Forest soil CO$_2$ flux: uncovering the contribution and environmental responses of ectomycorrhizas

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Abstract

Forests play a critical role in the global carbon cycle, being considered an important and continuing carbon sink. However, the response of carbon sequestration in forests to global climate change remains a major uncertainty, with a particularly poor understanding of the origins and environmental responses of soil CO$_2$ efflux. For example, despite their large biomass, the contribution of ectomycorrhizal (EM) fungi to forest soil CO$_2$ efflux and responses to changes in environmental drivers has, to date, not been quantified in the field. Their activity is often simplistically included in the ‘autotrophic’ root respiration term. We set up a multiplexed continuous soil respiration measurement system in a young Lodgepole pine forest, using a mycorrhizal mesh collar design, to monitor the three main soil CO$_2$ efflux components: root, extraradical mycorrhizal hyphal, and soil heterotrophic respiration.

Mycorrhizal hyphal respiration increased during the first month after collar insertion and thereafter remained remarkably stable. During autumn the soil CO$_2$ flux components could be divided into ~60% soil heterotrophic, ~25% EM hyphal, and ~15% root fluxes. Thus the extraradical EM mycelium can contribute substantially more to soil CO$_2$ flux than do roots. While EM hyphal respiration responded strongly to reductions in soil moisture and appeared to be highly dependent on assimilate supply, it did not respond directly to changes in soil temperature. It was mainly the soil heterotrophic flux component that caused the commonly observed exponential relationship with temperature. Our results strongly suggest that accurate modelling of soil respiration, particularly in forest ecosystems, needs to explicitly consider the mycorrhizal mycelium and its dynamic response to specific environmental factors. Moreover, we propose that in forest ecosystems the mycorrhizal CO$_2$ flux component represents an overflow ‘CO$_2$ tap’ through which surplus plant carbon may be returned directly to the atmosphere, thus limiting expected carbon sequestration from trees under elevated CO$_2$.

Keywords: ectomycorrhizas, lodgepole pine, mesh collar, mycorrhizal respiration, $Q_{10}$, root respiration, soil moisture, soil respiration, substrate supply, temperature

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Introduction

The rate of human-induced climate change is critically dependent on the carbon (C) sequestration potential of terrestrial ecosystems, especially forests (Bolin et al., 2000; Liski et al., 2003). It is generally believed that increasing atmospheric CO$_2$ enhances forest photosynthesis (Ainsworth & Long, 2005), resulting in net ecosystem C uptake, thus dampening the rate of hu-
man-induced climate change. However, recent work has questioned whether forests will continue absorbing CO₂ at the current rate, suggesting that stimulation of soil respiration may reduce the sequestration of root-derived C under elevated CO₂ (Heath et al., 2005). Soils store 1500–2000 Gt of organic C (Bolin et al., 2000) and temperate and boreal forest soils alone store 33% more C than the total C storage of tropical forests (Bolin et al., 2000). Each year ~70 Gt of C is respired from soils, equal to ~10% of atmospheric CO₂ and equal to 10 times annual global fossil fuel emissions (Grace & Rayment, 2000). Consequently, over time, even small changes in soil respiration may cause major changes in the net C balance of forests, potentially turning a global C sink into a net C source with feedback implications for the global C cycle (Cox et al., 2000).

However, there is major uncertainty associated with soil respiration responses to global change, as this is a complex flux (Qi et al., 2002), resulting from the activity of roots and their associated mycorrhizal fungi (autotrophic or better mycorrhizosphere respiration), and of free-living microbes and soil animals (heterotrophic respiration). The relative contributions of these flux components and their likely individual dependence on substrate supply and abiotic factors, such as soil temperature and moisture, will ultimately determine future forest C-sequestration potential. There remain clear contradictions in the literature with some studies suggesting that autotrophic respiration is more temperature sensitive than heterotrophic belowground respiration (e.g. Boone et al., 1998) while more recently it has been shown that autotrophic respiration is highly dependent on substrate supply from photosynthesis (Högberg et al., 2001; Lipp & Andersen, 2003) and may show little direct response to changes in soil temperature (Bhupinderpal-Singh et al., 2003). It is therefore critical to understand the magnitude of the different component soil CO₂ fluxes and to identify controlling factors.

