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A possible role for saprotrophic microfungi in the N nutrition of ectomycorrhizal *Pinus resinosa*

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Abstract

We determined whether *Pinus resinosa*, selected ectomycorrhizal and saprotrophic microfungi have access to various organic nitrogen sources commonly found in the forest. Vector analysis demonstrated nitrogen limitation of the *P. resinosa* in the plantation from which most of the fungi were isolated, establishing this study's relevance. Nonmycorrhizal *P. resinosa* seedlings did not absorb significant N from amino acids. The ectomycorrhizal fungi, including *Pisolithus tinctorius*, *Suillus intermedius* and *Tylopilus felleus*, obtained substantial N from amino acids, a limited amount of N from glucosamine, and essentially no N from protein–tannin complex. In contrast, *Penicillium* and *Trichoderma* readily acquired N from protein–tannin and glucosamine. Thus, there was an increasing ability to obtain N from complex organic N sources from plant to ectomycorrhizal fungi to saprotrophic fungi. Furthermore, N mineralization from an organic N source by *Penicillium* depended on the C:N ratio. We conclude that acquisition of relatively simple organic N sources by *P. resinosa* is likely to be largely indirect via ectomycorrhizal fungi, and that more complex organic N sources may become accessible to ectomycorrhizal fungi (and thus possibly their host plants) following mineralization by saprotrophic fungi such as *Penicillium* or *Trichoderma* when C:N ratios are sufficiently low.

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1. Introduction

Nitrogen (N) is a major limiting resource for plant growth in many temperate forest ecosystems (Gosz, 1981; Aber et al., 1989; Stump and Binkley, 1992). In such ecosystems the largest pool of N is typically organic, occurring within living organisms and as components of detritus in the forest floor (Johnson, 1992). Amino acids and proteins are among the most abundant forms of organic N in the soil (Grov, 1963a,b; Sowden and Ivarson, 1966; Abuarghub and Read, 1988; Turnbull et al., 1996; Schulten and Schnitzer, 1998; Johnsson et al., 1999). Amino sugars may also comprise a significant fraction of organic nitrogen in soils (Johnsson et al., 1999; Milchalzik and Matzner, 1999; Rodionov et al., 2001; Dai et al., 2002; Turrión et al., 2002; Praveen-Kumar et al., 2002; Xu et al., 2003) because fungi, including ectomycorrhizal fungi, contribute a very large fraction of microbial biomass in forest ecosystems (Högberg and Högberg, 2002) and because the cell walls of fungi contain large concentrations of chitin, a polymer of the amino sugar acetyl glucosamine.

Nitrogen mineralization is conventionally thought to be necessary to convert organic N into forms that are available to plants (Myrold, 1998), but recently there has been considerable discussion about the importance of mineralization in N cycling (Chapin, 1995; Kaye and Hart, 1997). There are three major reasons for this. First, some plant species are themselves able to absorb simple organic N compounds such as amino acids (Chapin et al., 1993; Kielland, 1994; Schimmel and Chapin, 1996; Turnbull et al., 1996; Näsholm et al., 1998), although this

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phenomenon is by no means universal (Stribley and Read, 1980). Second, some ECM fungi may be able to transfer N to their hosts after absorbing from the forest floor simple organic compounds such as amino acids or small peptides (Abuzinadah et al., 1986; Turnbull et al., 1995, 1996), which route was probably first suggested by Frank (1894). It should be noted, however, that there is considerable interspecific and intraspecific variability among ECM fungi in their ability to do this (Abuzinadah et al., 1986; Keller, 1996; Sawyer et al., 2001). Third, some ECM fungi have the ability to acquire some substances, notably phosphate, directly from basidiomycete, wood-decaying fungi (Lindahl et al., 1999). Lindahl et al. (2002) suggest that such transfer occurs via 'combative interactions' between ECM and wood decay fungi. These combative interactions could potentially lead to transfer of N as well as P.

