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The unexpected versatility of plants: organic nitrogen use and availability in terrestrial ecosystems

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Abstract The recently recognized importance of organic nitrogen (ON), particularly amino acids, to plant nutrition in many types of agricultural and natural ecosystems has raised questions about plant-microbe interactions, N availability in soils, and the ecological implications of ON use by plants in the light of climate change and N pollution. In this review we synthesize the recent work on availability and plant uptake of amino acids with classic work on ON in soils. We also discuss recent work on the use of natural abundance levels of ^{15}N to infer N sources for plants. Reliance on ON is widespread among plants from many ecosystems. Authors have reached this conclusion based on laboratory studies of amino acid uptake by plants in pure culture, amino acid concentrations in soils, plant uptake of isotopically labeled amino acids in the field and in plant-soil microcosms, and from plant natural abundance values of ^{15}N. The supply of amino acids to plants is determined mainly by the action of soil proteolytic enzymes, interactions between amino acids and the soil matrix, and competition between plants and microbes. Plants generally compete for a minor fraction of the total amino acid flux, but in some cases this forms a significant N resource, especially in ecosystems where microbial biomass undergoes large seasonal fluctuations and contributes labile ON to the soil. A quantitative understanding of ON use by plants is confounded by incomplete data on partitioning of ON between plants, mycorrhizal fungi, and competing soil microbes. Further research is needed to predict the ecological implications of ON use by plants given the influence of climatic change and N pollution.

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Introduction

Plant roots have long been known to absorb amino acids (Virtanen and Linkola 1946; Miettinen 1959), but this fact was not considered to be ecologically relevant because of the belief that soil microbes out-compete plants for amino acids. It eventually became accepted that ON was an important N source for plants with ericoid and ectomycorrhizal symbioses (Stribley and Read 1980; Bajwa and Read 1985; Abuzinadah and Read 1989), but only recently has the potential ecological importance of ON uptake by non- and vesicular-arbuscular mycorrhizal plants been highlighted (Schobert and Komor 1987; Chapin et al. 1993; Jones and Darrah 1994; Kielland 1994; Raab et al. 1996). This awareness came about, in part, because of the observation that the availability of inorganic N (IN) (i.e., net N mineralization rates) is insufficient to account for annual plant N uptake in alpine, arctic and boreal forest ecosystems (Rehder and Schafer 1978; Fisk and Schmidt 1995; Kaye and Hart 1997). This indicated that other N sources are important for plant N nutrition. The potential importance of ON in plant nutrition has now been demonstrated for certain species in every ecosystem in which the authors looked for it, ranging from alpine and arctic tundra to a subtropical rainforest to ephemeral pools in the Namibian desert (Table 1). Amino acids are absorbed efficiently by plants in many plant families and with every possible mycorrhizal status. This unexpected versatility of plants in acquiring N has presented new challenges to the understanding of the terrestrial N cycle. The traditional view holds that plant roots cannot effectively compete with soil microbes for uptake of nutrients from the soil. ON molecules such as amino acids are excellent C and N sources for microbes, and thus only inorganic N in excess of microbial requirements would be available to plants. Studies of N availability to plants have therefore relied main-

Table 1 Terrestrial plant communities in which ON has been demonstrated to be potentially important to plant N nutrition

Community/Ecosystem	Reference
Agricultural	Jones and Darrah 1994; Yamagata and Ae 1996; Näsholm et al. 2000
Alaskan dry heath	Kielland 1994
Alaskan wet meadow	Kielland 1994
Alaskan tusock tundra	Kielland 1994
Alaskan shrub tundra	Kielland 1994
Boreal coniferous forest	Bajwa and Read 1985; Abuzinadah and Read 1989; Näsholm et al. 1998
Colorado alpine dry meadow	Raab et al. 1996, 1999
Colorado shortgrass steppe	Raab et al. 1999
Colorado subalpine fen	Raab et al. 1999
Desert ephemeral pools (Namibia)	Schiller et al. 1998
Heathland (UK)	Stribley and Read 1980; Abuarghub and Read 1988
Subantarctic herbfield	Schmidt and Stewart 1999
Subtropical coral cay	Schmidt and Stewart 1999
Subtropical rainforest	Schmidt and Stewart 1999
Subtropical wet heathland	Schmidt and Stewart 1999
Semiarid mulga woodland	Schmidt and Stewart 1999
Tropical savanna woodland	Schmidt and Stewart 1999

ly on pool sizes and fluxes of inorganic N (IN). Where ON availability has been studied, it has generally been in the context of availability to soil microbes for the process of N mineralization. The measurement of ON availability to plants is a new and complex issue that involves understanding supply rates of ON, interactions of ON with the soil matrix, and plant-microbial and plant-plant competition for ON. Other questions that have arisen include determining how widespread ON use is among different ecosystems and plant species, and what implications ON use by plants will have for our understanding of other processes such as climate change, interspecific competition, and N pollution.

The purpose of this review is to synthesize the recent work on availability and plant uptake of ON with classic work on ON in soils, and to integrate plant ON uptake into the current understanding of the terrestrial N cycle. We also review the methodologies used to assess plant ON use in ecosystems, including the recent work on the use of natural abundance levels of ^{15}N to infer N sources for plant species. While some studies have shown that ericoid mycorrhizal fungi may allow their host plants access to N from amino sugars and nucleic acids (Chalot and Brun 1998), the majority of recent studies of ON uptake by plants have focused on amino acids. Therefore, this review will be restricted mainly to the discussion of amino acids as N sources for plants.

