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Review

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Uptake of organic nitrogen by plants

Author for correspondence:
Torgny Näsholm
Tel: +46 90 786 8205
Fax: +46 90 786 8163
Email: torgny.nasholm@seksko.slu.se

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Torgny Näsholm[1], Knut Kielland[2] and Ulrika Ganeteg[3]

[1]Department of Forest Ecology and Management, Swedish University of Agricultural Sciences, SE-901 83 Umeå, Sweden; [2]Institute of Arctic Biology, University of Alaska, Fairbanks, Alaska 99775-0180, USA; [3]Department of Forest Genetics and Plant Physiology, Umeå Plant Science Centre, Swedish University of Agricultural Sciences, SE-901 83 Umeå, Sweden

Contents

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Summary

Languishing for many years in the shadow of plant inorganic nitrogen (N) nutrition research, studies of organic N uptake have attracted increased attention during the last decade. The capacity of plants to acquire organic N, demonstrated in laboratory and field settings, has thereby been well established. Even so, the ecological significance of organic N uptake for plant N nutrition is still a matter of discussion. Several lines of evidence suggest that plants growing in various ecosystems may access organic N species. Many soils display amino acid concentrations similar to, or higher than, those of inorganic N, mainly as a result of rapid hydrolysis of soil proteins. Transporters mediating amino acid uptake have been identified both in mycorrhizal fungi and in plant roots. Studies of endogenous metabolism of absorbed amino acids suggest that L- but not D-enantiomers are efficiently utilized. Dual labelled amino acids supplied to soil have provided strong evidence for plant uptake of organic N in the field but have failed to provide information on the quantitative importance of this process. Thus, direct evidence that organic N contributes significantly to plant N nutrition is still lacking. Recent progress in our understanding of the mechanisms underlying plant organic N uptake may open new avenues for the exploration of this subject.

I. Introduction

Plant nitrogen (N) nutrition is a topic that challenges the researcher with a number of problems not encountered in other areas of plant mineral nutrition research. The diversity of N forms present in the soil, their interconversions, their different chemical and physical characteristics and not the least the multitude of adaptations and acclimatizations that plants display to optimize acquisition of various N forms all contribute to the complexity of plant N nutrition. Thus, plants can use a wide array of chemical N forms, ranging from simple inorganic N compounds such as NH_4^+ and NO_3^- to

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polymeric N forms such as proteins (Paungfoo-Lonhienne *et al.*, 2008). In spite of this ability of plants to use a wide range of N forms, research on plant N nutrition has had a strong focus on inorganic N forms. This focus was motivated by the prominent role of inorganic N in many arable soils and the dependence of many crop plants on this N source. It was also, naturally, motivated by the abundance of inorganic N fertilizers for agricultural use.

After the widely discussed human perturbation of the global carbon (C) cycle, anthropogenic alteration of global N turnover is the second most important driver of global change (Vitousek *et al.*, 1997; Galloway *et al.*, 2008). This perturbation is to a large extent driven by increased use of chemical fertilizers, that is, inorganic N produced from atmospheric N through the Haber–Bosch process (Gruber & Galloway, 2008), because of the dependence of modern agriculture on the production and use of inorganic N fertilizers in crop production (Matson *et al.*, 1997; Miller & Cramer, 2004). In this context, knowledge of the basic mechanisms through which plants acquire this element is a fundamental requirement. Plant uptake of N from soil is a key process in the global N cycle and the extent to which this process involves other chemical forms than those supplied in inorganic fertilizers is therefore an important issue.

The concept of plant organic N nutrition relies, to a large degree, on studies of amino acids. Thus, amino acid N is in many cases used as a synonym for organic N. Whereas the soil solution may contain a vast array of organic N compounds, free amino acids generally only account for a small fraction of this pool (e.g. Schulten & Schnitzer, 1998; Yu *et al.*, 2002; Andersson & Berggren, 2005). By contrast, peptide- and protein-bound amino acids may contribute more than half of the organic N pool of the soil solution (Senwo & Tabatabai, 1998). These polymeric N forms are, however, sources for the production of the monomeric forms, and rapid turnover of amino acids in soils suggests that this group of compounds may be more important as N sources than their share of the dissolved organic N would suggest. Many plant species form intimate symbioses with fungi. The capacity of mycorrhizal fungi to degrade polymeric N compounds is well established, as is the function of amino acid absorption (cf. Smith & Read, 2007). Nonmycorrhizal plants have, however, received less attention as regards organic N acquisition. Nevertheless, our understanding of organic N acquisition by such plants has recently taken a leap forward. In this review, we discuss amino acid uptake by both mycorrhizal and nonmycorrhizal plants, although, in some sections, with a strong emphasis on the latter.

There are three fundamental requirements for a specific N compound to function as an N source for plants.

- Availability – access to the compound in the soil.
- Uptake – the presence of a regulated uptake system directly or indirectly dependent on free energy input.
- Metabolism – the presence of an endogenous metabolism allowing utilization of the absorbed N compound in the synthesis of various N-containing metabolites.

The quantitative importance of a specific N form in plant N nutrition is thus a function of soil parameters as well as plant parameters.

Plant uptake of organic N has been studied for more than a century and has also been covered by several recent and older reviews (see e.g. Read, 1991; Lipson & Näsholm, 2001; Näsholm & Persson, 2001; Neff *et al.*, 2003; Schimel & Bennett, 2004; Rentsch *et al.*, 2007). In spite of the numerous studies showing the capacities of plants to absorb organic N compounds through roots, and demonstrations of how plants acquire such compounds also in field settings, the issue is still a matter of intense debate. The purpose of this review is therefore to scrutinize some of the evidence suggesting that plants may acquire significant amounts of N through uptake of organic N as well as the criticisms that these studies have received. It is also our purpose to identify the major knowledge gaps and the type of studies required to fill these gaps. In this context, we also suggest new approaches that can be taken to further our knowledge in this area.

II. Availability

1. Production and consumption of soil amino acids

In a variety of ecosystems, rates of amino acid production appear to be much higher than those of N mineralization (Chapin *et al.*, 1988; Fisk *et al.*, 1998; Raab *et al.*, 1999; Lipson & Näsholm, 2001). Although the production rates often exceed apparent plant requirements, a large proportion of these amino acids probably become unavailable to plants as a result of adsorption processes and uptake by soil microbial biomass (Lipson & Näsholm, 2001). High concentrations of organic N in the form of amino acids appear to be sustained through high proteolytic activity (Weintraub & Schimel, 2005a; Berthrong & Finzi, 2006; Kielland *et al.*, 2007; Fig. 1). This activity results from exudation of proteolytic enzymes by free-living microbes, mycorrhizal fungi and plant roots (Bajwa & Read, 1985; Abuzinadah & Read, 1986a,b; Schmidt *et al.*, 2003; Godlewski & Adamczyk, 2007; Paungfoo-Lonhienne *et al.*, 2008). Proteolysis appears to be under strong pH control, as well as being correlated with soil organic matter pools and soil protein concentrations. For example, across a primary successional sequence in interior Alaska, gross amino acid production increased nearly 10-fold from deciduous shrub communities to late successional black spruce (*Piecea mariana*) forests (Kielland *et al.*, 2007). This observation can in part be explained by marked increases in soil acidity in late successional, mature forests which favour proteolysis to a greater extent than net N mineralization (Bajwa & Read, 1985; Chapin *et al.*, 1988; Leake & Read 1989). Decreased soil pH may also accelerate amino acid turnover. For example, in an upland black spruce ecosystem, amino acid turnover increased 4-fold with a drop in soil acidity of less than half a pH unit (Jones & Kielland, 2002). Amino acid production

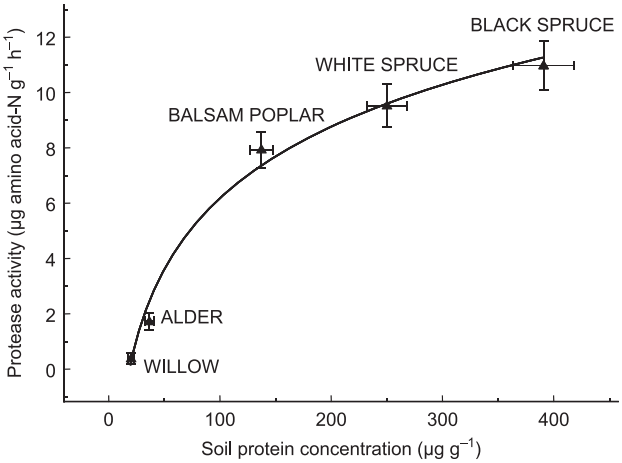

Fig. 1 High concentrations of amino acids found in many soils appear to be sustained through high proteolytic activity. The graph shows the rate of proteolytic activity as a function of soil protein concentration of boreal forest soils across a primary successional gradient in interior Alaska encompassing black spruce (*Picea mariana*), white spruce (*Picea glauca*), balsam poplar (*Populus balsamifera*), alder (*Alnus tenuifolia*) and willow (*Salix* sp.) forests. Reproduced with kind permission from Springer Science + Business Media: Kielland *et al*. (2007).