In contrast with wood decaying fungi, the common soil microfungi may interact with ECM fungi in a different manner owing to their inability to form rhizomorphs and thus their inability to translocate resources over great distances. This inability could lead to local carbon limitations (low C:N ratios) and the resultant mineralization of N (Lindahl et al., 2002). If the saprotrophic microfungi were able to mineralize N from organic sources that are less directly accessible to ectomycorrhizal fungi, one might predict the existence of either additive or synergistic interactions between coexisting microfungi and ectomycorrhizal fungi in terms of host plant N or P content. Indeed, previous microcosm experiments of ours showed that saprotrophic organisms in the forest floor could act additively or synergistically with an ectomycorrhizal fungus (Pisolithus tinctorius) to influence N or P contents of P. resinosa seedlings (Koide and Kabir, 2001). We were also able to show that saprotrophic microfungi isolated from a P. resinosa plantation could mineralize protein–tannin complex (Wu et al., 2003). Our objective in the present research was to compare common saprotrophic microfungi and ectomycorrhizal fungi in their ability to acquire N from organic sources using a broader range of both ectomycorrhizal fungi and organic N sources. Moreover, we wanted to determine the dependence of mineralization of organic N by common saprotrophic microfungi on the C:N ratio.

The soils of many temperate forests typically contain large concentrations of phenolic compounds, including humic acids and tannins (Coulson et al., 1960; Kuiters and Sarink, 1986), and their presence can strongly affect the capacity of at least some ECM fungi to utilize organic forms of N. This can occur either because of their direct effects on fungal physiology (Koide et al., 1998), or indirectly by interaction with the organic N compounds (Horner et al., 1988; Bending and Read, 1996). Naturally occurring tannins form hydrogen bonds with proteins, causing them to precipitate (Hagerman, 2002). In addition, phenolic compounds may interact with various compounds without causing them to precipitate (Qualls et al., 1991). The effects of interactions among phenolic compounds and organic N sources in the forest floor on the availability of N to saprotrophic and ECM fungi are poorly understood, but they may be important when considering whether organic N sources are directly or even indirectly available to plants. Therefore in some of our tests we combined tannin with the organic N source to determine its effect on the bioavailability of N.

2. Materials and methods

2.1. Study 1. Vector analysis of plantation trees

This study was carried out in an approximately 65-year-old plantation of P. resinosa Ait (red pine), located in State College, Centre County, PA, USA, and previously described in Koide et al. (2000). Vector analysis is a method of determining nutrient deficiencies by analyzing the effect of fertilizer application on the nutritional status and weight of leaves (Weetman, 1989; Kiefer and Fenn, 1997). We established four treatments on 29 October 1997 including nitrogen (N), phosphorus (P), dolomitic lime, and control, and these were arranged in a complete block design with eight replications. Within each block four trees were selected for uniformity. The average distance between selected trees within a block was 17.4 m. Treatments were randomly assigned to the selected trees. At least one untreated tree separated trees of the various treatments. All treatments were applied to the forest floor in a 2.25 m radius around an individual tree (16 m^2). N was applied as type 120R, 4-month, controlled release urea (86% urea, 14% coating, ExxonMobil Research and Engineering, Houston, TX, USA) at 500 kg N ha^{-1}. P was applied as triple super phosphate (0-46-0, Setre, Inc., Hazelton, PA, USA) at 500 kg P ha^{-1}. Dolomitic lime (White Stone Co., Paradise, PA, USA) was applied at 3570 kg lime ha^{-1}. The control trees received no amendment.

On 27 October 1998, a branch from the upper region of each tree (receiving direct sunlight) was clipped using a pole pruner with extensions. Needles were taken from each branch. The weight of 100 fascicles was determined following drying at 70 °C. All samples were then ground, and subsamples were digested at 400 °C in a 1:1 mixture of concentrated H$_2$SO$_4$ and 30% H$_2$O$_2$. N concentrations were determined using the Nessler method (Jensen, 1962). P concentrations were determined using the molybdo-phosphate method of Watanabe and Olsen (1965). Ca and Mg concentrations were determined by atomic absorption spectrometric methods (Richards, 1993).

2.2. Study 2. Potential N sources for red pine seedlings

Seeds of red pine were obtained from F.W. Schumacher Co. (Sandwich, MA, USA). They were surface-sterilized in