ON availability in soils

Forms of ON in soils

Amino acid N is the most common form of N in soils. Acid hydrolysis of soils typically yields 20–50% of the total N as amino N (Senwo and Tabatabai 1998). Acid hydrolysis underestimates the total amount of amino N because some of the ammonium liberated during hydrolysis originates from the amides asparagine and glutamine, and because the non-hydrolyzable fraction also contains proteinaceous materials that are physically protected by occlusion or binding to clay minerals (Leinweber and Schulten 1998). A recent review of soil ON estimates the following breakdown of soil ON: 40% proteinaceous materials, 35% heterocyclic compounds (e.g., nucleic acids), 5–6% amino sugars, 19% ammonium (Schulten and Schnitzer 1998). A recent study found that the distribution of amino acid species in soil peptides was fairly uniform, with the acidic amino acids, aspartate and glutamate, together with their amides, asparagine and glutamine, and the two neutral amino acids, glycine and alanine, being most common (Senwo and Tabatabai 1998).

Only a small fraction of the total proteinaceous amino acid N pool is in the form of individual amino acids dissolved in the soil solution (generally referred to as "free amino acids"). A variety of solvents have been used to extract amino acids from soils, including water, ethanol, ammonium acetate, barium hydroxide, and sulfuric acid. Reported values fall in the range of 0.04–24 μg N g^{-1} soil (Abuarghub and Read 1988; Kielland 1995; Lipson et al. 1999b; Matsumoto et al. 1999; Schmidt and Stewart 1999). Amino acids in lysimeter samples of soil pore water have been detected in several environments at concentrations up to 158 μmol l^{-1} (Raab et al. 1996, 1999). A wide variety of free amino acids are found in soil extracts and pore water. Aspartate, glutamate, and glycine commonly dominate the amino acid profile (Kielland 1995; Raab et al. 1996, 1999; Turnbull et al. 1996), although the basic amino acids lysine (Paul and Schmidt 1961), arginine (Kielland 1995; Schmidt and Stewart 1999), and histidine (Abuarghub and Read 1988), and neutral amino acids such as alanine, serine, asparagine, glutamine and leucine (Abuarghub and Read 1988; Kielland 1995; Turnbull et al. 1996; Schmidt and Stewart 1999) are sometimes present in relatively high concentrations. The recovery of amino acids in soil extracts depends on the solvent used. For example, water effectively extracted acidic and neutral amino acids, but not basic amino acids from a silt loam, while the use of

ammonium acetate was more effective in extracting basic amino acids (Paul and Schmidt 1960).

The free amino acid pool is small and dynamic because amino acids are rapidly taken up by soil microorganisms and plants. Reported half-lives for amino acids in soils are in the range of 1.7–28.7 h (Hadas et al. 1992; Martens and Frankenberger 1993; Kielland 1995; Jones 1999; Lipson et al. 2001). Also, amino acids are difficult to extract because of strong interactions with the soil particle surfaces. Therefore, the concentration, alone, of amino acids in extracts or soil pore water samples may not be the best index of availability for plant roots and soil microbes. On the other hand, the majority of total soil amino N is protected by minerals and humus and so turns over very slowly. The ON pool that is most indicative of amino acid availability to plants is probably one that is extractable by some aqueous solvent. Electroultrafiltration or extraction with hot water or calcium chloride yields a high molecular weight ON pool that is correlated with net N mineralization and plant N uptake (Serna and Pomares 1992; Appel and Mengel 1993). Different types of salt solutions allow extraction of different fractions of organic matter. Easily extractable protein concentrations in soils are generally an order of magnitude higher than free amino acid concentrations (Turnbull et al. 1996; Lipson et al. 1999b; Matsumoto et al. 1999). The process of proteolysis is frequently substrate-limited in soil (Lipson et al. 1999b), so the presence of proteins implies a steady source of free amino acids. Mayer et al. (1995) developed a method to estimate bioavailability of amino acids in which sediments were incubated with proteolytic enzymes and the release of amino acids was monitored.

Sources of free amino acids

Amino acids are released into the soil solution by a variety of mechanisms. Bacteria accumulate amino acids such as γ-aminobenzoic acid (GABA), proline and glutamic acid as compatible solutes either constituitively or during osmotic stress (Measures 1975). These amino acids are released during lysis of cells, or by excretion as cells switch to trehalose accumulation in the next phase of osmotic adjustment (Csonka and Hanson 1991). Hence, increased soil amino acid concentrations have been observed after drying-rewetting events (Lipson and Monson 1998). High concentrations of GABA were observed after waterlogging in a tropical heathland, although the authors believed the source was plant roots under oxygen stress (Schmidt and Stewart 1997). Amino acid released from sediments upon incubation with proteases was initially dominated by osmolytes such as taurine (Mayer et al. 1995). Freeze-thaw events can also release amino acids from cells and from sites where amino acids are occluded inside aggregates (Ivarson and Sowden 1966; Edwards and Cresser 1992; Winter et al. 1994; Mayer et al. 1995). However, these same physical factors can also damage plant roots, and so may not result in significant opportunities for amino acid uptake by plants (Lipson and Monson 1998).

