

# Below-ground ectomycorrhizal community in natural *Tuber melanosporum* truffle grounds and dynamics after canopy opening

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**Abstract** The ectomycorrhizal fungus *Tuber melanosporum* fruits in association with *Quercus* in natural forests of Spain. Some of these stands are managed to keep an open canopy and meet the habitat requirements of the fungus. However, there are few quantitative studies analysing in these forests the relationship between soil environment and *T. melanosporum*. Eight forest stands which produce *T. melanosporum* have been monitored for 6 years in order to characterise the below-ground ectomycorrhizal community and to assess its temporal dynamics after experimental canopy opening. The *brûlé*, the ground where *T. melanosporum* fruits, shows a distinct ectomycorrhizal community, characterised by lower density of active ectomycorrhizal tips, lower morphotype richness per soil volume, higher abundance of *T. melanosporum* and lower abundance of *Cenococcum geophilum* than soil closest to the trunk of the host *Quercus ilex*. Opening the canopy has not stimulated an increase in *T. melanosporum*, suggesting that a shift in the soil environment alone will not trigger the formation of new *truffières* in the short term. The dry climate of these *truffières* may be a factor as *T. melanosporum* abundance appears to be sensitive to annual weather conditions.

**Keywords** *Tuber melanosporum* · *Quercus ilex* · Ectomycorrhiza · *Brûlé* · Wild *truffière* · Silviculture

## Introduction

The European black truffle (*Tuber melanosporum* Vitt.) is an ectomycorrhizal (EM) fungus extensively cultivated due to its gastronomic value. The ground where it fruits, the so-called *brûlé*, is characterised by a scarce plant cover, due to the phytotoxic activity of the fungus (Splivallo 2008). In the wild, *T. melanosporum* frequently fruits in association with several *Quercus* species in the framework of forest dynamics. Such dynamics is not completely understood yet, but it is widely accepted that it implies the evolution of the root system of the truffle tree (i.e. the host tree), the physical environment of the soil, soil organic matter and composition of the EM community (Ricard et al. 2003; Sourzat et al. 2004). The occurrence of *T. melanosporum* ectomycorrhizas in wild *brûlés* and in non-productive grounds remains less explored (Mello et al. 2006), as well as the influence of the environment on the below-ground EM community of these soils (Hall et al. 2003). Such information would help to clarify the ecological role of *T. melanosporum* and would be useful for the management of the forest stands where *T. melanosporum* fruits (i.e. wild *truffières*).

Koide et al. (2011) propose that EM communities are structured first by a host plant filter, then by an abiotic filter, followed lastly by competitive interactions. In this regard, *T. melanosporum* appears to thrive best in an early-seral environment of open forests, and fruiting has been found to decline as the forest canopy closes (Olivier et al. 2002; Reyna et al. 2004). To prevent this decline, canopy opening around the *brûlé* is widely recommended (Gregori

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et al. 2001; Sourzat et al. 2004) to maintain the appropriate abiotic conditions and minimise competitive displacement by other EM fungi. However, the effects of these cuttings on the below-ground EM community remain largely unexplored, and the abundance of fruiting bodies is considered to poorly reflect the composition of the below-ground EM community (Gardes and Bruns 1996). It is known that clearcutting alters the abundance of roots and changes the soil environment, affecting the EM community of the regenerating forest stand (Parsons et al. 1994; Jones et al. 2003a) even in the advance regeneration (Hagerman et al. 2001). On the other hand, in the patches of retention trees the EM community does not seem to change in the short term (Jones et al. 2008).

In this study, we aim to characterise the below-ground EM community of wild *truffières* that have undergone a canopy opening treatment and particularly to assess the occurrence of *T. melanosporum* ectomycorrhizas. We hypothesised that in the various environmental conditions of the *truffière*, the density and richness of the EM community would differ, as well as the occurrence of *T. melanosporum* ectomycorrhizas. A secondary aim of our study is to assess the effect of the canopy opening on the below-ground EM community. We hypothesised that the root gap created by the cutting would provoke (1) an increase in the density of root tips of the trees remaining and (2) a shift in the occurrence of the various EM species. Our study is part of a larger research effort trying to assess the feasibility of sustaining truffle yield of forests through silviculture.

