Do nutrient additions alter carbon sink strength of ectomycorrhizal fungi?

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Summary

• Carbon sink strength differences are examined here between ectomycorrhizal fungi in interaction with additions of ammonium and apatite (a phosphorus- and calcium-containing mineral).
• Pinus muricata associated with Paxillus involutus and four suilloid isolates (Suillus pungens and members of three Rhizopogon section Amylopogon species group) were used in microcosm nutrient addition experiments.
• The associations differed in ectomycorrhizal biomass, mycelial growth rate, biomass and respiration. Paxillus involutus produced the lowest biomass of ectomycorrhizal connections to Pinus muricata, but it consumed proportionally more carbon per connection and transferred more than twice as much ammonium to the host per unit mycorrhizal biomass. Paxillus also colonized the soil more rapidly and intensely than the other fungi, but its mycelial respiration was lowest. Ammonium and apatite addition resulted in a marked increase in respiration and mycelial biomass, respectively, by the suilloid fungi.
• The high carbon cost of ammonium uptake is suggested as one explanation for reduced sporocarp production and mycelial growth by ectomycorrhizal fungi commonly found after high levels of nitrogen addition.

Key words: ammonium, apatite, carbon, ectomycorrhiza, mycelium, respiration.

Introduction

Mycorrhizas are strong sinks for photosynthetically-derived carbon (Smith & Read, 1997). However, carbon budget comparisons between different ectomycorrhizal (EM) associates of a single host species using a common experimental system have not been previously reported. Two carbon budgets for isolates of single EM species have been estimated (Rygiewicz & Andersen, 1994; Ek, 1997). However, species comparisons are essential to understand specialization, diversity, and successional dynamics in ectomycorrhizal systems. In this study, we compared carbon budgets of Pinus muricata associated with Suillus pungens, members of three Rhizopogon section Amylopogon species groups, or Paxillus involutus. We used mycorrhizal microcosms modified from the original design by Ek (1997) to study host carbon allocation to EM fungi under the influence of fertilization with nitrogen and apatite (a phosphorus- and calcium-containing mineral).
are the exclusive associates of the two largest myco-heterotrophic monotrope plants, Sarcodes sanguinea and Paxillus involutus (Monotropoideae, Ericaceae) (Kretz et al., 2000). The monotropes are nonphotosynthetic and depend entirely on their fungal associate for carbon, which is ultimately derived from neighbouring green plants (Björkman, 1960). Callings et al. (1996) have speculated that these fungi have been targeted by the monotropes because they have a high carbon sink strength. *Paxillus involutus* is a cosmopolitan generalist EM fungus, but little is known of its ecological relationships. Ek (1997) reported comparable host carbon demand by *Hebeloma crustuliniforme* (c. 7%) (Rygiwiec & Andersen, 1994), Colpaert et al. (1992) compared extramatrical fungal biomass of *P. involutus*, *Suillus luteus* and *S. bovinus* in a soilless system and found that four out of five *Suillus* isolates produced higher biomass than *P. involutus*. Differences in growing conditions and plants between these studies makes comparisons difficult.

The resource unit of an EM fungus is the individual host root tip (Deacon & Fleming, 1992) and thus ectomycorrhizal root biomass constitutes the focus of the vast majority of recent ecological studies. Högström (1989) reported nitrogen flow rates into EM roots of *Pinus sylvestris* and *Picea abies* as only a per root tip measure. The growth forms of EM roots are hardly comparable among EM fungi, and keeping with the recent mycorrhizal literature, we chose to compare on the basis of EM root tip biomass. Carbon budgets per colonized root biomass may be similar among EM fungi but the proportional investment on basidiocarp production (or in supplying carbon to an epiparasite) vs vegetative growth could vary. Alternatively, specialist suillid fungi may have different abilities to obtain carbon per mycorrhizal connection (i.e. carbon sink strength) than nonsuillid generalists and this is then reflected in different allocation patterns of reproduction vs vegetative growth. Furthermore, fungi with high carbon sink strength may or may not return more nutrients to their host per mycorrhizal connection.