Plant roots exude amino acids, though whether this efflux is a net source in the rhizosphere depends on the amino acid uptake capacity of the plant (Jones and Darrah 1994). It is possible that plant species with high affinities for amino acids could scavenge amino acids from the rhizosphere of plant species with weaker amino acid uptake capacity. As a possible example, levels of soil N and the abundance of certain plant species are positively correlated with the distribution of the N-fixing species *Trifolium dasyphyllum* in the Colorado alpine (Thomas and Bowman 1998). This effect could simply be caused by high litter N concentrations, but legumes are known to secrete proteins and other forms of ON into soils (Rougier and Chabot 1985; Sawatsky and Soper 1991). Additionally, many perennial plants store N in the form of free amino acids in belowground structures such as roots and rhizomes (Lipson et al. 1996; Nordin and Näsholm 1997). Leakage from these structures or decay of old tissues could release appreciable amounts of free amino acids to the soil solution.

The largest and most reliable source of free amino acids for plants is probably the hydrolysis of proteins and peptides by extracellular enzymes. As discussed above, the soil protein and peptide pool is relatively large, and this pool is continuously replenished by the turnover of microbial biomass and plant tissues. Murien found in bacteria cell walls contains a variety of amino acids, notably glycine, lysine, and both D- and L-isomers of glutamic acid and alanine (Stanier et al. 1986). Also, polymers of D-glutamic acid and L-glutamine are associated with cell walls of various bacteria (Kandler et al. 1983). Based on mineralization rates in soil, D-amino acids probably represent a small fraction of the soil amino acid flux compared to L-isomers, with the possible exception of D-alanine (Hopkins et al. 1994). Mayer et al. (1995) found that amino acids released from sediment after incubation with proteases were depleted in methionine, and concluded that microbial coat proteins were an important source. Soil microbial biomass N typically turns over several times a year (Davidson et al. 1992; Fisk et al. 1998), and produces a labile N pool that is rapidly degraded (Marumoto et al. 1982; van Veen et al. 1987; Groffman et al. 1993; Mengel 1996). Maximum soluble protein concentrations and/or protease activities are often observed immediately after a peak and decline in microbial biomass (Ladd and Paul 1973; Nannipieri et al. 1979; Asmar et al. 1994; Lipson et al. 1999b). When ^{15}N-labelled microbial biomass was incubated in soils, the largest fraction (38.5%) of ^{15}N was found in the amino acid pool (Marumoto et al. 1982). It is likely that amino acid availability increases during periods of microbial biomass turnover, especially in systems where seasonal microbial dynamics are linked to a flush of available N to plants (Singh et al. 1989; DeLuca et al. 1992; Lipson et al. 1999b).

The proteolysis of soil proteins and peptides is generally considered to be the rate-limiting step in N mineral-

ization (Ladd and Paul 1973; Cunningham and Wetzel 1989; Asmar et al. 1994). However, potential protease rates are usually several orders of magnitude higher then net N mineralization rates, even allowing for the fact that these protease rates were often measured with unlimiting substrate and at high temperatures (Alef et al. 1988; Chapin et al. 1988; Smith et al. 1989; Asmar et al. 1994; Watanabe and Hayano 1995; Yamagata and Ae 1999). Two studies in the Colorado alpine that measured rates of proteolysis of native substrate only, without protein amendments, and adjusted the rates for field temperature estimated amino acid fluxes of 42–109 μg N g^{-1} soil $year^{-1}$ (Raab et al. 1999; Lipson et al. 2001), values which are slightly greater than estimates of gross N mineralization rates for the ecosystem (Fisk et al. 1998), and much greater than net N mineralization (Fisk and Schmidt 1995). Published rates of soil protease activity using a variety of techniques and incubation temperatures usually range from 0.1 to 4 μmol g^{-1} soil h^{-1} amino acid released (Chapin et al. 1988; Smith et al. 1989; Asmar et al. 1994; Yamagata and Ae 1999). These studies show that fluxes of amino acid N can be much greater than plant N requirements in most ecosystems, but the majority of the amino acid flux is sequestered by microbial biomass and interactions with the soil matrix. The deciding factor in amino acid availability to plants is what modest fraction of this large flux plant roots and their mycorrhizae are able to divert from these competing sinks.

Interactions of ON with the soil matrix

The non-biological component of soils needs to be considered when predicting plant uptake of ON. Soil minerals and humus protect ON from degradation and slow its diffusion through soil. Studies of N partitioning in high-OM alpine soils found that 17–65% of added $^{15}NH_4^+$ (Jaeger et al. 1999) and 43% of added ^{15}N -glycine (Lipson and Monson 1998) ended up in a non-biomass soil fraction. Amino acids interact extensively with humic substances. In one study, 7–71% of added ^{15}N-labeled glycine was fixed into sterile humus after a 24 h incubation, with the greatest fixation occurring at neutral to high pH (Nommik 1970). Amino acids are fixed by humic substances more rapidly than are nucleic acids and other ON forms, and amino acid-N also appears to turn over relatively quickly in the humic acid fraction (Kuzyakov 1997). As such, humic substances act as both a sink and a resevoir for ON. Humics bind to proteins, potentially interfering with proteolysis, although significant catalytic activity of protease-humic acid complexes has been observed (Rowell et al. 1973). Northup et al. (1996) concluded that polyphenols in forest litter inhibited N mineralization, thus increasing the ON/IN ratio. Clay minerals also bind proteins, and can slow or completely halt bacterial degradation of protein, depending on the clay:protein ratio (Marshman and Marshall 1981). Amino acids interact extensively with soil minerals, particularly amorphous minerals in clay fractions (Schnitzer and Kodama 1992). In the aforementioned study it was found that neutral amino acids were especially associated with silicon-rich minerals, and acidic amino acids with aluminum-rich minerals.

