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Controls over mycorrhizal uptake of organic nitrogen

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ABSTRACT

Mycorrhizal plants from a variety of ecosystems have the capacity to take up organic forms of nitrogen, yet the fraction of plant nitrogen demand met by organic N (ON) uptake remains unclear. ON uptake by mycorrhizal plants is a biochemical process that involves multiple steps, including breakdown and uptake of soil ON by mycorrhizal fungi, internal transformation of ON, and transfer of N to the host plant. We present hypothetical mechanisms controlling each of these steps and outline predictions for how these mechanisms structure patterns of ON uptake by mycorrhizal plants in ecosystems. Using a synthesis of published data, we found that uptake of amino acids by mycorrhizal fungi is related to the relative abundance, N content, and carbon structure of the amino acid. We hypothesize that the bond strength and structural diversity of soil ON controls the breakdown of polymeric ON by mycorrhizal fungi. In addition, the availability of carbon resources for the mycorrhizal fungus influences the capacity for mycorrhizal fungi to assimilate amino acids and produce extracellular enzymes that catalyze the breakdown of polymeric ON.

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Contents

Introduction . . . 169
The nature of soil ON . . . 170
Direct uptake of mono- and oligomeric ON . . . 172
Breakdown of polymeric ON . . . 174
Transformation of ON within fungi. . . . 175
Transfer of ON to plants . . . 175
Hypotheses for ON uptake in ecosystems . . . 175
Conclusion . . . 176
Acknowledgements . . . 177
References . . . 177

Introduction

Organic nitrogen (ON) accounts for up to 95% of the soluble N pool in soils (Nemeth et al., 1987; Abuarghub and Read, 1988). This pool could be an important component of plant N budgets, given that inorganic N pools in soil are insufficient to account for annual plant N uptake in many alpine, arctic, boreal, and temperate ecosystems (Dyck et al., 1987; Johnson, 1992; Kielland, 1994). Plants that associate with mycorrhizal fungi are predicted to have greater access to ON compared to non-mycorrhizal plants (Schimel and Bennett, 2004). In fact, all mycorrhizal fungal species examined to date can use at least one form of ON as an N source (Table 2). Likewise, a broad array of ON compounds can be used by at least one mycorrhizal fungus. Nonetheless, plant growth is N-limited in most terrestrial ecosystems around the world (Vitousek and Howarth, 1991; LeBauer and Treseder, 2008) (Fig. 1).

Why is symbiosis with mycorrhizal fungi insufficient to alleviate N-limitation in plants, even though ON is abundant and can be targeted by mycorrhizal fungi? The uptake and use of ON by mycorrhizal fungi – and subsequent transfer to plants – involves multiple steps including breakdown of polymer ON in soil solution, direct uptake of mono- and oligomer ON into mycorrhizal fungi, internal transformation of ON, and transfer across the fungal–host plant interface (Fig. 2). Each of these steps is a biochemical process driven by physical, chemical, and biological mechanisms operating on the molecular level. Identifying which mechanisms most strongly control each of

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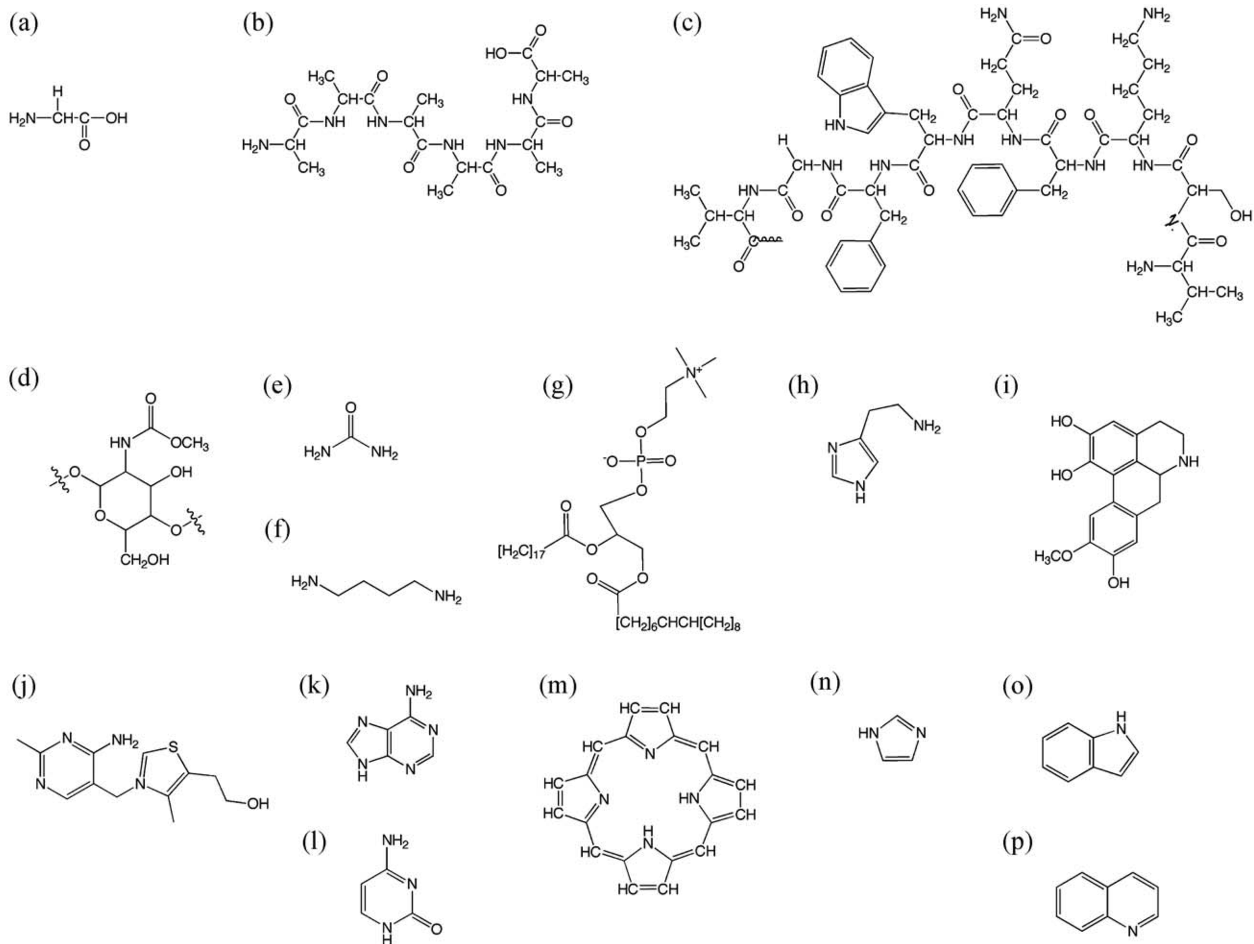