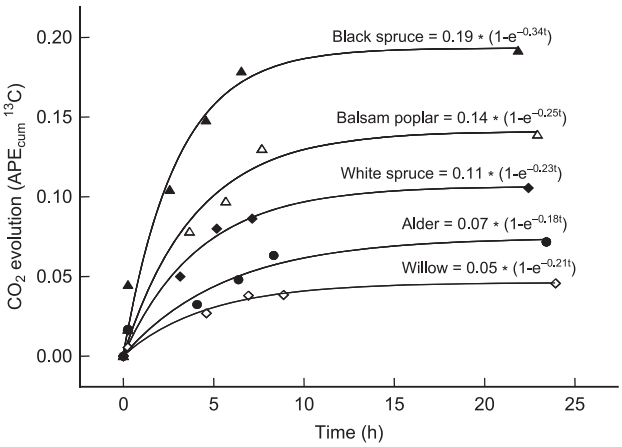

Fig. 2 High rates of amino acid turnover are sustained in late successional coniferous ecosystems in spite of the recalcitrant soil organic matter and low soil temperatures found in these ecosystems. The graph shows the time course of ^{13}C-carbon dioxide evolution *in situ* from successional black spruce (*Picea mariana*), white spruce (*Picea glauca*), balsam poplar (*Populus balsamifera*), alder (*Alnus tenuifolia*) and willow (*Salix* sp.) forest soils amended with L-Asp, Gly, and L-Ala. Values are expressed as atom% enrichment of ^{13}C-CO_2. Reproduced with kind permission from Springer Science + Business Media: Kielland *et al*. (2007).

could also be stimulated by high microbial amino acid demand, as indicated by the inverse relationship between soil N concentration and proteolytic activity found in some studies (Weintraub & Schimel, 2005b).

Soil organic N serves both as an important mineralization substrate (Keilland, 1995; Jones, 1999; Jones & Hodge, 1999) and as a direct source of N for a variety of plant species in arctic (Kielland, 1994; Schimel & Chapin, 1996; Kielland, 1997; Henry & Jefferies, 2002; Nordin *et al*., 2004), boreal (Näsholm *et al*., 1998; Nordin *et al*., 2001; Persson & Näsholm, 2001a; McFarland *et al*., 2002; Bennett & Prescott, 2004), temperate (Falkengren-Grerup *et al*., 2000; Finzi & Berthrong, 2005; Rains & Bledsoe, 2007), Mediterranean shrubland (Hawkins *et al*., 2005) and alpine ecosystems (Raab *et al*., 1996, 1999; Lipson *et al*., 1999a; Miller & Bowman, 2002). Moreover, many agricultural species also readily absorb organic N (Yamagata & Ae, 1996; Näsholm *et al*., 2000, 2001; Okamoto *et al*., 2003). Whereas the primary focus of many recent ecological studies of organic N in northern ecosystems has been on the uptake of amino acid by plants, the dynamics of amino acids in soil has received only modest attention, with a few notable exceptions (Weintraub & Schimel 2005a,b; Kielland *et al*., 2006, 2007).

Contrary to biochemically well-founded predictions, the recalcitrant soil organic matter and low soil temperatures in late successional coniferous ecosystems (VanCleve *et al*., 1983; Kielland *et al*., 2006) do *not* result in low *in situ* rates of organic N turnover (Kielland *et al*., 2007; Fig. 2). In particular, the nearly 2-fold increase in the rate of amino acid turnover between early successional shrub communities and late successional black spruce forests, despite a nearly 10°C difference in ambient soil temperatures, suggests that cold, late successional soils exhibit an apparent temperature compensation for amino acid turnover. Normalizing amino acid turnover for *in situ* differences in soil temperature (as fractional turnover per °C) magnifies the difference between early (warm) and late (cold) successional soils. Analogous relationships with temperature have been observed for organic matter turnover in both laboratory and field experiments (Kirschbaum, 2004), and may also be pertinent to soil N turnover.

The traditional perspective on plant N relationships in terrestrial ecosystems has been that there is an absolute dependence of plants on mineral N for uptake and metabolism. A challenge to the generality of this view has emerged over the last 15 yr, based upon work in plant physiology, biogeochemistry and ecosystem ecology. The fundamental argument rests on the finding that annual plant requirements for N greatly exceed the annual inorganic N supply. This observation has been made in arctic (Giblin *et al*., 1991; Kielland, 2001), alpine (Labroue & Carles, 1977; Fisk & Schmidt, 1995), and taiga ecosystems (Ruess *et al*., 1996; Kielland *et al*., 2006; Kranabetter *et al*., 2007). Although the varied methodologies for estimating both supply and demand clearly affect the accuracy of these parameters, the sheer magnitude of the discrepancy (2- to 6-fold; Kielland, 2001) provides a reasonable justification for the idea that many plant species must derive their N supply from additional sources other than NH_4^+ and NO_3^-.

2. Soil nitrogen composition

Boreal forest soils have a high organic matter content and thus high concentrations of total soil N. Most of this N is in

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the form of N in humic material (Stevenson, 1982), but concentrations of soluble proteins can be high; on the order of 0.5 mg g^{-1} soil (corresponding to *c.* 0.08 mg protein N g^{-1} soil; Kielland *et al.*, 2007). Concentrations of dissolved organic N (DON) are typically about an order of magnitude greater than those of NH_4^+ and NO_3^- (DON 16–32 kg ha^{-1} and dissolved inorganic N (DIN) 0.9–15 kg ha^{-1}; Kranabetter *et al.*, 2007), but the effective bio-availability of DON may be < 20% (Jones & Kielland, 2002; Neff *et al.*, 2003). High concentrations of amino acids and high rates of amino acid production are also characteristic of some temperate forests. Thus, Berthrong & Finzi (2006) found that, in two out of three studied sites, amino acids dominated over inorganic N in the soil solution of the organic horizon.

3. Control of plant amino acid uptake in the field

As is the case for all plant nutrients, the effective limitation of plant acquisition is controlled more strongly by soil processes in the rhizosphere (especially diffusion) than by specific physiological properties of a given plant species (Nye, 1977). However, uptake of soil N, including amino acids, is a concentration-dependent process under control of transporters in the plasmalemma. The concentration of amino acids in the bulk soil is controlled by proteolysis, as described above, and the buffer capacity of the soil, which in turn is affected by the charge distribution on individual amino acids. Basic amino acids such as L-Arg and L-Lys tend to be less mobile than neutral amino acids such as Gly and L-Ala (Owen & Jones, 2001). Amino acid concentrations in soil are also, naturally, controlled by uptake and release (efflux) both by plant roots and by various symbiotic and free-living micro-organisms. While mycorrhizal fungi may improve the uptake capacities of plant roots (Sokolovski *et al.*, 2002), free-living microbes may stimulate the efflux of amino acids through release of specific compounds such as 2,4-diacetylphloroglucinol, phenazine and zearalen (Phillips *et al.*, 2004). Thus, amino acid concentrations close to root surfaces may be very dissimilar from those of the bulk soil, and elucidation of the dynamics of N sources in general, and of amino acids in particular, is therefore warranted.

III. Uptake

1. Uptake of amino acids by roots

All tested plant species, including plants from all major mycorrhizal types and nonmycorrhizal species, have been found to possess the capacity to take up amino acids (cf. Lipson & Näsholm, 2001). Early work (Hutchinson & Miller, 1911; Brigham, 1917; Virtanen & Linkola, 1946; Ghosh & Burris, 1950; Wright, 1962) indicated that plants could absorb amino acids as N sources. Following these early demonstrations of plant amino acid utilization, a number of studies focussed on aquatic plants such as *Lemna* sp. (e.g. Joy, 1969; Holst & Yopp, 1979; Borstlap *et al.*, 1986) and showed that such plants were able to utilize several amino acids for growth and even perform better on mixtures of amino acids and inorganic N than on inorganic N only (Joy, 1969). Further, characteristics of the uptake system(s) were studied in *Lemna*, showing high rates of uptake and high affinities for uptake in this plant (Datko & Mudd, 1985). Several studies have also focussed on the characterization of root uptake of amino acids in land plants (Soldal & Nissen, 1978; Bright *et al.*, 1983; Shobert & Komor, 1987; Jones & Darrah, 1994; Kielland, 1994; Heremans *et al.*, 1997; Schmidt & Stewart, 1999; Persson & Näsholm, 2001a; Persson *et al.*, 2003, 2006; Thornton, 2001; Thornton & Robinson, 2005; Jämtgård *et al.*, 2008). These studies used a wide range of compounds and experimental procedures, but common to all of them was the notion that plants efficiently absorb amino acids from test solutions.

For the purpose of this review, four questions are of great significance regarding root uptake capacities.

