Mycorrhiza (2006) 16: 213–217
DOI 10.1007/s00572-006-0037-x

SHORT NOTE

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Limited transfer of nitrogen between wood decomposing and ectomycorrhizal mycelia when studied in the field

Received: 19 August 2005 / Accepted: 23 December 2005 / Published online: 6 April 2006

Abstract Transfer of ^{15}N between interacting mycelia of a wood-decomposing fungus (*Hypholoma fasciculare*) and an ectomycorrhizal fungus (*Tomentellopsis submollis*) was studied in a mature beech (*Fagus sylvatica*) forest. The amount of ^{15}N transferred from the wood decomposer to the ectomycorrhizal fungus was compared to the amount of ^{15}N released from the wood-decomposing mycelia into the soil solution as ^{15}N-NH_4. The study was performed in peat-filled plastic containers placed in forest soil in the field. The wood-decomposing mycelium was growing from an inoculated wood piece and the ectomycorrhizal mycelium from an introduced root from a mature tree. The containers were harvested after 41 weeks when physical contact between the two foraging mycelia was established. At harvest, ^{15}N content was analyzed in the peat (total N and $^{15}NH_4^+$) and in the mycorrhizal roots. A limited amount of ^{15}N was transferred to the ectomycorrhizal fungus and this transfer could be explained by $^{15}NH_4^+$ released from the wood-decomposing fungus without involving any antagonistic interactions between the two mycelia. Using our approach, it was possible to study nutritional interactions between basidiomycete mycelia under field conditions and this and earlier studies suggest that the outcomes of such interactions are highly species-specific and depend on environmental conditions such as resource availability.

Keywords Antagonistic interactions · *Hypholoma* · Nitrogen transfer · Mycorrhiza ^{15}N · *Tomentellopsis*

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Introduction

Nitrogen uptake by forest trees was traditionally considered to be dependent on the mineralizing activity of saprotrophic organisms in the soil. Research conducted during the last three decades has, however, shown that the ectomycorrhizal fungi that live in symbiosis with tree roots have access to more complex nitrogen forms than ammonium and nitrate (Read and Perez-Moreno 2003). Many of these fungi possess the enzymatic capability to degrade complex organic compounds such as proteins (Abuzinadah and Read 1986) and chitin (Lindahl and Taylor 2004), enabling mobilization of amino acids and amino sugars from organic matter (Bending and Read 1995). Aided by their mycorrhizal symbionts, many plants can take up amino acids directly (Näsholm et al. 1998), making plants less dependent on the mineralizing activities of decomposers to obtain nitrogen (Read 1991).

Recent results further showed that nutrient dynamics in forest soil dominated by fungi might be even more complex. Boddy and Watkinsson (1995) proposed that nutrients are conserved within mycelia of saprotrophic fungi and released mainly in connection with antagonistic interactions with other fungi, rather than through mineralization and release to the soil solution. Leake et al. (2001) demonstrated in laboratory microcosms that carbon allocation to foraging ectomycorrhizal [*Suillus bovinus* (L. ex Fr.)] mycelia was greatly inhibited by the presence of a wood-decomposing fungi [*Phanerochaete velutina*, DC: (Pers.) Parm.]. Lindahl et al. (1999) demonstrated that the ectomycorrhizal fungi *Suillus variegatus* (Fr.) Kuntze and *Paxillus involutus* (Batsch) Fr. could obtain radiolabeled P from mycelia of the wood-decomposing fungus *Hypholoma*

fasciculare (Huds. ex Fr.) during antagonistic interactions in microcosms. Further experiments by Lindahl et al. (2001) demonstrated that P transfer may take place in both directions and that the flux rates of P were dependent on the size of the wood block (resource size) available to the wood decomposer. The wood decomposer became more competitive for P when it grew out from a larger wood block. A large amount of work was performed on P uptake and transport within mycelia of wood decomposers, reflecting the methodological advantages of using the radiolabeled isotopes of P. N transport was studied mainly in ectomycorrhizal mycelia (Finlay et al. 1988, 1989). Only recently were translocation studies conducted with the focus on N redistribution within decomposer mycelia (Tlalka et al. 2002, 2003). Nutritional interactions involving transfer of N between these two fungal groups are likely to be of more ecological significance than transfers of P because N is usually the limiting nutrient in boreal forests (Tamm 1991).