Fig. 1. Examples of common ON compounds found in plant litter, microbial biomass, and soils. Compounds include (a) glycine, (b) six-unit alanine peptide, (c) one domain of endoglucanase (a cellulose-degrading enzyme), (d) N-acetylglucosamine (monomeric unit of chitin), (e) urea, (f) putrescine, (g) phosphatidylcholine, (h) histamine, (i) aporphine, (j) thiamine, (k) adenine, (l) cytosine, (m) porphyrin, (n) imidazole, (o) indole, and (p) quinoline. Compounds (n)–(p) are typical products of pyrrolysis of whole soils.

these steps will help us predict the extent of ON use by mycorrhizal plants and how it may vary by species and environment.

In this review, we examine potential controls over the various stages of ON use by mycorrhizal fungi to better understand the cycling of N among soils, mycorrhizal fungi, and plants. We focus on the most common mycorrhizal associations found in nature (ecto-, ericoid, and arbuscular mycorrhizal fungi). We hypothesize that the extent to which ON is taken up by plants via mycorrhizal fungi will be determined by the abundance and N content of ON compounds due to the cost of investment in membrane transfer proteins by mycorrhizal fungi; by the size, bond strength, and bond diversity of ON compounds due to cost of investment in extracellular enzymes and internal biochemical transformations by mycorrhizal fungi; and by degree of mutualism with the host plant due to carbon growth requirements of mycorrhizal fungi (Fig. 2). Where possible, we test these hypotheses with data synthesized from published studies of ON pools and mycorrhizal fungi.

The nature of soil ON

Mycorrhizal roots are exposed to a diverse and dynamic mixture of ON compounds that are derived primarily from plant and microbial litter (necromass). Plants alone are estimated to synthesize over 15,000 different N-containing compounds (Baxter et al., 1999). These ON compounds can remain in leaves and roots after senescence (Rosenthal and Janzen, 1979; Waterman and Mole, 1994) and are then deposited onto soils through leaf and root litter (Jenny, 1980). The major classes of ON compounds in soils include aliphatic-N, like amino-N and polysaccharide-N, and aromatic-N, such as the compounds present in soil humus (Table 1, Fig. 1). These compounds are found in whole soils as well as in soil solution (Chen and Xu, 2006; Roberts and Jones, 2008). On average, ON constitutes 50% of the N dissolved in soil solution or extractable by strong salt solutions (Jones et al., 2005; Chen and Xu, 2006), but can comprise up to 90% and 95% of these respective N pools in some ecosystems (Dou et al., 2000; Jones et al., 2004). The high ON content of soil solution indicates that the exposure of mycorrhizal roots to ON can be equal to or greater than exposure to inorganic N in most soils.

Soil ON compounds vary widely in chemical properties such as molecular weight, bond strength, and structural diversity, which should influence their bioavailability to mycorrhizal fungi. One of the greatest differences is the size of ON compounds, which varies from monomers and oligomers as small as 31 g/mol to polymers on the order of one million g/mol. Some monomers (such as amino acids) and oligomers (such as small peptides) can be taken up directly by mycorrhizal roots. In contrast, larger polymers often must be broken down into mono- or oligomeric products by extracellular enzymes prior to uptake by mycorrhizal fungi. Some fungi can grow on amino acids or other monomeric compounds as a sole N source, but cannot grow as well or at all on larger

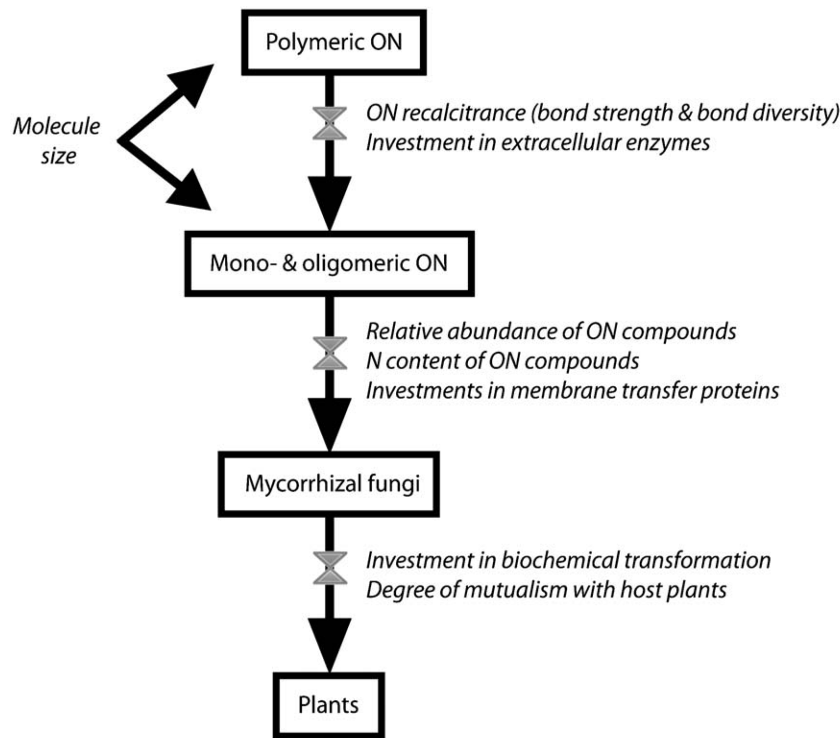

Fig. 2. Hypothesized controls over ON use by mycorrhizal fungi.

