Relation between oak tree phenology and the secretion of organic matter degrading enzymes by *Lactarius quietus* ectomycorrhizas before and during bud break

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Abstract

The ectomycorrhizas (ECM) formed by *Lactarius quietus*, an ECM fungus specifically associated with *Quercus* spp., are abundant all the year long. Root and stem growth, beginning before bud break in oak, are strong carbon sinks partially fulfilled with carbohydrate reserves. We hypothesized that *L. quietus* contributes to providing trees with carbon at bud break through enzymatic activities before photosynthesis begins. Activities of eight secreted enzymes (xylosidase, glucuronidase, cellobiohydrolase, \(\beta\)-glucosidase, \(N\)-acetyl-glucosamine, leucine aminopeptidase, acid phosphatase and laccase) relevant to carbon cycling and the release of phosphorus and nitrogen from soil organic matter were measured on *L. quietus* ECMs before, during and after the bud break. Phenological, climatic and pedoclimatic parameters were also measured. Laccase, glucuronidase, cellobiohydrolase and \(\beta\)-glucosidase activities proved to be significantly related to tree reactivation and climate. All these activities can help the formation of new tissues by supplying carbon. *L. quietus* can behave saprotrophically, using soil organic matter as substrate. This is consistent with the hypothesis that it provides the oak trees with carbon when demand is high and photoassimilates are not yet available.

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Keywords: *Lactarius quietus*; Ectomycorrhizal fungi; Bud break; Stem growth; Secreted enzymes; Organic matter; Carbon; Mixotrophy; Mycoheterotrophy

1. Introduction

In Central Europe, pedunculate (*Quercus robur* L.) and sessile (*Quercus petraea* Matt. Liebl.) oaks are the most important deciduous forest tree species, ecologically and economically. Tree phenology changes seasonally due to both climate and endogenous rhythms. In oak, a ring-porous species, the early wood including large vessels and a part of the annual radial stem growth is achieved before leaf expansion in spring (Dougherty et al., 1979; Hinckley and Lassoie, 1981; Bréda and Granier, 1996).

Oak stem growth starts 10 days before bud burst (Bréda and Granier, 1996; Barbaroux and Bréda, 2002) and about 30\% of the total annual stem increment is added before bud burst (Hinckley and Lassoie, 1981). Primary root elongation, related to changes in external climatic variables (soil temperature and soil water potential) (Teskey and Hinckley, 1981) and endogenous factors including water and hormone transfer (Lachaud, 1989), begin before bud swell and last until bud burst when trees are leafless.

Root and stem growth, as well as bud burst, are strong carbon sinks (Ashworth et al., 1993). Total nonstructural carbohydrate reserves (TNC: starch and sugars) provide the most important part of the energy needed for tree reactivation (Hinckley and Lassoie, 1981). However, measurements of carbohydrate reserves show that TNC are depleted after bud burst (Barbaroux...
and Bréda, 2002; Hoch et al., 2003) and not sufficient to supply spring carbon reactivation needs (Barbaroux et al., 2003).

Fine roots are important compartments of the trees, especially in terms of nutrient and water uptake. Oak fine roots are colonized by symbiotic fungi, which form ectomycorrhizas (ECM). The ectomycorrhizal roots are located in the top 20 cm of the soil which are enriched in organic matter and where nutrients are concentrated (Schenk and Jackson, 2002). Many ECM fungi possess saprotrophic capabilities, which enable nutrient capture from complex organic sources, which the plant does not have direct access by itself (Smith and Read, 1997; Chalot and Brun, 1998). ECM fungi are thus able to mobilize nutrients from organic substrates (proteins, amino acids, chitin, phosphomonoesters and phosphodiesters) or nutrients linked to organic residues by secreting extra-cellular enzymes (Dighton, 1983; Abuzinadah and Read, 1986a, b; Leake and Read, 1990; Guttenberger et al., 1994). The ability to secrete extra-cellular enzymes differs with EM fungal species and with season. Buée et al. (2005), Courty et al. (2006) even reported such activities in winter, when deciduous trees were leafless.

