while promoting the production of truffles in Prussia (Frank 2005). Since then, 'black truffle', *Tuber melanosporum* Vittad., cultivation has lead to advances in mycology and mycorrhizal science, including morphology and anatomy, ontogeny, biology, taxonomy, and molecular characterization.

Harvests of the 'black truffle' in France decreased dramatically from an average of 800 tonnes at the beginning of the 20th century to less than 50 tonnes nowadays (Hall and Zambonelli 2012). In the 1960s a significant research effort was carried out, aimed at reversing this trend by using a science-based management approach for truffle cultivation involving modification of soil and growing conditions, and use of seedlings inoculated with specific truffle species to

establish plantations (Olivier et al. 2012; Selosse et al. 2013). Thanks to the progress made by Prof. Mario Palenzona in the IPLA of Torino (Italy) and Prof. Gérard Chevalier in the INRA of Clermont-Ferrand (France) (Palenzona 1969; Chevalier et al. 1973) the use of inoculated mycorrhizal seedlings became widespread. Commercially harvested truffles can establish ectomycorrhizal relationships with different woody host species and many different combinations of truffle and host species mycorrhizal seedlings are produced in nurseries (Benucci et al. 2012).

For decades, most research efforts have focused on the cultivation of the 'black truffle' and the 'white truffle', *T. magnatum* Pico. Other truffle species such as the 'bianchetto' (*T. borchii* Vittad.) and 'summer truffles' (*T. aestivum* Vittad. and *T. uncinatum* Chatin) have been less widely studied but provide interesting alternatives due to their ecological plasticity, wide soil range requirements and resistance to dryness. Thus, the cultivation of these less studied species could be easier, more reliable and feasible than the more valuable species (Bencivenga 2007; Zambonelli et al. 2007). Other native *Tuber* species adapted to particular regions have also been cultivated and promoted, such as *T. gibbosum* Harkn. and *T. oregonense* Trappe, Bonito & Rawlinson (Lefevre 2012) in Oregon, USA, and *T. indicum* Cooke & Massee in China (Wang 2012).

Continuous advances in technical management of truffle plantations have made it possible to grow truffles in a wide range of field conditions, including outside of their natural range. The ability to produce European truffles in Australia and New Zealand, for example, allows marketing of these valuable fungi when they are not producing in Europe (Zambonelli and Bonito 2012). The development of molecular techniques and their application in truffle research has allowed significant advances including the publication of the complete genome of *T. melanosporum* (Martin et al. 2010), the disclosure of its life cycle (Riccioni et al. 2008; Rubini et al. 2011b), and the detection and quantification of its extraradical mycelium (Suz et al. 2006; Parladé et al. 2013).

Some truffle plantations produce large quantities of truffles, while others in apparently similar conditions do not. The management of every plantation should favor the persistence of the introduced truffle species, but, in many cases, suitable biological and ecological factors for truffle development are not always taken into account when establishing a new plantation (Vellinga et al. 2009; Kües and Martin 2011). Unfortunately, to date factors favoring the dominance of a particular truffle species are not completely understood (Chevalier and Sourzat 2012; García-Barreda and Reyna 2012).

The persistence of truffle ectomycorrhizae introduced in the field on inoculated seedlings is affected by competitive relationships with other ectomycorrhizal species (Zambonelli et al. 1998; Águeda et al. 2010b). The replacement of ectomycorrhizae formed by the targeted truffles by native ectomycorrhizal fungi is one of the most important causes of truffle cultivation failures (Hall et al. 2007; Chevalier and Sourzat 2012; Olivier et al. 2012). The available studies on fungal diversity in truffle plantations have brought a considerable amount of information about the communities of mycorrhizal fungal species that coexist with valued truffles in natural plant communities and truffle plantations. Although many of the fungi have been identified via morphotyping and molecular techniques, little is known about their ecology and how they can influence truffle development.

The main objective of this study is to compile and summarize the available information on ectomycorrhizal fungal diversity and dynamics with natural and managed truffle hosts with the goal of developing preliminary insights into the influence of ectomycorrhizal fungus communities on the productivity of commercial *Tuber* plantations.

Ectomycorrhizal fungus diversity in truffle plantations

Before truffle inoculated seedlings are planted into the field, various ectomycorrhizal fungi also colonize inoculated nursery plants in addition to targeted truffle fungi and are considered contaminants. Among these are *Tuber* species, such as *T. brumale* Vittad., *T. aestivum*, and *T. borchii*, as well as other fungal species commonly found in nurseries such as *Sphaerosporella brunnea* (Alb. & Schwein.) Svrček & Kubička, *Pulvinula convexella* (P Karst.) Pfister (=*P. constellatio*), *Thelephora terrestris* Ehrh. and *Laccaria* Berk. & Broome species (Meotto and Carraturo 1988; Bencivenga et al. 1995). The origin of these undesired species include the use of contaminated truffles, the accidental use of rejected or misidentified truffles as inoculum, contamination in the greenhouse by common fungi present there, contamination of plants during transit or during storage on site, or fungi introduced on farm machinery (Chevalier et al. 1982; Hall et al. 2007; Olivier et al. 2012).

In addition to competitive pressure from undesired ectomycorrhizal fungi on nursery inoculated seedlings, more competition arises when inoculated seedling are planted in the field. Once planted, competing fungi can colonize seedling roots from fungal propagules in the soil, from ectomycorrhizal fungi associated with surrounding trees, or ectomycorrhizal fungus spores dispersed by mycophagous animals, particularly hypogeous fungi. Zambonelli et al. (2012) emphasized the important effect of competition by resident ectomycorrhizal fungi during the initial years after planting, when introduced species are most vulnerable to displacement, especially if seedlings are poorly colonized by the targeted truffle species at the nursery stage (Iotti et al. 2012). Thus, it is important that we examine the ectomycorrhizal fungal community in truffle plantations so that we can identify potential competitors and fungal indicators of potential truffle productivity.

Based on research by Dominik (1959), Fontana and Centrella (1967), Luppi and Gautero (1967), Palenzona (1969), Palenzona et al. (1972), Chevalier (1973), Chevalier et al. (1973) and Chevalier and Delmas (1975), Giraud (1979) described 50 morphotypes of ectomycorrhizae in 'black truffle' plantations in a pioneer study. Among them are the often cited AD and SB types, as well as the original drawings of these morphotypes that have been used as reference in a number of studies. Ten years later, Gregori et al. (1990) described for the first time 17 ectomycorrhizal types in *T. magnatum* stands, including natural and plantation areas. This is also an important pioneering study in *T. magnatum* culture because it was the first to compare ectomycorrhizal fungus diversity in natural areas and plantations. These studies by Giraud (1979) and Gregori et al. (1990) detected several species that occur in productive truffle areas: 17 types associated with *T. melanosporum* producing trees and ten types associated with *T. magnatum* producing trees. They specifically identified *T. aestivum-uncinatum*, *T. excavatum* Vittad., *T. mesentericum* Vittad., *Cenococcum geophilum* Fr. (=*C. graniforme*), *Hymenogaster vulgaris* Tul. & C. Tul., *Melanogaster variegatus* (Vittad.) Tul. & C. Tul., and *Tomentella* sp. in productive truffle areas, and suggested that these fungal species do not necessarily have a negative effect on truffle production.