The two nutrient elements most commonly found to be limiting in conifer forest systems are nitrogen and phosphorus. In particular, the ability of EM fungi to weather phosphorus-containing minerals has been shown to vary among EM fungi (Wallander, 2000). Nitrogen assimilation is a process requiring large amounts of energy and carbon skeletons for amino acid synthesis. Wallander (1995) suggested that the uptake of ammonium by EM fungi would lead to less carbon available for vegetative growth. This change in carbon allocation within a fungus could explain the reduced growth of extramatrical mycelium and formation of sporocarps by ectomycorrhizal fungi observed after high levels of nitrogen addition (Wallander, 1995; Wallander & Kortke, 1998). In this study, we tested the hypotheses that there are carbon sink strength differences between EM fungi and that carbon sink strength responds to ammonium and apatite additions.

### Materials and Methods

#### Mycorrhizal synthesis

Ectomycorrhizal associations were synthesized (Finlay et al., 1988) between *Pinus muricata* (Pinaceae) seedlings and the following fungi: *Suillus pungens* (Boletales) isolated from a basidiocarp collected in a *P. muricata* forest at Point Reyes, California, USA; *Rhizopogon* 2272 (Boletales, species group I in Kretz et al., 2000) isolated from an ectomycorrhiza formed in a seedling bioassay of soil collected in a *P. muricata* forest at Pt. Reyes; *Rhizopogon* 378 (species group IV in Kretz et al., 2000) isolated from a monotropoid mycorrhiza of *P. sylvestris* (Ericaceae) in a *P. ponderosa* (Pinaceae) forest at the Sierra National Forest, California; *Rhizopogon* 571 (species group II in Kretz et al., 2000) isolated from a monotropoid mycorrhiza of *Sarcodes sanguinea* (Ericaceae) in an *Abies magnumifica* (Pinaceae) forest at the Sierra National Forest, and *Paxillus involutus* 86.017 (Boletales) (same isolate used by Ek et al., 1994, 1997).

In this study, we refrain from using the inadequate current specific epithets available within *Rhizopogon* section *Amylophorus* (M. I. Bidartondo & T. D. Bruns, unpublished) or those provisionally used by Kretz et al. (2000). Isolates are maintained in the Bruns Laboratory collection at Berkeley or at the Department of Microbial Ecology collection at Lund.

#### Mycorrhizal microcosms

Each mycorrhizal or nonmycorrhizal seedling (to be used as a control) was transferred to a transparent polystyrene microcosm made from a TC Dish 245 × 245 × 25 mm (Nunc A/S, Roskilde, Denmark). These microcosms (Fig. 1) are a variation of those used by Ek (1997). Plexiglas® barriers (1 cm wide) were used to divide each microcosm into one root compartment (which could also include mycorrhizas and mycelium) and two fungal compartments (from which roots were excluded). Three narrow perforations (1.5 mm diameter) through the root barrier between the root compartment and each fungal compartment allowed the mycorrhizal mycelium to connect them. Once one perforation per fungal compartment was crossed by the mycelium, the other two were plugged. Sandy soil collected from a *Pinus sylvestris* stand served as substrate (Ek et al., 1994). We sieved the soil through a 2.5-mm mesh and microwaved it to 90°C twice with a 2–3-d interval to eliminate propagules from mycorrhizal fungi. Afterwards, the soil was not maintained under aseptic conditions. The microcosms were wrapped in aluminium foil, placed inside a ventilated plastic bag, and maintained at 200 µmol m⁻² s⁻¹ photosynthetically active radiation (PAR), 80% rh, and an 18/6 h, 18/16°C, day/night cycle.

