Ectomycorrhizal identity determines respiration and concentrations of nitrogen and non-structural carbohydrates in root tips: a test using *Pinus sylvestris* and *Quercus robur* saplings

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Received November 30, 2009; accepted February 3, 2010; published online March 18, 2010

Summary Fine roots play a significant role in plant and ecosystem respiration (RS); therefore, understanding factors controlling that process is important both to advancing understanding and potentially in modelling carbon (C) budgets. However, very little is known about the extent to which ectomycorrhizal (ECM) identity may influence RS or the underlying chemistry that may determine those rates. In order to test these relationships, we examined RS, measured as O2 consumption, of first-order ECM root tips of *Pinus sylvestris* L. and *Quercus robur* L. saplings in relation to their ECM fungal symbionts and associated nitrogen (N), C and non-structural carbohydrate concentrations. Roots of *P. sylvestris* were colonized by *Rhizopogon roseolus*, *Tuber* sp. 1 and an unknown species of Pezizales. Fungal species colonizing *Q. robur* roots were *Hebeloma* sp., *Tuber* sp. 2 and one unidentified ECM fungus described as *Tuber*-like based on ECM morphology. ECM RS rates for different host species were significantly different and more than 97% of the variation in RS within a host species was explained by ECM root tip N concentrations. This may indicate that some of the variability in fine root RS–N relationships observed between and within different host species or their functional groups may be related to intraspecific host species differences in root tip N concentration among ECM fungal associates.

Keywords: ectomycorrhiza, morphotype, nitrogen, pedunculate oak, respiration, Scots pine.

Introduction

Mycorrhizal fine roots are very dynamic and play a crucial role in forest ecosystem functioning and carbon dioxide fluxes (Fisk et al. 2004, Heinemeyer et al. 2007, Malcolm et al. 2008). Mycorrhizal fungal symbions aid plants by enhancing the uptake of water and nutrients, but often at an increased carbon (C) cost (Smith and Read 2008). Mycorrhizas are also important modifiers of root turnover and organic nitrogen (N) acquisition (Fogel 1980, Chalot and Brun 1998). Nearly all known plant families form mycorrhizas with soil fungi; therefore, plant mycorrhizas should receive greater attention when estimating N and C cycling in forest ecosystems.

Fine root respiration (RS) is associated with three major energy-requiring processes: root growth, maintenance of root biomass and function and uptake of mineral nutrients (Bryla and Eissenstat 2005). Loss of C through root and rhizosphere RS of recent photosynthate may vary from <10 to >90% of total soil CO2 efflux, with the modal root contribution between 40 and 50% (Hanson et al. 2000, George et al. 2003, Volder et al. 2005). While many factors affect mycorrhizal root RS (Hanson et al. 2000, Ohashi et al. 2000, Höögberg et al. 2001, Volder et al. 2005), a potentially important but seldom explored factor is differences in species of mycorrhizal fungi (Malcolm et al. 2008). The identity of mycorrhizal fungi could be important to C flux dynamics because different fungi could differ in the energy required for the maintenance and repair of fungal tissue and related cellular processes associated with the absorption, translocation and transfer of nutrients from the soil to the host (Bidartondo et al. 2001, Bryla and Eissenstat 2005). Mycorrhizal fungi, which are widespread and highly variable in type and abundance, thus may substantially contribute to driving variability in soil RS among species-dominated patches and among ecosystems (Langley et al. 2005).

In many ecosystem models, root RS is estimated as a simple function of soil temperature and total root biomass (Chapin and Ruess 2001). The models do not include type of root and coexisting organisms in the root and their functional
ECM identity determines RS and concentrations of N and TNC

The relationships between RS and N and carbohydrates for different ECM morphotypes.