- Can roots absorb amino acids from solutions containing field-relevant amino acid concentrations?
- How do the uptake characteristics of plants compare with those of soil micro-organisms?
- How does plant uptake of organic N compounds such as amino acids compare with that of inorganic N compounds?
- Do measured rates of gross uptake, for example through assessment of the increase in the content of isotopic label over time, accurately describe net uptake of amino acid N?

Can roots absorb amino acids from solutions containing field-relevant amino acid concentrations? Generally, most studies have measured uptake rates of roots at concentrations higher than those normally found in soils (Wright, 1962; Jones & Darrah, 1994; Schmidt & Stewart, 1999; Falkengren-Grerup *et al.*, 2000; Owen & Jones, 2001; Thornton, 2001; Thornton & Robinson, 2005). This is a serious drawback because many uptake systems have a lower concentration limit under which no net uptake occurs. Consequently, uptake as demonstrated at concentrations appreciably above realistic soil solution concentrations may be of little value for inferring uptake under field conditions. However, a few studies using tracers (Soldal & Nissen, 1978; Shobert & Komor, 1987; Kielland, 1994; Phillips *et al.*, 2004) or solution depletion (Jämtgård *et al.*, 2008) described root uptake characteristics at amino acid concentrations < 10 μM and showed uptake to occur also at these relatively low concentrations.

How do the uptake characteristics of plants compare with those of soil micro-organisms? With respect to affinity constants, different studies have come to vastly different conclusions, probably mainly reflecting the range of solution concentrations used rather than true species differences (Lipson & Näsholm, 2001). When root amino acid uptake has been studied at a soil-relevant concentration range, the affinity

constant (K_m) for different compounds has been found to fall within the range 10 μM (L-Arg; Soldal & Nissen, 1978) to 300 μM (L-Glu; Kielland, 1994). Published values for affinity constants for amino acid uptake by soil microbes also vary on the scale of several orders of magnitudes, but studies that have employed relatively low amino acid concentrations in the test solutions suggest that K_m should fall within the range 20–50 μM (cf. Jämtgård *et al.*, 2008). Wallenda & Read (1999) studied the kinetics of amino acid absorption of detached mycorrhizal root tips of *Pinus sylvestris* and *Fagus sylvatica* and found that K_m varied between 19 and 233 μM for various amino acids. Plassard *et al.* (2002) found that infection of *Pinus pinaster* roots with *Hebeloma cylindrosporum* greatly increased the plant perfomance on L-Glu. Sokolovski *et al.* (2002) compared uninfected and infected fine roots of *Calluna vulgaris* and concluded that infection with *Hymenoscypus ericae* greatly improved the capacity for root uptake of a range of amino acids. Affinity constants for infected *C. vulgaris* presented by Sokolovski *et al.* (2002) do, however, fall within the same range as that for, for example, barley (*Hordeum vulgare*; Soldal & Nissen, 1978; Jämtgård *et al.*, 2008), *Ricinus communis* (Shobert & Komor, 1987) and *Arabidopsis thaliana* (Svennerstam, 2008). From this, we can conclude that affinity constants of plant roots and mycorrhizas for amino acid uptake do not exclude the possibility that root uptake mechanisms have a role in plant capture of amino acids from soil solutions.

How does plant uptake of organic N compounds such as amino acids compare with that of inorganic N compounds? Uptake of NH_4^+ and NO_3^- is mediated by a range of transporters and, for both ions, high- and low-affinity transporters have been identified (cf. Williams & Miller, 2001; Miller & Cramer, 2004). Comparisons of rates of root uptake of amino acids, NH_4^+ and NO_3^- have been made for several species and the general conclusion from these comparisons is that NH_4^+ is absorbed at the highest rates, followed by amino acids, while the lowest rates of uptake are usually displayed for NO_3^- (Falkengren-Grerup *et al.*, 2000; Öhlund & Näsholm, 2001; Thornton, 2001; Thornton & Robinson, 2004; Finzi & Berthrong, 2005), although in some cases uptake of amino acids has been shown to be higher than that of both NH_4^+ and NO_3^- (Persson *et al.*, 2006; Kielland *et al.*, 2006). This picture changes slightly when uptake of different N forms is measured and when several N sources are present simultaneously. Specifically, uptake rates of both NH_4^+ and NO_3^- seem to be decreased more than rates of uptake of amino acids when roots are exposed to mixtures of these ions both in soil (Öhlund & Näsholm, 2001) and in solution (Thornton & Robinson, 2004). The 'preferences' displayed in short-term studies of root uptake from aqueous media may be eliminated by interactions between these N sources and the soil. Thus, diffusion rates in the soil may vary by several orders of magnitude among these N sources, with NO_3^- displaying a 10–100-fold higher diffusion rate than NH_4^+, while for amino acids a wide range of diffusion rates exists, from that for small uncharged compounds such as Gly to that for large cations such as L-Arg (Owen & Jones, 2001; Miller & Cramer, 2004). We conclude that rates of uptake of amino acids are probably lower than those of NH_4^+ but higher than those of NO_3^- for most plant species studied to date.

Do measured rates of gross uptake, for example through assessment of the increase in the content of isotopic label over time, accurately describe net uptake of amino acid N? It is well established that net uptake, that is, the increase in the amount of a given element in the plant, depends on both influx and efflux (Britto & Kronzucker, 2004; Szczerba *et al.*, 2006). Thus, for such compounds, rates of net uptake cannot be deduced from data on influx rates only, but must be determined from the balance between influx and efflux processes. Whereas influx carriers have been identified for both NO_3^- and NH_4^+, available information on the nature of efflux mechanisms *per se* is rather limited (Gaymard *et al.*, 1998). Efflux of anions such as NO_3^- can occur through passive leakage via anion channels, while proton movement in the opposite direction must accompany efflux of NH_4^+. It appears that the efflux to influx ratio of any ion increases with increasing concentrations of the compound studied in the root medium and high efflux rates are thus typical for low-affinity transport systems (Britto & Kronzucker, 2006). Thus, a study of white spruce (*Picea glauca*), Douglas-fir (*Pseudotsuga menziesii*) and trembling aspen (*Populus tremuloides*) showed that, at an external concentration of 1.5 mM NH_4^+, efflux constituted 35, 85 and 78% of the influx of the respective species (Kronzucker *et al.*, 2003).

Most studies of amino acid absorption by plant roots have only measured gross influx rates and not net rates of amino acid uptake. This is because the majority of studies have utilized labelled amino acids and assessed the rates at which roots acquire label over time but not acknowledged the possibility of a leakage component, which could affect the calculations of net uptake rates. A few studies have compared rates of uptake of label and depletion of amino acids from bathing solutions (Persson & Näsholm, 2001b; Warren, 2006; Jämtgård *et al.*, 2008), but in these studies significant rates of efflux of the tested amino acids was not recorded. Consequently, labelling studies represent an adequate methodology with which to infer rates of net amino acid absorption by plants.

Efflux of amino acids from roots has, however, been shown to occur in several species (e.g. Jones & Darrah, 1994; Paynel *et al.*, 2001; Phillips *et al.*, 2004, 2006; Lesuffleur *et al.*, 2007). The molecular mechanisms involved in amino acid efflux in plants remain to be elucidated. Hypothetically, energy-dependent carriers in the plasma membrane may mediate the efflux of amino acids. In bacteria such translocators have been found to be responsible for export of amino acids (Eggeling & Sahm, 2003). Amino acids have been shown to be exported by

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exocytosis in yeast, transport into intracellular vesicles being mediated by the weak acid and quinidine resistance gene (AQR1; Velasco *et al*., 2004), a member of the major facilitator superfamily. Polar cell-to-cell transport of auxin in plants has been postulated to be mediated by a similar mechanism, involving auxin influx and efflux carriers, for example the PIN-formed (PIN) family of proteins (Baluska *et al*., 2003; Blakeslee *et al*., 2005). As auxin is a derivative of the amino acid L-Trp, it is possible that amino acid export in plants is also mediated by exocytosis. However, available information suggests that efflux may not be carrier-mediated but may simply represent leakage from roots, as a result of the high concentration gradient of amino acids across the plasmalemma. Leakage would thus involve the movement of amino acids over root plasma membranes, down a concentration gradient. For example, root cells may exhibit amino acid concentrations of c. 1–10 mM (Jones & Darrah, 1994) whereas the soil solution exhibits concentrations of individual amino acids in the range of 0.1–10 μM, resulting in a concentration gradient of three to five orders of magnitude between the living root and its soil environment. This provides a strong driving force for diffusion of amino acids out of root cells. Except for the magnitude of the driving force, the resistance exerted by the plasmamembrane against movement of amino acids must be taken into account when calculating potential rates of leakage from root cells. In general, uncharged and hydrophobic compounds should display higher flux rates over lipid bilayers than charged hydrophilic substances. In accordance with this theory, measured permeability coefficients for amino acids over lipid bilayers range from 0.5×10^{-11} cm s^{-1} for Gly, Ser and Lys to 25 cm s^{-1} for Phe and 41 cm s^{-1} for Trp (Chakrabarti, 1994).