The importance of interactions between wood decomposing and ectomycorrhizal mycelia as a pathway for nutrient cycling led Lindahl et al. (2002) to formulate a new model of nutrient cycling in forest ecosystems with low nutrient availability and a fungus-dominated microbial community. This model puts less emphasis on the role of inorganic nutrient pools in nutrient cycling than traditional models but focus instead on the competition between saprotrophic and mycorrhizal fungi for organic nutrients.

The outcomes of the interactions between fungal mycelia appear to be variable (Baar and Stanton 2000) and the direction of potential nutrient fluxes will depend on the fungal species involved (Holmer and Stenlid 1997) and the resource availability of the interacting fungi (Lindahl et al. 2001). The use of small seedlings in microcosms does not clearly correspond to the conditions prevailing in nature because mature trees have a much larger capacity to assimilate carbon. To achieve a better understanding of the ecological significance of these fungal interactions, it is therefore essential to perform experiments in the field under natural conditions.

In the present study, we used ^{15}N to estimate transfer of N from a wood-decomposing fungus (*H. fasciculare*) to an ectomycorrhizal fungus [*Tomentellopsis submollis*, (Svrcek) Hjortst.] in the field. The experiment was performed in a mature beech (*Fagus sylvatica* L.) forest where roots colonized by *T. submollis* were carefully led into peat-filled plastic containers with wood pieces colonized by ^{15}N-labeled *H. fasciculare* mycelium. The containers were harvested after 9 months when physical contact between the two foraging mycelia was established. The aim of the study was to evaluate the potential for competition for nitrogen between saprotrophic and mycorrhizal fungi by comparing the transfer of N from saprotrophic mycelium to mycorrhizal roots with the net N mineralization in systems without roots. We hypothesized that little inorganic N would be released from the wood-decomposing mycelia when it was growing alone while a significantly larger amount would be transferred to the ectomycorrhizal fungus as a result of antagonistic interactions when they were growing together.

Materials and methods

The study was performed in a beech (*F. sylvatica* L.) forest south of Åsljunga in Southern Sweden (lat. 56°17′ N, long. 13°25 E). The organic layer was approximately 10-cm-thick including a 6- to 8-cm litter layer in the form of intact or partly decomposed beech leaves. When removing beech leaves carefully, roots colonized by mycorrhizal fungi were exposed between the leaves. The roots, still intact and connected to the tree, could easily be lifted and placed inside plastic containers (see below). In an initial study, we placed several different types of mycorrhizal roots inside plastic containers and we found that abundant external mycelia grew out from mycorrhizal roots with a pink color. The fungus colonizing these roots was identified as *T. submollis* based on the description by Agerer (1987–1998) and Koljalg et al. (2002). A thorough description of the pink morphotype is also given by Wallander et al. (1997). Branched fine roots colonized by *T. submollis* were carefully excavated and used in the experiment described below.

The experiment was performed during 2000–2001. *H. fasciculare* was cultivated on agar plates and the composition of the nutrient media was: $^{15}NH_4SO_4$, 1 g l^{-1}; KH_2PO_4, 0.5 g l^{-1}; $MgSO_4 \times 7H_2O$, 0.5 g l^{-1}; glucose, 5 g l^{-1}; and malt extract, 5 g l^{-1}. Pieces of beech wood (~0.3 g dry weight) were placed on the agar surface and became completely colonized by the ^{15}N-labeled fungus within 2 weeks. Sixty rectangular plastic containers (1×2×20 cm) were filled with a