## Materials and methods

### Study site

The study was conducted in El Toro, in the Valencian Community (eastern Spain; 39°59' to 40°2' N, 0°44' to 0°47' W, 990–1,050 masl). The climate is Continental Mediterranean, with a mean annual rainfall of 500–550 mm and a mean annual temperature of 11.9–12.4°C. The soils are calcixercept developed on a quaternary calcareous glais with little slope (less than 5%), sandy clay loam texture, pH ranging from 7.7 to 8.3 and 2–3% organic matter.

Before 1958, *Quercus ilex* L–*Quercus faginea* Lam. coppices and cereal cultures dominated the landscape. In the latter, *Quercus* were also present in field boundaries. Around 1955, the local population began to harvest the truffles, most of which associated with *Q. ilex*. From 1958 to 1969, the site was reforested with *Pinus nigra* Arnold. By the 1990s, the reforestation had formed a close canopy (density, 900–2,500 trees ha<sup>-1</sup>; canopy cover, 60–95%) and the fruiting body production had sharply decreased. The *Quercus* survived as suppressed trees under the pine

overstorey, and a seedling bank of *Q. ilex*–*Q. faginea* spontaneously established under the pine reforestation. Over all these years, *T. melanosporum* had been intensively exploited as a common-pool resource and no management practices had been carried out.

In 2000–2001, silvicultural treatments were executed with the aim of opening the canopy and improving the habitat for *T. melanosporum* fruiting. Circular clearcuts were created around the *brûlés*, with radius depending on the height of the tree layer and ranging 14–50 m. Pines were systematically cut down since they do not encourage *T. melanosporum* fruiting, all shrubs were removed and *Quercus* were preserved and slightly pruned (less than 10% of the live crown was removed) (Reyna et al. 2004). A canopy cover less than 50% was obtained within a radius of 15 m from the *brûlé*, whereas in the surrounding clearcut canopy cover was less than 30%.

### Experimental design

We have selected eight *truffières* with *Q. ilex* as the truffle tree. The *truffières* produce fruiting bodies of *T. melanosporum*, but not of other species of *Tuber*. Four of them had been treated in 2000 (January–April) and four in 2001 (January–April). In each *truffière*, we have sampled soil cores along two transects that orient north to south and run from the trunk of the truffle tree (the centre of the plot) through the *brûlé* to the untreated pine stand. Four positions have been sampled within each transect: (1) near the trunk of the truffle tree (NTTT), (2) the centre of the *brûlé*, (3) the centre of the clearcut gap surrounding the *brûlé* and (4) the control pine stand. In each position, the roots of a *Q. ilex* have been sampled. The roots sampled both in the *brûlé* and NTTT correspond to the truffle tree, but crown shading, distance to the trunk and leaf litter accumulation differ (Table 1). The differences between these positions are clearly depicted in truffle plantations: when the tree is young, the *brûlé* typically appears closely around the trunk, but as the tree grows it moves away. In the context of this dynamics, the clearcut gap constitutes a potential space for the spread of the *brûlé* and for the formation of new *truffières* (Sourzat et al. 2004). In the clearcut gap, the most frequent EM trees that remained after the cutting are the juvenile (height ≤ 1.3 m) *Q. ilex* that previously formed a seedling bank under the pine stand. In the control pine stand, where isolated adult *Q. ilex* and a *Q. ilex* seedling bank coexist, both adult and juvenile trees have been sampled (Table 1).

### Ectomycorrhizal root sampling

From 2001 to 2006, the *truffières* have been annually sampled in late spring (May–June) since it is an optimum

**Table 1** Environmental conditions in the four sampled spatial positions and characteristics of the sampled trees

	NTTT	<i>Brûlé</i>	Clearcut gap	Pine stand
Direct insolation on the soil surface	No	Yes	Yes	No
Leaf litter accumulation	Yes ( <i>Quercus</i> )	No	No	Yes ( <i>Pinus</i> )
Fruiting of <i>T. melanosporum</i>	No or occasional	Yes	No	No
Sampled tree species	<i>Q. ilex</i>	<i>Q. ilex</i>	<i>Q. ilex</i>	<i>Q. ilex</i>
Tree height in 2001 (m)	4–8	4–8	0.2–0.7	Juveniles, 0.1–1 Adults, 2–7
Distance from the sampling point to the tree (m)	0.5–1.5	4–7	0.5×height	Juveniles, 0.5× height Adults, 0.5–1.5