Table 1
Major classes of ON compounds found in plant and microbial biomass and soils using a variety of analytical techniques.

Compound class	Example[a]	Molar weight (MW: g/mol)	Extracellular enzyme required	Relative abundance (% soil N)[b]	Bond strength[c]	Structural diversity[d]	% ECM species[e]
Amino-N							
Free amino acid	a	75–204	None – direct uptake	< 1%	Low	Low	20–100% (5–20)
Synth. oligopeptide	b	267–534	None – direct uptake	0%	Low	Low	75% (4)
Protein	c	> 5000	Hydrolase	13–85%	Medium	Medium	87% (53)
Polysaccharide-N							
Amino sugars	d	≥ 179	Hydrolase	≤ 30%	Low	Low	75%(28)
Other aliphatic-N							
Urea	e	60	None/hydrolase	< 1%	Low	Low	54%(84)
Plant and microbial amines	f	31–581	Hydrolase	< 1%	Low	Medium	0% (3)
Phospholipids	g	≥ 61	Hydrolase	≤ 20%	Medium	Medium	24% (90)
Aromatic-N							83% (65)
Plant and microbial amines	h	31–581	Hydrolase/ oxidase	< 1%	Low	Medium	n.d
Alkaloids	i	71–1057	Hydrolase/ oxidase	< 1%	Medium/high	Medium	n.d
Vitamins	j	122–1580	Hydrolase/ oxidase	< 1%	High	High	100% (5)
Purines/ pyrimidines	k–l	≥ 80	Hydrolase/ oxidase	< 8%	High	Medium	80% (5)
Other aromatic-N	m–p	≥ 67	Hydrolase/ oxidase	10–50%	High	High	n.d

[a] Illustrated in Fig. 1. Examples represent compounds that are commonly found in plant biomass, microbial biomass, or soil organic matter.
[b] Stevenson (1994), Schulten and Schnitzer (1997), and Knicker (2004) and references therein.
[c] Based on number of C=N and C=C bonds.
[d] Based on number of repeating N–x bonds.
[e] For individual amino acids, see Table 2. Numbers in parentheses indicate number of species examined. Data synthesized from How (1940), Lindeberg (1948), Norkrans (1950), Melin (1953), Lundeberg (1970), Giltrap (1982), Ramstedt and Soderhall (1983), Abuzinadah and Read (1986), El-badaoui and Botton (1989), Hutchison (1990), Leake and Read (1990a), Maijala et al. (1991), Cao and Crawford (1993), Gunther et al. (1998), Kanunfre and Zancan (1998), Chambers et al. (1999), Gebauer and Taylor (1999), Sarjala (1999), Burke and Cairney (2002), Sawyer et al. (2003a, b), Hatakeyama and Ohmasa (2004), Pritsch et al. (2004), Courty et al. (2005, 2006, 2009), Buee et al. (2007), and Artz et al. (2009).

substrates of similar structure that require extracellular breakdown before uptake (Haider and Martin, 1975; Abuzinadah and Read, 1986). One example is the ectomycorrhizal fungus *Laccaria laccata,* which grows well on alanine, but cannot grow when supplied with a hexa-alanine peptide as a sole N source (Abuzinadah and Read, 1986). These findings suggest that breakdown of polymeric ON and direct uptake of mono- and oligomeric ON could be controlled by different mechanisms.

The difference in bioavailability of monomeric and oligomeric ON compared to polymeric ON is reflected in the relative concentrations of these ON compounds in soil solution. Similar to total soil N, the majority of ON in soil solution (over 95% in some cases) is polymeric, with molecular weights >1000 g/mol (Jones et al., 2004, 2005). Low-molecular weight monomeric and oligomeric compounds (primarily amino acids) can comprise up to 20% of total extractable N in soils, but usually comprise less than 5% total soil solution N (Jones et al., 2004, 2005). These low concentrations result from low inputs of free amino acids into soils, as well as rapid uptake or mineralization of mono- and oligomeric ON compared to polymeric ON. Mycorrhizal fungi that can take up amino acids directly from soil solution may be able to compete with free-living soil microbes and plant roots for amino acid-N (Jones and Kielland, 2002; Jones et al., 2005). To form hypotheses about the likelihood of mycorrhizal fungi competing successfully for this ON, it is necessary to consider the mechanisms regulating amino acid uptake and polymer breakdown by mycorrhizal hyphae.

Direct uptake of mono- and oligomeric ON

Research on plant ON uptake has focused almost exclusively on root absorption of small ON compounds, particularly amino acids. A number of studies have shown that mycorrhizal as well as non-mycorrhizal plants from a variety of biomes have the capacity to take up low-molecular weight ON directly as amino acids from culture or soil solution (e.g. Weigelt et al., 2005). The high affinity of mycorrhizal hyphae for amino acids (Chalot and Brun, 1998) and the large volume of hyphae produced by arbuscular and ectomycorrhizal fungi in soils (Leake et al., 2004) suggest that plant amino acid uptake could be greatly facilitated by association with these mycorrhizal fungi.