Since these pioneering studies, considerable research has targeted the ectomycorrhizal fungal communities associated with natural truffle areas and plantations. From our examination of 85 studies on this subject, we have recorded a total of 100 species of ectomycorrhizal fungi; around 25 species were found in the majority of sites studied (Table 1). Detailed information on all reviewed records on diversity of mycorrhizae in truffle plantations is provided in Table S1. The countries with most studies on this subject are France, Italy and Spain, with 15, 25 and 40 records, respectively. A few papers refer to truffle plantations in the USA, Canada and New Zealand.

Among the 85 reviewed papers, 64 identify the ectomycorrhizae of truffle plantations only according to morphological aspects (morphotypes), emphasizing the difficulties in identifying the ectomycorrhizae to species level. To facilitate the recognition of other ectomycorrhizae coexisting with targeted *Tuber* species in the field, especially in truffle plantations, many efforts have been made to describe the ectomycorrhizae of *Tuber* species based on inoculated mycorrhizal seedlings under laboratory conditions (De Román et al. 2005).

With the advent of DNA sequencing methodology, it became possible to identify species using molecular techniques. Zambonelli et al. (2000a) were the first to combine morphological and molecular tools. Studies combining both methodologies have increased significantly since then, with 18 records registered to date (Table S1). Molecular tools have proven essential to identify common morphotypes, particularly the AD type, described by Giraud (1979) and mentioned in numerous studies. Baciarelli Falini et al. (2006) revealed its identity as *Trichophaea woolhopeia* (Cooke & W. Phillips) Boud., and subsequently Águeda et al. (2008, 2010a) and Rubini et al. (2011a) reported on its biology and morphology in detail.

Similar to ectomycorrhizal fungus community studies worldwide, scientists studying the ectomycorrhizal fungus diversity of truffle plantations have been challenged by the lack of identified fungal sequences to compare their findings in databases, such as GenBank® (http://www.ncbi.nlm.nih.gov/nucleotide) (Benson et al. 2005) or UNITE (http://unite.ut.ee/) (Kõljalg et al. 2013), although a considerable effort has been made to annotate reliable sequences in public databases

Table 1 Species, genera, families and groups of ectomycorrhizal morphotypes mentioned in *Tuber melanosporum* plantations reported more than four times

Ectomycorrhizal morphotypes	Times reported
Astraeus hygrometricus (Pers.) Morgan	4
Basidiomycetes Winter	12
Cenococcum geophilum Fr.	30
Genea Vittad.	15
Hebeloma* (Fr.) P. Kumm.** (=Hebeloma***-*Cortinarius*)	44
***Hymenogaster* Vittad.**	26
Melanogaster variegatus (Vittad.) Tul. & C. Tul. (=*Melanogaster* sp.)	4
Pisolithus arhizus (Scop.) Rauschert (=*P. tinctorius* (Pers.) Coker & Couch	20
Quercirhiza squamosa (Palfner and Agerer 1996)	13
***Scleroderma* Pers.**	35
Sphaerosporella brunnea (Alb. & Schwein.) Svrček & Kubička	13
Thelephora Ehrh. Ex Willd.	5
Thelephoraceae Chevall.	6
***Tomentella* Pers. ex Pat.**	10
Tomentella galzinii Bourdot (=SB; =forma 14) (=*Quercirhiza fibulocistidiata*)	25
Tomentella subtestacea Bourdot & Galzin	5
Trichophaea woolhopeia (Cooke & W. Phillips) Boud. (=AD; Forma 2) (=*Quercirhiza quadratum* (Águeda B et al. 2008))	53
***Tuber* Micheli ex Wiggers**	10
***Tuber aestivum* Vittad.** (=*T. uncinatum* Chatin)	55
***Tuber borchii* Vittad.** (=*T. albidum* Pico)	24
***Tuber brumale* Vittad.**	52
***Tuber melanosporum* Vittad.**	55
Tuber mesentericum Vittad.	25
***Tuber rufum* Pico**	30
***Xerocomus* Quél.**	6

Data in *bold* also appear in *T. magnatum*, *T. borchii* and *T. aestivum* plantations. All unidentified mycorrhizae (unidentified Morphotypes [UM], *Formas*, types, tipos, morphotypes, unknown mycorrhizae and other ectomycorrhizal species [OE]), are not included

(Tedersoo et al. 2011). Some ectomycorrhizal databases like DEEMY (http://www.deemy.de/) (Agerer and Rambold 2004–2013), an interactive database for identification of ectomycorrhizae, with entries for 554 taxa, and the Database of Descriptions of Ectomycorrhizae of the British Columbia Ectomycorrhizal Research Network (http://forestry-dev.org/biodiversity/bcern/cde/index_e.html), have proven useful to identify and study ectomycorrhizal fungus communities in truffle sites. An online interactive key useful for the identification of *Tuber* ectomycorrhizae on inoculated commercial seedlings, called 'TuberKey' (http://www.truffle.org/tuberkey) (Zambonelli et al. 2000b) is also available. The new 'ectomycorrhizal fungus community database' (eMyCo, http://emyco.uniss.it/index.php) (Lancellotti et al. 2011), collects and analyzes ecological data on ectomycorrhizal fungi, allowing comparative studies on ectomycorrhizal communities.

The incorporation of metagenomics in the studies of fungal communities has represented a significant step forward in global identifications and data processing (Zambonelli et al. 2012). Mello et al. (2011) used 454 pyrosequencing to conclude that Ascomycota were the dominant fungal phylum in *T. melanosporum* truffle brûlés and that their frequency of occurrence decreased from inside to outside the brûlé, while the number of Basidiomycota increased. Recently, Leonardi et al. (2013) combined morphological and molecular tools to study the ectomycorrhizal communities in a *T. magnatum* stand, with interesting results: they report 129 operational taxonomic units (OTUs), and identified many species and genera that are poorly known in truffle plantations due to the lack of morphological descriptions of their ectomycorrhizae.