NTTT near the trunk of the truffle tree

moment for the observation of *T. melanosporum* ectomycorrhizas (Bonet et al. 2001). In the *brûlé*, either a stone layer or a physical crust (1–3 cm) covers the soil. No fine roots grow either in the crust or in the leaf litter that accumulates on NTTT and the pine stand, so these layers have been discarded before taking the soil cores. The cores are cylindrical, 100 ml in volume and 10 cm in depth.

The samples have been gently washed with water and stored at 4°C for a maximum of 7 days. With the aid of binocular and optical microscopes, the active and the senescent fine root tips have been counted. Senescent tips are typically darkly coloured, with a wrinkled surface. The active tips have been classified as mycorrhized (turgescient live tips, covered by fungal mantles) or non-mycorrhizal. The latter have not been statistically analysed because they appear in only 8% of the samples. The ectomycorrhizas have been sorted in morphotypes according to the methodology of Agerer (1987–2002) and with the aid of the descriptions in De Román (2003).

The below-ground EM community has been characterised through the density of active ectomycorrhizas, the morphotype richness (per 100-ml core sample), the morphotype diversity (Shannon index) and the frequency of appearance (per sample) for the most common morphotypes. The occurrence of the most frequent species, *Cenococcum geophilum* Fr. and *T. melanosporum*, has been examined through its relative abundance (percentage of the total number of active EM tips).

## Statistics

The aforementioned traits have been analysed through conventional and generalised linear mixed models (LMM) that include the position within the *truffière* and the orientation of the transect as fixed predictor variables and the *truffière* as a random block variable ( $n=376$ ).

Time from the canopy opening is treated as a repeated-measures variable: a linear and a quadratic component have been included. The width of *P. nigra* latewood (calculated

as the mean for 20 trees in the study site) is included as a proxy for the annual weather conditions. The latewood section has been chosen instead of the earlywood or the total ring because it shows a better correlation with the conditions of the current year (Martín-Benito et al. 2008). Since the density of active root tips significantly differs among positions, this variable is included as a covariate in the LMMs that analyse the morphotype richness and frequency.

In the LMMs that analyse the occurrence of *T. melanosporum*, the number of digs made for harvesting *T. melanosporum* fruiting bodies (annual mean from 2001 to 2006) is included as a proxy for the fruiting body productivity of each *truffière*. Harvesters are guided by trained dogs to the ripe sporocarps, which are dug out with a small spade. In a previous study in the area, it was found that the annual number of digs in ten *truffières* during four different fruiting seasons showed a significant and positive correlation with the annual weight of truffles harvested (Pearson's  $r=0.83$ ,  $P=0.003$ ).

In each LMM analysis, the covariance structure has been chosen following the restricted log-likelihood criterion. In order to avoid overparameterised models, LMMs have been simplified by means of the log-likelihood criterion. The response variable is transformed when the model assumptions are violated. Significant differences among treatments have been identified with an LSD test with Bonferroni correction.

## Results

### Density and diversity of the ectomycorrhizal community

According to the LMM, the density of active EM tips shows a significant relation with the position ( $P<0.001$ ; Table 2) and the quadratic component of time ( $P<0.001$ ), which reflects an initially negative trend, followed by a recovery from the fourth year (Fig. 1a). The density of

**Table 2** Mean characteristics of the below-ground EM community across the spatial positions of the *truffière*

	NTTT	<i>Brûlé</i>	Gap	Pine stand
Density of active ectomycorrhizas ( $\Gamma^{-1}$ ) <sup>a</sup>	951 a	65 c	94 bc	220 b
Morphotype richness <sup>b</sup>	2.8	2.9	2.5	2.4
Shannon index <sup>a</sup>	0.75 a	0.66 ab	0.45 c	0.53 bc
Frequency of <i>T. melanosporum</i> <sup>b</sup>	0.28 b	0.62 a	0.24 bc	0.12 c
Abundance of <i>T. melanosporum</i> (%) <sup>a</sup>	3.8 b	29.7 a	—	—
Abundance of <i>C. geophilum</i> (%) <sup>a</sup>	68.2 a	22.3 b	59.6 a	68.2 a