#### Experimental conditions

Within 4 months, the mycorrhizal mycelium had colonized most of the root compartment, and within 8 months approximately...
half of all the fungal compartments had been invaded. Chambers with fungal compartments colonized were selected and transferred to a constant temperature chamber at 18°C, approx. 200 µmol m⁻² s⁻¹ PAR, and 14/10 h day/night cycle. We replaced the soil in the fungal compartments with 75 g of uncolonized soil. A 1.5-ml plastic container was placed in each compartment to be used as a CO₂ trap. One day after transfer, we sealed the polystyrene microcosm lid with Terostat VII Sealing Profile (Teroson AG, Heidelberg, Germany), so that each compartment was gas-tight from the others. Sealed perforations (2 mm diameter) in the lids allowed sampling gases, exchange of solutions in CO₂ traps, and addition of water (using a syringe) to maintain soil moisture. We filled the CO₂ traps with 1.0 ml 1.5 M NaOH solution. We set up soil-only controls in completely sealed fungal compartments without fungal mycelium. The numbers of microcosms and fungal compartments are listed in Table 1. On the fourth day after sealing the microcosms, one of the two fungal compartments in a microcosm received 1.0 ml distilled water while the other received either apatite mineral or ammonium. The ammonium (1.0 mg N as (NH₄)₂SO₄) was dissolved in 1.0 ml of distilled water. The ammonium sulphate was 98% ¹⁵N (Cambridge Isotope Laboratory, Andover, MA, USA). The apatite mineral (25 mg of apatite mineral ground to a particle size of 50–250 µm, 160 P mg g⁻¹) was suspended in 1.0 ml of distilled water. Solutions were applied by spraying over the entire area of the fungal compartments. In a preliminary experiment adding apatite to soil-only boxes (n = 15), we found that this amount and particle size range produced a negligible pH effect over a 10-day period. Microcosms with only one fungal compartment colonized and the soil-only control compartments received 1.0 ml distilled water each. To estimate fungal growth rate, the expanding edge of the mycelium in the fungal compartments was manually traced on the lid every 2 d after sealing the chambers.

Gas analysis

The NaOH solution in each CO₂ trap was collected and replaced daily using a syringe, and immediately injected into a 15-ml N₂-flushed sample vial containing 5 ml of 1.5 M H₂SO₄. The vials were equilibrated for 24 h before analysis. CO₂ concentration in the headspace of each vial was determined by gas chromatography with an HP6890 GC System (Hewlett Packard, Palo Alto, CA, USA) using helium as the carrier gas. We estimated daily respiration from the difference

Table 1 Number of microcosms used in this study

<table>
<thead>
<tr>
<th>Microcosms</th>
<th>Fungal compartments</th>
<th>Apatite controls</th>
<th>Apatite addition</th>
<th>NH₄ controls</th>
<th>NH₄ addition</th>
<th>No treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhizopogon 2272</td>
<td>6</td>
<td>12</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Rhizopogon 371</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Rhizopogon 378</td>
<td>7</td>
<td>14</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Paxillus involutus</td>
<td>9</td>
<td>18</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Suillus pungens</td>
<td>8</td>
<td>14</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>nonmycorrhizal</td>
<td>3</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>soil-only</td>
<td>6</td>
<td>12</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

¹Pinus muricata mycorrhizal microcosms with two fungal compartments colonized were separated into apatite and NH₄ microcosms. On the fourth day after sealing the microcosms, one fungal compartment in the apatite microcosms received 1.0 ml of distilled water (apatite control) and the other 25 mg of apatite mineral suspended in 1.0 ml of distilled water (apatite addition). In the NH₄ microcosms, one fungal compartment received 1.0 ml of distilled water (NH₄ control) and the other 1.0 mg N as (NH₄)₂SO₄ dissolved in 1.0 ml of distilled water (NH₄ addition). Soil-only control microcosms were treated as above. Some microcosms with only one fungal compartment received 1.0 ml distilled water (no treatment). Nonmycorrhizal microcosms did not have fungal compartments.
in respiration between the fungal or root compartments and the soil-only control compartments. One day prior to harvest, we estimated oxygen concentration in the headspace of randomly chosen root and fungal compartments using an azide modification of the iodometric method for dissolved oxygen (Inland Waters Directorate, 1974). The mean oxygen concentration was 18% (SD 3, n = 8). Immediately before harvest, we examined the net carbon assimilation rate by the plants. Each sealed microcosm with its extruding shoot was placed inside a gas-tight Plexiglas® chamber connected to an EGM2 infra-red CO₂ gas analysis system (PP Systems, Hertfordshire, UK) and the CO₂ concentration (ppm) measured over a 10-min period.

