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LETTER

Amino acid uptake: a widespread ability among boreal forest plants

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Abstract

Amino acids constitute a potentially important source of nitrogen for plants in boreal forest ecosystems. Accordingly, it may be suggested that distinct plant species differing abilities to take up amino acids constitutes an important factor in determining plant ecosystem composition. Using GC-MS and isotopically labelled amino acids, we measured the simultaneous uptake of 15 different amino acids by 31 common boreal forest plant species. The results from this study show that all plant species tested, representing a wide variety of plant types, have the ability to take up amino acids from an incubation solution. Furthermore, uptake rates were unrelated to mycorrhizal associations as well as habitat soil amino acid concentrations and plant nitrogen availability dependence as measured by Ellenberg nitrogen indicator values. These results suggest that mycorrhiza is of minor importance for discrete plant amino acid uptake rates and further points out the potential importance of amino acids to plant nitrogen nutrition in boreal forest ecosystems.

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INTRODUCTION

During the last decade, a number of studies have confirmed earlier reports showing uptake of amino acids by plants (e.g. Miller & Schmidt 1965; Newton 1974; Chapin *et al*. 1993). Although the ability of plants to take up and use amino acids as a source of nitrogen is becoming widely accepted, the mechanisms behind this uptake as well as its actual importance in the field are not well understood.

Nitrogen is generally a limited resource in the boreal forest, and nitrogen availability is one of the factors regulating growth in these ecosystems. Since amino acids constitute a relatively large part of plant available nitrogen in boreal forest soils (Ivarson & Sowden 1969; Näsholm *et al*. 1998), it may be argued that the ability to take up these compounds may be an important factor in determining plant ecosystem composition in the boreal forest.

In order to test the importance of the type of mycorrhizal connections, habitat and Ellenberg's nitrogen indicator values for amino acid uptake rates by different plant species, we conducted a study testing uptake of 15 different universally (U-^{13}C, ^{15}N) labelled amino acids simultaneously by 31 different plant species. The different species were taken from six different habitats of the boreal forest exhibiting differing soil amino acid content. The species were chosen to represent a wide range of plant types and to include natively nonmycorrhizal, as well as arbuscular mycorrhizal, ericoid- and ectomycorrhizal species. The total amino acid uptake rates of the different species were subsequently related to mycorrhizal connections, habitat soil amino acid concentrations and Ellenberg's nitrogen indicator values as a measure of plant dependence on nitrogen availability.

The results show that amino acid uptake is a common feature of plants growing in boreal forest habitats of greatly varying types. Furthermore, uptake of amino acids was not correlated to either mycorrhizal type, soil amino acid concentrations or Ellenberg's nitrogen indicator values.

MATERIALS AND METHODS

Fine roots from 31 differing plant species from six different locations were carefully removed from the soil and kept moist during transport to the laboratory. Roots were cleaned and carefully rinsed under tap water and thereafter incubated in a solution containing 15 universally (U-^{13}C, ^{15}N, >98%) labelled amino acids at a total concentration of 941 μM for 20 min. The amino acid concentration used is within range of K_m found for amino acid uptake of a range of differing plant species (e.g. Kielland 1994; Boorer & Fischer 1997).

Individual amino acid concentrations in the incubation solution were; Ala 98 μM, Gly 99 μM, Val 52 μM, Leu 97 μM, Ile 37 μM, Pro 68 μM, Met 8 μM, Ser 47 μM, Thr 46 μM, Phe 33 μM, Asp 86 μM, Glu 85 μM, Lys 112 μM, Arg 47 μM and Tyr 26 μM.

In order to keep root functioning intact for all of the widely differing plant species used in this study, pH of the incubation solutions was set to 6.8 (Schubert *et al.* 1986). Each species was replicated 4–5 times ($n = 4$ or 5).

Root cleaning, extraction, SPE-purification and GC-MS analysis was performed according to Persson and Näsholm (in press). In short, root amino acids were extracted using 10 mM HCl and the resulting extracts were purified using strong cation exchange (SCX) SPE-cartridges. The resulting purified amino acid extracts were derivatized to their *tert.*-butyltrimethylsilyl (tBDMS) derivatives. The derivatized amino acids were separated on a 30-m Chrompack CP-Sil 5 MS capillary column and subsequently mass analysed on a Varian Saturn 2000 ion trap mass spectrometer. Amino acid uptake was quantified by measuring the mass peak resulting from the added universally labelled amino acids in the mass spektra from each individual amino acid.