In addition, the contributions of different soil biota to soil respiration are largely unknown (Luo et al., 2001) and, given their potential importance in both nutrient and C cycling (Read et al., 2004), the ubiquitous mycorrhizal fungi are an especially poorly quantified group. In northern coniferous forests, which play a major role in the terrestrial C sink (Bolin et al., 2000), primary productivity is especially dependent on the activity of ectomycorrhizal (EM) fungi (Read et al., 2004). Although extraradical EM mycelia can reach 8000 m per metre length of root (Leake et al., 2004), and make up one-third of soil microbial biomass in coniferous forests (Högberg & Högberg, 2002), their contribution to below-ground respiration and response to environmental variables have not been quantified in situ in the field. Moreover, difficulties in separating root from mycorrhizal hyphal respiration in the field have resulted in EM hyphal respiration invariably being included in the ‘autotrophic’ soil flux component. Consequently, root respiration sensu stricto is frequently overestimated, whereas EM fungal activity is largely ignored (Pendall et al., 2004), even though we are beginning to appreciate that mycorrhizal fungi may respond uniquely to environmental change (Staddon et al., 2002; Heinemeyer et al., 2006; Moyano et al., 2007) and elevated CO₂ (Gorissen & Kuyper, 2000; Alberton et al., 2005). If mycorrhizal mycelia represent a substantial component of belowground respiration in forest systems, and respond uniquely to environmental variables, then modelling of belowground respiration may need to be re-evaluated. In this study, we used continuous monitoring of soil CO₂ efflux in a Lodgepole pine (Pinus contorta Douglas ex Loudon) forest with mycorrhizal ingrowth mesh collars as suggested by Hobbie (2006), using a similar design to that developed previously for arbuscular mycorrhizal hyphae in grasslands (Johnson et al., 2001). We aimed to (i) determine whether the respiration of extraradical mycelium contributed substantially to belowground respiration, (ii) compare the relative contributions of root and extraradical mycelium to ‘autotrophic’ respiration, and (iii) investigate whether the different components of belowground respiration differed in their responses to changes in soil temperature, soil moisture and substrate supply from aboveground.

Materials and methods

Site description

The field site, an ~1 ha, 15-year-old Lodgepole pine forest [with scattered silver birch (Betula pendula Roth.),] is located at ~20 m above sea level at 53°54′34″N; 0°59′48″W, approximately 5 miles south of York, United Kingdom, and was chosen for its lack of understorey vegetation, which permitted investigation of the responses of pine roots and associated EM fungi in isolation. The soil type is a well-draining sandy gley soil (Holme Moor series) with a superficial organic layer (O₀ and O₁ to 3 cm deep) overlaying a 3 cm deep Ah horizon (see Fig. 1). Hourly soil temperature in the litter layer, at 5 and 10 cm, soil moisture at 5 cm in the mineral soil were measured on site. Further, 10 min readings of photosynthetically active radiation (PAR), wind speed and rainfall were monitored (all Delta-T Devices, Cambridge, UK) in a clearing at 50 m distance.
Experimental design

We established eight experimental blocks within the pine forest for soil respiration monitoring, a schematic treatment design is shown in Fig. 1. Each block contained two different mesh collar treatments, one that permitted and one that excluded EM extraradical hyphal in-growth; both treatments were designed to exclude roots. Within each block, two 20 cm diameter and 30 cm deep (inserted 25 cm into the soil), open-ended PVC collars (Plumb Centre, Wolseley, UK; Ripon, UK) were established. Each collar had four 5 cm x 5 cm windows cut into their side which were covered with 41 µm mesh which excluded roots but permitted the ingrowth of EM extraradical hyphae; S collars also had four 5 cm x 5 cm windows cut in their side but these were covered in 1 µm mesh which excluded roots and EM extraradical hyphal in-growth). The deep collar did not have any mesh windows. Each block contained one each of the RMS, MS and S collars and two deep collars.

Fig. 1 Diagram showing one of eight blocks with the four different collar treatments and collar insertion depth (cm) in relation to the different soil layers. RMS collars were shallow surface collars, which did not cut any roots, and thus contained all soil respiration flux components i.e. roots (dark lines), ectomycorrhizal (EM) extraradical hyphae (thin lines) and soil heterotrophs (dotted pattern). MS collars had four 5 cm x 5 cm windows cut in their side which were covered with 41 µm mesh which excluded roots but permitted the ingrowth of EM extraradical hyphae; S collars also had four 5 cm x 5 cm windows cut in their side but these were covered in 1 µm mesh which excluded roots and EM extraradical hyphal in-growth). The deep collar did not have any mesh windows. Each block contained one each of the RMS, MS and S collars and two deep collars.
tional contributions of extraradical EM mycelium, root and soil respiration to total soil CO₂ efflux.