Harvest

After 10–12 d, we harvested the microcosms in randomized order. This period was found to be sufficient to observe nutrient addition effects by Ek (1997). Each plant was divided into shoot, mycorrhizal, and nonmycorrhizal roots (by examination with a dissecting microscope). Mycorrhizal roots were detached from rhizomorphs. All tissues were then frozen, lyophilized and weighed. The weights of soil in the different compartments were recorded. A total of c. 2 g of soil was removed from five random points colonized by mycelium in each compartment, frozen, lyophilized and weighed.

Ergosterol analysis

We determined the amount of ergosterol in the soil to estimate extramatrical mycelial biomass. Methods, reagents, and instruments are described by Wallander (2000). Soil from Pinus muricata microcosms (n = 3) grown under similar conditions as the experimental plants but without EM fungal inoculation was used to correct for background ergosterol. Ergosterol to fungal biomass conversion factors were determined for each fungus from 20-d-old mycelial cultures grown on cellobiose overlaid on modified Melin–Norkrans agar medium (supplemented with 1.25 g l⁻¹ glucose and 5 g l⁻¹ malt extract). The conversion factors (µg ergosterol mg⁻¹) were: S. pungens 12.5 (SE 0.2; n, 6); R. 378 10.4 (SE 0.3; n, 6); R. 2272 10.5 (SE 0.3; n, 9); R. 371 10.2 (SE 0.6; n, 9). For P. involutus we used 9.6 µg ergosterol mg⁻¹ (Ek, 1994). For converting fungal biomass to fungal carbon, a carbon content of 45% was assumed (Ek, 1997). We estimated the biomass increase in each fungal compartment from the slope of the area colonized by fungal mycelium plotted against time (data not shown).

¹⁵N analysis

Total N and ¹⁵N atom percentage were determined in pooled mycorrhizas, roots, and the shoot of each microcosm, ground to < 0.5 mm particle size in a Cyclone Mill (Udy Corp., Ft. Collins, CO, USA), with an Automated Nitrogen Carbon Analyser-Man Spectrometer (PDZ Europa Ltd, Cheshire, UK). Nonmycorrhizal controls (n, 3) were used to correct for background ¹⁵N.

Statistical analysis

All tests were performed using JMP (SAS Institute, Cary, NC, USA). If variances were unequal, we tested on log-transformed values. However, untransformed means are reported. Microcosms were treated as replicates. Pair-wise comparisons could not be made since pairs of fungal compartments were not always obtained. Significant (P < 0.05) ANOVA tests were followed by means comparisons using Tukey’s honestly significant difference (HSD) tests.

Results

At harvest, none of the Pinus muricata seedlings showed signs of nutrient deficiencies. Total nitrogen (g) differed significantly between plants associated with different fungi or nonmycorrhizal (P < 0.001, means followed by different letters are significantly different): Rhizopogon 378, 0.032 a; S. pungens, 0.031 a; P. involutus, 0.013 b; Rhizopogon 2272, 0.011 b; nonmycorrhizal, 0.009 b. Rhizopogon 371, no fertilization data available. Plant biomass did not differ significantly (Table 2). Two chambers with Rhizopogon 378 produced mature sporocarps (1.5 cm diameter) in the root compartments.

Fungal hyphae (and hyphal aggregations, here called rhizomorphs) proliferated rapidly within the fungal compartments after crossing the barrier. Within the fungal compartment, the presence of rhizomorphs was most pronounced in Rhizopogon species and P. involutus. The areas colonized by each isolate (cm² d⁻¹) differed significantly (P < 0.0001, means labelled as above): P. involutus, 5.2 a; Rhizopogon 378, 4.2 a; Rhizopogon 2272, 2.0 b; S. pungens, 1.3 c; Rhizopogon 371, 1.2 c. P. involutus and Rhizopogon 378 had completely colonized the fungal compartments by the time of harvest.