The ECM morphotype formed by Lactarius quietus (Fr.) Fr., an ECM fungus specifically associated with Quercus spp., is abundant all the year long in many European oak forests. In this work, the main objective was to study the contribution of L. quietus contributes to providing trees with carbon and other nutrients at bud burst through its exo-enzymatic activities involve in plant litter decomposition, at a time when photosynthesis has not yet begun. We addressed this issue in an oak forest in north-eastern France by sampling fine roots in two soil horizons in spring. Eight exo-enzymatic activities, relevant to carbon cycling and to the release of phosphorus and nitrogen from soil organic matter (hydrolase and oxidase activities), were measured on L. quietus ECMs.

2. Materials and methods

2.1. Site and forest stand

The experimental site was a 100-year-old oak even aged stand from natural regeneration with a continuous oak canopy of Q. petraea and Q. robur and a hornbeam understory (Carpinus betulus L.) in north-eastern France (48°75′/N, 6°35′/E, elevation 250 m). Floristic composition of the understory was diverse and reflected the position of the understory was diverse and reflected the Quercetum carpinetum association. The soil is a luvic cambisol (pH [H2O] of 4.6) with a loamy texture in the A1 (0–5 cm) and A2 horizons (Courty et al., 2005, 2006). Total soil depth occupied by oak roots was 2 m, but root vertical distribution was strongly reduced by a clay layer at 50–60 cm deep. Soil extractable water was 165 mm (Bréda et al., 1995). The topography was flat, mean annual temperature was 9.2°C and average annual precipitation was 744 mm.

2.2. Root sampling and identification of ECM types

Roots were sampled in four 2.5 m × 1.5 m plots. Each plot was located at a distance of 1.5 m from a dominant oak and successive samples from a same plot were 0.5 m apart. In each plot, one soil core (5 cm in diameter and 20 cm deep) was extracted every second, third or fourth day from March 27, 2004 (day of the year 85) to May 24, 2004 (day 146). They were immediately transported to the nearby laboratory (INRA Center, 5 km away) and processed the same day. The samples coming from the top (0–5 cm) corresponded to the A1 horizon and were enriched in organic matter and contained a high density of fine roots. The 5–20 cm layer, corresponding to the top of the A2 horizon, was separated from the upper layer. Fine roots were soaked in tap water for 15 min before being gently washed and then observed in water with a stereomicroscope (×40). The ECMs due to L. quietus were identified morphologically according to Agerer (1987–1998) and molecularly by sequencing the ITS region of the ribosomal DNA of the fungal symbionts according to the methods previously described by Buée et al. (2005) and Courty et al. (2005, 2006). DNA sequencing was performed in our lab. L. quietus forms a smooth ECM morphotype with few emanating hyphae. It belongs to the “contact exploration type” (Agerer, 2001) and binds dead leaves, forming a close contact with them.

2.3. Enzymatic activity profiling of the L. quietus ECMs

Fourteen randomly picked tips of L. quietus ECMs from each sample were used to determine potential enzymatic activities, using the high-throughput photometric and fluorimetric microplate assays described by Pritsch et al. (2004) and Courty et al. (2005). Each well of the 96-well micro-titration plate contained a single ECM tip. Six activities were first sequentially measured on seven tips: acid phosphatase (EC 3.1.3.2), β-glucosidase (EC 3.2.1.3), N-acetyl-glucosaminidase (a chitinase: EC 3.2.1.52), glucuronidase (EC 3.2.1.31), xylolysidase (EC 3.2.1.37) and cellobiohydrolase (EC 3.2.1.91). Then, the seven remaining tips were used to measure leucine aminopeptidase (EC 3.4.11.1) and laccase (EC 1.10.3.2) activities. See Courty et al. (2005) for the detailed procedures. The eight enzymatic activities are related to carbon cycling and the release of P and N from organic macromolecules of the litter and the humus. All measured activities were calculated per mm² of projected areas of individual ECM tips against the incubation time (min). The projected areas, determined with the automated image analysis software WINRHIZO 2003b (Regent Instruments, Inc., Quebec, Canada; Buée et al., 2005), are linearly correlated with the surface areas of the ECMs considered as cylinders.
2.4. Oak growth and phenology