Soil samples ($n = 4$) from each sampling location were collected and extracted by 10 mL of sterilized water. Soil extracts were analysed for amino acid content by HPLC according to Näsholm *et al.* (1987). Labeled amino acids were obtained from Cambridge Isotope Laboratories (Cambridge, MA). Mycorrhizal connections were deduced after Harley & Harley (1987). Ellenberg nitrogen indicator values were taken from Ellenberg *et al.* (1992).

Plant amino acid uptake rates were grouped and made subject to ANOVA. Uptake rates of the individual amino acids from all species were pooled and made subject to correlation analysis. All statistics were performed using Statview 5.0 statistical software.

RESULTS

Roots from all species acquired amino acids from the solution at total rates varying between 0.25 and 9.2 μmol g^{-1} DW h (2.71 ± 0.47 μmol g^{-1} DW h; mean ± SE) (Table 1). All plant species did not take up all of the different amino acids (data not shown). Total uptake of amino acids was not related to plant-mycorrhizal connections (nonmycorrhizal, arbuscular-, ericoid- or ectomycorrhizal) (Fig. 1a). Nor could we find any relation between total amino acid uptake and habitat soil amino acid concentrations or Ellenberg nitrogen indicator values (Fig. 1b, c).

Uptake rates of the individual amino acids were not correlated to abundance in the uptake solution. The uptake of Asp, Glu and Tyr was very low (0.033 ± 0.007, 0.004 ± 0.004 and 0.004 ± 0.002 μmol g^{-1} DW h, mean ± SE, respectively) by all species. Apart from the low uptake of the acid amino acids, no correlation between charge or polarity and uptake of the individual amino acids was found (data not shown).

Normalized relative uptake rates (corrected for relative abundance in the incubation solution) of all amino acids are shown in Fig. 2. Normalized uptake rates of Gly, Val, Leu, Ile, Pro, Ser, Thr, Phe and Lys exhibited strong positive correlation to each others ($r = 0.783$–0.973) as well as to total uptake rates ($r = 0.777$–0.931) (data not shown).

DISCUSSION

The results presented in this study indicate that the ability to acquire exogenous amino acids is a widespread character among boreal forest plants. This supports earlier studies suggesting amino acid uptake to be a common feature of plants from widely differing habitats (e.g. Kaye & Hart 1997; Schmidt & Stewart 1999). Although varying greatly between individual species, mean amino acid uptake rates found in this study (2.71 ± 0.47 μmol g^{-1} DW h) are in the range of uninduced nitrate and ammonium uptake rates at similar concentrations recorded in several studies of a number of differing plants (e.g. Aslam *et al.* 1992; Kielland 1994; Høgh-Jensen *et al.* 1997; Min *et al.* 2000). The present study does not account, however, for soil–amino acid interactions or plant–plant and plant–microorganism competition. Therefore the uptake rates recorded here cannot be used as a measurement of the importance of amino acids for plant nitrogen nutrition in the field.

Amino acid uptake rates varied greatly between plant species, but were not significantly related to the type of mycorrhizal connections of the plant. Plant maintaining arbuscular-, ericoid or ectomycorrhiza as well as nonmycorrhizal plants apparently took up amino acids at similar rates. Thus, this study implies that the type of plant–mycorrhizal connections have an inferior role in determining the ability of plant roots to take up amino acids.

These results, however, do not rule out an important role of mycorrhiza for long-term plant uptake of amino acids in the field, since the ability of ericoid- and ectomycorrhiza to release exogenous proteolytic enzymes (Chalot & Brun 1998) may be of great importance for the release of amino acids from complex soil compounds. This ability, combined with the surface-extending role of mycorrhiza for plant roots, may be of great importance in the exploitation of amino acids for plant nitrogen nutrition.

Although the varying uptake rates recorded between species could be an effect of the expression of amino acid transport systems with differing affinities (Fischer *et al.* 1998) due to growth in habitats with differing soil amino acid concentrations, we could not find any relation between habitat soil amino acid concentrations and measured uptake rates (Fig. 1b).