In the context of root amino acid leakage, it has been suggested that root uptake mechanisms may primarily be involved in retrieval of amino acids that have leaked out of root cells (Jones *et al*., 2005). Phillips *et al*. (2006), studying *Lolium multiflorum*, *Zea mays* and *Medicago truncatula*, showed that influx rates exceeded efflux rates by 94–374%. Lesuffleur *et al*. (2007), however, studying a range of crop plants, found that all species, and in particular the N-fixing *Trifolium repens* and *Medicago sativa*, displayed high efflux rates of Gly and L-Ser and that efflux rates were considerably higher than influx rates. The reason for the very high efflux rates recorded for these two amino acids in this study is unclear: other amino acids occurring at higher concentrations in root cells did not display such high rates of efflux and, as stated above, permeability coefficients for Gly and L-Ser diffusion over lipid bilayers are small. Notably, the N leakage represented by the efflux rates recorded by Lesuffleur *et al*. (2007) would account for a loss of *c*. 0.5–1 mg N g^{-1} root dry weight h^{-1}, thus severely depleting roots of N even over short time periods. Other studies have found efflux rates to be small or insignificant (Shobert & Komor, 1987; Phillips *et al*., 2004; Jämtgård *et al*., 2008). Clearly, more experimentation is needed to resolve the issue of whether a significant efflux component exists.

If the role of root amino acid uptake systems is primarily to recapture amino acids that have leaked out of root cells (cf. Owen & Jones, 2001; Jones *et al*., 2005), thereby offering a means by which rhizospheric bacteria and fungi can be controlled (Phillips *et al*., 2004, 2006), then mutants with restricted capacities for root amino acid uptake could offer new ways of studying these functions. To date, transporters known to be active in root amino acid uptake include lysine histidine transporter 1 (LHT1; Hirner *et al*., 2006; Svennerstam *et al*., 2007), amino acid permease 1 (AAP1; Lee *et al*., 2007) and amino acid permease 5 (AAP5; Svennerstam *et al*., 2008) (see the next section, 'Transporters mediating root amino acid uptake'). Hirner *et al*. (2006) performed growth tests on *A. thaliana LHT1* knock-out mutants and found these plants to display reduced growth on fertilized soil, growth conditions that would probably supply large amounts of inorganic N to the plants. This result therefore suggests that loss of this transporter could affect growth through increased losses of amino acids from roots, also implying that such losses could be of quantitative significance and thereby corroborating the idea that amino acid transporters may have an important function in recapturing amino acids. However, LHT1 was also shown to function in retrieval of apoplasmic amino acids in leaves. When the *LHT1* gene was re-expressed in the knock-out plants under the control of a leaf-specific promoter, the growth phenotype disappeared, suggesting that the function of acquiring amino acids from apoplasmic fluids in the leaves is a critical determinant of the phenotype. Moreover, plants with *LHT1* re-expressed in leaves showed a strong growth phenotype when supplied with L-Asp as an N source, underscoring the role of this transporter in root uptake of amino acids. Recent studies (Svennerstam *et al*., 2008) of *lht1*aap5* mutants failed to show that leakage of amino acids was elevated compared with wild-type plants. These results suggest that, for *A. thaliana*, leakage of amino acids from root cells is not of a sufficient magnitude to affect growth. The lack of a growth phenotype of plants with greatly reduced capacities for root amino acid uptake hence does not support the theory that the main physiological function of root uptake mechanisms pertains to re-capture of amino acids, although more experimentation is definitely needed to confirm this conclusion.

2. Transporters mediating root amino acid uptake

Studies of root uptake rates have provided important information on the capacity of roots to acquire amino acids from solutions. A debate on the identity of the actual carriers mediating root amino acid uptake, and their numbers, specificities, characteristics and regulation, was initiated as early as the 1970s. It was, at that time, not known whether amino acid transport was mediated by one or several transport systems. In their comprehensive review, Reinhold & Kaplan (1984) argued that most studies suggested that a single system was accountable for amino acid transport. Kinraide (1981)

analysed the findings of a range of root uptake studies and inferred from this analysis that two major transport systems, one mediating transport of neutral and acidic amino acids and another mediating transport of basic amino acids, could account for root amino acid uptake. This conclusion was supported by the findings of Datko & Mudd (1985), who used *Lemna gibba* as a model plant, and by those of Shobert & Komor (1987), who studied *Hordeum vulgare*. By analysing amino acid transport into plasma membrane vesicles isolated from *Beta vulgaris*, Li & Bush (1990, 1991) identified four amino acid symport systems, two for neutral, one for acidic and one for basic amino acids.

A new direction of research, applying methods of molecular biology, allowed this long-term debate to be settled. Frommer *et al.* (1993) cloned and identified a plant amino acid transporter (AAP1) using complementation of a transport-deficient yeast mutant strain with an expression library from *A. thaliana*. Molecular cloning and functional complementation in yeast have, since then, revealed that plants express a multitude of different amino acid transporters. However, it is not until recently, with the use of modern molecular biology tools and the advent of the genomics era, with the sequencing of full genomes, that we have been able to grasp the abundance of amino acid transporters in plant genomes. Amino acid transporters in plants belong to at least five gene families (Rentsch *et al.*, 2007), comprising at least 67, 134 and 96 genes annotated as being, or putatively being, amino acid transporters in *A. thaliana*, *Populus trichocarpa* and *Oryzae sativa*, respectively (Rentsch *et al.*, 2007; The Arabidopsis Information Resource (TAIR); http://arabidopsis.org; The DOE Joint Genome Institute (JGI); http://genome.jgi-psf.org/Poptr1_1/Poptr1_1.home.html; Tuskan *et al.*, 2006; The Institute for Genomic Research (TIGR); http://www.tigr.org; Yuan *et al.*, 2005; Ouyang *et al.*, 2007).

It is believed that plant amino acid transporters are functionally defined by distinct spatial and temporal expression patterns and substrate specificities (Liu and Bush, 2006). However, the possibility cannot be excluded that there is functional redundancy among some transporters, possibly explaining that, for example, *A. thaliana* mutants of the amino acid transporter AAP3 cannot be distinguished from wild-type plants (Okumoto *et al.*, 2004).

However, the resolution of one issue – that of whether a single or several different amino acid transport systems exist – resulted in new questions. What are the functions of this multitude of different transporters *in planta*? To what extent do they represent redundancy in function, and to what extent do they fulfil specific roles? In trying to answer these questions, the physiology, biochemistry and molecular biology of plant amino acid transporters have been extensively studied, mainly using *A. thaliana* as a plant model system. The collective knowledge generated by these studies has recently been reviewed (Liu & Bush, 2006; Rentsch *et al.*, 2007). In the context of the present review, however, these questions pertain to the number and functions of amino acid transporters mediating root amino acid uptake. Resolving these issues is of central importance not only for disentangling the mechanisms underpinning the uptake processes but also for understanding how, under field conditions, different amino acids interact in the uptake process, to what extent different compounds compete for uptake and to what extent their uptake is complementary.

Studies aiming at pinpointing the identity of transporters involved in root amino acid uptake have utilized a variety of techniques, including both forward and reverse genetics. Basically, root uptake of neutral and acidic amino acids was shown to depend on functional expression of LHT1 (Hirner *et al.*, 2006; Svennerstam *et al.*, 2007; Table 1). Hirner *et al.* (2006) selected five mutant lines that were deemed likely to be involved in root amino acid uptake. Svennerstam *et al.* (2007) used forward genetics to obtain mutant lines affected in root amino acid uptake as well as a reverse genetic screen and concluded that both screening strategies resulted in the identification of LHT1 as crucial for root uptake of acidic and neutral amino acids. Similarly, Lee *et al.* (2007) using a combination of forward and reverse genetics, identified AAP1 as important for root amino acid uptake although the phenotype of the mutants was only displayed when plants were exposed to relatively high amino acid concentrations (0.15–10 mM). Moreover, neither LHT1 nor AAP1 was found to mediate root uptake of the cationic amino acids L-Lys and L-Arg, suggesting the existence of additional transporters active in root amino acid uptake. Thus, using a reverse genetic screen of 23 T-DNA lines mutated in genes with potential roles in this process, Svennerstam *et al.* (2008) found AAP5 to be crucial for root uptake of these two amino acids. In the same study, double mutants of LHT1 and AAP5 displayed reduced uptake rates for all tested amino acids when these were present at a concentration of 10 μM and an overall reduction in amino acid uptake of 78 %. Results of studies of the root amino acid uptake kinetics of AAP1, LHT1 and AAP5, in the concentration range 2–50 μM, confirm that LHT1 and AAP5 are the crucial components of the root amino acid uptake process in *A. thaliana* within the concentration ranges relevant for field conditions (Svennerstam, 2008), while the function of AAP1 may be to mediate amino acid uptake at higher external concentrations. Moreover, these data suggest the overlap between LHT1 and AAP5 regarding substrate spectra to be small, so that LHT1 mediates uptake of all amino acids except L-Lys and L-Arg while AAP5 is only active in the uptake of these two cationic amino acids. If this model is valid, there are some direct consequences for our understanding of plant utilization of amino acids as N sources under natural conditions. Firstly, uptake of L-Lys and L-Arg is largely independent of uptake of neutral and acidic forms and thus plants could potentially regulate uptake of the cationic forms differently from the uptake of other forms. Secondly, the available soil amino acids would, from the plant perspective, be divided into two separate pools: the neutral + acidic pool and the basic pool.