Mycorrhizal fungi acquire compounds from soil solution by producing membrane transport proteins that target those compounds. These transporters can be general enough to take up all 20 protein amino acids (Chalot et al., 1996; Nehls et al., 1999; Wipf et al., 2002), or specific enough to target one particular type or class of amino acids with specific chemical properties (Cappellazzo et al., 2008). Amino acid transporters have been empirically assessed in three ectomycorrhizal fungal species, and each of these transporters can bind the 20 common amino acids (Chalot et al., 1996; Nehls et al., 1999; Wipf et al., 2002). One amino acid transporter can also bind a variety of other non-protein amino acids (Chalot et al., 1996). By contrast, a recent study of an amino acid permease in the arbuscular mycorrhizal fungus *Glomus mosseae* found that this transporter is restricted to

Table 2
Species of mycorrhizal fungi that can use of the 20 common amino acids as a sole nitrogen source in culture or in symbiosis with a host plant. Plus signs indicate positive growth on the amino acid compared to a no-nitrogen control; minus signs indicate no significant positive growth compared to a no-nitrogen control. Blank spaces indicate no test for a species–amino acid combination. Amino acids are listed by their common abbreviation (Leu, leucine; Ala, alanine; Gly, glycine; Val, valine; Ser, serine; Glu, glutamic acid; Ile, isoleucine; Arg, arginine; Thr, threonine; Asp, aspartic acid; Lys, lysine; Pro, proline; Asn, asparagine; Phe, phenylalanine; Gln, Glutamine; Tyr, tyrosine; Met, methionine; His, histidine; Cys, cysteine; and Trp, tryptophan).

Species	Leu	Ala	Gly	Val	Ser	Glu	Ile	Arg	Thr	Asp	Lys	Pro	Asn	Phe	Gln	Tyr	Met	His	Cys	Trp	Reference
Ectomycorrhizal																					
Amanita alboverrocosa			+			+		+		+			+		+			+			1
Amanita conicoverrucosa						+		+		+			+		+			–			1
Amanita fuscosquamosa			+			+		+		+			+		+			–			1
Amanita muscaria	+	+	+	+	+	+	+	+	+	+	+	–	+	–	+	–	–	+	–	–	2, 3, 4
Amanita nauseosa			+			+		+		+			+		+			–			1
Amanita ochrophylla			+			+		+		+			+		+			–			1
Amanita pyramidifera			+			+		+		+			+		+			+			1
Cenococcum geophilum	+	+		–	+	+		+			+	–			+	+		+			2, 5, 6
Hebeloma crustuliniforme	+	+	+	+	+	+	+	+	–	+	+	–	+	–	+	+	–	–	–	+	3, 7
Hebeloma cylindrosporum	+	+	+	+	+	+	+		+	+	–		+	+	+		+	–	–	+	8
Laccaria laccata		+																			2
Lactarius rufus		+	+			+		+		+			+		+						2, 9, 7
Paxillus involutus		+	+			+		+		+			+		+						2, 7, 9, 10, 11
Pisolithus albus		+				+		+		+			+		+			+			12
Pisolithus marmoratus		+				+		+		+			+		+			+			12
Pisolithus tinctorius		+																			2
Rhizopogon roseolus		+																			2
Suillus bovinus	+	+	+	+	+	+	+	+	–	+	+	–	+	+	+	–	–	–	–	–	3, 7
Suillus variegatus		+				+		+		+					+						9
Tricholoma fumosum	+	+	+										+								13
Tricholoma imbricatum	+	+	+										+								13
Thelephora terrestris		+				+		+					+								10
Ericoid																					
Oidiodendron sp.		+				+		+			+	+	+		+			+	+		14
Rhizoscyphus ericae	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15, 16, 17, 18
Arbuscular																					
Glomus intraradices			+												+						19
Glomus mosseae	+	+									+		+			+					20

1. Sawyer et al. (2003a); 2. Abuzinadah and Read (1986); 3. Abuzinadah and Read (1988); 4. Sawyer et al. (2003b); 5. Melin and Mikola (1948); 6. Lilleskov et al. (2002); 7. Chalot and Brun (1998); 8. Guidot et al. (2005); 9. Sarjala (1999); 10. Finlay (1992); 11. Chalot et al. (1995); 12. Anderson et al. (2001); 13. Norkrans (1950); 14. Whittaker and Cairney (2001); 15. Leake and Read (1990b); 16. Bajwa and Read (1986); 17. Cairney et al. (2000); 18. Midgley et al. (2006); 19. Hawkins et al. (2000); 20. Cappellazzo et al. (2007).

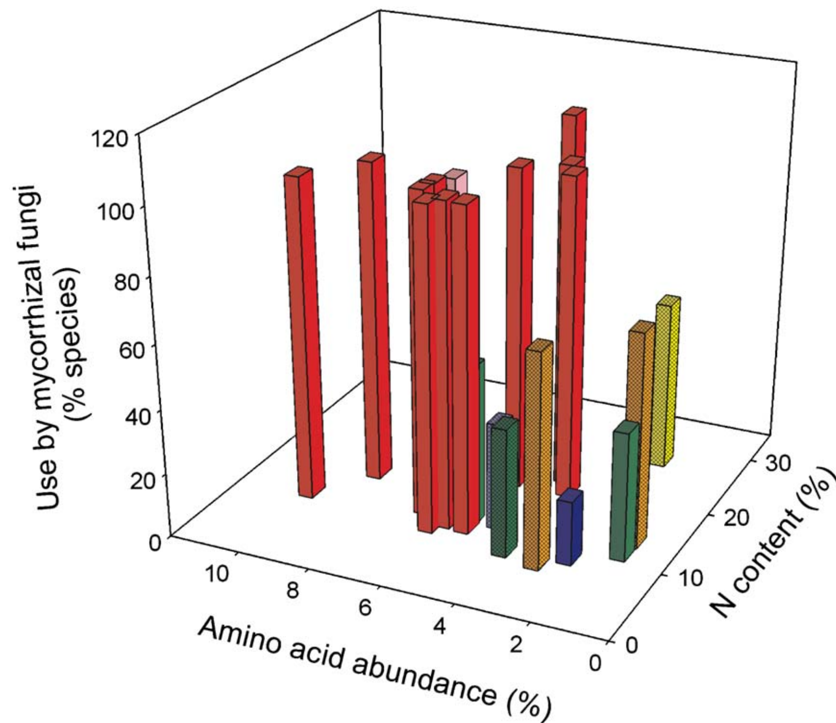

Fig. 3. Relationships between the extent of amino acid use among mycorrhizal fungi and the abundance and N content (% molar mass) of amino acids. Amino acid use was synthesized from published, culture-based studies (Table 2). Amino acid abundance was calculated from the sequences of all protein products in the NCBI reference sequence database as of 17 January 2006 (www.ncbi.nlm.nih.gov). Based on a multivariate logistic regression (r^2=0.49, P=0.003 overall), extent of amino acid use was significantly related to amino acid abundance (P=0.004) and marginally significantly related to %N (P=0.092). Each bar represents one amino acid. Bar color is proportional to the %species that can use an amino acid, which ranged between 20% and 100%. Bar pattern represents the carbon structure of the amino acid; solid bars indicate acyclic amino acids, hatched bars indicate cyclic amino acids.

neutral and non-polar, hydrophobic amino acids (Cappellazzo et al., 2008). Membrane transport proteins serve as a control point over the use of ON by mycorrhizal fungi, especially if certain fungal species lack the physiological capability to construct particular transporters owing to evolutionary constraints.