Materials and methods

Plant materials and growing conditions

Seeds of Scots pine (P. sylvestris L.) and acorns of pedunculate oak (Q. robur L.) were sown in 2500 cm³ pots filled with 3:1 (v/v) mix of substrate collected from the top 20 cm of soil collected at a forest plantation in Zwieziec near Kórnik, Poland in pine or oak forests and peat (pH 5.5). Pots were kept in a shadow tent that reduced sunlight by 40% for the first growing season. Pots were kept under full sunlight for the rest of the experiment. Seedlings were fertilized (2 kg/1000 dm³) with granulate Osmocote slow-release (5–6 months) fertilizer containing: 15% N (7% NO₃⁻ and 8% NH₄⁺), 9% P (P₂O₅), 12% K (K₂O) and 2.5% Mg (MgO). Saplings of P. sylvestris were transplanted to 5-dm³ pots in spring in the fifth year of growth. Pots were kept outside throughout the whole year and every winter pots were covered with sawdust to prevent roots from freezing.

Ectomycorrhizal fungal identification and respiration of collected morphotypes

Roots were taken in October and November 2006 from 6–10 3-year-old pedunculate oak and 6-year-old Scots pine. Only visibly healthy saplings were chosen. We excised roots from different parts of the root systems and rinsed them with distilled water. Root tips were examined under a dissecting microscope and classified into mycorrhizal morphotypes based on macroscopic features (Agerer 1987–2003). We found three different morphotypes on both P. sylvestris and Q. robur saplings. DNA extraction, PCR and sequencing of ITS regions were performed as described previously (Trocha et al. 2007). Sequences were compared with sequences deposited in the GenBank and UNITE databases using blastn. Neighbour-joining analyses were conducted using MEGA version 4, applying the Kimura two-parameter model (including transitions and transversions) with 1000 bootstrap replicates.

RS of each morphotype was measured with an oxygen electrode system at 25 °C. Potential differences between the chamber and probe.
We examined six to seven replicates to estimate RS for each morphotype. There was no effect of sample mass on the respiratory rates (data not shown). The buffer used in the chamber contained: 1 mM CaSO₄, 5 mM 2-(N-Morpholino)ethansulfonic acid, adjusted with KOH to pH 5.5. RS (in nanomoles of O₂ per gram per second) was expressed based on the dry mass of each ECM root tip sample.

Measurements of N, C and total non-structural carbohydrate concentrations

N and C in the morphotypes studied were measured for samples of dried (65 °C for 48 h) and powdered tissue. For N and C analyses, the samples were analysed using the Elemental Combustion System CHNS-O 4010 (Costech Instruments, Italy/USA). Total non-structural carbohydrate (TNC) concentrations were determined by a modification of the method described by Hansen and Møller (1975) and Haissig and Dickson (1979). Sugars were extracted from oven-dried (65 °C, 48 h) ground tissue in methanol–chloroform–water, and tissue residues were used for starch content determination. For N, C and TNC measurement, we used the root tip samples of each morphotype used for RS measurements. Because RS can be determined on a smaller sample than the chemical analyses, we randomly selected three to four RS samples of each morphotype and pooled them for the determination of N, C and TNC. This resulted in two samples for each morphotype where N, C and TNC were determined, which were then compared with a weighted estimated of the RS based on the RS of the individual subsamples.

Statistical analyses

For all variables, statistical differences among host species and ECM species/morphotypes were calculated by analysis of variance (GLM procedures). Relationships between the traits studied were made using correlation and regression analyses. All analyses were made using statistical analysis software (JMP version 7.0.2, SAS Institute Inc., Cary, NC).

Results

Morphotyping and molecular identification of ECM fungi

In total, we described three different morphotypes on each host tree species (Table 1). Based on GenBank and UNITE accession numbers, the closest matches for Scots pine morphotypes were: Rhizopogon rubescens (DQ068965) and Rhizopogon roseolus (UDB001619) for morphotype 1, Pezizales species (FJ901319) and Peziza sp. (UDB001572) for morphotype 2 and Tuber sp. (EU379679), Tuber puberulum (UDB00122) for morphotype 3.

Table 1. Description of ECM morphotypes based on Agerer 1987–2003, exploration types based on Agerer 2001 and ECM fungal symbiont identity based on ITS sequencing (n = 2–3).