Table 1 Reduction of root amino acid uptake in *Arabidopsis thaliana* with altered expression of amino acid transporter

Mutant	*lht1*		*aap1*	*aap5*	*lht1* × *aap5*
Method	^{14}C-Aa[a]	Depl[b,d]	^{14}C-Aa[c]	Depl[d]	Depl[d]
Concentration (mM)	5.0	0.025	10.0	0.010	0.010
Duration (h)	1.5–3	4–5	48	1–4	1–4
Amino acid	Reduction of amino acid uptake (%)				
Ala		65	*c*. 40*	ns	74
Met			*c*. 50		
Phe			*c*. 60		
Pro		78[d]	*c*. 50	ns	79
Trp			*c*. 30		
Val		83[d]		ns	84
Asn		56[d]		ns	62
Gly		84	*c*. 50	ns	81
Gln	85	46	*c*. 60	ns	65
Ser		100		ns	87
Asp	82	40[d]	ns	ns	46
Glu	73	ns	*c*. 30, *c*. 50*	ns	ns
His		42	*c*. 30	ns	63
Arg		ns		87	88
Lys		ns	ns	90	85

Amino acid uptake was measured as uptake of labelled amino acid (^{14}C-Aa) or as depletion of amino acids from a solution (Depl). ns, nonsignificant.
[a]Hirner *et al*. (2006); [b]Svennerstam *et al*. (2007); [c]Lee *et al*. (2007) *150 μM; [d]Svennerstam *et al*. (2008).
aap, amino acid permease; *lht*, lysine histidine transporter.

Many transporters are expressed in different tissues at different developmental stages, and are therefore hypothesized to have multiple functions in plants (Liu & Bush, 2006). This has also been shown for LHT1, AAP1 and AAP5 which, in addition to their function in root amino acid uptake, have been shown to be involved in redistribution of amino acids in mesophyll cells (LHT1; Chen & Bush, 1997; Hirner *et al*., 2006), transport of amino acids for development and accumulation of storage proteins (AAP1; Frommer *et al*., 1993; Kwart *et al*., 1993; Fischer *et al*., 1995; Hirner *et al*., 1998) and phloem loading of amino acids in mature leaves (AAP5: Fischer *et al*., 1995). Tissue-specific expression patterns of *LHT1* and *AAP1* in *A. thaliana* have been investigated by histochemical analysis of plants carrying the reporter gene for β-glucuronidase under the control of an *LHT1* or *AAP1* promoter (Hirner *et al*., 2006; Lee *et al*., 2007). The results showed that *LHT1* is expressed in roots, mainly in the rhizodermis of emerging roots and lateral roots. *AAP1* was shown to be expressed in the root epidermis, cortex and endodermis as well as the vascular cylinder. Two gene expression maps of *A. thaliana* roots have been recently published. Birnbaum *et al*. (2003) created a global expression map based on 15 different root zones, corresponding to five cell types at three progressive developmental stages. In a recent study (Brady *et al*., 2007) the resolution was enhanced to cover 13 developmental zones and 19 cell types. *LHT1* is expressed mainly in the lateral root cap and in the epidermis. *AAP5* is expressed in all tissues, but mainly in the cortex, endodermis and lateral root cap, and *AAP1* mainly in the endodermis, cortex and stele. As amino acid transporters other than LHT1, AAP1 and AAP5 are also expressed in root tissue, the possibility cannot be excluded that other amino acid transporters also participate in root amino acid uptake. Even so, based on the abovementioned studies of *LHT1*, *AAP1* and *AAP5* gene expression, and the demonstrated importance of these transporters, we present a hypothetical model of amino acid transport in roots (Fig. 3).

For mycorrhizal plants, uptake of nutrients, including organic N, is to a large extent mediated by the fungal partner (cf. Chalot *et al*., 2002; Smith & Read, 2007). Studies of ectomycorrhizal (EM) fungi have identified several transporters active in amino acid uptake from the soil. Chalot *et al*. (1996) showed that amino acid transport by the EM fungus *Paxillus involutus* displayed a broad substrate specificity for amino acids and a K_m between 7 and 27 μM depending on the specific amino acid. Transport was shown to be pH-dependent and sensitive to protonophores, suggesting active transport mediated by specific transporters. Nehls *et al*. (1999) identified an amino acid transporter with high affinity for a wide range of amino acids in the EM fungus *Amanita muscaria* and named it AmAAP1. Wipf *et al*. (2002), working with the fungi *Hebeloma cylindosporum*, concluded that the general amino acid transporter HcGAP1 (General Amino Acid Permease 1) could mediate active uptake of a broad spectrum of amino acids. A recent study (Cappellazzo *et al*., 2008) also suggested that arbuscular

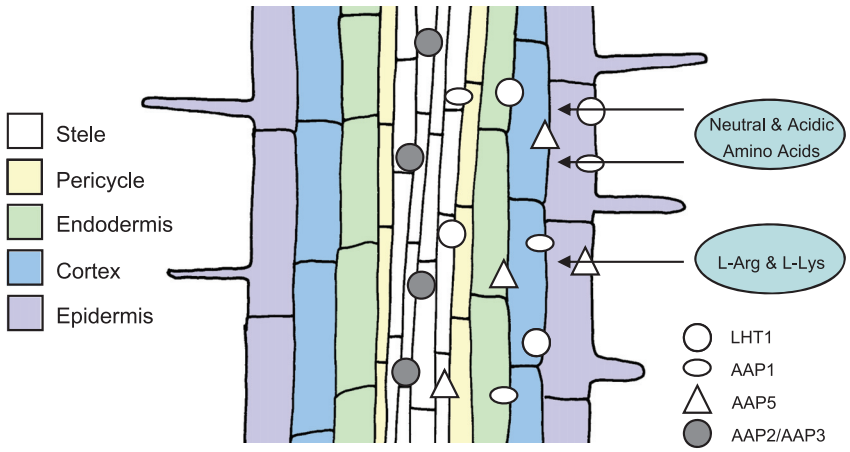

Fig. 3 Hypothetical model of root amino acid uptake in nonmycorrhizal plants. Two pools of amino acids, neutral/acidic and basic (L-Arg/L-Lys), are transported into the symplast of the root epidermis, cortex and/or endodermis by the amino acid transporters lysine histidine transporter 1 (LHT1) and amino acid permease 5 (AAP5), respectively (Hirner *et al*., 2006; Svennerstam *et al*., 2007, 2008). Both transporters are also expressed in the stele, suggesting a function in transport of amino acids from the apoplast into cells in, for example, the pericycle and/or phloem tissue. It is hypothesized that AAP1 is also involved in these processes (Lee *et al*., 2007). However, whereas LHT1 and AAP5 have been shown to be involved in root uptake of amino acids at low concentrations (2–50 μM; Svennerstam, 2008), AAP1 was found to mediate uptake of amino acids at higher concentrations (150–10 000 μM; Lee *et al*., 2007). Thus, it is unclear whether AAP1 is involved in root amino acid uptake at concentrations relevant for field conditions, and more studies are hence needed to clarify this issue. Phloem loading of amino acids for transport to root sink tissues is believed to involve AAP2 and AAP3 (Hirner *et al*., 1998; Okumoto *et al*., 2004).

mycorrhizal fungi express amino acid transporter genes by reporting the identification of an amino acid permease in *Glomus mosseae* (GmAAP1). In addition, a comprehensive analysis of N-compound transporters in *Laccaria bicolor* identified 29 gene models belonging to the amino acid-polyamine-organocation superfamily (Lucic *et al*., 2008). Thus, in fungi as well as in plants, transporters controlling uptake of amino acids have been shown to involve a wide range of compounds. Identification and characterization of the transporters involved at the plant–fungus interface represent a key step in understanding the processes of nutrient exchange occurring between the plant and the fungus. Therefore, for mycorrhizal plants, the hypothetical model in Fig. 3 may be modified, taking symbiotic features and fungal amino acid transporters into consideration.