Bud development was recorded at the same time as root sampling according to a six-stage scale (Bréda and Granier, 1996). The points (in parentheses) corresponding to each stage were (1) closed bud (0), (2) bud swelling (27), (3) breaking up of the scales (36), (4) scales and bud open (63), (5) emergence of the first leaf (90), (6) leaf expansion (99). The observations were performed every second or third day, in the third upper part of the crowns using binoculars from the ground level. For each date of observation, a budburst index, corresponding to the mean of the points given individually to 100 buds per tree was given on a sample of 15 oaks. Bud burst was achieved when the bud burst index reached 80. Tree growth was monitored in a nearby 45-year-old sessile oak stand, growing on a similar soil (Barbaroux and Bréda, 2002). Self-made automatic micro-dendrometers using precision potentiometers (Megatron, Allinges, France) were set 2 m high on the stems of six dominant oaks. Temperature 1 cm under the bark of the same trees was monitored with copper/constantan thermocouples. Stem diameter fluctuations and under bark temperatures were collected every minute on a CR10X data logger (Campbell Scientific Inc, Leicestershire, UK). Half-hourly averages were calculated. For the progression of growth, only the daily midnight value was considered, avoiding interaction with sap flux transfer.

2.5. Climate data

Soil temperature and soil water potential were measured in the 100-year-old stand with 20 psychrometric probes PST-55-15-SF (5 probes/plot) and a millivoltmeter (Wescor HR-33 T, Logan, UT, USA). Probes were set 10 cm deep in the A2 horizon and measurements were made in the morning three times per month.

Climate data were obtained from the INRA weather station of Champenoux, 5 km from the experimental site. Global radiation (MJ m\(^{-2}\)), minimum and maximum air temperature (°C) and rainfall (mm) were collected every minute and half hourly averages were recorded. Daily maximum or minimum air temperatures from the 48 half hourly values were used in the regression analysis. Global radiation was cumulated over 24 h. Potential evapotranspiration (PET, mm) was calculated according to Penman formula (Penman, 1948) and cumulated daily.

2.6. Statistical analysis

Multiple linear regression using the ascending stepwise technique (StatView 5; SAS Institute Inc., USA) was used to examine contribution of climate and enzymatic activities to variability in phenology and tree growth (i.e., tree reactivation). A correlation matrix was constructed among activities of the eight enzymes. Coefficients of correlation between enzyme activities and tree/soil parameters were also calculated. Significance was noted if the explained variance was significantly higher than residual variance (\(p < 0.05\)).

3. Results

3.1. Oak growth, phenology and climate

The stem girth increment reached 13 mm by day 270 and stayed there until the end of the year (Fig. 1a). During winter, two periods of stem shrinkage (end of January and end of February) occurred when below bark temperature dropped below zero. The third stem shrinkage event (end of April) was not associated with negative temperature and corresponded to water movement within the stem wood: this shrinkage was the first index of processes of reactivation. The following results focused on the period of stem growth reactivation during spring (Fig. 1b). Three phases were observed on the bud burst index curve. Buds remained dormant until day 101, when bud swelling was observed. The index increased slowly from day 101 (Index = 4.5) to day 111 (Index = 16.3). Bud break and emergence of the first leaves happened within 10 days, between days 111 and 122. Bud break was achieved on day 122 when the index reached 80.3. Afterwards, leaf expansion started. The index reached then its maximum (index = 91.8), without ever reaching 100 because part of the buds stayed dormant or died.