Of the literature reviewed, 69 references focus on *T. melanosporum* plantations. Due to its high commercial value, this was the first *Tuber* species established in plantations with mycorrhizal inoculated seedlings in many countries outside its natural distribution area (Chevalier 2008; Reyna 2012). In contrast, because of the difficulties in obtaining inoculated mycorrhizal plants, the development of *T. magnatum* cultivation has been slow, and, thus, the studies have been focused on natural Italian forest environments (Zambonelli et al. 2012). Only eight studies on ectomycorrhizal diversity in *T. magnatum* grounds have been carried out. Finally, nine references examine other edible cultivated truffles which have attracted interest in the last few decades, especially in Italy: *T. borchii* and *T. aestivum-uncinatum* are prominent among these studies and show a great potential for cultivation (Zambonelli et al. 2007; Chevalier 2010; Reyna 2013).

Our extensive compilation of ectomycorrhizal fungi identified in truffle plantations worldwide reveal more than 100 species corresponding to 31 fungal genera: *Asterostroma* Massee, *Astraeus* Morgan, *Boletus* L. (including *Xerocomus* Quél.) *Cantharellus* Adans. ex Fr., *Cenococcum* Moug. & Fr., *Cerrena* Gray (=*Sistotrema* Pers.), *Clavulina* J. Schröt., *Cortinarius* (Pers.) Gray, *Cyanthus* Haller (=*Peziza* L.), *Genea* Vittad., *Hebeloma* (Fr.) P. Kumm., *Hygrophorus* Fr., *Hymenogaster* Vittad., *Inocybe* (Fr.) Fr., *Laccaria* Berk. & Broome, *Lactarius* Pers., *Melanogaster* Corda, *Pachyphloeus* Tul. & C. Tul., *Pisolithus* Alb. & Schwein., *Pseudotomentella* Svrček, *Pulvinula* Boud., *Russula* Pers., *Scleroderma* Pers., *Sebacina* Tul. & C. Tul., *Sphaerosporella* (Svrček) Svrček & Kubička, *Suillus* P. Micheli ex Adans., *Thelephora* Ehrh. ex Willd., *Tomentella* Pers. ex Pat., *Tricholoma* (Fr.) Staude, *Trichophaea* Boud., and *Tuber* P. Micheli ex F.H. Wigg., among which *Tuber* and *Tomentella* stand out, with 19 and 11 species, respectively.

Table 1 summarizes the 25 most frequent ectomycorrhizal morphotypes by taxon mentioned in the 85 references reviewed. Ectomycorrhiza descriptions for many of these species and morphotypes can be found in the work of Agerer (1987–2012), Agerer et al. (1996–2008), and Agerer and Rambold (2004–2013), or in other studies summarized by De Román et al. (2005). Below, we highlight what we consider the important fungal species commonly found in truffle fungal communities. When information is available, we also comment on their suspected competitive interactions and potential effects on truffle productivity.

Tuber ectomycorrhizae

The appearance and frequency of 'black truffle' mycorrhizae in orchards is reported in many studies (Table S1) as is the presence of other *Tuber* species. Studies by Chevalier (1994), Iotti et al. (2005), and Di Massimo et al. (2010) indicate that different *Tuber* species are the most important competitors in 'black truffle', 'white truffle' and 'summer truffle' stands. All *Tuber* morphotypes belong to the short-distance exploration type, with distinct emanating hyphae and rather limited growth into the surrounding soil (Agerer 2001, 2006).

Tuber brumale ectomycorrhizae typically occur in the early stages of truffle plantation establishment. Such early establishment and competition by *T. brumale* cause great disappointment to truffle managers and has lead to management options to control it in plantations. For example, Sourzat (2011) indicates that the appearance of *T. brumale* in France is favored by some cultural practices such as frequent tilling with tractor-drawn tools. In New Zealand, *T. brumale* may be accidentally introduced as a contaminant on imported *T. melanosporum* mycorrhizal seedlings (Guerin-Laguette et al. 2010, 2013). In Spain, *T. brumale* does not appear to be a serious problem yet, although it can be found in some black truffle orchards. It remains unclear whether its presence in these plantations is due to contamination of inoculated nursery plants, wild invasion, or inappropriate cultural practices (Reyna 2012).

Tuber aestivum also frequently occurs in truffle stands in Europe. In Italy, some studies reveal the high competitiveness of *T. aestivum* in forests with closed canopies (Sourzat 2011; Belfiori et al. 2012). The dominance of *T. aestivum*

ectomycorrhizae can actually change the trajectory of some plantations from 'black truffle' to 'summer truffle' (Bencivenga et al. 1992; Granetti and Angelini 1992; Sáez García Falces and De Miguel Velasco 2008).

Other *Tuber* species can also be present but not as abundant as the previous ones. For example, *T. rufum* Pico grows in conditions similar to *T. melanosporum* and has been repeatedly noted in French and Spanish plantations and, to a lesser extent, in Italy and the USA. Olivier et al. (2012) report that its appearance is an indicator of future good production of *T. melanosporum*. *T. borchii* has also been reported many times in French, Spanish and Italian truffle plantations, but information is lacking about its competitiveness. Finally, *T. mesentericum* has been found in Europe but only in 'black truffle' grounds.

The 'Asian black truffle' (*Tuber indicum*) is considered a threat to *T. melanosporum* truffle culture due to its presumed competitiveness and broad host compatibility outside its native Asian range. Murat et al. (2008) used molecular tools to experimentally confirm its presence in a truffle plantation in Piedmont (Italy), although truffles of this species have never been harvested. It was found fruiting in a forest in Oregon, USA, and was detected as ectomycorrhizae within a truffle orchard established with trees supposedly inoculated with *T. melanosporum* (Bonito et al. 2011). The ecological consequences of introducing *T. indicum* into *T. melanosporum*-producing areas in Europe and elsewhere remain unknown. The close phylogenetic relationship between *T. indicum* and *T. melanosporum* poses some questions of scientific relevance such as the possibility of inbreeding and/or the replacement of *T. melanosporum* in truffle grounds (Murat et al. 2008).

Other important ectomycorrhizal fungi

Other ectomycorrhizal morphotypes such as AD and SB and ectomycorrhizal species of *Scleroderma*, *Pisolithus*, and *Hebeloma* have been detected regularly in truffle plantations. The AD Type (in French *angle droite*), identified as *Trichophaea woolhopeia* (Águeda et al. 2008; Rubini et al. 2011a), is the most frequently mentioned type (Table 1). It was first described by Giraud (1979, 1988) in his studies on productive and non-productive truffle trees and is listed as a common fungal associate in French handbooks on truffle cultivation (Verlhac et al. 1990; Sourzat 1994). In Italy, it was first noted by Bencivenga et al. (1995) and Granetti and Baciarelli Falini (1997) as a strong and undesirable competitor in nurseries. This species is also known from truffle plantations in New Zealand (Hance 2009, pers. com.) and Canada (Berch 2011). In Spain it was first mentioned by Sáez and De Miguel (1995) in truffle plantations and in nurseries by Di Massimo et al. (1996). Since then, *T. woolhopeia* has been regularly found in Spanish truffle orchards (Águeda et al. 2001; Domínguez Núñez et al. 2005; Sánchez 2008, 2012). The ectomycorrhizae of *T. woolhopeia* belong to the short-distance exploration type according to Agerer (2001) with distinct emanating hyphae and rather limited growth into the surrounding soil (Águeda et al. 2008). Although *T. woolhopeia* is a common member of the fungal communities in truffle stands, it does not seem to interfere with the production of 'black truffle' sporocarps as it is found with both productive and non-productive trees (Sánchez 2012).