Respiration measurements

We measured collar respiration rates immediately after insertion and on two further occasions before the onset of continuous monitoring, using a Li-Cor 8100 soil respiration system (Li-Cor, Lincoln, NE, USA) equipped with a 20 cm survey chamber (Model: 8100-101). Subsequent continuous high frequency monitoring was completed using a Li-Cor 8100 infra-red gas analyser linked to a custom-built multiplexed gas handler multiplexing unit (Electronics Workshop, Biology Department, University of York, UK), with automatic opening and closing chambers measuring up to 12 collars on an hourly cycle. This allowed continuous monitoring of four replicates for each treatment during Campaigns 1 and 2 but only three each during Campaign 3. As the chambers remained open, apart from 1.5 to 2.5 min h⁻¹ when fluxes were measured (depending on seasonality of flux rates), rainfall could enter the collars and soil temperature was unaffected by the presence of the chamber. The air temperature and humidity inside each chamber were also recorded. As only 12 chambers were available, the eight blocks were divided into two four-block sets (Set 1: blocks 1-4 and Set 2: blocks 5-8), which were rotated between at regular 1- to 2-week intervals between Campaigns 1 and 2. Campaign 3 used blocks 1, 3 and 4 and ran until 30 December 2005 (these blocks were chosen to give an EM extraradical hyphal flux contribution representative of the mean of all the blocks measured in Campaigns 1 and 2). Chambers were rotated after and during rainfall by 90° as to avoid dry patches developing under the open chamber position.

Validation of soil only flux measurements at the S collars

Using collars of limited depth will not exclude all the roots as they only cut to the insertion depth. Soil profiles dug on site confirmed that our 30 cm deep collars cut nearly all fine and most horizontal roots (only thick lateral and tap roots penetrated much deeper than 25 cm). However, to verify successful exclusion of root respiration from the mesh collars, on 29 April 2005 we established two additional deep collars (75 cm, 10 cm diameter, no mesh) in each block which cut all remaining horizontal roots within the soil profile as this was the depth beyond which there is a very hard (compacted) C horizon (Fig. 1). Respiration rates at all deep collars were monitored manually on six occasions between 17 May and 3 August using a second Li-Cor 8100 connected to a 10 cm survey chamber (Model: 8100-102).

The collar which most reflected the mean of all deep collar measurements, was selected for short periods of continuous monitoring, leaving the manual survey system in place during periods with no or limited rainfall. Further, this comparison enabled to test for a possible flux artefact as roots and fungi growing against the mesh outside might cause an increased flux.

Data analysis

Flux components. For Campaign 3, the respiration rate at the RMS collars was considered to represent total below-ground respiration (see Fig. 1) and the contributions of roots [Eqn (1)], EM extraradical fungi [Eqn (2)] and soil heterotrophic respiration [Eqn (3)] were calculated as follows:

\[
\text{Root\%} = \left( \frac{\text{RMS}_{\text{resp}} - \text{MS}_{\text{resp}}}{\text{RMS}_{\text{resp}}} \right) \times 100, \quad (1)
\]

\[
\text{EM\%} = \left( \frac{\text{MS}_{\text{resp}} - \text{S}_{\text{resp}}}{\text{RMS}_{\text{resp}}} \right) \times 100, \quad (2)
\]

\[
\text{Soil\%} = \left( \frac{\text{S}_{\text{resp}}}{\text{RMS}_{\text{resp}}} \right) \times 100, \quad (3)
\]

where Root\% is the percentage contribution of roots, EM\% is the percentage contribution of EM fungal extraradical hyphae, Soil\% is the percentage of soil respiration, and where RMS\text{resp} is the mean rate of respiration at the RMS collars, MS\text{resp} is the mean rate of respiration at the MS collars and S\text{resp} is the mean rate of respiration at the S collars.