3. Metabolism, allocation and growth

The abundance of studies on root uptake of amino acids is in sharp contrast to the very few studies actually devoted to following post-uptake conversions of absorbed compounds. Glycine has become the model compound in many studies of plant organic N uptake and therefore the metabolism of absorbed Gly has been investigated more extensively than that of other amino acids. Schmidt & Stewart (1999) studied the uptake of Gly by a number of Australian plants and the metabolism of absorbed Gly in *Hakea actities* (Proteaceae). Using inhibitors of the serine hydroxymethyltransferase/glycine decarboxylase pathway and inhibitors of aminotransferase activity they showed that metabolism of root-absorbed Gly was primarily via an aminotransferase, possibly serine glyoxalate aminotransferase, resulting in the majority of absorbed Gly-N being transferred to L-Ser, followed by synthesis of L-Gln, L-Glu and L-Ala. Interestingly, high ^{15}N enrichment was also found in Gly in the xylem, suggesting that a fraction of absorbed Gly was not metabolized by roots but was directly transported to shoots. This is consistent with the high concentration of glycine found in the xylem sap of many species (Sauter, 1981; Kielland, 1994). Thornton (2001) studied the uptake and metabolism of Gly in *Lolium perenne* and found, in agreement with Schmidt & Stewart (1999), that a large fraction of Gly-N was transferred to L-Ser, followed by synthesis of L-Gln, L-Glu, L-Asn and L-Ala. Persson *et al*. (2006) studied the uptake and assimilation of L-Ala and L-Glu as well as that of NO_3^- and NH_4^+ in nonmycorrhizal *Pinus sylvestris*. For the two inorganic N forms and for L-Glu, 62–75% of the total label in the amino acid pool was recovered in L-Gln, while for L-Ala the L-Glu pool also displayed significant ^{15}N incorporation. From these studies it seems that metabolism of absorbed amino acid N may primarily depend not on de-amination followed by incorporation of released N within the GS/GOGAT (Glutamine synthetase/Glutamate-2-oxoglutarate aminotransferase) cycle, but rather via transaminations, as a significant fraction of the ^{15}N label typically showed up in L-Ser (Gly-fed plants) and L-Glu (L-Ala-fed plants). Moreover, it seems possible that a fraction of absorbed amino acid N is directly transferred to shoots (Schmidt & Stewart, 1999; Persson *et al*., 2006).

A special case of plant amino acid metabolism pertains to the D-enantiomeric forms. Although some reports have stated that compounds such as D-Ala (Ogawa *et al*., 1978) and D-

Trp (Gamburg & Rekoslavskaya, 1991) are metabolized, both older and more recent studies suggest that this ability is not well developed in plants (Valle & Virtanen, 1965; Pokorny *et al.*, 1970; Erikson *et al.*, 2004, 2005; Forsum *et al.*, 2008), resulting in accumulation of D-amino acids in plants exposed to such compounds (Brückner & Westhauser, 2002). The low capacity of plants to metabolize D-amino acids is in sharp contrast to the situation in most other organisms. For example, genes encoding D-amino acid oxidase are found in bacteria, fungi and animals (Friedman, 1999; Pilone, 2000) but surprisingly not in any of the plant genomes published to date (*A. thaliana*, *Z. mays*, *O. sativa* and *Populus tricocarpa*). The low capacity to metabolize compounds such as D-Ala and D-Ser results in these compounds having strong toxic effects on plants (Erikson *et al.*, 2004, 2005). Transgenic *A. thaliana* encoding a D-amino acid oxidase from the yeast *Rhodotorula gracilis* could, however, detoxify D-Ala and could even grow well on this amino acid (Forsum *et al.*, 2008). The introduction of D-amino acid oxidase into the plant created a new pathway in which absorbed D-amino acids were metabolized into NH_4^+, keto acids and H_2O_2 and the NH_4^+ produced could thereafter be utilized for growth. This illustrates how a single metabolic step may restrict the suitability of an organic N form as an N source.

It is well known that the short-term fates of absorbed NH_4-N and NO_3-N differ. Many plant species will directly allocate a fraction of absorbed NO_3^- to shoots while most of the absorbed NH_4-N is incorporated into amino acids before transport occurs (Raven & Smith, 1976; Andrews, 1986; Bloom *et al.*, 1992). It has also been noted that absorbed amino acid N is allocated to shoots at a slower rate than NO_3-N (Schmidt & Stewart, 1999; Persson *et al.*, 2006), that is, that amino acid N, at least in the short term, is allocated similarly to NH_4-N. Moreover, short-term allocation is under the control of exogenous and endogenous cues so that, for example, pretreatment with high N concentrations in the root medium simultaneously decreases instantaneous uptake rates and increases allocation of absorbed amino acid N to shoots (Thornton, 2001; Persson *et al.*, 2006). The different allocation patterns of different N compounds and the effect of N status on allocation must be taken into consideration when results from labelling studies are interpreted. Field studies where labelled compounds are applied and only shoots are sampled to assess plant uptake of the various N forms may not give unambiguous results because the pattern of labelling is complicated by the above-mentioned differences in allocation.

A range of studies have investigated the extent to which plants can grow on amino acids as N sources. Generally, growth on the amides L-Gln and L-Asn is relatively rapid while other amino acids may not sustain growth or may even inhibit growth (e.g. Bollard, 1966; Forsum *et al.*, 2008). Comparisons between noninfected and infected plants of species normally displaying high densities of mycorrhization of roots (Stribley & Read, 1980; Turnbull *et al.*, 1995; Smith & Read, 2007) also suggest that plants exhibit a restricted capacity to use amino acids as N sources but upon infection this capacity is increased dramatically. There is ample evidence (see above) that a number of plant species, irrespective of the type of mycorrhiza (and including nonmycorrhizal species such as *A. thaliana*), have well-developed capacities for root amino acid uptake. The studies referred to above also suggest, with the exception of D-enatiomeric amino acids, that metabolism of root-absorbed amino acids is rapid. Why, then, do only a few amino acids function as N sources for (nonmycorrhizal) plants? To the best of our knowledge, all growth tests have supplied amino acids at concentrations of 1–10 mM, that is, 2–4 orders of magnitude higher than those recorded in the soil solution (see above). Thus, although plants can absorb and metabolize amino acids, growth may be hampered by a high concentration, a case that has similarities with plant NH_4^+ nutrition (Britto & Kronzucker, 2002). Notably, the growth inhibitory effect differs markedly among different amino acids. Generally, amino acids present at low endogenous concentrations in plants display stronger growth-reducing effects than those present at higher concentrations (Forsum *et al.*, 2008). Noctor *et al.* (2002) studied the leaf content of free amino acids in potato (*Solanum tuberosum*), barley (*H. vulgare*) and wheat (*Triticum aestivum*) grown under different photosynthetic conditions and noted that one group of amino acids found at low concentrations seemed to be present at constant ratios, irrespective of the actual concentration, and therefore suggested that the concentrations of members of this group of amino acids were co-regulated. It should be noted that, within this group of 'minor' amino acids (Noctor *et al.*, 2002), none of the compounds is effective as an N source, while for the second group of compounds ('major' amino acids), which were present at high concentrations but did not vary in concert, all but one are effective as N sources for *A. thaliana* (Forsum *et al.*, 2008). We conclude that the low capacity of some (nonmycorrhizal) plants to utilize amino acids for growth is probably not an effect of restricted capacities for root absorption, or caused by restricted metabolism of absorbed compounds in the sense that these substances can be transformed within the plant and N derived from root-absorbed amino acids used for protein synthesis. Instead, we speculate that the specific growth settings, with high concentrations of single amino acids, used in most studies may result in accumulation of the tested compounds within plants, causing inhibition of the synthesis of other amino acids (Bonner & Jensen, 1997). Growth tests on *Lemna minor* (Joy, 1969) confirm that single amino acids may inhibit, but protein (casein) hydrolysates may efficiently sustain, the growth of plants.

Mutants defective in transporters mediating root amino acid uptake offer a new and interesting way of assessing the importance of amino acids for plant N nutrition. *Arabidopsis thaliana* can use several amino acids for growth and the amides L-Gln and L-Asn are especially effective (Forsum *et al.*, 2008). *Arabidopsis thaliana* mutants defective in the

Table 2 Growth phenotypes on L-Gln of transgenic *Arabidopsis thaliana* with altered expression of lysine histidine transporter 1 (LHT1)

Mutants	L-Gln concentration (mM)	Growth (% of wild type)
lht1-1, 2; lht1RNAi3, 4[a]	5	ns
lht1-3 – lht1-5[b,c]	1	68
	0.5	53
	1	66
	1.5	91
P35S-LHT1[a]	5	ns
35SLHT1-1, 2[c]	0.5	*c.* 300
	1	*c.* 150
	1.5	*c.* 125

[a]Hirner *et al*. (2006); [b]Svennerstam *et al*. (2007); [c]Forsum *et al*. (2008). ns, nonsignificant.

LHT1 transporter displayed decreased growth on L-Gln. These effects were most pronounced at low amino acid concentrations. Interestingly, plants overexpressing this transporter displayed 300–400% increased growth when cultivated on 0.5 mM L-Gln (Forsum *et al*., 2008; Table 2). This demonstrates that growth on amino acids may be hampered by a low capacity to absorb such compounds and that genetic engineering may be used to improve plant amino acid nutrition. This effort may be of considerable relevance in agricultural regions, which are trying hard to reduce the use of fertilizers (Yamagata *et al*., 2001). It should also be noted, however, that similar attempts to increase plant growth through increased expression of transporters mediating uptake of inorganic N have not been successful (Britto & Kronzucker, 2004; Lea & Azevedo, 2006).