In culture studies, a number of ectomycorrhizal fungi have demonstrated the ability to use a broad range of amino acids as their sole N source, as well as some ericoid and arbuscular mycorrhizal fungi (Table 2). Nevertheless, of the 20 protein amino acids, some only supported growth of a fraction of the species tested. Species that do not use a particular amino acid may not possess membrane transport proteins targeting that amino acid. Alternately, those species may not contain the biochemical machinery necessary to process that amino acid internally (see below).

The evolution and extent of production of particular membrane transport proteins may be determined by a balance between costs of protein construction versus benefits of uptake of the mono- or oligomer in question. The construction of membrane transport proteins may be costly in terms of N, since proteins contain 8–32% N by weight. Membrane transport proteins, together with other proteins, represent about 50% of the mass of fungal cell membranes (Gooday, 1994), and so these transporters could represent a notable investment of N. Thus, production of a given membrane transporter protein may be evolutionary advantageous only under conditions that favor this N investment. We would expect this to be true for ectomycorrhizal, ericoid, and arbuscular mycorrhizal fungi alike.

For example, a mycorrhizal fungus may construct a membrane transport protein only if the targeted ON compounds are sufficiently abundant in the environment to offset the costs of construction. Organic N compounds vary in their relative abundance in soils; classes of compounds such as vitamins and alkaloids can represent less than 1% of soil N while proteins account for as much as 85% (Table 1). Likewise, amino acids vary in their frequency within proteins, with leucine, glycine, and alanine most common; and tryptophan, cysteine, and histidine rarest (http://www.ncbi.nlm.nih.gov). We found that the percentage of ericoid, arbuscular, and ectomycorrhizal fungal species capable of using a given amino acid (Table 2) was significantly and positively related to the relative abundance of that amino acid in proteins (Fig. 3, r^2=0.396, P=0.004) (The aromatic versus cyclic structure of the amino acids could also influence this relationship, as we discuss in "Transformation of ON within fungi", below). Relative abundance also appears to control breakdown of polymeric ON. More species of ectomycorrhizal fungi can grow on naturally produced protein (87% of species) as a sole N source compared to a synthetic oligopeptide composed of repeating units of alanine (75% of species) (Table 1). In addition, Ramstedt and Soderhall (1983) observed that proteases from *Suillus variegatus* and *Piloderma croceum* have over 10 times higher activity towards the protein casein compared to synthetic peptides, even though the peptides were composed of a variety of amino acids. These patterns lead to the hypothesis that mycorrhizal fungi have become adapted to take up ON that is relatively prevalent in their soil environment.

The N content of soil compounds could also determine the extent to which mycorrhizal fungi invest in their uptake. All else being equal, membrane transport proteins that target N-rich compounds may acquire N more efficiently than would those that target N-poor compounds. We observed that the percentage N, by molar mass, of a given amino acid was marginally significantly

related to the extent to which that amino acid was used among species of mycorrhizal fungi (Fig. 3, r^2=0.107, P=0.092). Together, percentage N and relative abundance of individual amino acids explained 49% of the observed variance in use by mycorrhizal fungi (r^2=0.49, P=0.003). If this pattern extends to other classes of compounds, then the physiological capacity for ON uptake by mycorrhizal fungi within ecosystems may be somewhat predictable based on soil chemistry. Specifically, we hypothesize that ecosystems in which soil ON is distributed among a few common, N-rich compounds may support mycorrhizal communities with greater capacity for ON uptake, compared to ecosystems in which soil ON is distributed among diverse, less abundant, N-poor compounds.

The availability of C to mycorrhizal fungi could also determine the extent of investment in ON uptake. If C supplies from a host plant are abundant, then stoichiometric requirements may induce N-limitation in mycorrhizal fungi (Sterner and Elser, 2002), with subsequent acquisition of ON. Nitrogen uptake kinetics in arbuscular and ectomycorrhizal fungi and plant roots often increase with a supply of C-rich hexoses (Pearson and Jakobsen, 1993; Logan et al., 1997; Javelle et al., 1999; Foyer et al., 2003). Furthermore, there is evidence that C availability increases uptake of amino acids by ericoid mycorrhizal fungi. The ericoid mycorrhizal fungus *Rhizoscyphus ericae* acquired 91% of N from glutamine when supplied with high concentrations of glucose (medium C:N of 39:1) compared to 72% when grown on low concentrations of glucose (medium C:N of 9:1) (Grelet et al., 2005).

Breakdown of polymeric ON

The depolymerization of soil organic matter has been proposed as a critical rate-limiting step in plant N availability (Schimel and Bennett, 2004). Since mycorrhizal uptake of low-molecular weight ON compounds is concentration-dependent (Nasholm et al., 2009), mycorrhizal fungi that decompose and assimilate N from polymeric ON compounds in soils should increase plant access to ON. In theory, these mycorrhizal fungi could control the supply of plant-available N without relying on N mobilization and mineralization by saprotrophic microbes.