Significant differences among levels ( $\alpha=0.05$ ) are shown with letters in each row

NTTT near the trunk of the truffle tree

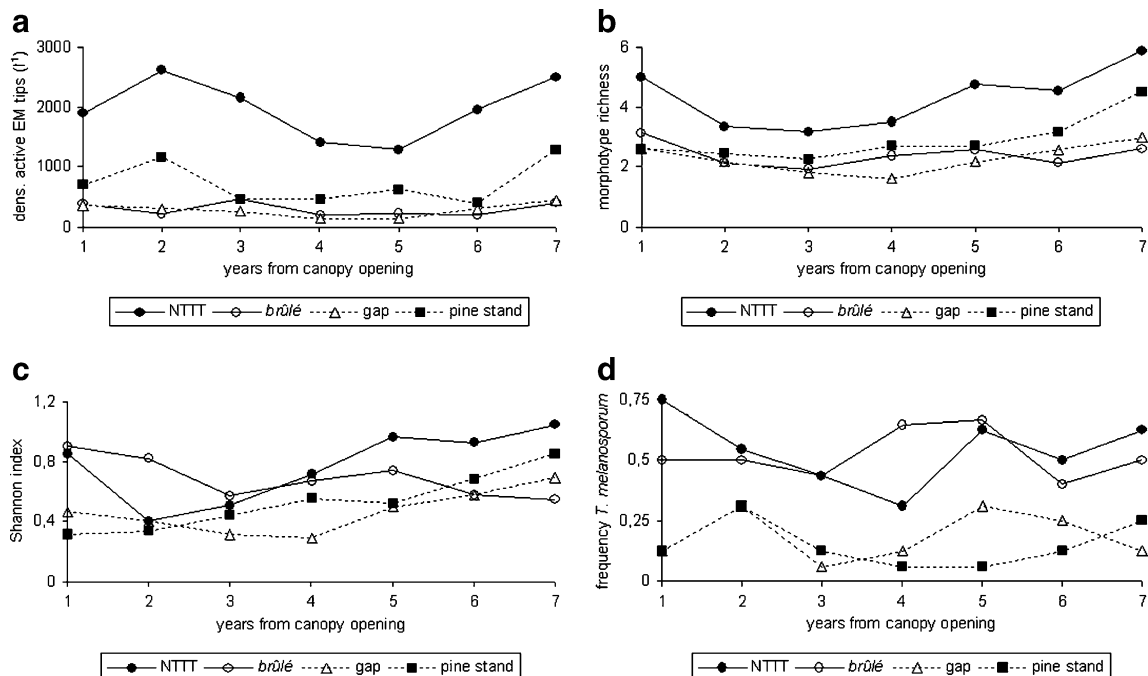
<sup>a</sup> Predicted means at year 3 and width of latewood ring equal to the mean value from 2001 to 2006

<sup>b</sup> Predicted means at year 3, density of active root tips in the 100-ml sample=265 tips  $\Gamma^{-1}$  and width of latewood ring equal to the mean value from 2001 to 2006

active EM tips also shows a positive relation with the latewood width of the year ( $P<0.001$ ) and significant differences among *truffières* ( $P=0.018$ ). To assess the effect of the size of the sampled *Q. ilex*, an additional LMM has been performed solely on the data of the pine stand. Tree height has been added as a covariate, and it shows a positive and significant relation with the density of active EM tips ( $P=0.036$ ).

According to the LMM, morphotype richness shows a significant relation with the interaction between position and density of active root tips ( $P<0.001$ ). The slope of the simple regression between density of active root tips and

morphotype richness is positive and significant in the four positions studied, but it is significantly higher in the *brûlé* (0.74) than in the rest (NTTT, 0.40; gap, 0.50; pine stand, 0.48). After removing the effect of the density of active root tips, morphotype richness shows a significant relation with the quadratic component of time ( $P<0.001$ ), reflecting a continued decrease that becomes more pronounced with time. To assess the effect of the size of the sampled *Q. ilex*, an additional LMM has been performed solely on the data of the pine stand. Tree height has been added as a covariate, and it shows a positive and significant relation with morphotype richness ( $P=0.016$ ).