Temperature sensitivity and environmental data. During Campaign 2 (9 September to 11 October), when temperature explained a large proportion of the variance in below-ground respiration and the relationship was clearly exponential, a \(Q_{10}\) model [Eqn (4)] was fitted to the respiration data with the values of \(R_{10}\) and \(Q_{10}\) that minimized the sum of the squared deviation from the model being identified using the ‘Solver Tool’ in MS Excel. The sum of squared deviation from the model and the total squared deviation from the mean were used to calculate an \(R^2\) value for the exponential relationships:

\[
R_t = R_{10} \times Q_{10}^{(T-10)/10}, \quad (4)
\]

where \(R_t\) is the rate of respiration at a measured soil temperature \(T\), \(R_{10}\) is the respiration rate at 10 °C and \(Q_{10}\) is the increase in the rate of respiration for a 10 °C increase in temperature.

For the analysis of diurnal cycles of CO₂ release from the different collar types, mean hourly rates of respiration and soil temperature were calculated across days for Sets 1 and 2 in Campaign 1 and for all
four blocks during Campaign 2 (e.g. the rate of respiration recorded between 00:00 and 01:00 hours each day was averaged across the 32 days in Campaign 2). Campaign 2 was split into days of high EM fungal activity (days during which $M_{\text{diff}}$ was greater than the period average) and low activity (days during which $M_{\text{diff}}$ was below the period average). It should be noted that, during Campaign 1, EM fungal activity was generally high when Set 1 was monitored and low when Set 2 was monitored, likely due to the timing of a ‘drought’ event.

Statistical analysis

Statistical analyses were carried out using SPSS (Version 11, SPSS Science, Birmingham, UK) with Kolmogorov–Smirnov and Levene’s tests being used to test for normality and homogeneity of variances. All data were normally distributed within a collar treatment or flux component (e.g. $M_{\text{diff}}$). Two-way analyses of variance (ANOVAs) on collar treatment and block were carried out before continuous-monitoring commenced, in order to determine whether the respiration rates at the different collar treatments differed on the three measurement dates. Repeated-measures ANOVAs were used to determine whether the respiration rates in the different collar treatments changed over time.

During the ‘drought’ period in Campaign 1, repeated-measures ANOVAs, on the daily mean respiration rates, were used to determine whether there was a significant effect on the rate of respiration at the different collar treatments. To investigate the response of respiration to the rainfall ending the drought, repeated-measures ANOVAs were carried out, on the different respiration rates and the contribution of $M_{\text{diff}}$ for the 24 measurements made during 28 July. For the diurnal cycle investigations, repeated-measures ANOVAs, with collar treatment as the between-subject factor, were used to determine whether the respiration rates changed significantly over the course of a day and whether there were differences between collar treatments. Linear regressions were used to investigate the relationship between the diurnal cycles of soil temperature and the respiration rates at the different collar treatments, and the relationship between the diurnal cycles of soil temperature and $M_{\text{diff}}$.

Results

Establishment of mycorrhizal mesh collars

Immediately after establishment (18 May 2005), there were no significant differences in CO$_2$ efflux between treatments (Table 1). However, 21 days later, the CO$_2$

<table>
<thead>
<tr>
<th>Date</th>
<th>Respiration rate (μmol CO$_2$ m$^{-2}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 May</td>
<td>1.38 ± 0.07</td>
</tr>
<tr>
<td>6 June</td>
<td>1.72 ± 0.10</td>
</tr>
<tr>
<td>23 June</td>
<td>1.82 ± 0.13</td>
</tr>
</tbody>
</table>

Mean respiration rates ± 1 SE ($n = 8$) are shown. Within a date, rates labelled with a different letter differ significantly (paired samples t-test, $P < 0.05$).

Table 1 The rate of respiration measured at the MS and S collars immediately following establishment and on two further dates before continuous monitoring began

<table>
<thead>
<tr>
<th>Campaign</th>
<th>RMS</th>
<th>MS</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.93 ± 0.13</td>
<td>1.49 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.48 ± 0.19</td>
<td>1.12 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.07 ± 0.08</td>
<td>0.98 ± 0.15</td>
<td>0.70 ± 0.04</td>
</tr>
</tbody>
</table>

Mean rates of respiration and standard errors are shown (Campaign 1: $n = 8$, Campaign 2: $n = 5$, Campaign 3: $n = 3$).