An important control over nutrient availability and plant-microbe competition is the diffusion rate of nutrients through the soil. The diffusion rate of amino acids in soils is strongly controlled by their tendency to adsorb to the soil matrix. This phenomenon is generally described by adsorption isotherms in which equilibrium concentrations in solid phase and soil solution are related to each other over a range of initial concentrations (Sposito 1989). Adsorption isotherms for amino acids in soils have been reported for pure montmorillonite and illite (Greenland et al. 1965), a B-horizon from a conifer plantation (Jones et al. 1994), a sandy loam Eutric cambisol (Jones and Hodge 1999), and a Pergellic cryumbrept (Raab et al. 1999). Isotherms are either linear, or saturate at high amino acid concentrations (Langmuir type isotherm). In general for the soils tested, lysine and other basic amino acids are adsorbed most strongly, due to their positive charge, and neutral and acidic amino acids are less strongly sorbed. Reported values for the buffer power (β_e) or the solid-liquid partition coefficient (K_d) (both related to the slope of the isotherm and inversely proportional to the diffusion rate) of basic amino acids range from 0.7 to 141 l kg^{-1}, and 0.3 to 24 l kg^{-1} for other amino acids. These values can be used to predict diffusion rates in soil, assuming that amino acids rapidly reach equilibrium with clay surfaces. This may not always be the case, however, and factors other than the equilibrium solid-liquid partition coefficient might become important (Darrah 1991). A study of amino acid mobility using thin layer soil chromatography found that smaller amino acids, such as glycine, tended to move more slowly, probably due to less steric hindrance in interactions with clay surfaces (Kumari et al. 1987). These results are contrary to predictions based on sorption data. In the aforementioned study, the presence of calcium carbonates or alkaline salts severely slowed movement of amino acids through soil. There are no studies, to our knowledge, that have measured adsorption or diffusion of amino acids in highly oxidized soils with significant anion exchange capacity. Amino acids would probably behave differently in such soils.

Amino acid uptake kinetics by plants, mycorrhizae and soil microbes

There are now many studies that report kinetic parameters for amino acid uptake by plant roots, mycorrhizal fungi in culture, mycorrhizal root tips, isolated microbes, and soil microbial biomass (Table 2). It has been established that amino acid uptake by plants occurs through active proton symport (Reinhold and Kaplan 1984). Recent studies on *Arabidopsis thaliana* confirms that a range of amino acid transporters are present in plant

Table 2 Amino acid uptake kinetic parameters for plants, mycorrhizal root tips, axenic mycorrhizal fungi, isolated soil microbes and soil microbial biomass (*n/a* data not available, V_{max} maximum uptake rate)

Organism	K_m (μmol l^{-1})	V_{max}	Reference
Plant roots			
Chamaegigas intrepidus	16	0.255 μmol g^{-1} FW h^{-1}	Schiller et al. 1998
	159	1.369	
Zea mays	4.8	6–19	Jones and Darrah 1994
Arabidopsis thaliana	35	n/a	Frommer et al. 1995
	1,700	n/a	Breitkreuz et al. 1999
	12,900	n/a	
Ricinus communis	30–50	0.8–1.2[a]	Schobert and Komor 1987
	1,200	n/a	Schobert et al. 1997
	1,500	n/a	
	2,100	n/a	
Hordeum vulgare	1.6	0.22	Soldal and Nissen 1978
	61	5.62	
	160	11.86	
	1,700	35.0	
Betula nana[b]	13–96	3.9–6.8	Kielland 1994
Carex aquatilis	9	1.3	
C. bigelowii	7	1.2	
Eriophorum angustifolium	143	2.4	
E. vaginatum	12	2.0	
Ledum palustre[b]	9	2.6	
Salix pulchra[b]	19–87	4.6–7	
Fagus sylvatica[b]	21–233	0.52–5.7	Wallenda and Read 1999
Picea abies[b]	42–135	0.8–22.5	
Carpinus betulas[b]	96	45	Chalot and Brun 1998
Aesculus hippocastoneum[b]	253	19.4	
Mycorrhizal fungi			
Paxillus involutus	7.2–27	14.5–42.8 μmol g^{-1} DW h^{-1}	Chalot et al. 1996
Lactarius subdulcis	197	22.8	Wallenda and Read 1999
Russula ochraleuca	233	3.1	
Xerocomus chrysenteron	84	18.7	
Unidentified dark septate	1.6–8.0	n/a	Mullen 1995
Heterotrophic microbes			
Aspergillus niger	180	0.24 μmol g^{-1} DW h^{-1}	Roos 1989
Penicillium cyclopium	24	19.2	
Escherichia coli	0.5–5	0.3–4.08 μmol g^{-1} protein h^{-1}	Schellenberg and Furlong 1977
Unidentified soil bacteria	7.4	n/a	Lipson et al. 1999a
Soil microbial biomass			
Alpine dry meadow	39–46	240–390 nmol g^{-1} soil h^{-1}	Lipson et al. 1999b
Experimental garden loam	500–1,000	20–70	Jones and Hodge 1999