<table>
<thead>
<tr>
<th>Host tree</th>
<th>Morphotype number</th>
<th>Morphotype description</th>
<th>Exploration type</th>
<th>Best GenBank and UNITE accession numbers</th>
<th>ECM fungal identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. sylvestris</td>
<td>1</td>
<td>White and woolly mantle; white, short, dense extramatrical hyphae; white, dense rhizomorphs; dichotomous, coralloid or tubercle-like</td>
<td>Long distance</td>
<td>DQ068965 R. rubescens; UDB001619 R. roseolus</td>
<td>R. roseolus</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Orange or light brown and irregular mantle; thin ECM root tips, dichotomous or coralloid</td>
<td>Contact</td>
<td>FJ901319 Pezizales, UDB001572 Peziza sp.</td>
<td>Pezizales</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Orange and slightly grainy mantle; ECM root tips robust and wider at the top; dichotomous or coralloid</td>
<td>Contact</td>
<td>EU379679 Tuber sp., UDB001122 T. puberulum</td>
<td>Tuber sp. 1</td>
</tr>
<tr>
<td>Q. robur</td>
<td>4</td>
<td>Orange or brownish smooth mantle; short, loose and whitish extramatrical hyphae; robust and short ECM root tips; short light cystidia</td>
<td>Contact</td>
<td>FM999595 unidentified fungus</td>
<td>Tuber-like</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Beige or light orange, smooth mantle; robust, short ECM root tips; short light cystidia</td>
<td>Contact</td>
<td>FJ554524 T. borchii, UDB001122 T. puberulum</td>
<td>Tuber sp. 2</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Light orange mantle covered with dense white spots of hyphae; dense woolly extramatrical hyphae; long ECM root tips; irregularly pinnate</td>
<td>Short distance</td>
<td>GQ267472 Hebeloma sp., UDB003191 H. cavipes</td>
<td>Hebeloma sp.</td>
</tr>
</tbody>
</table>

We examined six to seven replicates to estimate RS for each morphotype. There was no effect of sample mass on the respiratory rates (data not shown). The buffer used in the chamber contained: 1 mM CaSO₄, 5 mM 2-(N-Morpholino)ethansulfonic acid, adjusted with KOH to pH 5.5. RS (in nanomoles of O₂ per gram per second) was expressed based on the dry mass of each ECM root tip sample.
the latter accession numbers are from the UNITE database for all the above ECM fungal species. Pedunculate oak also had one unidentified symbiont with a sequence that matched the fungus FM9959595 in GenBank but that matched no sequence in the UNITE database. The comparison of ITS sequences allowed us to identify the ECM symbionts as: R. roseolus (=R. rubescens) for morphotype 1, Pezizales for morphotype 2 and Tuber sp. 1 for morphotype 3, all three on P. sylvestris. Morphotype 5 on pedunculate oak was formed by Tuber sp. 2 and morphotype 6 on pedunculate oak was formed by Hebeloma sp. (Table 1). The ECM fungus that formed morphotype 4 on pedunculate oak was unidentified, but was classified as Tuber-like based on ECM morphology. Close phylogenetic relationships of ITS sequences of morphotype 3 on Scots pine and morphotype 5 on pedunculate oak with sequence of T. puberulum from the UNITE database, but with different Tuber species sequences from GenBank, did not allow us to assign exact species identity. Phylogenetic analyses show that ITS sequences from morphotypes 3 and 5 form individual clades with ITS sequences from GenBank and the bootstrap values are higher for these sequences than for those from the UNITE database (data not shown).

RS and N, C and sugar concentrations in different ECM species/morphotypes

Analysis of variance showed that RS of the six ECM species/morphotypes described were significantly different (Table 2). Tuber sp. 1 had the highest RS rate (34.0 nmol O2 g−1 s−1) followed by R. roseolus (28.1 nmol O2 g−1 s−1), whereas the lowest RS rate was noted for Hebeloma sp. (7.3 nmol O2 g−1 s−1; Table 2). Overall, the average RS rate of Scots pine ectomycorrhizas was almost twice that of pedunculate oak (25.4 vs 13.5 nmol O2 g−1 s−1, P = 0.02; Table 2). Within host species, differences in RS among different ECM species/morphotypes were also statistically significant (P ≤ 0.008, Table 2).