The higher rate of respiration at the MS collars relative to the rate at the S collars was maintained throughout all three campaigns (Table 2). Partitioning of fluxes identified a substantial contribution of EM extraradical hyphae. Figure 2 summarizes treatment changes in respiration rates over time during the three measurement campaigns and also provides an example of the high frequency data that was obtained from this respiration system during a 4-day period in late June/early July. During Campaigns 1 and 2, mean extraradical hyphal respiration contributed ~25% of soil respiration at the MS collars (Table 2; Fig. 2) and Campaign 3 revealed proportional contributions of ~65% from soil, ~25% from extraradical hyphae and ~10% from roots (Figs 2 and 3). However, whereas the EM hyphal contribution tended to decrease after the main fruiting body production in late autumn (Fig. 1), root CO$_2$ flux contributions increased towards around 15% but never exceeded the extraradical hyphal contribution. Consequently, the ‘autotrophic’ flux...
component remained fairly constant at around 35% throughout Campaign 3 (maximum and minimum ‘autotrophic’ contributions of 46.3% and 26.2%, respectively, were observed during this campaign but no clear seasonal trend was detected; Fig. 3).

Environmental control over component soil respiration fluxes

Clear relationships between the flux rates of the different components and the measured climatic variables were identified. During Campaign 1, the contribution of extraradical hyphae ranged between 0% and 45% of total soil efflux (excluding root respiration, i.e. MS collar respiration) but was unrelated to soil temperature; temperature explained little of the variance in either of the two treatments ($R^2 < 0.25$), probably due to low rainfall during this period. The only slight exception was for litter temperature and S collar respiration in Set 1 ($P < 0.001$, $R^2 = 0.33$). Further, during Campaign 2, although there was a strong exponential relationship [Eqn (4)] between soil temperature (at 5 cm) and respiration in the S ($R^2 = 0.88$) and MS ($R^2 = 0.68$) treatments (Fig. 4), extraradical hyphal respiration ($M_{diff}$) was unaffected by soil temperature ($R^2 < 0.01$; Fig. 4). This produced a lower $Q_{10}$ value for MS collar respiration compared with S collar respiration.

In marked contrast, soil moisture content was found to be critical in controlling EM extraradical mycelial activity, limiting the rate of below-ground respiration during a ‘drought’ period. After 7 July, there were 18

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**Fig. 2** Mean hourly respiration rates at the MS (■) and S (○) collars as measured over each of the three Campaigns, including RMS collar measurements (□) in Campaign 3. Inset shows a typical run of high frequency measurements h (hourly mean values) made at MS (■) and S (○) collars during Campaign 1 between 29 June and 3 July. Error bars represent ±1 SE (n = 4). The mushroom symbol indicates main basidiomycete fruiting body activity observed at the site.

**Fig. 3** Relative contributions of root (□), ectomycorrhizal extraradical fungal (■) and soil (○) respiration during Campaign 3 between 25 October and 29 December. Contributions are calculated as a percentage of the mean rate at the shallow (RMS) collars (n = 3). The mushroom symbol indicates main basidiomycete fruiting body activity observed at the site.
consecutive warm days without rainfall, which caused a large drop in soil moisture to <15%. This resulted in a reduction in the respiration rate at all collar treatments \((P<0.001)\). However, extraradical hyphal respiration appeared to be more affected than soil respiration. During Campaign 1, the extraradical fungal contribution \((M_{\text{diff}})\) to soil respiration fell significantly from \(\sim 25\%\) to <10% during this ‘drought’ period \((P<0.001; \text{Fig. 5})\). However, when the ‘drought’ finally ended, a rapid and highly significant increase in the contribution of \(M_{\text{diff}}\) was observed \((P<0.001)\). The contribution of \(M_{\text{diff}}\) increased from \(\sim 10\%\) to >25% within 2–5 h of the rain commencing and continued to rise to >30%, 11 h after the rain began. However, soil moisture at a soil depth of 5 cm did not increase as rapidly as \(M_{\text{diff}}\) \((P<0.001; \text{Fig. 5})\). Further, \(M_{\text{diff}}\) correlated significantly with chamber air relative humidity \((P = 0.003, R^2 = 0.31)\).

\[\text{Diurnal cycles of respiration}\]