Ectomycorrhizae of epigeous Basidomycota genera, like *Scleroderma*, *Hebeloma*, *Cortinarius* and *Pisolithus* readily develop in truffle orchards; most of these species produce rhizomorphs and abundant emanating hyphae and sporocarps. Sourzat et al. (1993), Sourzat (1994), Sáez García Falces and De Miguel Velasco (2008) and Olivier et al. (2012) report that *Hebeloma* is one of the genera that could endanger truffle production due to its high competitiveness. Species in this genus often display a broad host range and an extensive distribution in temperate forests. Marmeisse et al. (1999) consider *Hebeloma* a cosmopolitan genus that appears in both early and late stages of plantations, and is probably favored by the open space created in the stand management. Sourzat et al. (1993) and Sourzat (1994) mentioned that the appearance, abundance, and competition of *Hebeloma* in truffle orchards can lead to the lack of truffle productivity. On the contrary, according to Donnini et al. (2010) and Sourzat (2011), neither *Scleroderma* nor *Pisolithus* ectomycorrhizae seem to diminish truffle production in plantations. The easily identified Basidiomycota genera *Russula* and *Xerocomus* occasionally occur in truffle plantations but typically not in abundance.

Sporocarps of hypogeous genera like *Hymenogaster*, *Melanogaster* and *Genea* are usually found in truffle plantations. *Hymenogaster* ectomycorrhizae were first described by Bencivenga et al. (1992) and noted as frequent in 'black truffle' plantations but sporadic in others. *Melanogaster* ectomycorrhizae were first described in France by Giraud (1979, 1988) and Verlhac et al. (1990) and later in Spain by Suz et al. (2010). *Genea* ectomycorrhizae, described by Jakucs and Bratek (1998) and Jakucs et al. (1998), are not abundant but easy to recognize in truffle plantations and have been reported in plantations in Spain (De Miguel et al. 2001; Águeda Hernández et al. 2005; González-Armada et al. 2010a), France (Sourzat 2011) and Italy (Belfiori et al. 2012).

Astraeus is a cosmopolitan genus frequently found in dry environments and also in boreal woodlands of planifolious termo-xerofilous forests (Courtecuisse and Duhem 2005; Llamas Frade and Terrón Alfonso 2005). Its easily recognized epigeous sporocarps can fruit in the burned area (the *brûlé*), but its ectomycorrhizae have received little attention. Giraud (1988), Verlhac et al. (1990), and Reyna (1999) indicated that *Astraeus* species contribute to producing the *brûlé* itself, with a herbicidal-like action, similar to truffle mycelium (Giraud 1988). *Astraeus* ectomycorrhizae have also been reported in Spanish plantations (Suz et al. 2010; Sánchez 2008, 2012).

Thelephoraceae species are often important members of the ectomycorrhizal fungus communities in boreal forests (Kõljalg 1996; Kõljalg et al. 2000, 2001) and often noted for their dominance (Horton and Bruns 2001). Mediterranean forests of *Quercus ilex* L. also support a high incidence and diversity of Thelephoraceae ectomycorrhizae (Richard et al. 2005). Thus, it is not surprising that members of the Thelephoraceae are commonly found in truffle plantations. Gregori et al. (1990) confirmed the identity of 'Mycorrhizic form 3' found in truffle plantations in Italy to *Tomentella* as deduced by the detection of spores from this genus within the hyphae of the ectomycorrhiza. Subsequently, De Miguel et al. (2001) and Águeda Hernández et al. (2005) reported the presence of Thelephoraceae in *T. melanosporum* plantations in Spain. In Italy, Murat et al. (2005) cited Thelephoraceae in *T. magnatum* natural grounds, Benucci et al. (2011) in *T. aestivum* plantations and Iotti et al. (2010) in *T. borchii* grounds. In the USA, Pruett et al. (2008) found them in *T. aestivum* plantations.

Tomentella galzinii Bourdot was first called the SB type (in French *spinules buclés*) by Giraud (1979). Jakucs (2002) described its ectomycorrhizae; it was among the first Thelephoraceae species identified in Spanish truffle plantations (Sáez and De Miguel 1995; De Miguel and Sáez 1997, 1998, 2005a, b; De Román and De Miguel 2001; Sánchez 2008, 2012; Agueda et al. 2010c; García-Barreda and Reyna 2012), but appears less abundant in other countries (Verlhac et al. 1990; Donnini and Bencivenga 1995; Zambonelli et al. 2005; Leonardi et al. 2013). Other *Tomentella* species have been identified in truffle stands by use of molecular tools including: *T. badia* (Link) Stalpers, *T. bryophila* (Pers.) M. J. Larsen, *T. coerulea* (Bres.) Höhn. & Litsch., *T. ellisii* (Sacc.) Jülich & Stalpers, *T. ferruginea* (Pers.) Pat., *T. lilacinogrisea* Wakef., and *T. subtestacea* Bourdot & Galzin (Pruett et al. 2008; Iotti et al. 2010; Suz et al. 2010; Benucci et al. 2011; De Miguel et al. 2011; Leonardi et al. 2013). *Pseudotomentella* species have only been identified in Spanish 'black truffle' plantations and in Italian 'summer truffle' orchards (De Miguel et al. 2011; Benucci et al. 2011). Other ectomycorrhizal morphotypes of a thelephoroid nature, described as *Quercirhiza squamosa* (Palfner and Agerer 1996), *Q. cumulosa* (De Román et al. 2002a), and *Q. stellata* (De Román et al. 2002b), have also been found in Spanish truffle plantations (De Miguel and Sáez 2005a; González-Armada et al. 2010a, b; Sánchez 2012). *Q. squamosa* is an abundant ectomycorrhiza in mature plantations (Sánchez 2012). Thelephoraceae are a highly diverse group appearing in both productive and non-productive trees in truffle grounds (Iotti et al. 2010; Sánchez 2012; Leonardi et al. 2013) and thus seem not to interfere in truffle sporocarp production.

Sphaerosporella brunnea, a cup-forming Ascomycota, is frequently mentioned as a common ectomycorrhizal fungus on nursery seedlings (Danielson 1984; Meotto and Carraturo 1988; Di Massimo et al. 1996). Although this species is readily displaced in the field on outplanted seedlings, it is also found in mature stands and truffle plantations, but does not interfere with truffle colonization or fructification (Sánchez 2012).