**Fig. 1** Density of active EM tips (a), morphotype richness in the 100-ml core samples (b), Shannon index (c) and frequency of appearance of *T. melanosporum* (d) across the spatial positions of the *truffière* after the canopy opening. Each point is the mean of 16 samples

The interaction between position and time shows a significant effect on Shannon index ( $P<0.001$ ). The time trend is significantly more negative in the *brûlé* (simple slope,  $-0.04$ ) than in the other positions (simple slopes for NTTT  $-0.10$ , the gap  $-0.05$  and the pine stand  $-0.09$ ; Fig. 1c). Shannon index also shows a negative and significant relation with the latewood width of the previous year ( $P<0.001$ ).

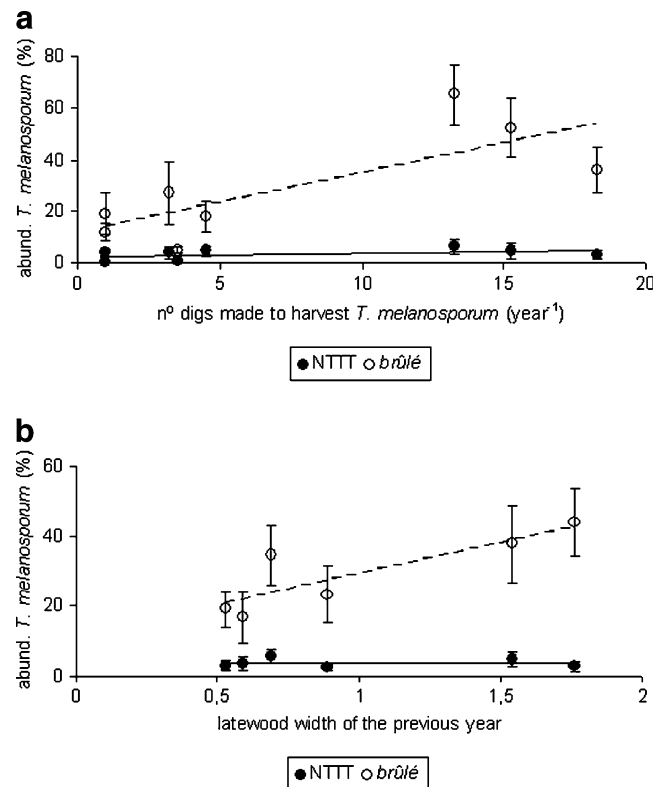
### Community composition

Overall, *T. melanosporum* has appeared in 33% of the samples, being the most frequent species in the *brûlé*. According to the LMM, its frequency of appearance shows a positive and significant relation with the density of active root tips ( $P<0.001$ ) and the number of digs made to harvest *T. melanosporum* fruiting bodies in the *truffière* ( $P=0.025$ ), as well as a significant relation with the position ( $P<0.001$ ; Table 2). The orientation of the transect shows a significant effect on the frequency of *T. melanosporum* ectomycorrhizas ( $P=0.023$ ), which is higher to the south.

The relative abundance of *T. melanosporum* ectomycorrhizas has been analysed solely in the *brûlé* and NTTT due to its low frequency in the remaining positions. It shows a significant relation with the interaction between position and the abundance of harvesting digs ( $P=0.002$ ) and a significant relation with the interaction between position and latewood width of the previous year ( $P=0.001$ ; Fig. 2). Neither the relative abundance nor the frequency of appearance shows a significant relation with the linear or the quadratic components of time (Fig. 1d).

*C. geophilum* is the most frequent morphotype overall (Table 3). The relative abundance of its ectomycorrhizas shows a significant relation with the position ( $P<0.001$ ; Table 2) and a negative relation with the linear component of time ( $P<0.001$ ).