No girth growth was recorded from days 85 to 111. The stem girth variation started on day 111, 10 days before bud break was achieved. The stem (at breast height, 1.3 m) first shrank from days 112 to 118. From day 118 tree growth began simultaneously with leaf expansion with girth increase reaching 2.6 mm on day 145. The temperature under the bark ranged from 6.5 to 17.5 °C during this period.

During the entire observation period, soil temperature varied from 7.9 to 13.1 °C and soil water potential varied from −0.18 to −0.43 MPa. Precipitation was moderate and regular (mean = 3.9 mm day\(^{-1}\)) except on days 105, 106, 110 and 115 when precipitations were more than 10 mm day\(^{-1}\).

Stepwise regression analysis showed that bud break and tree growth were highly positively correlated to mean air temperature cumulated from day of year 85 (90.6%, \(r = 0.95\), \(F = 156.3\) and 87.5%, \(r = 0.94\), \(F = 113.5\) of variance explained, respectively).

3.2. Enzymatic activities, climate and tree reactivation

The ECM activity profiles were similar in both horizons but with lower amplitudes in the A2 horizon. We therefore present the data from the A1 horizon only. Extracellular enzymes involved in the mobilization of carbon, nitrogen and phosphorus showed different patterns.

\(\beta\)-Glucosidase, cellobiohydrolase and to a lesser extent, xylosidase activities showed similar patterns and remained...
constant, with the exception of a synchronous peak of activity on day 103, just at the beginning of bud burst (Fig. 2a). A second maximum occurred at the end of the observation period, on day 144, for β-glucosidase and cellobiohydrolase activities. For the rest, β-glucosidase and cellobiohydrolase activities were strongly positively correlated \( (r = 0.892) \) (Table 1). These activities were also significantly positively linked to tree growth (Table 2): \( r = 0.484 \) and \( r = 0.528 \) for cellobiohydrolase and β-glucosidase activities, respectively. Moreover, β-glucosidase activity was also related to xylanase activity \( (r = 0.609) \), PET \( (r = 0.492) \) and cumulated global radiation between two dates of enzyme activities measurement \( (r = 0.496) \) (Tables 1–3). In contrast, glucuronidase activity was very low from days 85 to 124. On day 124, this activity increased quickly to achieve a maximum on day 135 before decreasing until the end of the observation period. However, it was positively correlated to PET \( (r = 0.743) \), the global radiation \( (r = 0.743) \) and rainfall \( (r = 0.649) \) cumulated between two successive sampling dates. Glucuronidase activity was also related to budburst \( (r = 0.711) \), tree growth \( (r = 0.779) \) and cumulated below bark temperature \( (r = 0.719) \).

Chitinase activity and phosphatase activity showed similar patterns (Fig. 2b) and were strongly positively correlated \( (r = 0.700) \) (Table 1). A first maximum was reached a few days before budburst, corresponding to a strong rainfall event (22 mm). A second maximum of activity of these two enzymes occurred jointly with oak stem increment.

Leucine amino-peptidase and laccase activities showed increases of activity beginning at budburst. Low activity

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**Fig. 1.** (a) Changes in oak stem girth increment and under bark temperature during 2005. Note changes during the growing season. The enzyme study was conducted from day of year 85–145 (circled). (b): Stem girth increment, budburst index (left axis, bold symbols) and temperature under the bark (right axis, light line) during the period of stem girth reactivation. Budburst index was divided by ten for scale facilities.
was detected for leucine aminopeptidase between days 85 and 104 (Fig. 2c). From day 104 to the end of the bud burst, day 122, the activity increased rapidly to reach a maximum. During the same period, laccase activity, insignificant from days 85 to 96, increased slowly to reach a maximum on day 120. Laccase activity was positively and significantly correlated with all climatic and phenology factors (Tables 1–3).