IV. Field studies of plant amino acid uptake

The previous section has established the mechanisms underlying root amino acid uptake. Importantly, it suggests that roots and mycorrhizas have high-affinity systems for the uptake of all tested proteinaceous amino acids. However, from the abovementioned findings, it cannot be inferred that plant N nutrition to a significant degree involves the acquisition of organic N molecules. There are a number of uncertainties that constrain our ability to scale the laboratory results showing uptake of amino acids by plant roots and the soil studies showing the presence of amino acids in the root environment to actual field settings.

During the last decade, a large number of field studies have investigated the possibility that the potential of plant roots to absorb amino acids is realized under realistic growth conditions and instrumental to this work has been the use of dual labelled (^{13}C, ^{15}N) amino acids (Schimel & Chapin, 1996; Näsholm *et al*., 1998). Inferences regarding amino acid uptake based on recovery of added label and the molar ratios thereof, such as ^{13}C:^{15}N, are particularly useful to assess the extent to which amino acids were mineralized before uptake (Näsholm *et al*., 1998; Näsholm & Persson, 2001). However, comparison of *in situ* uptake of different N forms, such as ^{15}N amino acids vs ^{15}N-NO_3^-, may be misleading if conclusions regarding relative uptake are solely based on recovery of ^{15}N in the target tissue (e.g. roots). Differences in ambient (unlabelled) concentrations of N forms must be corrected for, either through adjustments in isotopic labelling or mathematically through post-labelling corrections against differential isotopic dilution. For example, in a study on cycling dynamics of NH_4^+ and amino acids in a mid-successional taiga forest, it was concluded that these ecosystems rely approximately equally on NH_4^+ and amino acids (McFarland *et al*., 2002). This study failed to take into consideration differences in the concentration of soil amino acids compared with NH_4^+, which could have had a significant effect on the estimate of relative uptake of the two forms of N in these forests. Similar inferences based solely on recovery of ^{15}N have been made in other studies (e.g. Schimel & Chapin, 1996; Näsholm *et al*., 2000; Persson *et al*., 2003; Nordin *et al*., 2004) which would tend to negatively bias uptake estimates of the N form with the higher concentration in the soil. Few field studies presenting compound-specific ^{15}N values in the context of comparing the uptakes of different N forms have explicitly recognized that these data alone cannot be used to compare rates of uptake of endogenous soil N.

We recognize that to calculate the magnitude of isotopic dilution used to estimate N uptake it is important to identify the appropriate N pool. For an experiment involving several amino acids, the appropriate N pool may be the total soil free amino acid pool. However, in single-amino acid experiments it may be argued that the appropriate N pool would be the concentration of that particular amino acid. Alternatively, the total free amino acid concentration could be used if a correction factor can be applied for the uptake of the amino acid in question relative to other amino acids. Such data may be difficult to come by, but information pertinent to such an approach is available for selected amino acids, plant species, and ecosystems (Kielland, 1994; Lipson *et al*., 1999a; Persson & Näsholm, 2001b). From what has been described above regarding root uptake characteristics for amino acids it may be argued that any correction for dilution of introduced label into the soil amino acid pool should be based on the substrate specificity of the transporters mediating plant uptake of amino acids. This argument implies that studies using neutral or acidic amino acid tracers should calculate dilution based on the total pool of these in the soil solution, while studies using basic amino acids should use the sum of L-Lys and L-Arg concentrations.

The development of techniques for detecting intact labels in the root (gas chromatography–mass spectrometry (GC-MS); Persson & Näsholm, 2001a) allows direct measurement of the label in the root, but requires an experimental design that lets

the investigator track metabolic conversions and translocation of the label over time. The GC-MS technique will provide conclusive qualitative information on whether a test plant acquired intact amino acids or not but will not give a quantitative estimate of this process. Neither of the aforementioned methodologies, however, addresses the potential errors in uptake estimation produced by differential isotope dilution.

1. Sampling schedule

Most uptake studies under field conditions rely on measurements of isotopic enrichment in root tissue, because extensive transamination, deamination and decarboxylation of amino acids before translocation to shoots would make interpretation of shoot isotopic enrichment dubious (Persson & Näsholm, 2001b). The design of a sampling schedule to recover doubly labelled amino acids for uptake estimation in the field is governed by several factors, including anticipated process rates, specific scientific questions, and logistical challenges. Thus, it is not surprising that investigators have sampled on a variety of time schedules ranging from minutes to days. However, given the high turnover rate of amino acids in most soils and the rapid metabolic conversions within the root (see 'Metabolism, allocation and growth' in the previous section), inferences regarding uptake of intact amino acids become increasingly difficult in proportion to the time interval between applications of label and label recovery, irrespective of current methodologies (GC/MS, molar ration regression, etc.). For example, the molar ratios of ^{13}C:^{15}N in roots of injected amino acids decrease in an exponential fashion as a function of the time elapsed since injection (Fig. 4). Thus, small differences in sampling intervals may yield large differences in molar ratios, resulting in different interpretations of the fate of the label and

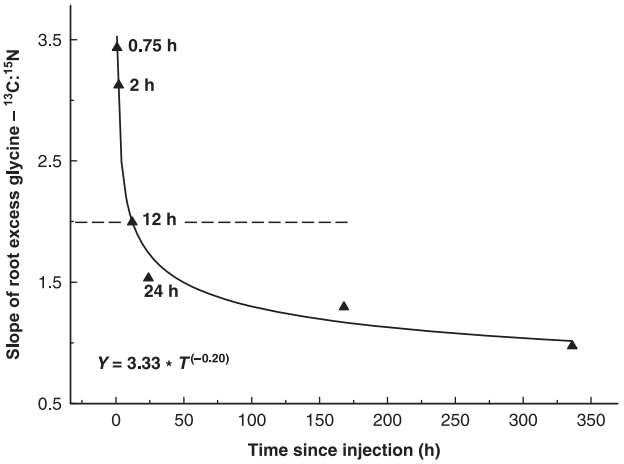

Fig. 4 The molar ratios of ^{13}C:^{15}N in roots of injected amino acids decrease exponentially as a function of the time elapsed since injection. Thus, differences in sampling intervals may result in large differences in the estimates of the proportion of amino acids absorbed intact. Data are derived from McFarland *et al*. (2002).

the assessment of how much of the label was absorbed intact. Consequently, an independent assessment of amino acid turnover/metabolic conversions in the root is required to accurately evaluate the degree of absorption of intact amino acids. A reasonable compromise may be to select two sampling periods, one shortly after label injection (1–6 h) and one much later (24–72 h). Alternative approaches have relied on the constancy of charge balance as indicated by acidification of the rhizospere (Chapin *et al.*, 1993), although measurement of this would be a difficult feat to pull off in a field study.

2. Inferential scope regarding competition between plants and microbes

Many of the recent ecological studies of organic N uptake *in situ* have cast this inquiry in the context of plant–microbe competition. This is indeed a laudable approach, which has spurred a discussion of these ecological interactions from both a theoretical and an empirical perspective. However, the inferences that have hitherto been made regarding competition are not without their caveats, as a consequence of both methodological and ecological considerations. In general, the amount of label added to soils in nearly all field studies of amino acid uptake has greatly increased N availability to the extent that the experiment no longer addresses uptake under ecological (limiting) conditions, but rather reflects differences in uptake under saturating (nonlimiting) conditions. Thus, such experiments do not adequately address ecological processes but rather potential differences in the capacity for resource acquisition under conditions that rarely occur in the field. Moreover, these levels of fertilization (dosage rates) effectively negate competition inasmuch as such an experimental design alleviates any semblance of N limitation. Well, no N limitation – no competition, and inferences pertaining to competition therefore have no meaning. The capacity of plants to acquire labelled amino acids was also shown to be rate dependent: plants compete better for this resource at high external concentrations (Jones *et al.*, 2005). *In situ* studies using double-labelled amino acids are constrained by the high dilution of the ^{13}C isotope, generally approximately two orders of magnitude higher than for the ^{15}N isotope (Näsholm & Persson, 2001). To be able to detect any ^{13}C label in a plant tissue, relatively large amounts of tracers must have been absorbed and thus relatively large amounts of label need to be applied. This methodological shortcoming may partly be circumvented by the use of compound-specific isotope analysis (GC-MS or GC-IRMS (gas-chromatography-isotope ratio mass spectrometry)) or through the use of ^{14}C, ^{15}N-labelled amino acids (Xu *et al.*, 2006, 2008) and thus future studies aiming at assessing competition for organic N could possibly study this process under more realistic conditions through application of lower concentrations of isotopes.