[a] Uptake rates were linear at concentrations >1,000 μmol l^{-1}. V_{max} given is for 0–500 μmol l^{-1} range
[b] Mycorrhizal root tips

roots (cf. Fischer et al. 1998). Some of these amino acid transporters show broad substrate specificity implying that roots should possess the capacity to absorb both acidic, neutral and basic amino acids. Plant roots often exhibit multiphasic kinetics, and so values reported for the half-stauration constant K_m, the value at which half the maximum rate occurs, can depend on the range tested (Soldal and Nissen 1978; Reinhold and Kaplan 1984). In some cases, multiple transporters with different affinities exist (e.g., Schobert et al. 1997; Schiller et al. 1998; Breitzkreuz et al. 1999), although multiphasic kinetics can also be caused by cooperativity of the transporter, or by diffusional effects (Reinhold and Kaplan 1984). Because of this phenomenon, the range of K_m values reported is quite large. Nonetheless, there are numerous reports of plant roots with high-affinity uptake systems for amino acids in the range of amino acid concentrations found in soil. Mycorrhizal roots and axenic mycorrhizal fungi generally also absorb amino acids with high affinity, but the range of K_m is not much lower than non-mycorrhizal plant roots. The effect of increased absorptive area and proteolytic activity may often be more important than uptake affinity in the improved acquisition of ON by mycorrhizal infection (Chalot and Brun 1998). Soil bacteria and fungi generally have very high affinities for amino acids, but the "K_m" reported for soil microbial biomass absorbing amino acids from a slurry or soil solution are higher. This is probably because (1) soil adsorption decreases the effective concentration of amino acids in the soil solution (Sposito 1989), and (2) uptake

by soil microbial biomass follows multiphasic kinetics (Jones and Hodge 1999), so the K_m reported depends on the concentration range studied.

Plant-microbe competition for ON

It has been assumed that microbes outcompete plants for uptake of nutrients because of their ubiquitous distribution throughout the soil, and their higher surface to volume ratios, substrate affinities, and specific growth rates compared to plant roots. The traditional measure of N availability to plants, net mineralization, assumes that plants can access only inorganic N that is released in excess of microbial N requirements. Amino acids serve as excellent C and N sources for microbes, and so will almost never exist in excess of microbial demand. That plants absorb amino acids from the soil implies direct competition between plants and microbes. The study of plant-microbe competition has, therefore, recently become a topic of great interest (Eviner and Chapin 1997; Kaye and Hart 1997).

The belief that microbes are strong competitors with plants is generally borne out in the literature. For example, the addition of glucose to soil stimulated N immobilization into the microbial biomass and reduced N uptake by plants, whereas sterilization of the soil increased N uptake by plants (Schmidt et al. 1997). Several recent studies have directly measured competition between plants and microbes for isotopically labeled N from soil. In general, short-term experiments (up to 2 days) show that plants are outcompeted by microbes for uptake of inorganic N by a factor of 4.2–532 for NH_4^+, and a factor of 1.7–106 for NO_3^- (Jackson et al. 1989; Schimel et al. 1989; Zak et al. 1990; Groffman et al. 1993). One of the major problems with studies of competition between plants and microbes for N is the difficulties to separate the microbial fraction into one that is linked to plants (the mycorrhizal fungi), and one that actually competes with plants. The techniques used for extracting microbial N tend to combine these functionally different groups of organisms (Eviner and Chapin 1997). If mycorrhizal hyphae absorb a substantial fraction of labeled N, part of this label would be expected eventually to end up in plants. In support of this, longer-term experiments (weeks to months) usually show that plants perform better than in short-term studies, sometimes outcompeting microbes (Jackson et al. 1989; Schimel and Chapin 1996; Kaye and Hart 1997; Jaeger et al. 1999). These results can also be explained by the more rapid turnover of microbial biomass compared to plant roots. During competition with soil microbes for amino acids, plants capture a minority of the added N: *Kobresia myosuroides* absorbed 0.9–4% (Lipson and Monson 1998; Lipson et al. 1999a), *Eriophorum vaginatum* 1–3.8% and *Carex aquatilis* 12% (Schimel and Chapin 1996). However, given the potentially large flux of amino acids in soils, these modest levels of competition could result in a significant N gain for plants. Taken together, the studies cited above show that short-term competition for N is dominated by microbes but the failure to distinguish between N uptake by symbiotic and non-symbiotic microorganisms restricts the value of such data. Moreover, the cited studies also indicate that plants compete equally well for ON and for IN.