Within host species, C/N ratio and N, C, starch, glucose and TNC concentrations differed significantly among the ECM species/morphotypes examined (Table 2). The highest concentration of N was in ectomycorrhizas formed by Tuber sp. 2 on pedunculate oak (24.7 mg g−1), and the lowest concentration of N was in ectomycorrhizas formed by Pezizales on Scots pine (13.1 mg g−1). There were statistically significant differences between ECM species within each host species (P < 0.0001; Table 2). Within each host sapling, ECM species/morphotypes with the highest RS (Tuber sp. 1 for pine and Tuber sp. 2 for oak) also had the highest carbohydrate concentrations. However, overall relationships between carbohydrates and RS within host species were not significant (data not shown). Highly significant correlations (r² ≥ 0.97) were found between carbohydrate concentration and RS rate of different ECM species/morphotypes within each host species (Figure 1).
It is accepted that RS (i.e., RS per unit dry mass or specific RS rate) and mass-based N concentrations are functionally related in leaves, stems and fine roots of higher land plants (Ryan 1991, Burton et al. 2002, Tjoelker et al. 2005, Reich et al. 1996, 2006, 2008). However, it was unknown whether RS–N relationships of mycorrhizal root tips will follow the same pattern. To our knowledge, this is the first study to address whether different ECM fungi occurring in sympo diff er in RS and if these differences within host species are related to N concentration (Figure 1).

It is likely that a RS–N relationship exists in ECM root tips because polymer chitin that composes much of the fungal cell wall contains N. Synthesis of chitin is an energy-consuming process, and ~60% of maintenance RS supports repair and replacement of cell walls (Penning de Vries 1975, Koide and Malcolm 2009). Our measurement of RS is likely mostly related to maintenance RS, as the buffer solution contained no N for ion uptake and growth RS of these fully formed tips was likely low.

It is interesting to note the strong RS–N relationships in ECM root tips within host species and the large difference in those relationships between the two host species (Figure 1). In all cases, the RS at a similar N level was higher for Scots pine than pedunculate oak root tips, indicating that, along with other factors such as growth rate or tissue type (Penning de Vries 1975), host species-related ECM identity may affect the RS–N relationship, although reasons for this are unclear. Clearly, more studies are needed to reveal whether observed differences may be attributed to the taxonomic position of the plant species studied (i.e., angiosperms vs gymnosperms), successional status of symbionts or host trees (early successional pine and late successional oak) or to other factors. The overall RS of ECM communities of both host species differed significantly (P = 0.02), with Scots pine having a higher RS rate by 46%. One literature review of fine root (<2 mm in diameter) RS showed a >10% higher RS rate for gymnosperm than angiosperm tree species (George et al. 2003), but data presented by Reich et al. (2008) showed no differences on average between woody angiosperm and gymnosperm fine root RS. However, those data show a greater RS per unit N in gymnosperms, consistent with our data for Scots pine and pedunculate oak.

Our data also revealed marked differences in RS among ECM fungal symbionts associated with a particular host. Some ECM taxa may be more costly to host plants than others (Gorissen and Kuyper 2000, Lerat et al. 2003). In oak and pine, the highest RS as well as N and carbohydrate concentrations were exhibited by root tips colonized by Tuber sp. (Table 2). Montanini et al. (2002) found that T. borchii mycelium had a high-affinity ammonium transporter that may make this species (and possibly other Tuber species) highly effective in N uptake, and ion uptake RS may be a significant component of total RS (Veen 1980, Bouma et al. 1996). In addition, a high level of nitrate reductase gene expression may lead (apart from those transported to the host plant) to N assimilation into different fungal compounds (Guescini et al. 2003).