Studies of plant and microbial uptake of inorganic and organic N sources show that plants are inferior to microbes,

irrespective of N form (Harrison *et al.*, 2007, 2008). Thus, we can conclude that, in many short-term studies, plants appear to be poor competitors for any N source. Studies following the fate of added tracers over longer time periods do, however, show that plants acquire a gradually increasing fraction of supplied N (Harrison *et al.*, 2007; see also Kaye & Hart, 1997). Unfortunately, such studies will not be informative regarding the actual compounds absorbed by plants over the experimental period. This transfer of N would probably involve both the production of NH_4^+ (and possibly NO_3^-) by microbes and the production of organic N via lysed microbial cells, but the relative contributions of these two routes are unknown and could be expected to vary among different soils. It is also important to stress that the competitive ability of any organism is not a constant but can be expected to vary depending on physiological status as well as the conditions under which its competitive ability is assessed (e.g. Lipson & Monson, 1998; Lipson *et al.*, 1999b).

As stated above, the absorptive surface of plant roots may be smaller than that of soil microbes, but both may show great spatial and temporal variations. Also, rates of transpiration by plants are strongly variable and, at high transpiration rates, plants may ease the competition through induced mass flow in the root environment. Such mechanisms would favour uptake of N forms present at high concentrations in the soil solution, in particular NO_3^-, but may be of importance also for other N forms.

Another aspect of plant–microbe competition that needs scrutiny pertains to the nature of the microbial community and its relation to plants. To the extent that plants and soil microbes consume similar resources, and that the process of resource acquisition in one has a negative impact on the other, plants and microbes represent distinct recipients of the soil N 'pie'. However, most wild plant species form intimate relationships with microbes, both bacteria and fungi, resulting in a blurring of the distinction between 'competitors' and 'co-operators' (Eviner & Chapin, 1997). For example, in some boreal forest ecosystems, the extramatrical ectomycorrhizal mycelium contributes one-third of the microbial biomass (Högberg & Högberg, 2002). Surely the 'loss' of an injected N label into the latter pool does not entirely represent a fraction of soil N that is unavailable to (mycorrhizal) plants! The apparent inferiority of plants in competition with microbes may, for mycorrhizal plants, partly be explained by our inability to separate symbiontic and nonsymbiotic micro-organisms. The temporal pattern of increased plant acquisition of ^{15}N over time from added inorganic or organic sources may, similarly, reflect gradual allocation from the fungal partner to the host plant.

V. Conclusions and future perspectives

1. Organic N – a significant N source for plants?

As stated in the previous sections, it is inherently difficult to assess the dependence of any plant on uptake of any N source, organic or inorganic. Currently, we lack *direct* evidence that organic N contributes significant amounts of N to plant nutrition in any ecosystem. This is a critical shortcoming as no single experiment has been able to explicitly show that plant N to a significant degree can be accounted for by organic N uptake. This is also, naturally, the single most important challenge for future studies. Having said that, we can also conclude that several lines of evidence suggest that plants inhabiting some ecosystems may to a significant degree rely on organic N forms (Kielland, 1994; Lipson *et al.*, 2001). These lines of evidence have been discussed above and include the following.

Missing N. There is a strong discrepancy between measured rates of production of inorganic N forms and annual plant N uptake, suggesting that plants must acquire sources of N other than the inorganic forms.

Soil N composition. High rates of production of monomeric organic N compounds such as amino acids in the soil and concentrations of free amino acids comparable to, and in some ecosystems higher than, those of inorganic N have been found.

Uptake. Both mycorrhizal and nonmycorrhizal plants have evolved capacities to acquire organic N such as amino acids via root uptake. These capacities are fully comparable to, and share many features with, inorganic N uptake capacities and we are now in the process of identifying the key players in organic N acquisition.

Metabolism. Absorbed organic N is metabolized and N derived from this uptake is used for synthesis of a range of protein amino acids. The exception pertains to D-enantiomers of amino acids for which plants seem to have a very restricted capacity for metabolism.

Field labelling. Dual labelled amino acids supplied to soil have been shown to occur within plants, illustrating that the above capacities are utilized in field settings. Labelled amino acids have also been traced with GC-MS, firmly establishing that plants in the field do absorb amino acids.

The most obvious argument against organic N substantially contributing to plant N nutrition is that plants are outcompeted by microbes for this resource. The larger surface to volume ratios of microbes and the fast turnover rates of microbes compared with plant roots mean that microbes could scavenge the soil for organic N. These arguments are, however, complicated by the following issues.

The problem of separating the microbial community. Microbes include both free-living and symbiotic organisms and the current techniques cannot separate these categories. For a mycorrhizal plant, uptake of organic N by a symbiotic partner would eventually mean that this N would come to benefit the plant as well.

The problem of measuring competition. Competition between plants and microbes is, in many ecosystems, equally strong for organic and inorganic N. Short-term labelling shows that microbes outcompete plants for any form of N. Long-term labelling studies show that plants over time

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acquire more and more of the tracer, possibly as a result of the higher turnover rates of microbes.

2. Is it important to know?

Assuming that organic N sources such as amino acids can make a substantial contribution to plant N nutrition, we may outline the biochemical, physiological and ecological ramifications of this process. It is well known that, if plant N nutrition is based on either NH_4^+ or NO_3^- uptake, this has a range of secondary effects on processes such as pH homeostasis energetic costs for uptake, assimilation and transport, differences in plant and root morphology, and differences in soil pH effects (Marschner, 1995). The pH effects of either NH_4^+ or NO_3^- nutrition are a result of both uptake and assimilation processes, so that NH_4^+ results in excess proton production and NO_3^- results in excess proton consumption (Raven & Smith, 1976). Amino acids are absorbed through proton symport with either one or two protons transported simultaneously with the amino acid (Bush, 1993). If plant N uptake was dominated by amino acids, this could then theoretically lead to a slight increase in rhizosphere pH. The metabolic costs of converting absorbed inorganic N into amino acid N may be substantial (Bloom *et al.*, 1992) so absorption of organic N could potentially lead to a substantial saving. This could potentially be important for growth rates of fine roots and mycorrhizas, structures that depend on transport of chemical energy from above-ground parts. As discussed above under the 'Metabolism, allocation and growth' part of Section III, the short-term allocation of absorbed N differs among NH_4^+, NO_3^- and organic N so that a larger fraction of absorbed NO_3^--N is allocated to above-ground structures than for either amino acids or NH_4^+. It is well known that the N status of plants has a great impact on their root mass fraction (Ingestad & Ågren, 1991). Furthermore, biomass allocation to shoots and roots has been shown to be influenced by the NO_3^- content of leaves, suggesting that NO_3^- may act as a signal for shoot allocation (Scheible *et al.*, 1997) as well as root branching (Zhang & Forde, 1998). It has also been shown that some amino acids such as L-Glu can affect root development (Walch-Liu *et al.*, 2006). The low diffusivity of most organic N forms, especially when compared with NO_3^- (e.g. Owen & Jones, 2001), would theoretically mean that plants would need a larger root (or hyphal) area to acquire a given amount of N as organic N. The degree (if any) to which plants may optimize uptake of a given N form and whether this optimization would be channelled through the overall N status of the plant are, however, unknown.

The growing interest in plant organic N uptake has also stimulated discussions about the possibility of niche separation between plants with respect to which N pools they tap. McKane *et al.* (2002) found evidence for the existence of such a niche separation between plants inhabiting an arctic tundra community, demonstrated as a correlation among species abundance, species preference for individual N forms (Gly, NO_3^- or NH_4^+) and the abundances of these N forms in the soil. Similarly, Miller & Bowman (2002) and Miller *et al.* (2007) found evidence for species partitioning of available N. However, Harrison *et al.* (2007, 2008) and Ashton *et al.* (2008) found no indication of species niche separation with regard to different N sources. As stated by McKane *et al.* (2002), niche separation may result from spatial or temporal divergences between individual, co-existing species but may also result from differences in uptake capacities ('preferences'). From a mechanistic viewpoint, 'preference' should ultimately be a result of the abundances and kinetics of individual transporters. Thus, preference for NH_4^+ should be mirrored by a high abundance of NH_4^+ transporters while a NO_3^- preference results from a high abundance of NO_3^- transporters. We cannot say if this simple relationship also holds for amino acids. Assuming (for nonmycorrhizal plants) that our model with two major transporters mediating uptake of amino acids is correct, it follows that plants should be able to display preference either for the basic amino acids L-Arg and L-Lys or for all neutral and acidic amino acids. It also follows that plants will not be able to specifically target uptake of, for example, Gly or L-Ser simply because these compounds share the same transporter.

3. Future challenges

The importance of N as a growth-limiting element in many terrestrial ecosystems and the necessity of supplying large amounts of N to sustain production rates of various crops underscore the importance of an accurate depiction of the process of plant N acquisition. This review has sought to gather and evaluate existing information concerning plant uptake of organic N to evaluate whether this route of N uptake is of importance for plant N nutrition today. Ever since the demonstration of plant uptake of organic N in laboratory and field experiments, the question of the quantitative importance of this uptake has been debated, and we conclude here that this question cannot yet be settled, but that this area of research needs to be revitalized through application of new approaches and techniques. We can identify three research fields where this would be especially critical: soil solution dynamics of inorganic and organic N compounds; plant N uptake under field conditions; and mechanistic understanding of root uptake processes. Merging of information from these three fields should enable better understanding of the ecology, physiology and molecular biology of plant N nutrition.

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