The high frequency of measurements made in this experiment allowed the different diurnal cycles of respiration at the different collar treatments to be investigated. Clear diurnal cycles in the measured respiration rates at the different collar types were observed \((P<0.001)\). However, the pattern of diurnal cycle often differed significantly between the different collar treatments. During Campaign 1, when Set 1 was measured highly significant changes in the rate of respiration were observed over the course of a day at both collar treatments \((P<0.001)\). In addition, a highly significant interaction between collar treatment and time of day was also identified \((P<0.001)\), indicating significant differences in the pattern of diurnal variation in the rate of respiration at the different collar treatments. When Set 2 was measured, significant diurnal cycles were again observed \((P<0.001)\) but did not differ between collar treatments \((P = 0.977)\). Again, it should be emphasized...
that due to the timing of the ‘drought’ event, EM fungal activity was generally low during Set 2 monitoring in Campaign 1, which may explain the lack of a significant interaction term between collar treatment and time of day. In Campaign 2 significant differences in the diurnal cycles of respiration at the MS and S collars were also observed during days of high EM fungal activity ($P = 0.009$) but not on days of low EM fungal activity ($P = 0.817$). Taking the results from Campaigns 1 and 2 together, it appears that differences in the diurnal cycles of respiration at the two collar treatments were only observed when extraradical hyphal activity was high.

When Set 1 was measured during Campaign 1, the diurnal cycle of $S$ respiration followed the diurnal cycle of temperature at a depth of 5 cm ($R^2 = 0.67$). However, temperature explained much less of the variation in the rate of respiration at the MS collars, although a significant correlation with temperature at 10 cm was observed ($R^2 = 0.30$). However, no significant relationship between $M_{\text{diff}}$ and soil temperature was observed ($P = 0.442$). When Set 2 was measured, both collar treatments showed highly significant correlations between respiration rate and the soil temperature at 10 cm ($MS: R^2 = 0.94; S: R^2 = 0.89$), suggesting that the layer where most of the CO$_2$ was being produced was deeper in the soil during the ‘drought’ period with low $M_{\text{diff}}$ flux rates.

During Campaign 2, on days when EM fungal activity was low, soil temperature at 5 cm explained a large proportion of the daily variation in $MS$ collar respiration ($P < 0.001$, $R^2 = 0.70$). In contrast, on days of high extraradical hyphal activity (Fig. 6), diurnal cycles of respiration correlated more strongly with soil temperature at the $S$ collar compared with the $MS$ collars ($R^2$ values: 0.63 and 0.33, respectively) and $M_{\text{diff}}$ was unaffected by temperature ($R^2$ value: 0.01). Further, clear differences in the temperature responses during daylight vs. night-time hours were observed for $MS$ collar respiration and $M_{\text{diff}}$ (at a given temperature, night-time fluxes were generally greater than daylight fluxes) but not for $S$ collar respiration (Fig. 6). Consequently, factors other than temperature appear to have been controlling the diurnal cycling of extraradical hyphal respiration (i.e. $M_{\text{diff}}$) at the $MS$ collars.

**Validation of soil only fluxes with deep collars**

Figure 7 shows the comparison of the mean hourly respiration rates measured at the 30 cm diameter $S$ mesh collars (excluding roots and mycorrhizas) and the selected deep collar (without mesh windows) during the period 30 October to 9 November 2005. There was very good agreement between the rate and diurnal cycles of respiration at the $S$ and deep collars. This confirmed that no roots grew through the mesh or entered the $S$ mesh collars from below and that any root or hyphal growth along the outer meshes did not increase measured fluxes.

**Discussion**

Mesh collars and the contribution of mycorrhizal fungal respiration

Laboratory studies (Rygiewicz & Andersen, 1994; Bidartondo et al., 2001) and the sparse field estimates...
of EM mycelial biomass (Högberg & Högberg, 2002) have hinted at a substantial EM fungal component in forest soil CO₂ flux. Recently, Fahey et al. (2005) attempted to estimate the contribution of mycorrhizal fungi and root exudation to total below-ground respiration in a hardwood forest. Using an indirect mass balance approach they estimated a highly uncertain contribution of ~12% mycorrhizal fungal flux. Our use of mycorrhizal mesh collars, coupled with high-frequency belowground respiration measurements, represents an important way forward for quantifying the relative rates of the key soil respiration components and their proportions in the field. For the first time we were able to directly measure the contribution of the respiration of EM mycelium to belowground respiration in the field. This partitioning method and the importance of using shallow collars to avoid severing roots, does not appear in the most recent soil respiration methodology review (Kuzyakov, 2006).