The extramatrical mycelial biomass of S. pungens in the root compartments was significantly lower than for the other fungi (Table 2). In the fungal compartments there was a significant overall negative effect of apatite on mycelial biomass (P < 0.016). The effect was particularly strong for S. pungens. A similar trend was observed in the NH₄ addition compartments. Significant results were obtained when the ergosterol to fungal biomass conversion factors were not used (data not shown). Mycorrhizal root biomass differed significantly: Rhizopogon 378 > Rhizopogon 371 = Rhizopogon 2272 > S. pungens > P. involutus. Shoot and nonmycorrhizal root biomass did not differ significantly between fungi (Table 2), nor did root : shoot ratios (data not shown).

The effect of the fertilization treatments applied to the fungal compartments on both respiration and fungal biomass are summarized in Table 3. Respiration means (from averaged daily measurements) before and after fertilization are shown.
Table 2  Biomass (mg) components at harvest in mycorrhizal microcosms of *Pinus muricata* grown with five ectomycorrhizal fungi

<table>
<thead>
<tr>
<th>Plant</th>
<th>Extramatrical mycelium</th>
<th>Shoot</th>
<th>NM roots</th>
<th>M roots</th>
<th>Root</th>
<th>NH₄</th>
<th>Apatite</th>
<th>Controls</th>
<th>ANOVA I[1] P &lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhizopogon</td>
<td>2272</td>
<td>810</td>
<td>420</td>
<td>190 b</td>
<td>47.0 a</td>
<td>25.4</td>
<td>14.7 a</td>
<td>13.5</td>
<td>0.26[3,23]</td>
</tr>
<tr>
<td>Paxillus</td>
<td>involutus</td>
<td>1600</td>
<td>1040</td>
<td>280 c</td>
<td>37.5 a</td>
<td>42.6</td>
<td>25.8 a</td>
<td>31.0</td>
<td>0.40[3,17]</td>
</tr>
<tr>
<td>Saillus</td>
<td>pungens</td>
<td>720</td>
<td>630</td>
<td>70 a</td>
<td>42.7 a</td>
<td>87.7</td>
<td>35.5 a</td>
<td>51.6</td>
<td>0.43[3,14]</td>
</tr>
<tr>
<td>ANOVA I[1] P &lt;</td>
<td>0.23[1,17]</td>
<td>0.07[1,17]</td>
<td>0.003[3,14]</td>
<td>0.03[3,20]</td>
<td>0.06[3,11]</td>
<td>0.01[1,19]</td>
<td>0.08[3,27]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1Microcosms with *PINUS MURICATA* and each of four ectomycorrhizal fungi after addition of apatite, ammonium, or distilled water to fungal compartments. From left to right: root, nonmycorrhizal roots in root compartment, mycorrhizal roots in root compartment. Biomass of fungal extramatrical mycelium estimated via ergosterol in microcosm compartments, from left to right: root, ammonium-fertilized, apatite-fertilized, distilled water controls. ANOVA I compares microcosms across different fungi, ANOVA II compares extramatrical mycelial biomass among ammonium, apatite, and distilled water controls across microcosms of a single fungus. Different letters following means within a column (a, b, c) or within a row (x, y) indicate significantly different (alpha = 0.05) means according to Tukey's HSD tests. NM, nonmycorrhizal; M, mycorrhizal; n.d., no data.

Table 3  The percent (%) increase in (a) respiration and (b) fungal biomass in mycorrhizal microcosms

<table>
<thead>
<tr>
<th>(a) Respiration</th>
<th>NH₄ control</th>
<th>Apatite control</th>
<th>NH₄ control</th>
<th>Apatite control</th>
<th>Apatite control</th>
<th>ANOVA II[2] P &lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhizopogon</td>
<td>2272</td>
<td>19</td>
<td>15</td>
<td>16 ax</td>
<td>205 by</td>
<td>15 ax</td>
</tr>
<tr>
<td>Rhizopogon</td>
<td>371</td>
<td>4</td>
<td>7</td>
<td>52 ax</td>
<td>257 by</td>
<td>45 ax</td>
</tr>
<tr>
<td>Paxillus</td>
<td>involutus</td>
<td>16</td>
<td>20</td>
<td>4 bx</td>
<td>68 cy</td>
<td>3 bx</td>
</tr>
<tr>
<td>Saillus</td>
<td>pungens</td>
<td>31</td>
<td>32</td>
<td>42 ax</td>
<td>328 by</td>
<td>45 ax</td>
</tr>
<tr>
<td>ANOVA II[2] P &lt;</td>
<td>0.28[3,16]</td>
<td>0.01[3,3]</td>
<td>0.091[3,11]</td>
<td>0.01[3,11]</td>
<td>0.07[3,19]</td>
<td>0.008[3,19]</td>
</tr>
</tbody>
</table>