Many studies have demonstrated that ericoid and ectomycorrhizal fungi can breakdown some of the most abundant ON polymers found in litter and soils. The majority of mycorrhizal fungi that have been tested are able to degrade protein in culture. Out of 53 species of ericoid and ectomycorrhizal fungi, 46 (87%) are able to produce protease or can grow on protein as a pure N source (Table 1). Ectomycorrhizal fungi also increase plant uptake of amino-N from protein (Finlay, 1992). In addition, certain ericoid and ectomycorrhizal fungi can decompose other classes of abundant polymeric ON like chitin (Bajwa and Read, 1986; Leake and Read, 1990a; Hodge et al., 1995; Chalot and Brun, 1998; Chen et al., 1999), and some produce oxidative enzymes that can catalyze the decomposition of more recalcitrant compounds such as aromatic-N (Meharg and Cairney, 2000). In fact, 83% of ectomycorrhizal fungi tested have the potential to produce polyphenol oxidases or lignin peroxidase (Table 1), enzymes that degrade various aromatic compounds.

The ecological significance of this polymer-degrading capability, however, is still a matter of debate (Nasholm et al., 2009). The polymeric ON forms that are most common to plant litter, dead microbial biomass, and soil organic matter vary in recalcitrance (i.e., resistance to degradation) by mycorrhizal fungi and other soil organisms. In theory, recalcitrance of a particular ON form should dictate the extent to which extracellular enzymes can depolymerize that compound in soils. Recalcitrance is a function of the physical and chemical properties of the substrate, and it depends partly on the bond strength and diversity of structural units in the compound (Baldock et al., 2004).

In terms of bond strength, more energy is required to decompose ON compounds that have double bonds or aromatic rings compared to compounds that are largely aliphatic. Aromatic polymers can be degraded by oxidative enzymes, which employ free-radical mechanisms (ten Have and Teunissen, 2001). These mechanisms are less specific in their target bond structures than the catalysis mechanism employed by hydrolytic enzymes. The inefficiency of this mechanism likely contributes to a high energetic cost of catabolizing conjugated or aromatic ON polymers. We hypothesize that this cost could reduce the extent to which mycorrhizal fungi breakdown aromatic-N in the environment.

Types of ON that lack defined secondary or tertiary structure, or in other words have a high diversity of bond types, may also have limited availability to mycorrhizal fungi. Compounds with high structural diversity may not be able to approach the enzyme active site, resulting in decreased catalytic efficiency of the oxidative enzyme compared to hydrolytic enzymes that specialize on more common bond structures (Allison, 2006). This mechanism has been invoked to explain why N-rich humus persists in soils (Allison, 2006). Unfortunately, few data are currently available to directly test the hypothetical importance of structural diversity and bond strength as factors controlling depolymerization of ON by mycorrhizal fungi.

In addition to the biochemical properties of the substrate, the physiological capacity of mycorrhizal fungi to produce extracellular enzymes under a given resource supply could limit the breakdown of polymeric ON. Extracellular enzyme production requires a significant expenditure of C and N (Schimel and Weintraub, 2003). Approximately 0.3–16.6% of C supply and 3.9–5.9% of N supply to microbes is allocated to extracellular enzyme production and maintenance (Frankena et al., 1988; Giuseppin et al., 1993; Christiansen and Nielsen, 2002). If C and N supplies to mycorrhizal fungi are low, the breakdown and uptake of polymeric ON by mycorrhizal plants may be reduced.

Experiments with intact mycorrhizal plants provide evidence that plant C supply to the fungal symbiont could control extracellular enzyme production and the acquisition of polymeric ON compounds. Intact ectomycorrhizal plants have been observed to rapidly colonize and mobilize N from patches of litter and soil organic matter in microcosms (Abuzinadah and Read, 1989; Bending and Read, 1995a, b; Perez-Moreno and Read, 2000). Furthermore, ericoid and ectomycorrhizal fungi acquire more N from high-molecular weight ON compounds when grown with plants versus without (Bajwa and Read, 1986; Dighton et al., 1987; Gunther et al., 1998). For example, Gunther et al. (1998) found that association with plants increased the capacity for the ectomycorrhizal fungi *Suillus granulatus* and *Paxillus involutus* to produce hydrolytic and oxidative extracellular enzymes. Additionally, *P. involutus* produced extracellular peroxidases only when grown in symbiosis with *Pinus sylvestris* seedlings (Gunther et al., 1998). Likewise, *L. bicolor* upregulates the gene expression of four chitinases (by 3–144-fold) and 13 secreted proteases (by 1.3–38.1-fold) upon colonization of poplar (Martin et al., 2008). These findings could have resulted from an increase in labile C availability to the fungi via the host plant. The effect of symbiosis on extracellular enzyme production by arbuscular mycorrhizal fungi has not been tested. As arbuscular mycorrhizal fungi are obligate symbionts, we hypothesize that C supplies from the host plant are crucial to enzyme production by these fungi.

In contrast to these host plant-based studies, C availability inconsistently affects enzymatic degradation of larger, polymeric ON by ericoid and ectomycorrhizal fungi in culture studies.

Elevated glucose concentrations in culture medium can increase mineralization of protein by some isolates of ericoid and ectomycorrhizal fungi (Zhu et al., 1994; Eaton and Ayres, 2002), but can also decrease the expression of extracellular proteases (Nehls et al., 1999) and chitinases (Leake and Read, 1990a; Bougoure and Cairney, 2006) by other ectomycorrhizal and ericoid mycorrhizal fungi. These variable responses imply strong regulation of extracellular enzyme activities by induction/repression mechanisms. For example, glucose induces protease production by *Rhizoscyphus ericae* in the presence of protein hydrolysate, a product of protein degradation. However, glucose represses protease production when the endophyte is grown with protein (Leake and Read, 1991). It has been hypothesized that catabolite repression may occur when mycorrhizal fungi are exposed to high concentrations of photosynthate in plant roots, where repression of extracellular enzyme production within colonized root cells would be favored (Leake and Read, 1991; Eaton and Ayres, 2002). However, the thresholds of these induction/repression mechanisms are largely unknown.