There have been several attempts to explain how plant-microbe competition might be minimized in natural systems. Jaeger et al. (1999) proposed that plants and microbes in the Colorado alpine partition N temporally, with microbes immobilizing N maximally in the fall and plants absorbing N in the early summer. This general pattern has been confirmed in other studies of this ecosystem (Fisk and Schmidt 1995; Lipson et al. 1999a), but microbes are still a significant competitive sink for amino acids during the plant growing season (Lipson and Monson 1998; Lipson et al. 1999a, 1999b). Lipson and Monson (1998) hypothesized that freeze-thaw and dry-rewet events reduce microbial competition and allow plants opportunities for amino acid uptake, but found that these events were more detrimental to plants, and that plants competed most efficiently under warm and moist conditions. There is now evidence for niche separation between microbes and plants in terms of specific amino acids. It has been widely observed that plants take up glycine faster than heavier amino acids (Kielland 1994; Schmidt and Stewart 1997; Lipson et al. 1999a), and that glycine is degraded by soil microbes at lower rates than are other amino acids (Alef and Kleiner 1986; Kielland 1995; Lipson et al. 1999a). In a microcosm experiment, *K. myosuroides* competed with soil microbes 3.25 times better for glycine than for glutamate (Lipson et al. 1999a). Based on these studies glycine is more available to plants because it is a poorer carbon source for microbes than other amino acids. The faster diffusion rates of glycine in soil (Raab et al. 1999) also probably contribute to its preferential uptake by plant roots, just as plants generally compete better for nitrate than ammonium because of its higher mobility in soil (Jackson et al. 1989; Schimel et al. 1989; Zak et al. 1990; Groffman et al. 1993).

The "rhizosphere effect" could alter the balance of plant-microbe competition for amino acids in microsites within the soil. Just as seasonal events cause the microbial biomass to turn over and release a flush of ON to plants, dynamics in root exudation and growth could cause similar events on smaller spatial and temporal scales. Exudation by a growing root tip can cause such microbial population dynamics (Darrah 1991), especially in the presence of protozoal grazers (Rutherford and Juma 1992). Another possible effect of the exudation by roots of organic acids and sugars is the preferential uptake by microbes of these compounds over rhizospheric amino acids, thus shifting the competitive balance of amino acid uptake towards plants. However, a recent study found that the addition of a tenfold excess of glucose or citrate had little effect on the microbial degradation of amino acids (Jones and Hodge 1999).

The mycorrhizal status of plants has obvious implications for the ability of plants to compete with soil mi-

crobes for amino acids. While the role of ericoid and ectomycorrhizae in transferring ON to plants is well established (Read 1991; Marschner and Dell 1994; Chalot and Brun 1998), the relative contributions of mycorrhizal and non-mycorrhizal roots is still an open question for many ecosystems. Ecto- and ericoid mycorrhizae improve the surface area (Rousseau et al. 1994) and affinity (Chalot et al. 1996; Wallenda and Read 1999) of plant roots for ON, and many of the plant species studied are unable to grow on ON sources without their mycorrhizal symbiont (Stribley and Read 1980; Bajwa and Read 1985; Abuzinadah and Read 1989; Finlay et al. 1992; Turnbull et al. 1995). However, because many non-mycorrhizal roots can also take up amino acids with high affinity, it is sometimes difficult to quantify the contribution of mycorrhizal versus non-mycorrhizal uptake of amino acid N. Microcosm and field experiments that measure competitive abilities of plants for labeled amino acids generally cannot distinguish between plant and mycorrhizal uptake of amino acid N (Näsholm et al. 1998; Lipson and Monson 1998; Lipson et al. 1999a). Using a two chamber system that excluded plant roots, fungal endophytes were shown to transfer 1.3% of glycine N, added to soil over 21 days, to the facultatively mycorrhizal alpine sedge, *K. myosuroides* (Lipson et al. 1999c). If it is valid to compare this result with shorter-term microcosm experiments in which plant roots are included, mycorrhizae might be responsible for 33–100% of glycine-N uptake by this plant. Quantification of the extent of mycorrhizal infection of roots and hyphal surface area in the soil is required to develop accurate mechanistic models of plant amino acid uptake in natural systems. Ectomycorrhizal fungal length has been estimated in various studies to represent 100–300 cm cm^{-1} root (Jones et al. 1990, willow), 504 cm cm^{-1} root (Rousseau et al. 1994, pine), and 1000–8000 cm cm^{-1} root (Read and Boyd 1986, pine). As reported in the literature, or converting from hyphal length using diameters of 3 µm for fungi and 400 µm for plant roots (Eissenstat and Yanai 1997), this represents 0.75–2.24, 3.16, and 7.5–60 cm^2 cm^{-2} root surface area contributed by ectomycorrhizal hyphae for the three studies, respectively.

It is likely that the determining factor in plant-microbe and plant-plant competition for amino acids is more frequently surface area rather than uptake kinetics. The values in Table 2 show that plants, mycorrhizae and soil microbes can have comparable affinities for amino acids. Moreover, given the strong adsorptive properties of soils, diffusion, rather than maximum uptake rates, generally will limit the supply of amino acids to all biological surfaces. Hodge et al. (1998, 1999b) observed increased root proliferation by grasses in patches where lysine was added to the soil, and found that N uptake from an organic patch was related to root density when the two grasses *Lolium perenne* and *Poa pratensis* were grown together (Hodge et al. 1999a). Thus, root density is particularly important in situations where multiple plant species are competing with each other.