Cenococcum geophilum is a well known cosmopolitan ectomycorrhizal fungus living in a wide range of environmental conditions (Trappe 1962; Voiry 1981; Pigott 1982). It is very abundant in truffle stands (Águeda et al. 2010b) and also appears to be the dominant ectomycorrhiza in old-growth Mediterranean forests dominated by holm oaks (*Q. ilex*) (De Román and De Miguel 2005a; Richard et al. 2005). Its common presence in plantations is related to its ubiquity on tree roots of the surrounding forest areas (Sánchez 2008; Águeda et al. 2010b). Giraud (1988) notes that truffle plantations established in forest soils often have problems in establishing the target truffle species due to competing root colonization by native ectomycorrhizal fungi present in the soil. Propagules of ectomycorrhizal fungi can persist in soil long after forest clearing or disturbance. For example, De Román and De Miguel (2005b) and Martínez de Aragón et al. (2012) found *C. geophilum* in holm oak truffle orchards several years after a fire. On the other hand, *C. geophilum* has not been found in Spanish and Italian 'black truffle' plantations established in formerly agricultural lands (De Miguel et al. 2001; De Miguel and Sáez 2005a; Baciarelli Falini et al. 2006).

Factors affecting ectomycorrhizal fungus communities in truffle plantations

In natural environments, ectomycorrhizal fungi form complex communities characterized by the coexistence of different species governed by competition (Kennedy 2010) and host availability (Tedersoo et al. 2012). Similar to natural forests, ectomycorrhizal fungal communities in truffle plantations are characterized by a few common morphotypes and a large number of rare types (Horton and Bruns 2001; Richard et al. 2005; Águeda et al. 2010b; Iotti et al. 2010).

Ectomycorrhizal fungus species richness increases with the age of the plantation (De Miguel 2004; Sánchez 2012), although no relationship has been established with the onset of sporocarp production. The average number of ectomycorrhizal types found is eight in 0- to 5-year-old plantations, 12 in 6- to 10-year-old plantations, 18 in 11- to 20-year-old plantations and 25 in more than 20-year-old plantations (Table 2). At maturity, mycorrhizal fungal communities stabilize with the Thelephoraceae becoming one of the dominant groups (Águeda et al. 2010b; Benucci et al. 2011; De Miguel et al. 2011; Leonardi et al. 2013). Some mycorrhizal types such as *T. woolhopeia* appear in any phase from mycorrhizal seedlings to adult plantations. On the other hand, *Sphaerosporella brunnea* appears in the nursery phase and disappears in the field in less than 3 years (Verlhac et al. 1990),

Table 2 All ectomycorrhizal morphotypes reported in *Tuber melanosporum* plantations by age (0–5, 6–10, 11–20, and over 20 years old), and by production

Age (years)	P/NP	References	Ectomycorrhizal morphotypes
0–5	NP	Chevalier et al. (1982)	*Tuber melanosporum*, *T. aestivum*, *T. albidum*, *T. brumale*, *T. mesentericum*, *T. uncinatum*, *Cenococcum*, *Hymenogaster*, *Hebeloma*
	NP	Sourzat et al. (1990)	*Tuber melanosproum*, *T. aestivum*, *T. brumale*, *T. mesentericum*, *T. rufum*, *Cenococcum graniforme*, *Hebeloma*, AD,1 UI
	NP	Granetti and Angelini (1992b)	*Tuber melanosporum*, *T. aestivum*, *Hebeloma*
	NP	Sáez and De Miguel (1995)	*Tuber melanosporum*, *T. aestivum*, *T. albidum*, *T. brumale*, *T. excavatum*, *T. mesentericum*, *T. rufum*, AD, SB
	NP	De Miguel and Sáez (1997)	*Tuber melanosporum*, *T. aestivum*, *T. brumale*, SB, AD, Basidiomicotina
	NP	Baciarelli Falini and Granetti (1998)	*Tuber melanosporum*, *T. aestivum*, *T. borchii*, *T. brumale*, *Cenococcum geophilum*, *Hymenogaster citrinus*, AD, Foma 1 (=Basidiomycete)
	NP	Etayo and De Miguel (1998)	*Tuber melanosporum*, *T. aestivum*, *T. borchii*, *T. brumale*, *T. rufum*, *Hymenogaster*, Basidiomycotina, AD, Other UI
	NP	Etayo and De Miguel (1999)	*Tuber melanosporum*, *T. aestivum*, *T. borchii*, *T. brumale*, *T. rufum*, *Hebeloma*, *Hymenogaster*, AD
	NP	Etayo and De Miguel (2001)	*Tuber melanosporum*, *T. aestivum*, *T. albidum*, *T. brumale*, *T. rufum*, *Hymenogaster*, Basidiomycetes, AD
	NP	Domínguez Núñez et al. (2005a)	*Tuber melanosporum*, *T. aestivum*, *Thelephora terrestris*, AD, 3UI
	NP	Domínguez Núñez et al. (2005b)	*Tuber melanosporum*, *T. brumale*, *Laccaria*, *Sphaerosporella*
	NP	Suz et al. (2010)	*Tuber melanosporum*, *T. brumale*, *T. oligospermum*, *T. rufum*, *Tuber* like, *Astraeus hygrometricus*, *Cenococcum*, *Melanogaster broomeanus*, *Quercirhiza quadratum* (AD), *Tomentella ellisii*, *Tomentella*, *Sphaerosporella*, *Suillus*, ECM1 to ECM7 (ECM2=Clavulinaceae)
	NP	Sánchez (2012)	*Tuber melanosporum*, *T. aestivum*, *T. brumale*, *Quercirhiza squamosa*, *Scleroderma*, *Sphaerosporella brunnea*, *Tomentella galzinii*, *Trichophaea woolhopeia*, Morfotype 2, 2 UI
	P	Sánchez (2012)	*Tuber melanosporum*, *T. aestivum*, *T. brumale*, *Trichophaea woolhopeia*, 1 Tomentella. 2 UI
6–10	NP	Granetti and Angelini (1992a)	*Tuber melanosporum*, *T. aestivum*, *Hebeloma pumilum*, Forma 1 (=Ascomycete), Forma 3 (Basidiomycete)
	NP	De Miguel and Sáez (1998)	*Tuber melanosporum*, *T. aestivum*, *T. brumale*, *T. mesentericum*, *T. rufum*, *Hebeloma*, *Hymenogaster*, AD, SB, *Basidiomycotina*, Type 1, Type 2
	NP	Murat et al. (2008)	*Tuber melanosporum*, *T. borchii*, *T. indicum*, 6 not *Tuber*
	NP	Sánchez (2008)	*Tuber melanosporum*, *T. aestivum*, *T. brumale*, *Tuber*, *Cenococcum*, *Genea*, *Hebeloma*, *Hymenogaster*, *Pisolithus tinctorius*, *Quercirhiza quadratum*, *Q. squamosa*, *Scleroderma*, *Sphaerosporella*, *Tomentella*, *T. galzinii*, *T. stuposa*, Tipo 2, Tipo 12, 18 UI
	NP	Berch (2011)	*Tuber melanosporum*, *T. aestivum*, *T. brumale*, other *Tuber*, *Trichophaea woolhopeia*
	NP	Belfiori et al. (2012)	*Tuber melanosporum*, *T. aestivum*, *Tomentella*
	NP	Sánchez (2012)	*Tuber melanosporum*, *T. brumale*, *Astraeus hygrometricus*, Basidiomycota, *Cenococcum geophilum*, *Pisolithus arhizus*, *Quercirhiza squamosa*, *Russula*, *Sphaerosporella brunnea*, 2 *Thelephoraceae*, 2 *Tomentella*, *Trichophaea woolhopeia*, 5 UI
	P	Sourzat et al. (1993)	*Tuber melanosporum*, *T. aestivum*, *T. brumale*, *T. mesentericum*, *T. rufum*, *Cenococcum graniforme*, *Hebeloma crustuliniforme*, *Scleroderma*, AD, Basidiomycete 'P', Basidiomycetes
	P	Granetti and Baciarelli Falini (1997)	*Tuber melanosporum*, *T. borchii*, *T. brumale*, 6 Forme (Forma 1=AD)
	P	Sáez and De Miguel (2000)	*Tuber melanosporum*, *T. aestivum*, *T. brumale*, *T. mesentericum*, *T. rufum*, *Hymenogaster*, *Scleroderma*, AD, SB, *Basidiomycotina*
	P	De Miguel et al. (2001)	*Tuber melanosporum*, *T. aestivum*, *T. albidum*, *T. brumale*, *T. mesentericum*, *T. rufum*, *Genea verrucosa*, *Hebeloma*, *Hymenogaster*, *Pisolithus tinctorius*, *Quercirhiza fibulocystidiata*, *Q. squamosa*, *Scleroderma*, *Sphaerosporella brunnea*, *Tomentella subtestacea*, AD, SB, Type B, Type C, Type D
	P	Etayo and De Miguel (2001)	*Tuber melanosporum*, *T. aestivum*, *T. albidum*, *T. brumale*, *Pisolithus*, *Scleroderma*, AD, Morphotypes 8 to 14