Evidence of the decomposer activity of ectomycorrhizal and ericoid mycorrhizal fungi has led to the prediction that these fungi access more ON compared to arbuscular mycorrhizal fungi, which in turn have greater ON uptake capability than non-mycorrhizal roots. One of the main reasons for this distinction is that there is little evidence that arbuscular mycorrhizal fungi produce extracellular enzymes that catalyze decomposition of polymeric ON. However, several studies have provided indirect evidence that arbuscular mycorrhizal fungi contribute to decomposition of polymeric ON. One notable study has shown that arbuscular mycorrhizal roots can accelerate decomposition of ON from *Lolium perenne* leaves (Hodge et al., 2001). More recently, Whiteside et al. (2009) demonstrated that arbuscular mycorrhizal fungi can absorb N from chitosan labeled with fluorescent quantum dots. In addition to providing evidence that arbuscular mycorrhizal fungi contribute to ON decomposition, these studies have employed new techniques that can be used in future studies to determine the extent of ON degradation and uptake among mycorrhizal fungi.

Transformation of ON within fungi

Once a mycorrhizal fungus acquires an ON compound, it often must be transformed in order to be transferred to the host plant root. The forms of ON that are transferred to the plant may vary among fungal and plant species, but evidence from ^{15}N-labeling studies has consistently implicated glutamine, asparagine, and alanine as the most common ON compounds involved in this process (Finlay et al., 1989, 1990; Smith and Smith, 1990; Arnebrant et al., 1993; Finlay et al., 1996; Chalot and Brun, 1998). Thus, mycorrhizal fungi must invest in the necessary biochemical machinery to convert the obtained ON compound to one of these transferred compounds. Not all fungal species may possess the physiological capability to transform every type of ON compound. For instance, most of the 20 common amino acids can be hydrolyzed by strong acid or catalytic enzymes, but aromatic amino acids are often resistant to hydrolysis (Stevenson, 1994). In our synthesis of published results, we found that cyclic amino acids were used by a significantly smaller fraction of mycorrhizal fungal species ($52 \pm 7\%$) than were aliphatic, non-cyclic amino acids ($87 \pm 7\%$) (Kruskal–Wallis, H=63.5, P=0.013). We hypothesize that this mechanism slows the use of even small ON molecules, if bond strengths within the molecules are high.

Transfer of ON to plants

Plant C supplies may offset many of the costs associated with ON uptake and transfer by mycorrhizal fungi, so an increase in allocation of photosynthate to mycorrhizal fungi may lead to greater ON use. Plants can allocate 10–20% of their C to mycorrhizal fungi (Harley, 1971) and this amount could vary depending on environmental parameters. Generally, plants increase investments in mycorrhizal fungi when belowground resources such as N and P are scarce, or when light or CO_2 are prevalent (Smith and Read, 1997; Whitbeck, 2001; Rillig et al., 2002; Treseder, 2004). The resulting improvement in C status of the mycorrhizal fungi could lead to greater exploitation of soil ON, but this response will only increase plant growth if ON transfer to plants increases as well.

Mycorrhizal fungi can be parasitic as well as mutualistic – they can consume C from host plants while transferring few nutrients in return (Johnson et al., 1997). In a greenhouse experiment, Johnson (1993) determined that arbuscular mycorrhizal fungi collected from fertilized fields were less beneficial to host plants than were those from unfertilized areas, possibly because the fungi transferred less N to the plant per unit C. Similarly, Hobbie et al. (2000) observed increases in foliar ^{15}N signatures as soil N availability increased across an Alaskan glacier chronosequence. Soil ^{15}N signatures did not vary with soil N availability, so one possible explanation of the results was that mycorrhizal fungi transferred a smaller fraction of acquired N to their host plants in more fertile areas (Hobbie et al., 2000).

An additional consideration is that high photosynthate allocation might select for fast-growing mycorrhizal fungi that invest more of their biomass in external hyphae than in root colonization structures (Treseder, 2005). These fungi would be more efficient at nutrient scavenging than transfer of nutrients to the host plant. Under these circumstances, mycorrhizal fungi would retain more N to construct their own biomass, transferring a smaller proportion of their acquired ON to plants. The benefit of mycorrhizal colonization to the host plant also varies by host plant genus (Karst et al., 2008) and species of mycorrhizal fungi (Jones et al., 2009). The degree to which mycorrhizal fungi are mutualistic with their plant hosts may depend on complex interactions between photosynthate allocations from host plants, cost-benefit relationships for mycorrhizal fungi, and plant and fungal community composition.

Hypotheses for ON uptake in ecosystems

The degree to which mycorrhizas acquire ON under natural conditions and within an intact microbial community remains unresolved (Nasholm et al., 2009). Read (1991) hypothesized that global-scale patterns of ON uptake by mycorrhizas were based on the distribution of soil organic N and mycorrhizal functional types. He suggested that ON uptake would increase with the percentage of nutrients bound in organic matter, which increases from low to high with latitude and altitude (Read, 1991). This hypothesis was developed from the observation that ericoid and ectomycorrhizal fungi are dominant in temperate, boreal, and arctic regions, while arbuscular mycorrhizal fungi are abundant in temperate grasslands and the tropics (Read, 1991).

Accumulating information on the global distribution of soil ON compounds contradicts Read's hypothesis. Soil protein content does not increase predictably with latitude (Sowden et al., 1977; Schmidt et al., 1999). In fact, total amounts of amino acid-N, amino sugar-N and recalcitrant aromatic-N in soils are similar between arctic, temperate, subtropical, and tropical sites

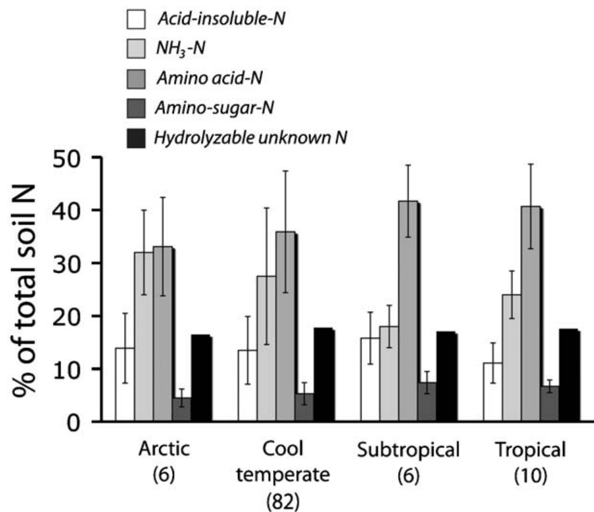

Fig. 4. Distribution of major ON fractions in soils of differing climatic regions. Numbers in parentheses indicate the number of soils. Acid-insoluble N refers to recalcitrant N compounds remaining in soil following acid hydrolysis. Hydrolyzable unknown N includes all N released by acid hydrolysis that is not NH_3, amino acid-N, or amino sugar-N (standard error not reported). Data re-illustrated from Stevenson (1994).