Table 1
Correlation coefficients showing relationships among activities of the eight enzymes

<table>
<thead>
<tr>
<th></th>
<th>Xyl</th>
<th>Glr</th>
<th>Nag</th>
<th>Cel</th>
<th>Gls</th>
<th>Pho</th>
<th>Leu</th>
<th>Lac</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xyl</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glr</td>
<td>−0.056</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nag</td>
<td>0.214</td>
<td>−0.026</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Cel</td>
<td>0.471</td>
<td>0.043</td>
<td>0.062</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gls</td>
<td>0.609</td>
<td>0.175</td>
<td>0.033</td>
<td>0.892</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pho</td>
<td>−0.119</td>
<td>−0.214</td>
<td>0.700</td>
<td>0.087</td>
<td>0.034</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leu</td>
<td>0.094</td>
<td>0.175</td>
<td>0.335</td>
<td>−0.072</td>
<td>−0.122</td>
<td>0.090</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Lac</td>
<td>−0.056</td>
<td>0.444</td>
<td>0.037</td>
<td>0.343</td>
<td>0.315</td>
<td>−0.110</td>
<td>0.158</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Values in bold are significant ($p<0.05$, $n=17$ dates).
Abbreviations for enzymes: Xyl, xylosidase; Glr, glucuronidase; Nag, N-acetyl-glucosaminidase; Cel, cellobiohydrolase; Gls, $\beta$-glucosidase; Pho, acid phosphatase; Leu, leucine aminopeptidase; Lac, laccase.

Fig. 2. Enzyme activities of *Lactarius quietus* ectomycorrhizas in the A1 horizon from days 85 to 145. Bars represent SE ($n=7$). Arrows define the bud burst period: (a) Cellobiohydrolase, glucosidase, xylosidase and glucuronidase activities. (b) Chitinase and acid phosphatase activities. (c) Laccase and leucine aminopeptidase activities.
Enzymatic activities measurements (day of year 85). Values in bold are significant ($p<0.05, n=17$ dates).

<table>
<thead>
<tr>
<th>Enzyme activities</th>
<th>Budburst (index)</th>
<th>Tree girth increment (mm)</th>
<th>Soil water potential (MPa)</th>
<th>Soil temperature ($^\circ$C)</th>
<th>Under the bark temperature ($^\circ$C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xyl</td>
<td>0.022</td>
<td>0.032</td>
<td>–0.372</td>
<td>–0.031</td>
<td>0.095</td>
</tr>
<tr>
<td>Gli</td>
<td><strong>0.711 (p = 0.0014)</strong></td>
<td><strong>0.779 (p = 0.0002)</strong></td>
<td>0.060</td>
<td>0.136</td>
<td><strong>0.719 (p = 0.0011)</strong></td>
</tr>
<tr>
<td>Nag</td>
<td>0.161</td>
<td>–0.006</td>
<td>–0.237</td>
<td>0.303</td>
<td>0.189</td>
</tr>
<tr>
<td>Cel</td>
<td>0.294</td>
<td><strong>0.484 (p = 0.049)</strong></td>
<td>–0.282</td>
<td>0.399</td>
<td>0.447</td>
</tr>
<tr>
<td>Gls</td>
<td>0.278</td>
<td><strong>0.528 (p = 0.029)</strong></td>
<td>–0.175</td>
<td>0.238</td>
<td>0.459</td>
</tr>
<tr>
<td>Pho</td>
<td>–0.151</td>
<td>–0.085</td>
<td>0.031</td>
<td>0.106</td>
<td>0.007</td>
</tr>
<tr>
<td>Leu</td>
<td>0.368</td>
<td>0.148</td>
<td>–0.412</td>
<td>0.198</td>
<td>0.325</td>
</tr>
<tr>
<td>Lac</td>
<td><strong>0.776 (p = 0.0003)</strong></td>
<td><strong>0.728 (p = 0.0009)</strong></td>
<td>–0.523 (p = 0.031)</td>
<td><strong>0.798 (p = 0.0001)</strong></td>
<td><strong>0.789 (p = 0.0002)</strong></td>
</tr>
</tbody>
</table>

Values in bold are significant ($p<0.05, n=17$ dates).