Table 2 (continued)

Age (years)	P/ NP	References	Ectomycorrhizal morphotypes
	P	Sáez and De Miguel (2001)	*Tuber melanosporum*, *T. aestivum*, *T. albidum*, *T. brumale*, *T. mesentericum*, *Trufum*, *Hebeloma*, *Hymenogaster*, AD, SB, Basidiomycetes
	P	Ricard and Bourrieres (2010)	*Tuber melanosporum*, *T. aestivum*, *T. brumale*, Type Truffe, *Cenococcum*, *Scleroderma*, AD, Basidiomycetes, UI
	P	Sánchez (2012)	*Tuber melanosporum*, *T. aestivum*, *T. brumale*, *Tuber*, *Astraeus hygrometricus*, *Cenococcum geophilum*, *Pisolithus arhizus*, *Quercirhiza squamosa*, *Russula*, *Sphaerosporella brunnea*, 5 *Thelephoraceae*, 2 *Tomentella*, *Trichophaea woolhopeia*, 2 UI
11–20	NP	Baciarelli Falini et al. (2006)	*Tuber melanosporum*, *T. aestivum*, *T. borchii*, *T. brumale*, *T. rufum*, *Tuber*, *Cortinarius*, *Scleroderma*, AD
	NP	Sánchez (2012)	*Tuber melanosporum*, *T. brumale*, *Astraeus hygrometricus*, *Cenococcum geophilum*, *Cortinarius*, *Hebeloma*, *Pisolithus arhizus*, *Scleroderma*, *Quercirhiza squamosa*, *Russula*, *Sphaerosporella brunnea*, *Tomentella galzinii*, *T. stuposa*, *Trichophaea woolhopeia*, Morfotipo 2, 18 *Basidiomycota*, 11 *Thelephoraceae*, 6 *Tomentella*, 20 UI
	P	Bencivenga et al. (1992)	*Tuber melanosporum*, *T. aestivum*, *T. albidum*, *Hymenogaster*, Forma 1, Forma 2, Forma 3
	P	Donnini and Bencivenga (1995)	*Tuber melanosporum*, *T. magnatum*, *T. aestivum*, *T. albidum*, *Cenococcum graniforme*, *Hymenogaster citrinus*, *Sphaerosporella brunnea*, 16 Forme (Forma 14=SB)
	P	Callot and Jaillard (1996)	*Tuber melanosporum*, *T. aestivum*, *T. borchii*, *T. brumale*, *T. rufum*, Type *Tuber*, *Cenococcum*, *Scleroderma verrucosum*, AD, 1 UI
	P	De Miguel et al.(2003)	*Tuber melanosporum*, *T. aestivum*, *T. brumale*, *T. mesentericum*, *T. rufum*, Tipo *Tuber*, *Genea verrucosa*, *Hebeloma*, *Hymenogaster*, *Pisolithus tinctorius*, *Quercirhiza cumulosa*, *Q. squamosa*, *Q. stellata*, *Scleroderma*, *Sphaerosporella brunnea*, *Tomentella galzinii*, *Tomentella*, AD, SB, Morfotipo 39 Morfotipo 53, Morfotipo 54
	P	De Miguel and Sáez (2005b)	*Tuber melanosporum*, *T. aestivum*, *T. brumale*, *T. mesentericum*, *T. rufum*, *Genea verrucosa*, *Hebeloma*, *Pisolithus tinctorius*, *Scleroderma*, Teleforoide, *Quercirhiza squamosa*, *Tomentella galzinii*, *T. subtestacea*, *Xerocomus chrysenteron*, AD
	P	De Miguel and Sáez (2005a)	*Tuber melanosporum*, *T. aestivum*, *T. borchii*, *T. brumale*, *T. mesentericum*, *T. rufum*, *Cenococcum geophilum*, *Genea verrucosa*, Tipo *Hebeloma-Cortinarius*, *Hymenogaster*, *Pisolithus tinctorius*, *Quercirhiza squamosa*, *Scleroderma*, *Tomentella subtestacea*, *T. galzinii*, *Xerocomus chrysenteron*, AD
	P	De Miguel et al. (2005)	*Tuber melanosporum*, *T. aestivum*, *T. borchii*, *T. brumale*, *T. mesentericum*, *T. rufum*, *Tuber* type, *Genea verrucosa*, *Hebeloma-Cortinarius*, *Hymenogaster*, *Pisolithus tinctorius*, *Quercirhiza cumulosa*, *Q. squamosa*, *Q. stellata*, *Scleroderma*, Thelephoroid, *Tomentella galzinii*, *T. subtestacea*, *Xerocomus chrysenteron*, AD, SB, Morphotype 39
	P	De Miguel et al. (2006)	*Tuber melanosporum*, *T. aestivum*, *T. brumale*, *Tuber*, *Boletus*, *Cenococcum*, *Cortinarius*, *Genea*, *Hebeloma*, *Hymenogaster*, *Lactarius*, *Pisolithus*, *Quercirhiza cumulosa*, *Q. squamosa*, *Q. stellata*, *Russula*, *Scleroderma*, *Tomentella galzinii*, *T. subtestacea*, *Tomentella*, *Xerocomus.* AD, 25 Thelephoroids
	P	González-Armada et al. (2010a)	*Tuber melanosporum*, *T. aestivum*, *T. brumale*, *T. mesentericum*, *Tuber*, *Cenococcum*, *Genea*, *Hebeloma-Cortinarius*, *Quercirhiza quadratum*, *Q. squamosa*, *Scleroderma*, *Tomentella*
	P	González-Armada et al. (2010b)	*Tuber melanosporum*, *T. aestivum*, *T. brumale*, *T. mesentericum*, *T. rufum*, *Tuber*, Boletal, *Genea*, *Hebeloma/Cortinarius*, *Hymenogaster*, *Pisolithus*, *Quercirhiza cumulosa*, *Q. squamosa*, *Scleroderma*, *Sphaerosporella*, *Tomentella*, SB, AD, T39
	P	Bonito et al. (2011)	*Tuber melanosporum*, *T. indicum*, *Tuber*, *Thelephora/Tomentella*, *Hebeloma*, *Tarzetta*, *Pachyphloeus.*, *Cortinarius*, *Inocybe*
	P	De Miguel et al. (2011)	*Pseudotomentella tristis*, *Tomentella badia*, *T. cinerascens*, *T. coerulea*, *T. galzinii*
	P	Belfiori et al. (2012)	*Tuber melanosporum*, *T. aestivum*, *T. brumale*, *T. maculatum*, *Inocybe*, *Trichophaea woolhopeia*, *Scleroderma cepa*, 5 *Tomentella*
	P	Guerin-Laguette et al. (2013)	*Tuber melanosporum*, *T. brumale*, *T. maculatum*, spiky *Tuber*, *Scleroderma*, OE (Other Ectomycorrhizal species)
	p	Sánchez (2012)	*Tuber melanosporum*, *T. aestivum*, *T. brumale*, *Astraeus hygrometricus*, *Basidiomycota*, *Hebeloma*, *Pisolithus arhizus*, *Quercirhiza squamosa*, *Russula*, *Scleroderma*, 2 *Tomentella*, *Trichophaea woolhopeia*, 1 UI
>20	P	Águeda B et al. (2001)	*Tuber melanosporum*, *Cenococcum geophilum*, *Hebeloma*, *Pisolithus*, *Cortinarius*, AD, Dark Type, 5 types
	P	Águeda Hernández B et al. (2005)	*Tuber nigrum*, *T. aestivum*, *T. brumale*, Boletal, *Cenococcum geophilum*, *Cortinarius*, *Genea*, *Hebeloma*, *Hymenogaster*, *Pisolithus tinctorius*, *Scleroderma*, 28 Thelephorales