Apart from these two species, 23 morphotypes have been detected, ten of which appear in more than 5% of the samples (Table 3). The frequencies of the most common types show a positive and significant relation with the density of active root tips (types *Genea*:  $P=0.003$ , *Thelephoroid*:  $P<0.001$ , *Scleroderma*:  $P<0.001$ , *Hebeloma-Cortinarius*:  $P<0.001$ , *Sebacina*:  $P=0.004$ , *Russula*:  $P<0.001$ , *Tuber brumale* Vitt.:  $P<0.001$  and *Tomentella galzinii* Bourdot:  $P=0.023$ ). After removing the effect of this variable, a significant effect of the position has been found only for the types *Scleroderma* ( $P=0.007$ ; significantly more frequent in the *brûlé* than in NTTT), *Hebeloma-Cortinarius* ( $P=0.009$ ; significantly more frequent in NTTT and the pine stand than in the *brûlé* and the gap), *Sebacina* ( $P=0.025$ ; significantly more frequent in the gap and the pine stand than in the *brûlé*), the type unidentified-1 ( $P=0.009$ ; significantly more frequent in NTTT than in the pine



**Fig. 2** Mean for the relative abundance of *T. melanosporum* ectomycorrhizas in the *brûlé* and near the trunk of the truffle tree (NTTT), according to the number of digs made to harvest *T. melanosporum* fruiting bodies (annual mean from 2001 to 2006) (a) and the latewood width of the previous year. Lines are regression estimates of mean abundance. Latewood width is expressed as a ratio to the mean in the period 1997–2008 (b). Bars indicate standard error

stand) and *T. brumale* ( $P=0.011$ ; significantly more frequent in the *brûlé* than in the rest). Among the various morphotypes, three distinct time trends have been found: (1) a significant quadratic trend, always reflecting an initially negative trend followed by a recovery from the third or the fourth year (types *Genea*:  $P<0.001$ , *Thelephoroid*:  $P<0.001$ , unidentified-1:  $P<0.001$  and *T. galzinii*:  $P=0.040$ ), (2) a positive and significant linear trend (types *Hebeloma-Cortinarius*:  $P=0.003$ , *Sebacina*:  $P<0.001$  and *Russula*:  $P<0.001$ ) or (3) no significant time trend. Two morphotypes show a significant relation with the latewood width of the current year (types *Thelephoroid*:  $P=0.001$  and *Scleroderma*:  $P=0.003$ ) and three with that of the previous year (types *Genea*:  $P<0.001$ , *Thelephoroid*:  $P=0.008$  and *Pisolithus*:  $P=0.030$ ).

### Discussion

#### EM community of the truffle tree

Knowing the factors that affect the EM community is fundamental to the management of sporocarp production in



**Table 3** Description and frequency of appearance of the 12 most common morphotypes. The morphotypes are ordered according to their global frequency in the sampling

Morphotype	Frequency	Colour	Mantle	Emanating elements
<i>Cenococcum geophilum</i> Fr.	0.77	Black	PL-type G	H: dark brown, thick-walled, non-ramified, non-clamped
<i>Tuber melanosporum</i> Vitt.	0.33	Orange to brown	PS-type M	C: yellowish-reddish, right angle-ramified, non-clamped
Type <i>Genea</i> (De Román 2003)	0.24	Reddish brown	PS-type L/K	C: yellowish-reddish, rigid, ramified, enlarged base, non-clamped
Type Theleforoid	0.23	Whitish grey to brown	PL-type D	C: hyaline, awl-shaped, non-ramified, with clamped (when only one) and non-clamped septa H: hyaline, Y-shaped ramification, anastomizing, with clamped and non-clamped septa
Type <i>Scleroderma</i> (De Román 2003)	0.22	Pale yellow	PS-type A	H: hyaline-yellowish, ramified, enlarged in the septa, non-clamped R: type D, whitish-yellowish, with knot-like structures in ramifications
Type <i>Hebeloma-Cortinarius</i> (De Román 2003)	0.19	Whitish rose to brown	PL-type B	H: hyaline, ramified, enlarged in the septa, clamped, anastomizing R: type A, hyaline, with fan-like connection to the mantle
Type <i>Sebacina</i> (De Román 2003)	0.15	Pale yellow to greyish-brown	PL-PS, type H	H: hyaline, thin, Y-shaped ramification with enlarged walls, scarce and non-clamped septa
Unidentified-1	0.12	Pale yellow to brown	PS-type P	C: hyaline, short, non-ramified, capitate, non-clamped H: hyaline-yellow, sometimes with ring-like shapes, ramified, non-clamped
Type <i>Russula</i> (De Román 2003)	0.10	Whitish to yellowish brown	PL-type B	C: hyaline, flask-shaped
Type <i>Pisolithus</i> (De Román 2003)	0.09	Golden orange	PL-type B	H: yellowish brown, ramified, sometimes ribbon-like, clamped R: type B, brown, ramified, with inflated cells
<i>Tuber brumale</i> Vitt.	0.09	Orange to brown	PS-type M	C: yellow, bristle-like, enlarged base, usually without septa
<i>Tomentella galzinii</i> Bourdot in Bourdot and Galzin	0.08	Yellowish to greenish brown	PS-type L	C: bristle-like, enlarged base, yellow below the first septa, clamped H: yellow, ramified, clamped R: type A, yellowish-greenish