Assessing plant ON use in ecosystems

Laboratory and field studies

The conclusion that amino acids are potentially important N sources for plants in many studies was based on the presence of free amino acids in the environment and the ability for plant roots to absorb amino acids (Chapin et al. 1993; Kielland 1994; Jones and Darrah 1994; Raab et al. 1996; Turnbull et al. 1996; Schiller et al. 1998; Schmidt and Stewart 1997, 1999). In many cases, plant species were shown to preferentially absorb amino acids over inorganic N sources (Table 3). Some workers have added isotopically labeled amino acids to soil and recovered them in plant tissues, showing that plants can compete successfully with microbes for amino acid N (Schimel and Chapin 1996; Lipson and Monson 1998; Näsholm et al. 1998; Lipson et al. 1999a, 1999c; Yamagata and Ae 1999). In some cases it was verified that the amino acids were taken up intact, rather than after mineralization to ammonium, by recovery of labeled C in plant tissues (Lipson and Monson 1998; Näsholm et al. 1998; Lipson et al. 1999a). Critical remarks on the potential use of ON by plants have also been presented. Jones (1999) argued that studies of amino acid uptake by plants performed either on detached roots or in solution cultures give little information about uptake of ON *in situ*. As turnover rates of a range of amino acids were shown to be rapid, and from published values on kinetic parameters of microbial amino acid transporters, the author concluded that microbes present in the rhizosphere would effectively filter any ON molecule diffusing toward root surfaces. Hodge et al. (1998, 1999b) studied root proliferation and plant N capture of five grass species grown in microcosm. Dual-labeled lysine was added to assess potential uptake of ON by plants. Because plant roots and shoots were found to be isotopically labeled with ^{15}N but not with ^{13}C, it was concluded that these plant species could not capture ON when competing with soil microbes. Schimel and Chapin (1996) recovered no excess ^{13}C in plants after injecting dual-labeled glycine into tundra soil, but found that more ^{15}N was recovered in glycine-fed plants than in ammonium-fed plants. These authors argued that plants competed well for amino acid N, but rapidly respired away the labeled C. Hence, different studies have arrived at divergent conclusions about plant utilization of ON although similar techniques have been used. These different conclusions might reflect a variation in plant ON utilization – some plants might be able to compete for these substances while others might not. The diverging results might also reflect differences in experimental setup and analytical techniques. For example, Hodge et al. (1998, 1999b) and Schimel and Chapin (1996) used amino acids labeled at the C1 (carboxyl) position, whereas the other previously mentioned studies used C2-labeled glycine. The carboxyl group of amino acids is more prone to rapid respiratory decarboxylation than the C2 atom (Fokin et al. 1993). Also, Hodge et al. (1998, 1999b)

Table 3 Plant species that take up amino acid at comparable or greater rates compared with inorganic N

Plant species	Native community	Reference	Method[a]
Kobresia myosuroides	Alpine	Raab et al. 1999	1
Carex rupestris	Alpine	Raab et al. 1999	1
K. simpliciulscula	Subalpine fen	Raab et al. 1999	1
C. ebenea	Subalpine forest	Raab et al. 1999	1
C. canescens	Subalpine forest	Raab et al. 1999	1
C. rhynchophysa	Subalpine forest	Raab et al. 1999	1
C. stenophylla	Shortgrass steppe	Raab et al. 1999	1
C. fillifolia	Shortgrass steppe	Raab et al. 1999	1
Cyperus strigosus	Temperate swamp	Raab et al. 1999	1
C. halpan	Dry tropical forest	Raab et al. 1999	1
Hakea sp. proteoid roots	Subtropical wet heathland	Schmidt and Stewart 1997	2
Pisonia grandis	Subtropical coral cay	Schmidt and Stewart 1997	2
Betula nana	Dry heath, tussock tundra	Kielland 1994	2
Carex bigelowii	Tussock tundra	Kielland 1994	2
Eriophorum vaginatum	Tussock tundra	Kielland 1994	2
Ledum palustre	Tussock tundra	Kielland 1994	2
Salix pulchra	Shrub and tussock tundra	Kielland 1994	2
Oryza sativa	Agricultural	Yamagata and Ae 1996	1
Zea mays	Agricultural	Jones and Darrah 1994	1

[a] Method: *1* intact plants in hydroponic solution, *2* excised roots

used the positively charged amino acid lysine, which binds strongly to the soil matrix (Jones and Hodge 1999), and is taken up more slowly by some plants (Jones and Darrah 1994).

Modeling uptake of amino acids by plants

Several approaches have been used to predict amino acid uptake by plants using mathematical models. Using Michaelis-Menten kinetics and soil amino acid concentrations, authors have estimated that amino acids contribute 60% (*E. vaginatum*, Chapin et al. 1993) and 10–82% (10 arctic tundra species, Kielland 1994) to the N budget of the plant species under study. Lipson et al. (2001) estimated a yearly flux of 109 g amino acid-N m^{-2} by adjusting measured protease rates to field conditions, and estimated that amino acids could make up at least 50% of the N requirements of *K. myosuroides* based on past studies of plant-microbe competition. Jones and Darrah (1994) modeled uptake of amino acids by *Zea mays* using initial bulk soil amino acid concentrations, diffusion rates, and Michaelis-Menten parameters from the literature. These authors concluded that amino acids could make up 30–90% of the plants N requirement, depending on the availability of inorganic N. Leadley et al. (1997) estimated uptake of ammonium, nitrate, and glycine by *E. vaginatum* by modeling supply rates and diffusion through soil to growing and non-growing root cylinders where Michaelis-Menten kinetics were used to describe root uptake. The supply rates and buffering capacity of the soils were poorly constrained, especially for glycine, but the simulation revealed the relative importance of the model parameters (supply rates>diffusion rates=root density>root uptake kinetics), and that in soils with high buffering capacity, equilibrium is reached slowly (~90 days) and dynamics in the supply rate are dampened.