Table 2 (continued)

Age (years)	P/NP	References	Ectomycorrhizal morphotypes
	P	Águeda et al. (2010b)	*Tuber melanosporum*, *T. aestivum*, *T. brumale*, *Cenococcum geophilum*, *Cortinarius*, *Genea*, *Hebeloma*, *Hymenogaster cf. citrinus*, *Pisolithus tinctorius*, *Quercirhiza quadratum*, *Scleroderma*
	P	Di Massimo et al. (1996)	*Tuber nigrum* (=*T. melanosporum*), *T. aestivum*, *Cenococcum geophilum*, *Sphaerosporella*, AD
	P	De Román and De Miguel (2001)	*Tuber melanosporum*, *T. brumale*, *T. mesentericum*, *Cenococcum geophilum*, *Hebeloma*, *Hymenogaster*, *Scleroderma*, *Sphaerosporella brunnea*, *Thelephora terrestris*, AD, SB, 6 UI
	P	Águeda et al. (2010c)	*Tuber melanosporum*, *T. aestivum*, *T. brumale*, *Cantharellus tubaeformis*, *Cenococcum geophilum*, *Cortinarius*, *Hebeloma* cf. *sinapizans*, *Hebeloma*, *Pisolithus arrhizus*, *Scleroderma*, *Tomentella galzinii*, AD, 8 (Thelephorales, Tuberales and Boletales)
	P	Belfiori et al. (2012)	*Tuber melanosporum*, *T. aestivum*, *T. rufum Cenococcum geophilum*, *Clavulina cinerea*, Clavulinaceae, *Cortinarius*, *Genea*, *Geopora arenicola*, *Hebeloma leucosarx*, *Hydnobolites cerebriformis*, *Inocybe umbrinella*, *Inocybe*, *Peziza succosa*, *Russula brevipes*, *Scleroderma cepa*, *Sebacina*, *Sistotrema muscicola*, *Tarzetta catinus*, *Thelephora terrestris*, 4 *Tomentella*, *Tricholoma scalpturatum*, *Trichophaea woolhopeia*
	P	García-Barreda and Reyna (2012)	*Tuber melanosporum*, *T. brumale*, *Cenococcum geophilum*, *Genea*, *Hebeloma-Cortinarius*, *Pisolithus*, *Russula*, *Scleroderma*, *Sebacina*, Thelephoroid, *Tomentella galzinii*, 14 UI

Species mentioned in the table that have not been cited with author previously in the text: *Cantharellus tubaeformis* Fr., *Clavulina cinerea* (Bull.) J. Schröt., *Genea verrucosa* Vittad., *Geopora arenicola* (Lév.) Kers, *Hebeloma crustuliniforme* (Bull.) Quél., *Hebeloma leucosarx* P.D. Orton, *Hydnobolites cerebriformis* Tul. & C. Tul., *Hymenogaster citrinus* Vittad., *Inocybe umbrinella* Bres., *Melanogaster broomeanus* Berk., *Peziza succosa* Berk., *Pseudotomentella tristis* (P. Karst.) M.J. Larsen, *Russula brevipes* Peck, *Scleroderma cepa* Pers., *Scleroderma verrucosum* (Bull.) Pers., *Sistotrema muscicola* (Pers.) S. Lundell, *Tarzetta catinus* (Holmsk.) Korf & J.K. Rogers, *Tomentella cinerascens* (P. Karst.) Höhn. & Litsch., *Tomentella stuposa* (Link) Stalpers, *Tricholoma scalpturatum* (Fr.) Quél., *Tuber maculatum* Vittad., *Tuber oligospermum* (Tul. & C. Tul.) Trappe, *Xerocomus chrysenteron* (Bull.) Quél.)