Our data unequivocally demonstrate the key role of the EM extraradical mycelium in the autotrophic soil CO₂ flux components of forest C cycling. The mesh collars showed a rapid colonization by EM fungi (MS collars showed 22% higher respiration rates 37 days after insertion), which supports published EM extraradical mycelia growth rates of up to 10 mm day⁻¹ (Donnelly et al., 2003; Leake et al., 2004). Throughout the first two campaigns, the proportional contribution of extraradical mycelium to below-ground respiration was ~25%. In Campaign 3, the contribution of EM fungi was especially high during the period of peak fruiting body production in late October and early November (~35%; Fig. 3). Moreover, although the overall soil respiration declined with lower soil temperatures in autumn, the ‘autotrophic’ respiration component (mycorrhizal roots and extraradical mycelium) stayed relatively constant at ~35% throughout. However, our mesh collars did not include the EM mantle tissue respiration; therefore, our EM fungal flux estimates might still have been underestimated. If there were any seasonal changes in the relative contributions of EM soil hyphae and mantle tissue to total EM fungal respiration, this error may not be constant. However, no technique currently exists to separate root and EM fungal mantle respiration and due to their major role in nutrient uptake, respiration of the soil exploring extraradical mycelium can be expected to be much higher than that of the mantel. Furthermore, the overall soil heterotrophic component was around 65%, which agrees well with a recent meta-analysis (Subke et al., 2006).

We consider these mean contributions to be representative of the situation throughout the year as during the first month of Campaign 3 soil temperatures remained high (approximately 11 °C at 5 cm depth) and root growth (i.e. new white root tips) was still observed in the monthly observations made throughout. In addition, the extraradical hyphal contribution was relatively constant between late June and late November (Fig. 2; Table 2). Published data suggest that the contribution of the ‘autotrophic’ soil respiration component does not decline substantially in the autumn in coniferous forests (Cisneros-Dozal et al., 2006); clearly, EM fungi are a major component of soil respiration and may in fact dominate the ‘autotrophic’ component, at least at certain times of the year. The importance of EM fungi might explain why scaled-up respiration measurements, using excised (mycorrhizal-deprived) roots, show low estimates of ‘autotrophic’ respiration when compared with girdling and trenching experiments, which include intact EM mycelia (Simard et al., 1997; Högberg et al., 2002).

**Environmental control of the mycorrhizal flux component**

The lack of an EM fungal respiration temperature response is in direct conflict with the short-term (1–3 days) laboratory study of Bååth & Wallander (2003), which noted that the temperature sensitivities of rhizosphere (including EM fungi) vs. soil heterotrophic respiration do not differ. However, longer time series of field measurements (2–3 weeks) show that autotrophic respiration is relatively unaffected by changes in soil temperature (Bhupinderpal-Singh et al., 2003; Olsson et al., 2005). Moreover, as heterotrophic soil respiration appeared to depend strongly on soil temperature (Fig. 4), our study demonstrates that the different components of soil respiration do not respond identically to changes in temperature.

The most likely explanation for the decoupling of the diurnal cycle of EM extraradical hyphal respiration and diurnal cycle of soil temperature (Fig. 6) is that fluctuations in the supply of above-ground photosynthates to the rhizosphere determines the rate of EM fungal activity, as suggested previously for autotrophic respiration in an oak savanna ecosystem (Tang et al., 2005) and arbuscular mycorrhizal fungal respiration (Heinemeyer et al., 2006; Moyano et al., 2006). In fact, our data suggest that the temperature independent dial variation in soil respiration observed in the study of Liu et al. (2006) was probably caused by the EM flux component. In ponderosa pine, white root respiration has been shown to respond to fluctuations in substrate supply from photosynthesis over a 24 h period (Lipp & Andersen, 2003) and, in a laboratory experiment, the severing of links between EM fungi and roots and between roots and shoots, suggested that EM fungal respiration may be even more dependent on substrate supply than root respiration (Söderström & Read, 1987). Further, studies
using $^{14}$C pulse labelling have indicated that EM fungal activity is highly dependent on the substrate supply from roots (Leake et al., 2001) and it has also been shown that EM fungal fruiting body production depends on the recent rate of photosynthesis (Högberg et al., 2001; Kuikka et al., 2003). Consequently, the soil CO$_2$ flux is a combination of a temperature sensitive component (soil heterotrophs) and a more photosynthetic dependent flux component (EM mycelia); thus applying a $Q_{10}$ function to model total soil respiration becomes questionable.