PL plectenchymatous, PS pseudoparenchymatous (type according to Agerer (1987–2002)), C cystidia, H hyphae, R rhizomorph

forests. Our results show that the below-ground EM community in wild *brûlés* is distinct from that existing close to the trunk of the truffle tree. The lower density of active EM tips, the higher ratio between morphotype richness and density of active root tips, the lower abundance of *C. geophilum* and the higher abundance of *T. melanosporum* appear as distinctive features of the *brûlé*. Besides, the time trend of EM diversity after the canopy opening is contrasting.

The lower density of active EM tips in the *brûlé* could be attributable to the distance to the trunk (Luoma et al. 2006), to the soil environment (Deschaseaux and Ponge 2001; Jones et al. 2003b) or to the higher phytotoxic activity of *T. melanosporum* since this species induces alterations on root growth (Pargney et al. 2001; Sourzat et al. 2004). It is

difficult to isolate the role of each factor as they usually covariate in the *truffière*.

The scarce abundance of *C. geophilum*, another distinctive feature of the *brûlé*, could be attributable to the distance to the trunk (Dickie and Reich 2005), to a founder effect (i.e. earlier colonisation by *T. melanosporum*) or to the competition with *T. melanosporum*. *C. geophilum* is considered a generalist fungus with a wide host and habitat range, indicating stressful environments (Dickie and Reich 2005). However, in our study, it is less abundant in the *brûlé* than in NTTT, where the soil environment is more similar to that characterising dense forests (Table 1) and the density of active EM tips is higher.

The contrasting density of active EM tips supports the theory that the dynamics of *truffières* is closely linked to the

evolution of the root system of the truffle tree and the evolution of the soil environment in the *brûlé* (Ricard et al. 2003; Sourzat et al. 2004). The development of a forest stand leads to reduced insolation of the soil surface (an abiotic effect), and an increase in fine root density (Claus and George 2005), likely a competitive effect with invading EM fungi. These trends depart from the characteristics of the EM community found in the *brûlé*, the *T. melanosporum*-preferred habitat. According to Ricard et al. (2003) and Sourzat et al. (2004), EM communities of truffle trees are highly structured, with *T. melanosporum* dominating in the *brûlé*, but preceded and followed by an entourage of other EM fungi. Lian et al. (2006) found a similar pattern in *Tricholoma matsutake* Sing. *shiros*. In our study, the pattern of occurrence of *T. melanosporum* ectomycorrhizas agrees with that idea, particularly in *truffières* with higher fruiting body production.

Our data reveal that the responsiveness of *T. melanosporum* ectomycorrhizas to annual weather is different in the *brûlé* from that in NTTT, pointing that the functioning of the population of *T. melanosporum* ectomycorrhizas could be determined by the interaction of abiotic factors and the degree of dominance of the fungus (biotic interactions).

In the *brûlé*, *T. melanosporum* ectomycorrhizas appear to be more related to latewood width of the previous year than to the current year. This could be due to: (a) propitious weather conditions favouring fruiting and the germinated spores colonising fine roots and forming new ectomycorrhizas and (b) the main shift in the relative abundance of *T. melanosporum* ectomycorrhizas from year to year occurs in autumn, when they have one of their two seasonal peaks (Bonet et al. 2001), whereas the sampling has been performed in spring. The seasonal patterns of EM populations differ across species (Courty et al. 2008) and our results indicate that the influence of weather is morphotype specific. This could also contribute to changing the relative abundance of *T. melanosporum* ectomycorrhizas from one season to another.