NP production not started, *P* production started, *UI* unidentified

whereas *Cenococcum* is only detected in mature plantations (De Román and De Miguel 2005b; Águeda et al. 2010b; Martínez de Aragón et al. 2012). Other species like *T. rufum*, *Pisolithus* spp., and *Scleroderma* spp. have been mentioned as forerunners of truffle production (Sáez García Falces and De Miguel Velasco 2008; Olivier et al. 2012).

Several host trees are used in truffle plantations. Mixed plantations, with several host tree species per orchard, show a higher diversity than monospecific ones (Iotti et al. 2010; Benucci et al. 2011).

From the very first work on truffle stands, rapid development of the host tree roots has been considered disadvantageous for the establishment and maintenance of truffle mycorrhizae, as it favors colonization by other species. Therefore, optimizing tree development is essential (Chevalier et al. 1973; Gregori et al. 1990; Granetti and Angelini 1992; Bencivenga et al. 1992, 1995; Donnini and Bencivenga 1995; Granetti and Baciarelli Falini 1997). The root system of *Corylus avellana* L. grows faster than that of *Quercus humilis* Mill. and *Q. ilex*, and may be easily colonized by native ectomycorrhizal fungi. Truffle productivity in *C. avellana* plantations is lower than that observed with other hosts (Sáez García Falces and De Miguel Velasco 2008; Chevalier and Sourzat 2012).

The diversity of ectomycorrhizal fungal communities is related to sporocarp productivity in a range of 'black truffle' plantations with different soil and climatic conditions. In general, more species are detected in productive plantations than in non-productive ones (Table 2). Among these species, there is no predominance of any of the exploration types described by Agerer (2001, 2006). Ectomycorrhizae belonging to short-, medium- and long-distance exploration types are equally represented, regardless of the productive or non-productive status of the plantations. No contact exploration types have been cited.

The preceding use of the plantation soil (Reyna et al. 2006) and the presence of forests near the plantation (Chevalier and Sourzat 2012) are factors clearly influencing the fungal diversity in *Tuber* orchards. Sánchez (2012) detected an increase in the frequency of appearance of *Q. squamosa* in plantations established in previously forest soils.

Plantation management has a clear influence on the ectomycorrhizal community. Many different methods have been proposed for plantation management (Chevalier and Sourzat 2012), due to the diverse geographical, climatic, edaphic or soil conditions in which the plantations are established. Each practice must be carefully monitored, generally in the long term and under different soil conditions (Sánchez 2012).

Among the management techniques tested to increase truffle production are mulching (Etayo and De Miguel 2001; Zambonelli et al. 2005; Ricard and Bourrieres 2010), calcareous amendments (García-Montero et al. 2012), pruning (Sourzat et al. 1993; Donnini and Bencivenga 1995),

irrigation (Olivera et al. 2011), fertilization (Suz et al. 2010) and canopy opening (García-Barreda and Reyna 2012). The lack of continuity of these studies, as well as the different environmental conditions, make it difficult to draw clear conclusions about the effects of management practices on the ectomycorrhizal community at this time. Some combined practices such as increasing organic matter, lower soil bulk density and no pruning seem to favor the presence of *T. woolhopeia*; and tillage seems to decrease *Scleroderma* sp. and other ectomycorrhizae belonging to the long-distance exploration type (Sánchez 2012) as rhizomorphs are broken in the process.

Conclusions and lessons learned

The available studies on ectomycorrhizal fungal communities in truffle plantations include a number of different experimental situations and methodologies. It is therefore difficult to compare them and to properly explain spatial and temporal fungal dynamics as well as their relationship with sporocarp production. Most of the studies reviewed (69 out of 85) deal with *T. melanosporum* and the associated ectomycorrhizal fungi.

Ectomycorrhizae of *T. melanosporum* are the most frequent in productive plots. *T. rufum* and members of the Boletales are only found in productive plots. Some authors include also *Pisolithus*, *Scleroderma* and *T. woolhopeia* within the species associated with good truffle sporocarp production. On the other hand, *Hebeloma*, *Laccaria* and *Russula* species have been mostly associated with unproductive plots. Approximately 25 % of the ectomycorrhizal fungal species described in the 85 reviewed studies were reported across most studies.

Molecular studies using next generation sequencing tools have demonstrated the predominance of Ascomycotina within the brûlés of *T. melanosporum* and the increase in Basidiomycotina outside. Other studies using metagenomic approaches allowed an increase in the described ectomycorrhizal types associated with *T. magnatum* grounds of up to 129 OTUs. Molecular techniques have been applied in only 11 of the 85 studies reviewed. It is expected that future combined morphological and molecular studies may give a better integrated and functional overview of the ectomycorrhizal communities than was possible with morphotyping alone.

The appearance of *T. brumale* ectomycorrhizae is generally associated with host tree species with rapid growth and inappropriate cultural practices at early stages of the plantation. The presence of *T. indicum* is still only a threat limited to some areas in Italy but its close phylogenetic relationship with *T. melanosporum* opens up the possibility of inbreeding or displacement of the target species.

Ectomycorrhizae belonging to Thelephoraceae are frequently found in mature truffle orchards and do not seem to interfere in sporocarp production. *Sphaerosporella brunnea* is commonly detected at the nursery stage and generally disappears after outplanting. *Cenococcum geophilum* mycorrhizae are related to the proximity of forests and are generally not found in plantations established in agricultural lands.

Several factors have been found to affect the ectomycorrhizal communities associated with truffle orchards. Among them are the age of the plantation (the number of species increases with the age of the plantation), the host species (rapidly growing species, such as *C. avellana*, host a higher number of ectomycorrhizal fungus species than slow-growing species like oaks), productivity (more diversity is found in productive orchards), the surrounding environment (forest soils and the presence of nearby forests increase the number of species) and management (combined practices such as increasing organic matter, lower soil bulk density and no pruning seem to favor the presence of *T. woolhopeia*; and tillage seems to decrease *Scleroderma* sp.).

Recent achievements such as the complete sequencing of the genome of *T. melanosporum* have significantly improved our knowledge about its biology and life cycle. However, the frequent failures in productive plantations indicate that this species is still far from being considered as 'domesticated' and there are many gaps in our knowledge about its reproduction and cultivation. Some of the missing answers could be related to interactions with other organisms within the rhizosphere. Understanding the fungal communities inhabiting different plantations may give us clues about the dynamics of the targeted truffles and the possibility of identifying species related to site productivity. Further multidisciplinary research is still needed to improve the success of truffle plantations and meet their owners' expectations.

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