Interestingly, the extraradical mycorrhizal fungal flux component was highly sensitive to soil moisture changes and seems to be an important factor explaining the reduction in overall soil respiration during drought periods (Fig. 5). We observed a threshold of about 15% soil moisture in the top mineral soil layer below which EM fungal activity was inhibited considerably. Other studies have also reported a higher soil moisture sensitivity of the autotrophic than the heterotrophic soil respiration component (Andersen et al., 2005; Sulzman et al., 2005). This is perhaps not surprising due to the fact that EM fungi tend to be most abundant near the soil surface and are thus particularly vulnerable to low soil moisture (Read et al., 2004). In fact, a recent study by Querejeta et al. (2007) shows a decline in hyphal densities under drought in the top soil. In our case chamber air relative humidity correlated with $M_{diff}$ fluxes, which probably reflects the moisture content of the litter and upper soil layers. However, litter layer humidity is difficult to measure directly without disturbance but seems to determine mycorrhizal hyphal activity more than deeper soil moisture. In addition, we detected a near instantaneous increase in EM extraradical fungal respiration after rainfall. This supports earlier findings by Pigott (1982) that certain EM fungi can withstand desiccation in an inactive state, making them ready to respond quickly to rewetting, possibly due to trees providing water to maintain EM mycelia during short periods of drought (Querejeta et al., 2003). In addition, the fact that mycorrhizal fungi responded so rapidly to the rain event, and largely before soil moisture at 5 cm had increased, also suggests that the moisture content of the surface soil layers is especially important. Without the high frequency of measurements made possible by the multiplexed Li-Cor 8100 system, this response would not have been observed.

The role of mycorrhizal fungal respiration for future terrestrial carbon storage

Plant responses are likely to become increasingly nutrient, rather than C, limited under elevated CO$_2$ and, therefore, more C may be allocated to EM mycelia. A recent meta-analysis demonstrated that EM hyphal growth is positively affected by elevated CO$_2$ (Alberton et al., 2005; not including Godbold et al., 2006) and, critically, it was found that the activity of extraradical mycelium was stimulated more than the overall plant response. In addition, when nutrient limitation has been relaxed in coniferous forests, soil respiration has been shown to decline (Olsson et al., 2005; Rodeghiero & Cescatti, 2006). The possibility of altered assimilate supply to EM mycelia may have major effects on below-ground C cycling and storage, such as increased EM fungal respiration (Gorissen & Kuiper, 2000) and the potential for rapid turnover of mycorrhizal C, as shown for arbuscular mycorrhizal fungi (Staddon et al., 2003). These results, taken together with the high rate of EM extraradical hyphal respiration identified here,
make it highly likely that increased EM hyphal respiration, linked to plant nutrient demand, is involved in the reduced sequestration of root-derived C observed under elevated CO₂ (Heath et al., 2005), even under observed increased hyphal densities (Godbold et al., 2006). In summary, EM mycelia respiration may represent a potential CO₂ ‘overflow tap’, through which any surplus plant C may be returned directly to the atmosphere, thus limiting expected C sequestration from trees under elevated CO₂ (Heath et al., 2005). Our findings highlight that further research is needed to clarify, whether increased EM activity does indeed limit C sequestration in forest ecosystems.

Conclusion

Our work outlines a new methodological approach for measuring respiration of EM fungal mycelium in situ, in the field. Together with our increasing understanding of mycorrhizal fungal responses to environmental variables and predicted global environmental change, these data highlight the need to re-think the way in which the flux components of forest soil respiration are studied and modelled. Our data support a number of recent studies (Fitter et al., 2000; Alberton et al., 2005) calling for a more ‘mycocentric’ approach; it is becoming increasingly clear that this poorly understood group of symbiotic-organisms plays a critical role in the global C-cycle. Rhizosphere C-cycling models may need to be adapted to reflect that root exudation and turnover are not the only major sources of soil-respired CO₂ and their seasonal variation using a unique radiocarbon tracer. Global Change Biology, 9, 1788–1791.

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FOREST SOIL RESPIRATION FLUX COMPONENTS