#### Temporal evolution after the canopy opening

Canopy opening increases the insolation into the *brûlé*, reduces leaf litter accumulation and kills pine roots. However, in our study site (with intensive exploitation but no cultural practices carried out), it has not triggered a positive dynamics of *T. melanosporum* ectomycorrhizas. Their abundance seems to be in a rather steady state, driven solely by weather fluctuations.

However, the density and the richness of the EM community have been affected by the canopy opening. They both initially decrease, approximating the EM community to the distinguishing features of the *brûlé*. This

decrease may be due to the loss of EM inocula provoked by pine mortality, but this factor does not seem relevant since non-mycorrhized active tips are scarce throughout all the monitoring. This indirectly points to the effect of the soil environment on the EM fungi (Jones et al. 2003a) or on the fine roots. In a subtropical forest, Jones et al. (2003b) found that the fine roots of pine and those of understorey species replaced each other over a pine density gradient, but when the understorey was removed the pine root density was primarily driven by abiotic factors.

In our *truffières*, the density of active EM tips recovers from the fourth year, suggesting an acclimation period to the new environment after which the fine roots and the associated ectomycorrhizas tend to compensate for the root gap left by the death of pines.

On the other hand, the morphotype richness decreases monotonously throughout all the monitoring, although its positive relation with the active root tips density partially compensates for this decrease (Fig. 1b). The contrasting time trends of the various morphotypes indicate that the canopy opening has exerted a distinct effect on the EM fungi, although it is not possible to discriminate which is the responsible factor.

In the model of truffle cultivation proposed by Sourzat et al. (2004), the gaps between the *brûlés* are considered a potential space for *T. melanosporum* spread. In the clearcut gaps of our study, *Q. ilex* regeneration is subjected to a physical environment of the soil similar to that in the *brûlé*. The density of active EM tips and the morphotype richness are low, like in the *brûlé*, but that is likely due to the small size of the sampled trees. This is supported by the fact that in the pine stand, tree size shows a positive effect on the density of active EM tips and morphotype richness.

The occurrence of *T. melanosporum* ectomycorrhizas, however, is much lower and has not shown a significant increase after the canopy opening (Fig. 1d), suggesting that the shift in soil environment alone does not trigger the formation of new *truffières* in the short term. The apparent absence of time dynamics might also be owed to an inadequate sampling intensity (Taylor 2002). In the study site, the silvicultural treatment was executed in 26 ha (with a mean density of 581 juvenile *Q. ilex* ha<sup>-1</sup>) and *T. melanosporum* fruiting bodies have been harvested in only four juvenile trees that were previously non-productive (Garcia-Barreda and Reyna, unpublished data). The feasibility of sustaining truffle yield of forests through canopy opening has not been scientifically evaluated yet. Whereas the empirical experiences show promising results at maintaining the existing *brûlés* (Granetti 2005, Sourzat 2008), the results with respect to the formation of new *truffières* are conflicting, with the best results obtained for adult *Q. ilex* of 10–20 years old (Sourzat et al. 2008).

In conclusion, we have proposed a clear picture of the below-ground EM community in wild *truffières* under Continental Mediterranean climate. The structure of this community has been evaluated in the context of truffle cultivation. Most truffling regions in France and Italy lie on more wet climates (Hall et al. 2007), where *Q. ilex* is likely to show higher colonising ability and the forest canopy closes faster (Lloret et al. 2004; Zavala 2004). In these regions, the EM community is likely to differ in the density of fine roots and the speed of the response after the silvicultural treatment.

On the other hand, in agricultural soils which are periodically tilled, the fine roots and the ectomycorrhizas distribute preferentially deeper in the soil (Barry-Etienne et al. 2008). Since the EM fungi differ in their preference for soil depth (Courty et al. 2008), the EM community is likely to differ from that in wild *truffières*. In addition, the abundance of *T. melanosporum* ectomycorrhizas in our study is lower than that generally reported in truffle plantations (which are usually established with nursery-inoculated seedlings on agricultural soils), and the richness of soil-borne EM fungi is higher (Sánchez-Durán et al. 2009; Águeda et al. 2010). Further study in non-productive trees is necessary to elucidate its potential role in the renovation of truffle production.

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