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Enzymatic activities of three ectomycorrhizal types of *Quercus robur* L. in relation to tree decline and thinning

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Abstract

In a declining oak forest, a thinning treatment was performed in August 2004, targeting trees belonging to two decline classes. The whole ectomycorrhizal (EM) community was dominated by the fungal symbionts *Clavulina cinerea*, *Tomentella sublilacina* and *Russula* spp. The potential activities of eight secreted enzymes, involved in mobilizing nutrients (N, P) from soil organic matter, were measured on these three EM types in winter and spring 2006 using multiwell microplate photometric and fluorogenic methods. The enzymatic activities recorded in winter were generally significantly higher than in spring. Most of the enzyme activities studied, and particularly phosphatase and β-glucosidase, changed according to both decline class and sylvicultural treatment. In spring, each anatomotype displayed different enzymatic profile according to the decline class. These results suggest that the potential enzymatic activity of ectomycorrhizae adapts to the changes resulting from the sylvicultural treatment and reacts to the anthropic disturbance by adjusting to the new resource structure.

Keywords: Ectomycorrhiza; Enzymatic activities; Forest decline; *Quercus robur*; Forest thinning

1. Introduction

Around one third of the microbial biomass in forest soils is constituted by ectomycorrhizal (EM) fungi (Högberg and Högberg, 2002) which are symbiotically associated with the roots of social tree species (i.e. forming mono-specific stands, such as pines, spruce, beech or oaks) in temperate and boreal forests. EM fungi have a great importance in tree nutrition due to their ability to weather minerals, to mobilize organic and mineral phosphorus, to transport iron and to release nitrogen entrapped in organic molecules by producing extra-cellular enzymes (Smith and Read, 1997).

The EM community structure associated with a given host tree changes with forest ageing (Last et al., 1987) and following disturbance such as fire (De Román and De Miguel, 2005), nitrogen deposition (Lilleskov et al., 2002) or thinning (Jones et al., 2003; Buée et al., 2005). Many studies have been carried out to analyse the structure and the dynamic of EM community (Tedersoo et al., 2003; Montecchio et al., 2004; Buée et al., 2005; Toljander et al., 2006). Nevertheless, it is now necessary to describe the functionality of EM communities to evaluate their role in forest ecosystems.

Methods have recently been proposed to evaluate the EM functionality in situ by directly measuring the metabolic activities of single etomycorrhizal tips. Buée et al. (2005) monitored phosphatase and laccase activities using a microwell microplate system, showing that these activities change with the fungal species, the season, soil temperature and water potential. The multiwell microplate assay has been further developed for measuring potential enzymatic activities of single EM root tips by Pritsch et al. (2004) using fluorescent substrates (4-methylumbelliferone and 7-amino-4-methylcoumarin). This method was then improved by Courty et al. (2005), who tried to increase the number of enzymatic activities tested without raising the

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number of EM tips used. Eight enzymatic activities, involved in the turnover of carbon and in the release of N and P from organic complexes, were monitored in two forest sites (beech and oak) of central Europe. Courty et al. (2005) found that EM activity profiles depended on the species of the fungal symbionts, on seasons and on soil horizons.

In this work, we hypothesized that the enzyme activity profile of an EM community, or of any EM type within this community, is also partly determined by the host trees themselves in terms of age, health and competitive status in the forest stand. We tested this hypothesis for two controlled factors in an oak forest: the state of decline of the target trees and the felling of neighbouring trees (thinning) resulting in alleviated competition. We addressed eight enzyme activities involved in nitrogen and phosphorus mobilization from soil organic matter.

2. Materials and methods

2.1. Site and sampling

The experimental site is a 60 year old declining lowland forest in northern Italy (described in more detail in Mosca et al., 2007), dominated by pedunculate oak (*Quercus robur,* 42% of the total basal area), with *Carpinus betulus* in the understorey. The brown soil has typical mull-type humus and a dark brown loamy A1 horizon (0–5 cm thick) above a deep clayey and mottled B horizon.

Sixteen trees with similar dimensions had been chosen in two decline classes (moderate and severe) according to canopy transparency, leaf discoloration, presence and density of epicormic twigs, longitudinal cracks in the bark and rhizomorphs or basidiocarps of *Armillaria* spp. at the base of the trunk (Montecchio et al., 2004). Eight trees in each decline class were released from competition in July 2004 by felling their closest neighbours, resulting in the following experimental design: 2 decline classes (moderate or severe) × two sylvicultural treatments (thinned or not) × 4 tree replicates (individuals).

Four samples were collected 1.5 m from the base of the trunk of each tree in 2006 (i.e., more than 1 year after the thinning treatment) by cutting blocks of the A1 horizon 20 × 20 cm wide and 15 cm deep, containing the A1 horizon (0–5 cm- and the top (5–15 cm) of the B horizon. Soil samples were placed in plastic boxes and stored a 4 °C for not more than 4 days before processing in the laboratory. Sampling was performed twice, on January 27 (during a short 5–6 °C period after 2 weeks of negative temperatures) and on May 8 (at oak bud break, with 16–18 °C mean daily temperature).

2.2. Laboratory analyses

Fine roots were removed from the soil blocks, soaked in water and gently washed. Healthy looking and turgid EMs were observed on oak roots using a stereomicroscope. Fourteen EM tips (2–4 mm long) of the three dominant EM types, *Clavulina cinerea*, *Tomentella sublilacina* and *Russula* spp. (Mosca et al., 2007, Table 1), were picked from each root sample to be subjected to multiple enzymatic tests according to the methods described by Pritsch et al. (2004), Buée et al. (2005) and Courty et al. (2005, 2006). Seven mycorrhizal tips (2–4 mm in length and 0.2–0.5 mm in diameter, depending on the anatomotype, with the average volume around 1 mm^3) were individually placed in the wells of a clear, flat bottom, 96-well microtitration plate, leaving the eighth well of the row

Table 1
Description of the main features of the three dominant anatomotypes and their molecular identification (Mosca et al., 2007)

ECM anatomotype	Macroscopic description	Outer mantel	Inner mantel	Emananting hyphae	Best match sequence	Similarity index (%)	Database and accession number
Clavulina cinerea	Pinkish white; monopodial-pinnate; cottony	Transitional type between plectenchymatous and pseudoparenchymatous, irregularly shaped hyphae form a coarse net	Plectenchymatous, hyphae arranged net-like, repeatedly and squarrosely branched	White; with clamps; open H-shaped anastomosis	*Clavulina cinerea*	99	UNITE UDB000074
Russula spp.	Yellowish white to pinkish white; in old parts light brown; monopodial pinnate; smooth	Plectenchymatous; hyphae arranged net like, repeatedly and squarrosely branched	Transitional type between plectenchymatous and pseudoparenchymatous irregularly shaped hyphae form a coarse net	Scarce; hyaline	*Russula* sp.	98	UNITE AF418629
Tomentella sublilacina	Light to dark brown; irregularly pinnate; smooth	Pseudoparenchymatous mantle with epidermoid cells	Plectenchymatous, hyphae rather irregularly arranged	Colourless; with clamps; open H-shape anastomosis Y-shape ramification	*Tomentella sublilacina*	100	NCBI GenBank AJ889982

The accession numbers and similarity indices refer to the nucleic acid sequences from the ITS region of the rDNA, compared with the UNITE (http://unite.ut.ee) and NCBI GenBank (http://www.ncbi.nih.gov/BLAST) databases using the BlastN algorithm.