Fig. 1 Photograph of *Hypholoma fasciculare* (*left*) and *Tomentellopsis submollis* (*right*) when growing in plastic containers in the field. The two fungi have different growth patterns; note the much denser growth of the wood decomposer *H. fasciculare*. The photographs are taken when roots were still attached to the trees (December 2001)

thin layer of compressed peat (5 mm). At one end of the container, a *H. fasciculare* inoculated piece of wood was placed. At the other end, a small opening (2-mm-wide) was made and an intact mycorrhizal root (colonized by *T. submollis*) from the forest floor was led into the container at the time when they were placed in the forest. Thirty reference containers where no roots were introduced were also included to estimate the net release of labeled nitrogen from the wood-decomposing mycelia in the absence of mycorrhizal roots. The containers were covered with a plastic lid and placed in the forest soil and thereafter covered by leaf litter on September 7, 2001. The containers were inspected on December 19, 2001 and photographs of some of the containers were taken at this time (Fig. 1). In most cases, the mycelia of the two species had not met and incubation was prolonged until June 13, 2002 when the containers were incubated for 41 weeks.

At harvest, we selected 14 containers out of the 60 containers with both *H. fasciculare* and *T. submollis*. In ten of these containers, the two fungi had formed extensive overlapping mycelia but the ectomycorrhizal mycelia had not reached the piece of wood and the wood-decomposing mycelia had not reached the mycorrhizal roots. In four of the containers, the fungi had not met but both fungi appeared healthy and actively growing. In the other residual 46 containers, the peat substrate had more or less dried out and growth had ceased before the fungi had met and these containers were discarded. In addition, we selected ten reference containers with extensive growth of *H. fasciculare* where no roots were introduced. The other 20 reference containers were discarded.

The roots were brushed clean of adhering peat and then lyophilized. All the peat was also lyophilized and thereafter milled in a ball mill. The mycorrhizal roots (2 to 6 mg) and subsamples of the peat were analyzed for ^{15}N content in a mass spectrometer (Finnigan MAT Delta plus, Thermo-Finnigan, San Jose, USA). Another subsample (0.5 g) of the peat was extracted with 30 ml 0.5 M K_2SO_4. Nonsoluble fractions were removed by centrifugation and the amounts of ^{15}N in extractable NH_4 were analyzed by steam distillation of the peat extract. The total amount of ammonium in the distillate was measured through titration with H_2SO_4 (Stevenson 1982). After evaporation of the distillate, the ^{15}N content of the remaining salt crystals was determined using mass spectroscopy. No attempt to make a total budget for added ^{15}N were made because the amount of ^{15}N in the whole host trees could not be quantified as only the mycorrhizal roots in the containers were sampled. In addition, the exact amounts of ^{15}N present in the wood blocks at the beginning of the experiment were not analyzed.

Analysis of variance (ANOVA) was performed to test for difference in total ^{15}N and $^{15}NH_4$ content in the peat between containers with the wood-decomposer alone and containers with both fungi. ANOVA was also performed to test for differences in ^{15}N content of mycorrhizal root tips in containers where the two fungi had met and containers were the two fungi had not met.

Results

The two fungi had different mycelial morphology; *T. submollis* formed a diffuse hyphal front while *H. fasciculare* grew more vigorously forming a dense hyphal front. Both species formed rhizomorphs a couple of centimeters behind the front (Fig. 1).

^{15}N originating from the decomposer mycelium could be detected in mycorrhizal roots of all systems including the ones where the two fungi had not met (Table 1). The average amounts tended to be lower in containers where the fungi had not met (0.10±0.07 μg) than in systems where the fungi had met (0.46±0.15 μg) (Table 1), although the differences were not statistically significant (p=0.07). The amounts of $^{15}NH_4^+$ retrieved from the peat exceeded the amounts of ^{15}N in mycorrhizal roots by an order of magnitude. In systems without introduced mycorrhizal roots, the average amount of $^{15}NH_4^+$ retrieved from the peat was approximately twice the amount in systems with mycorrhizal roots (p=0.056). The total amount of ^{15}N present in the peat, which we assume is mainly located in the fungal mycelia, was not significantly influenced by the presence of mycorrhizal fungus (Table 1).