(b) Fungal biomass

| Rhizopogon    | 2272        | n.d.            | n.d.        | 105 y           | 109 ay          | 106 y       | 210 bx     | 0.0084[1,14] |
| Rhizopogon    | 371         | n.d.            | n.d.        | 179 y           | 74 y            | 140 y       | 347 ax     | 0.0002[1,10] |
| Paxillus      | involutus   | n.d.            | n.d.        | 82 y            | 79 y            | 116 y       | 233 bx     | 0.0000[1,14] |
| Saillus       | pungens     | n.d.            | n.d.        | 215              | 50 b            | 178         | 164 b      | 0.24[3,18] |

1Microcosms with *Pinus muricata* and each of four ectomycorrhizal fungi after addition of apatite, ammonium, or distilled water to fungal compartments. From left to right: root compartments of microcosms where one fungal compartment received ammonium and the other distilled water, root compartments of microcosms where one fungal compartment received apatite and the other distilled water, fungal compartments that received distilled water in microcosms that received ammonium, fungal compartments that received ammonium, fungal compartments that received water in microcosms that received apatite, fungal compartments that received apatite. ANOVA I compares microcosms across different fungi, ANOVA II compares fungal compartments across microcosms of a single fungus. Different letters following means within a column (a, b, c) or within a row (x, y) indicate significantly different means according to Tukey’s HSD tests. n.d., no data.

Rates of net assimilated carbon were allocated to mycelial respiration and biomass increase in the fungal compartments (Table 5). The data for each fungus were pooled across treatments and controls. This is an underestimate because it does not include fungal respiration in the root compartment. Estimating allocation to the mycelium in the root compartment would require knowledge of the proportions of root, mycorrhizal root, and extramatrical mycelial respiration. However, since all mycelium in the fungal compartments was ultimately connected to mycorrhizal roots in the root compartment, we can estimate the proportion of
fixed carbon allocated to the fungal compartments and relate it to the total mycorrhizal root biomass available for carbon translocation. Biomass growth was significantly higher for *P. involutus* than for the other fungi leading to the higher total carbon allocation to *P. involutus* than to any other fungus (Table 5).

We determined the percentage of added 15N-labelled ammonium transferred from the fungal compartments to the plant and fungal tissues in the root compartment plus the shoot of *Pinus muricata* with *Paxillus involutus*, *Rhizopogon* 378, *Rhizopogon* 2272, and *Suillus pungens*. The mean respiration in soil-only compartments was subtracted from each mean. Vertical bars represent standard errors.

**Table 4** Mycelial respiration at harvest in the fungal compartments (µg C mg⁻¹ ergosterol h⁻¹)

<table>
<thead>
<tr>
<th>Treatment</th>
<th><em>Rhizopogon</em> 2272</th>
<th><em>Rhizopogon</em> 378</th>
<th><em>Rhizopogon</em> 378</th>
<th><em>Paxillus involutus</em></th>
<th><em>Suillus pungens</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₄ Apatite Controls</td>
<td>1.4 a 7.2 a 5.1 a</td>
<td>1.4 a 3.0 a 1.85 a</td>
<td>0.37 b 0.54 b 0.35 b</td>
<td>2.1 a 9.9 a 3.0 a</td>
<td></td>
</tr>
<tr>
<td>NH₄ Addition</td>
<td>0.37 b 0.54 b 0.35 b</td>
<td>2.1 a 9.9 a 3.0 a</td>
<td>0.02, p&lt;0.05</td>
<td>0.019, p&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

Different letters following means within a column (a, b) indicate significantly different means according to Tukey’s HSD tests. n.d., no data.