Abbreviations for enzymes: Xyl, xylosidase; Gli, glucuronidase; Nag, N-acetyl-glucosaminidase; Cel, cellobiohydrolase; Gls, $\beta$-glucosidase; Pho, acid phosphatase; Leu, leucine aminopeptidase; Lac, laccase.

### 4. Discussion

The activities of four of the eight enzymes studied were related to oak phenology: glucuronidase, cellobiohydrolase, $\beta$-glucosidase and laccase. They are involved in the degradation and mobilization of the major carbon components of the cell wall and dead plant material, through lignin degradation and cellulose and hemicellulose depolymerization.

According to Barbaroux et al. (2003), total nonstructural carbohydrates in the sapwood of oaks are not sufficient to support fine root respiration and elongation, bud break and stem growth. Stem growth begins before bud break and continues after bud break whereas leaves are not yet mature (Hinckley and Lassoie, 1981). According to Dickson (1987), deciduous trees take up both carbon and nitrogen (e.g., amino acids) that have cycled through the root and mycorrhizal associations. Carbohydrate transport to roots, nitrogen uptake, production of amino acids, and transport back to shoots are closely controlled by feedback cycles regulated by the demand of both shoots and roots (Dickson, 1989). Here, $\beta$-glucosidase, cellobiohydrolase and glucuronidase, which are related to oak stem growth, act together on cellulose and hemicellulose in substrates in the soil to quickly liberate carbon compounds easily assimilated as glucose.

Cellobiohydrolase and $\beta$-glucosidase are responsible for the hydrolysis of the $\beta$-1,4 glucosidic bonds in cellulose. These two enzymes recognize the presence of cellulose and degrade it into cellobiose and then into glucose (Henrisatt and Davies, 1997). The large amount of resulting glucose causes catabolic repression, which is directly responsible for negative regulation at the transcriptomic level (Ilmen et al., 1997). Catabolic repression prevents fungi from synthesizing an excess of cellulases when there is abundant easily assimilated carbon, such as glucose. This could explain the high correlation between the cellobiohydrolase and $\beta$-glucosidase activities secreted by L. quietus ECMs. Glucuronidase is responsible for the hydrolysis of the $\beta$-O-linkage between the D-glucuronic acid and the organic compound to which it is linked (Kuroyama et al., 2001). Thus, it provides access to glucuronic acid, one of the principal components of cell wall of leaves, as a carbon source which ECMs can metabolize. Here, the high activity of glucuronidase clearly corresponds to leaf expansion after budburst is completed, leading us to hypothesize the key role of this enzyme in providing carbon to the trees at the time of the formation of new leaves.
Lignins, another major part of the cell wall, are polymeric condensed compounds. They are degraded by different polyphenol oxidases (lignin and manganese peroxidases, laccases and glyoxal oxidases). Here, we focused our study on laccases, which are blue copper polyphenol oxidases (Gramms et al., 1998; Chen et al., 2003). Secreted fungal laccases are encoded by complex families of structurally related genes (Hoegger et al., 2003). Secreted fungal laccases are encoded by complex families of structurally related genes (Hoegger et al., 2004). Laccases isozymes can be either constitutively expressed (Kurk and Cullen, 1998) or induced/regulated by different factors. Pedoclimatic or climatic parameters such as temperature and soil pH (Criquet et al., 1999) are able to modify laccase expression in oak forest litter. Other factors such as the veratryl alcohol (Barbosa et al., 1996) or phenolic compounds (Farnet et al., 2002), which are components of plant cell walls, regulate laccase expression.