Discussion

The amount of $^{15}NH_4$ present in the peat collected from systems with only the wood-decomposer was more than twice as high (4.5 μg ^{15}N) as the amount in systems with both fungi (2.2 μg ^{15}N, Table 1), suggesting that inorganic nitrogen released by saprotroph mycelium was taken up by the mycorrhizal roots. However, only a minor amount of ^{15}N (0.46 μg) accumulated in the mycorrhizal roots, indicating that a major fraction of the absorbed nitrogen was passed on further to the tree host. As the amount of mineralized ^{15}N, by

Table 1 The amount of ^{15}N present in peat as total ^{15}N or as ^{15}N-NH_4 extracted with KCl and in roots colonized by *Tomentellopsis submollis* in containers with only the wood decomposer (*Hypholoma fasciculare*) and in containers with both fungi

Fungi present in containers	Physical contact (+/−)	Total ^{15}N in peat (μg)	$^{15}NH_4$ in KCl extract of peat (μg)	^{15}N in mycorrhizal root (μg)	No. of samples
H. fasciculare	–	42±5.9	4.5±0.9		10
H. fasciculare and *T. submollis*	+	40±2.6	2.2±0.7	0.46±0.15	10
H. fasciculare and *T. submollis*	–	n.m.	n.m.	0.10±0.07	4
ANOVA					
p value		n.s.	0.056	0.07	

The containers were incubated in the field for 41 weeks
n.m. not measured and *n.s.* not significant

large, exceeded the amounts recovered in the mycorrhizal roots, no indication that the mycorrhizal fungus had mobilized organic nitrogen during antagonistic interactions with the wood-decomposing mycelium could be found. The suggestion by Boddy and Watkinsson (1995) and Lindahl et al. (2002) that nutrients are released from basidiomycetous mycelia in soil mainly during competitive interactions between fungi could thus not be confirmed in our study.

The amount of ^{15}N released from the mycelium of *H. fasciculare* in the form of ammonium to the soil was larger than we expected (10% of the total amount of ^{15}N in the peat, which we assume is located in the fungal mycelia). Wood-decomposing fungi are usually very economical with nitrogen because this resource is extremely scarce in wood (Merrill and Cowling 1966). It is possible that some nitrogen was released from the wood-decomposing mycelia during the winter at periods of freezing and thawing and it is possible that soil animals grazing on the mycelia have had a significant role in the mineralization of nitrogen (Setälä 1995; Persson 1989). The reason why the ammonium was not reabsorbed by the saprotrophic mycelium during the following spring and summer may be that after inoculation on a rich medium, sufficient amount of nitrogen was provided in the inoculum.

Net transfer of nutrients between wood decomposing and ectomycorrhizal fungi is dependent on the resource availability of the fungal species involved in the interaction (Lindahl et al. 2001). A larger carbon supply for a particular fungus would make it more competitive for other nutrients in short supply such as N and P. In the present study, the potential carbon supply for the ectomycorrhizal fungus originates from a mature tree. In previous studies performed in the laboratory (Leake et al. 2001; Lindahl et al. 1999, 2001), the carbon supply for the fungus may differ considerably from natural systems because the plants were small and may have suffered from the artificial conditions in these systems such as limited space for root development. The carbon supply for the wood-decomposing fungi, on the other hand, was considerably smaller in the present study (a small wood piece) than the natural carbon sources for this fungus, which are usually logs or stumps of trees in the forest. Even though the carbon source was small in the experimental containers, growth and activity of wood-decomposing fungi are usually constrained by the low N and P availability in the wood (Merill and Cowling 1966; Boyle 1998) and it is likely that the high availability of mineral nutrients during inoculation of the wood stimulated the competitive ability of the decomposer fungus.

The present study demonstrates that nutritional interactions between basidiomycete mycelia can be investigated under more natural conditions than those obtained in laboratory studies. To increase our understanding of the ecological importance of such interactions, we strongly suggest that more studies should be performed under natural conditions using mature host plants and that the studies should include a larger number of species of both ectomycorrhizal fungi and wood and litter decomposing fungi.

Acknowledgements Funding was provided by the Royal Swedish Academy of Agriculture and Forestry (grants to H. Wallander). Thanks to Nancy Johnson for critical comments on the manuscript. Thanks to Stefan Olsson and von Reiserska Stiftelsen for giving us access to their forest and to Bo and Ingrid Wallander for letting us use Rye cottage as our field laboratory in the study.

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