ON use and natural abundance of ^{15}N

An approach different from those mentioned above to the problem of assessing the role of ON (and IN) for different plant species has been that of using the natural abundance of the stable N isotope, ^{15}N ($\delta^{15}N$). Michelsen et al. (1996, 1998) suggested that differences between plant species in their utilization of different N sources were mirrored in the $\delta^{15}N$ of leaves of the respective species. A large set of data from two subalpine plant communities showed consistent differences between plant functional types in $\delta^{15}N$. It was concluded that low $\delta^{15}N$ values of ericaceous shrubs and some ectomycorrhizal species were related to a large dependence of these plants on ON sources while the relatively high $\delta^{15}N$ values found in grasses and sedges was due to a larger uptake of IN. However, measurements of $\delta^{15}N$ of soil amino acids are not in agreement with the conclusion that ON is depleted in ^{15}N, but rather show soil amino acids to have positive $\delta^{15}N$ values (Ostle et al. 1999). Gebauer et al. (1994) found similar trends in $\delta^{15}N$ between lifeforms, but attributed the higher $\delta^{15}N$ values found in the grass, *Calamagrostis canadensis*, to a reliance on N from deeper soil horizons. Nadelhoffer et al. (1996) examined variations in $\delta^{15}N$ in several ecosystems in the arctic tundra. A similar pattern was found in $\delta^{15}N$ variation between plant functional groups to that found by Michelsen et al. (1996) but the authors stressed that many processes, such as plant rooting depth and fractionation during plant or mycorrhizal uptake of N, could lead to differences in plant $\delta^{15}N$. Hobbie et al. (1999a) found large differences in $\delta^{15}N$ between plants growing in forests of different successional stages. Based on model simulations these authors concluded that the most important fractionation step occurred during the transfer of N from mycorrhizal fungus to plants (Hobbie et al. 1999b). Fractionation during vesicular-arbuscular mycorrhizal N uptake and transfer to the host plant has been observed

(Handley et al. 1993), but further work is needed to clarify whether such fractionation occurs in ecto- and ericoid mycorrhizae, and to distinguish whether fractionation occurs during uptake or during transfer to the plant (Handley et al. 1993; Högberg 1997; Högberg et al. 1999). Other factors that could complicate natural ^{15}N abundance studies include spatio-temporal variation in the $\delta^{15}N$ of a specific N source within an ecosystem, and the fact that ON is not one N source but may include a range of substances that diverge in ^{15}N natural abundance. Measurements of $\delta^{15}N$ in individual amino acids have revealed both differences in fractionation during microbial metabolism (Macko et al. 1986, 1987), trophic interactions (Hoch et al. 1996), peptide hydrolysis (Silfer et al. 1992), and variations in $\delta^{15}N$ in steady state pools of plants (Yoneyama et al. 1997). A final consideration is that a substantial fraction of the N content of perennial plants is repeatedly recycled. Differences in $\delta^{15}N$ between plants could therefore depend on the fraction of recycled N in plants and the number of recycling events to which this N pool has been subjected. Fractionation during recycling of N could arise by: (1) differences in $\delta^{15}N$ between N pools that are remobilized and those that are not (such as cell wall proteins), (2) synthesis of specific transport substances during N remobilization, and (3) losses of N as NH_3 formed during hydrolysis of proteins or amino acids. Clearly, a number of processes can affect the $\delta^{15}N$ of plants (cf. Handley and Scrimgeour 1997; Högberg 1997). Currently, available information on the relative importance of these factors is too restricted to allow inferences of plant dependence on specific N sources from the natural abundance of ^{15}N.

Ecological implications of ON use by plants

The widespread importance of ON uptake by plants has important ramifications for our understanding of ecological processes. The most fundamental is the fact that virtually all research on plant N uptake in ecosystems has focused solely on ammonium and nitrate. The N cycle in many ecosystems needs to be revisited with a new perspective. Because N availability commonly regulates biomass production as well as species composition in terrestrial ecosystems, the role of ON is critical to our understanding of how ecosystems function and how they will be affected by environmental changes. For example, one of the most serious effects of N deposition in terrestrial ecosystems is that it causes vegetation changes (see Bobbink et al. 1998; Lee and Caporn 1998). While some attention has been paid to the importance of shifts in the relative abundance of NH_4^+ and NO_3^- for species compositions in plant communities (e.g., Diekmann and Falkengren-Grerup 1998; Näsholm 1998), the role of changes in plant available ON has not been properly addressed in discussions about N deposition effects on vegetation. N deposition could change the IN/ON balance both directly by adding IN, and indirectly by the inhibitory effect of IN on proteolytic activity in soils (Smith et al. 1989). Global warming could shift the IN/ON balance in a similar way. Higher temperatures could lead to a loss of soil organic matter, in turn leading to lower amino acid fluxes and higher rates of mineralization. Also climate change could change seasonal dynamics, thus affecting seasonal ON releases from microbial biomass. Increased CO_2 levels could increase rhizodeposition from plant roots (Körner and Arnone 1992; Paterson et al. 1999). This could change the cycling of ON in complex ways, either stimulating organic matter breakdown and N release by increasing microbial turnover rates in the rhizosphere (Clarholm 1985), or by increasing microbial immobilization of inorganic N (Michelsen et al. 1999). Clearly, the widespread use of ON by plants raises many questions that are central to the field of ecology, and which can only be answered if the the role of ON is routinely included in studies of terrestrial ecosystems.

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