**Discussion**

The isolates used in this study differed in mycorrhizal biomass, mycelial growth rate, biomass, and respiration. All of these factors contributed to differences in carbon sink strength (Table 5). However, due to the lack of competition in microcosms, it is not surprising that the total proportion of fixed carbon allocated to the fungi did not differ between isolates except for *P. involutus*. Each fungus had captured as many fine roots and created as much of a carbon sink as physiologically possible (under the prevailing nutrient conditions) over several months by the time a new carbon sink was created upon entering uncolonized, and sometimes fertilized, soil. It is surprising that a fungus such as *P. involutus* with comparatively low biomass of connections to the plant was able to extract proportionally more carbon per EM connection, thus making up for differences in standing EM root biomass among isolates. Furthermore, *P. involutus* transferred more than double the amount of ammonium to the host per EM biomass than *Rhizopogon* 378, *P. involutus*, and *S. pungens*.

Our results indicate that adding ammonium to EM mycelium can substantially increase the amount of carbon biomass, *P. involutus* transferred a significantly higher amount of ammonium per mycorrhizal biomass than *Rhizopogon* 378, *P. involutus*, and *S. pungens*.

**Fig. 2** Fungal respiration (nanomoles CO₂ s⁻¹) before (open columns) and after (closed columns) addition of ammonium, apatite or water (controls) to microcosms of *Pinus muricata* with *Paxillus involutus*, *Rhizopogon* 378, *Rhizopogon* 2272, and *Suillus pungens*. The mean respiration in soil-only compartments was subtracted from each mean. Vertical bars represent standard errors.
The smaller percentage of fixed carbon allocated to the fungal compartments transferred to *P. involutus* mycelium in our study compared with that found by Ek (1997) can be attributed to differences in plant host growth. At harvest, *Pinus muricata* had on average almost twice the biomass of the *Betula pendula* used by Ek (1997).

It is likely that differences in maximum potential carbon sink strength per EM connection will contribute, for instance, to determine fungal distribution patterns within forest soils where competition imposes limits on mycorrhizal root biomass. Comparatively low carbon sink strength could lead fungi such as *Rhizopogon* to behave as ruderal microsite specialists where large mycorrhizal biomass can be achieved and maintained in the absence of competitors.

The turnover of mycorrhizal biomass is another factor that may differ among the isolates we studied. It is apparent that *P. involutus* is an aggressive colonizer of soil, at least in terms of growth rate (Table 5), but its mycelial respiration was significantly lower than that of the suilloid fungi (Table 4). Some inferences can be made from our observations while harvesting EM roots from the microcosms. For instance, where most mycorrhizal root biomass appeared active and very few were degraded, we may infer a low turnover rate. *Rhizopogon* spp. had a low turnover and aggressively colonized all available roots, *P. involutus* had a low turnover and colonized only about one quarter of available roots, and *S. pungens* had a high turnover and colonized almost half of the available roots, but the majority of these had degraded. Sequential harvests would be required to quantitatively address turnover of mycorrhizal biomass.

The smaller percentage of fixed carbon allocated to the *P. involutus* mycelium in our study compared with that found by Ek (1997) can be attributed to differences in plant host growth. At harvest, *Pinus muricata* had on average almost twice the biomass of the *Betula pendula* used by Ek (1997). This emphasizes the importance of recognizing that mycorrhizae are dual systems when attempting comparisons across fungal species. Furthermore, the fraction of the respiration in the fungal compartments corresponding to bacterial respiration of fungal exudates is unknown.

Interspecific differences in investment in basidiocarp production vs EM root biomass have been reported from nature (Danielson, 1984; Natarajan et al., 1992; Gardes & Bruns, 1996; Jonsson et al., 1999). This study indicates that in laboratory conditions carbon sink strength can differ between EM fungi; this may be a key element to consider when interpreting below ground mycorrhizal biomass data. How this finding correlates with carbon use efficiency from a plant perspective remains to be determined. Even though *P. involutus* had the highest carbon sink strength of the fungi tested across experimental conditions, its respiratory activity increased comparatively less than for other fungi following addition of ammonium. Across isolates, the fungal response to apatite addition was mostly via biomass accumulation, while the response to ammonium addition was mostly observed via respiratory activity. In addition, ammonium transfer characteristics differed between the fungi examined.

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