Non-structural carbohydrate demand among the ECM species may vary due to differences in fungal structure. Ectomycorrhizas formed by Tuber species were robust, had a thick mantle and were characterized by high TNC concentration, whereas ectomycorrhizas of Pezizales were very thin and delicate (Table 1). The highest RS was observed for Tuber sp. 1 and R. roseolus on Scots pine and for Tuber sp. 2 on pedunculate oak (Table 2). These three morphotypes were characterized by the thickest and well-built mantles. However, Hebeloma sp. had the lowest respiration independent of the very thick extramatrical hyphae. It is likely that, during root preparation, we lost most of the delicate extramatrical hyphae, leading to an underestimate of the RS of this morphotype.

Since a considerable amount of photosynthate is required by mycorrhizas, at least half of which is used for respiratory processes (Bryla and Eisenstat 2005), additional studies would be necessary to answer questions regarding the extent to which observed differences in respiration and carbohydrate concentration among mycorrhizas may affect the C economy of individual plants. The results of our study have shown that, based on N concentration in ECM root tips, it may be possible to identify ECM fungi that are less metabolically expensive for the host species to maintain.

Although grown in very different nutrient conditions (to be taken as a caveat), comparisons with foliage N data from our prior study (Withington et al. 2006) showed that average N concentrations of ECM root tips were higher than in current-year needles for Scots pine (17.5 in roots vs 11.8 mg g⁻¹ N in needles) and similar in pedunculate oak (19.7 in roots vs...
18.6 mg g\(^{-1}\) N in foliage). On average, N concentrations of \(P.\ sylvestris\) and \(Q.\ robur\) ECM morphotypes in our study were roughly 50% higher than those for bulk fine roots (<2 mm in diameter) at the common garden planting in south-central Poland (Hobbie et al. 2007). Such high N concentrations in ECM root tips is most likely related to high enzymatic abilities of ECM fungi and the fact that most of the N pool in roots is involved in metabolically expensive processes such as ion uptake and transport, storage and conversion of sugars (Koide and Malcolm 2009). Because N concentration in ECM tissue is determined mainly by cell wall chemistry, it is relatively insensitive to substrate chemistry (Wallander et al. 2003, Koide and Malcolm 2009). Therefore, differences in ECM root tip N, C concentrations and C/N ratio observed in our study (Table 2) may be relatively stable. On average, C/N ratios of different ECM fungal species were about 25 and were in the range of C/N variation (14–29) of ECM mycelia growing in situ in Norway spruce forest soils (Wallander et al. 2003), indicating that the values seen in pure culture are consistent with those found in ECM morphotypes.

ECM morphotype RS declined with increasing C/N ratio (Table 2). This indirectly suggests that ECM morphotypes that are characterized by slow root RS, low N and high C/N ratios may also have longer life spans. Tjoelker et al. (2005) found that across forbs, grasses and legumes, long root life span was significantly associated with slow root RS, low specific root length and high C-to-N ratios. Similar tendencies between C/N and the life span of first-order and second-order fine roots was also found in a prior study with 11 north-temperate tree species (that included Scots pine and pedunculate oak) in a common garden in central Poland (Withington et al. 2006).

In summary, we have demonstrated significant host species-specific RS–N relationships for root tips with varying ECM fungal symbionts. Our findings suggest that the RS–N relationship is not only broadly robust across species and tissue type—foliage, stems and roots (Reich et al. 2008), but also within a plant species across ECM species associations. Since even small changes in C use in plants can result in large changes in C cycling at larger scales (Rygiewicz and Andersen 1994), our findings suggest that, in modelling studies, N concentration may be a useful surrogate of the contribution of ECM fungi in forests to RS and the C budget. Understanding how mycorrhizal root N concentration and root RS vary across different host and fungal associate species and environmental conditions may be necessary for revealing implications of variation in ECM respiratory activity.

Acknowledgements

This research has been partially supported by the grant PBZ-KBN-087/P04/2003 from the State Committee for Scientific Research (Poland) and National Science Foundation grant DEB-0128958. We are grateful to Roma Zytkowiak and Ewa Maderek for the assistance in chemical analyses.

References