Regarding the four enzymes involved in the degradation of plant cell wall, we hypothesized a succession of the activities. Just before the beginning of bud break, we noted an increase of the β-glucosidase, cellobiohydrolase, xylanase and laccase activities. Then L. quietus secreted enzymes involved in depolymerizing cellulose and hemicelluloses, such as cellobiohydrolase, β-glucosidase, xylanase and glucononidase, which mobilize carbon as glucose. At this stage, the ECM fungus acts as a brown rot fungus, which depolymerizes cellulose early in the wood decay process and degrades cellulose without prior removal of lignin (Evans et al., 1994; Blanchette, 1995). Next, at the end of bud break and during leaf expansion, the laccases are also activated, which maximizes soil carbon mobilization. In this case, L. quietus behaves more as a white rot fungus which breaks down lignin (Hatakka, 1994; Eggert et al., 1996). Thus, these activities can contribute to mobilize carbon that can be used by the oak host.

With respect to the other enzymes, the production of cell-wall bound acid phosphatase in soil depends on inorganic or organic phosphorus concentration and on soil temperature (Dighton, 1983; Tibbett et al., 2000). Concerning N-acetylglucosaminidases, they degrade complex amino sugars oligomer rich in carbon and nitrogen such as chitin. The ratio between carbon and nitrogen concentrations play a major role in the regulation of transcription and secretion of chitinases (Donzelli and Harman, 2001). Rapid modifications of nitrogen and carbon concentration in the soil could explain the cyclic curve of the chitinase activity due to catabolic repression.

Soil carbon is a nonlimiting nutrient source compared to nitrogen. Some plant species are chlorophyllous and heterotrophic, but not directly parasitic upon autotrophs (Leake, 1994, 2005). The carbon they use comes directly from mycorrhizal associations. Mixotrophic plants exploit fungal carbon as part of their carbon resources (e.g., the ground orchid Cephalanthera damasonium, Gebauer and Meyer, 2003) and mycoheterotrophic plant as its exclusive carbon source (Selosse et al., 2006). Here, oaks might act temporarily as mixotrophic or mycoheterotrophic plants during budbreak, when the leaves have not expanded and are not functional. This would be consistent with document two-directions carbon fluxes in ECM networks (Simard et al., 1997). Alternately, the secretion of exoenzymes involved in carbon mobilization can be a response of the mycorrhizal fungal symbiont to the need of carbon from the tree, which does not necessarily result in a soil–tree carbon flux.

A last hypothesis concerning the increase of activities during the bud break is that the carbon transfer from the plant to the fungus is certainly stopped and the fungus is starved in carbon. Because L. quietus behaves as a saprotrophic fungus, the presence of organic matter in soil releases catabolic repression and induces the synthesis and the secretion of hydrolytic enzymes involved in carbon mobilization (Donzelli and Harman, 2001).

These activities could also result from the mobilization of nutrients from recent dead roots or old ECMs, a phenomenon observed after clear-cutting (Jones et al., 2003).

To conclude, our results strongly suggest that, under certain conditions, some ECM fungal symbionts behave as saprobes, using litter and soil organic matter as substrates and providing the host trees with carbon at time when demand is high and photoassimilates are not yet available, such as spring for deciduous trees.

This hypothesis challenges the common paradigm of mycorrhizal symbiosis, where the fungal partner derives all its carbon from the photosynthetic plant. However, it is consistent with the transfer of carbon from the fungus to the plant in mycoheterotrophic or mixotrophic plants and with seasonal peaks of carbon need, as discussed in this work.

It is plausible that the marked seasonality of carbon fluxes in temperate, deciduous forest ecosystems, as well as the close evolutionary and phylogenetic proximity between saprotrophic and ectomycorrhizal fungi, led the latter to transfer carbon to their host trees. This is even more probable if we consider that ECM fungi are not rhizospheric organisms but extensions of the root tissues: the long coevolution resulted in optimized management of carbon resources.

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