(Sowden et al., 1977) (Fig. 4). Instead, soil protein appears to increase with increasing successional stage (Kielland et al., 2007). Soil proteolytic activity tends to increase with increasing soil protein content (Kielland et al., 2007), suggesting that old-growth systems could have higher rates of amino acid capture by mycorrhizal fungi compared to early-successional sites.

Our synthesis also indicates that plant ON uptake is a function of the chemical composition of the soil ON pool and the physiological capacity of mycorrhizal fungi to catabolize ON internally or via extracellular enzymes. For instance, systems in which soil protein consists of amino acids that are relatively common, acyclic, or N-rich should have high rates of amino acid uptake by mycorrhizal fungi. Noteworthy systems of this type include temperate deciduous forest and short grass/tall grass pasture systems in Nebraska, where soil protein contains high concentrations of arginine, an N-rich amino acid, and leucine, a relatively abundant amino acid (Martens et al., 2004). By contrast, soil ON in thermic and hyperthermic grasslands in Texas is distributed more evenly among a diverse set of amino acids, including relatively high concentrations of proline and tyrosine, two cyclic amino acids (Amelung et al., 2006). We hypothesize that amino acid uptake would be higher in the Nebraska ecosystems and lower in the Texan grasslands due to evolutionary constraints and stoichiometric requirements of mycorrhizal fungi, as well as the chemical recalcitrance of the amino acid pool.

Additionally, systems that are dominated by ericoid and ectomycorrhizal fungi that have the capability to produce a broad range of extracellular enzymes, such as *Rhizoscyphus ericae* (Leake and Read, 1990a, b; Leake and Miles, 1996; Cairney et al., 2000; Midgley et al., 2006) and *Cenococcum geophilum* (Lundeberg, 1970; Abuzinadah and Read, 1986; El-badaoui and Botton, 1989; Hutchison, 1990; Courty et al., 2005; Buee et al., 2007), may have particularly high rates of ON uptake. However, we expect that ON capture by mycorrhizal fungi, including those with strong decomposer capabilities, depends on C supplies from the plant hosts. We hypothesize that rates of mycorrhizal ON uptake increase under elevated levels of plant C allocation belowground, due to increased amino acid acquisition and production of extracellular enzymes by mycorrhizal fungi. Plant C distribution to roots occurs when the plant exhibits high rates of photosynthesis, such as under high light availability or low cloud cover (Talbot et al., 2008), or when the plant experiences elevated levels of CO_2 (Andrew and Lilleskov, 2009) or low levels of mineral nutrients in soils (Treseder, 2004). However, the extent to which mycorrhizal fungi transfer the acquired ON to the plant host is difficult to predict based on available data. Future studies on the effects of plant C supplies, fungal C and N allocation, and the distribution and community composition of mycorrhizal fungi on ON uptake will provide insight into the relative importance of each mechanism in controlling ON transfer to the host under different environmental conditions.

Conclusion

The contribution of ON uptake to total plant N nutrition has been a matter of debate for over 15 years (Nasholm et al., 2009). We introduce here a theoretical framework that highlights the biochemical mechanisms responsible for driving the ON uptake process. The size, abundance, N content, and structure of the amino acid can control the extent of amino acid uptake by mycorrhizal fungi. Furthermore, ON uptake by mycorrhizal fungi appears to increase under high levels of carbon availability to the fungus.

We hypothesize that the rate at which mycorrhizal fungi degrade large ON polymers in soils is also controlled by the plant C resources available to the fungi to construct extracellular enzymes, as well as the bond strength and structural diversity of the target ON compound. These expectations are consistent with the hypothesis that the chemical composition of ON is responsible for widespread N-limitation of net primary production (Vitousek et al., 2002). In ON compounds, nitrogen is bound to carbon via covalent bonds, which require substantial energy to break (such as that provided by enzymatic catalysis). By contrast, organic phosphorus and sulfur are often bound to oxygen, which is more easily dissociated in soils. Furthermore, N can easily become physically and chemically protected by recalcitrant carbon compounds in soils, such as tannins and lignins (Vitousek et al., 2002). Over 90% of soil N in most terrestrial systems is present in organic forms (Stevenson, 1994). Therefore, the cost of producing enzymes to catabolize the release of N from ON may constrain total plant N uptake.

The extent of ON uptake by mycorrhizal plants could have large-scale consequences for the cycling of N and carbon. Use of a given ON compound by mycorrhizal fungi may lead to an increase in carbon fluxes into an ecosystem, if that N is transferred to plants and used to support photosynthesis. Conversely, ON that is not targeted by mycorrhizal fungi could be taken up by asymbiotic microbes. If the N augments microbial activity or biomass, it may contribute to the release of CO_2 from soils to the atmosphere (Treseder, 2008). Identifying the mechanisms that drive ON uptake by mycorrhizal plants is therefore an important step towards determining the contribution of this process to biogeochemical cycling in terrestrial systems. Our study presents a new framework for the ON uptake process that emphasizes the importance of the chemical and biological mechanisms that control ON uptake by mycorrhizal plants at the molecular, physiological, and ecological level. Research focused on direct tests of these hypothetical mechanisms will help define the limits of plant ON uptake in ecosystems.

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