By using GC-MS and isotopically labelled amino acids, we were able to follow the uptake of 15 different amino acids simultaneously. This allows for a better simulation of field conditions where plant roots encounter a wide range of

Table 1 Root uptake rates, mycorrhizal connections, habitat soil amino acid concentrations and individual species Ellenberg nitrogen indicator values of the 31 species tested

Species	Uptake rate	Mycorrhiza (nmol g^{-1} soil DW)	Soil aa concentration	Ellenberg NIV (μmol g^{-1} root DW h)
Carex canescens	0.32 ± 0.17	non	5 ± 0.3	2
Carex magellanica	0.72 ± 0.05	non	5 ± 0.3	n.a.
Cornus suecia	4.07 ± 0.76	non	68 ± 4	2
Melampyrum pratense	0.74 ± 0.15	non	29 ± 2	2
Potentilla palustris	5.69 ± 0.81	non	92 ± 19	2
Rumex acetosella	1.90 ± 0.34	non	29 ± 2	2
Silene dioica	0.56 ± 0.04	non	27 ± 4	8
Achillea millefolium	2.22 ± 0.11	AM	5 ± 0.3	5
Agrostis capillaris	5.81 ± 0.77	AM	5 ± 0.3	4
Calamagrostis canescens	1.24 ± 0.17	AM	27 ± 4	5
Dechampsia caespitosa	4.32 ± 0.61	AM	27 ± 4	3
Juniperus communis	4.70 ± 0.48	AM/EM	29 ± 2	n.a.
Maianthemum bifolium	0.25 ± 0.06	AM	330 ± 124	3
Molinia caerulea	9.22 ± 1.32	AM	27 ± 4	2
Paris quadrifolia	1.00 ± 0.09	AM	27 ± 4	7
Ranunculus acris	2.52 ± 0.39	AM	5 ± 0.3	n.a.
Rubus idaeus	0.59 ± 0.12	AM	29 ± 2	6
Sorbus aucuparia	0.63 ± 0.02	AM	29 ± 2	n.a.
Trientalis europaea	2.17 ± 0.27	AM	330 ± 124	2
Viola epipsila	6.89 ± 0.28	AM	27 ± 4	2
Andromeda polifolia	8.13 ± 1.07	ErM	68 ± 4	1
Calluna vulgaris	1.28 ± 0.28	ErM	68 ± 4	1
Ledum palustre	2.76 ± 0.26	ErM	68 ± 4	2
Vaccinium myrtillus	3.76 ± 0.21	ErM	330 ± 124	3
Vaccinium oxycoccus	0.35 ± 0.11	ErM	92 ± 19	1
Vaccinium vitis-idaea	0.45 ± 0.04	ErM	29 ± 2	1
Betula pendula	7.57 ± 0.71	EM	330 ± 124	n.a.
Picea abies	2.29 ± 0.15	EM	330 ± 124	n.a.
Pinus sylvestris	0.65 ± 0.16	EM	29 ± 2	n.a.
Populus tremula	0.67 ± 0.07	EM	330 ± 124	n.a.
Salix repens	0.63 ± 0.02	EM	92 ± 19	n.a.

Aa denotes amino acid. Uptake rates are quoted in μmol g^{-1} root DW h. Non denotes nonmycorrhizal; AM, ErM and EM denotes arbuscular mycorrhizal, ericoid and ectomycorrhizal, respectively. Amino acid soil concentrations are quoted in nmol g^{-1} soil DW. N.a. in the Ellenberg NIV column denotes not applicable (plant species having no Ellenberg NIV). Values presented are means ± SE.

compounds, a large advantage over studies utilizing only one compound.

The uptake rate correlations found between differing amino acids when pooling all species suggest that similar, but not necessarily equal, uptake mechanisms or transport systems were responsible for uptake in most species. The very low uptake rates found for Asp and Glu can most likely be explained by the high pH (6.8) of the incubation solution (e.g. Borstlap *et al.* 1986). We cannot, however, see any similar simple explanation to the low general uptake rates of Tyr.

No significant correlation between Ellenberg's plant nitrogen indicator values and uptake rates could be found, indicating that plant amino acid uptake is a general ability found in many different types of plants from widely differing habitats. These results are further supported by several other studies showing amino acid uptake by a wide array of different plant species and types (e.g. Soldal & Nissen 1978; Schobert & Komor 1987; Schimel & Chapin 1996; Raab *et al.* 1999; Näsholm *et al.* 2000; Streeter *et al.* 2000).

Although amino acid uptake rates displayed significant interspecies variation in this study, this variation does not appear to be systematic in any lucid way. Since all species tested exhibited the ability to take up amino acids, this ability in itself does not appear to be an important determinant of plant ecosystem composition. Rather, availability of amino acids and the capacity to compete for these compounds appear to be more important factors regulating plant uptake of amino acids. Similar results have

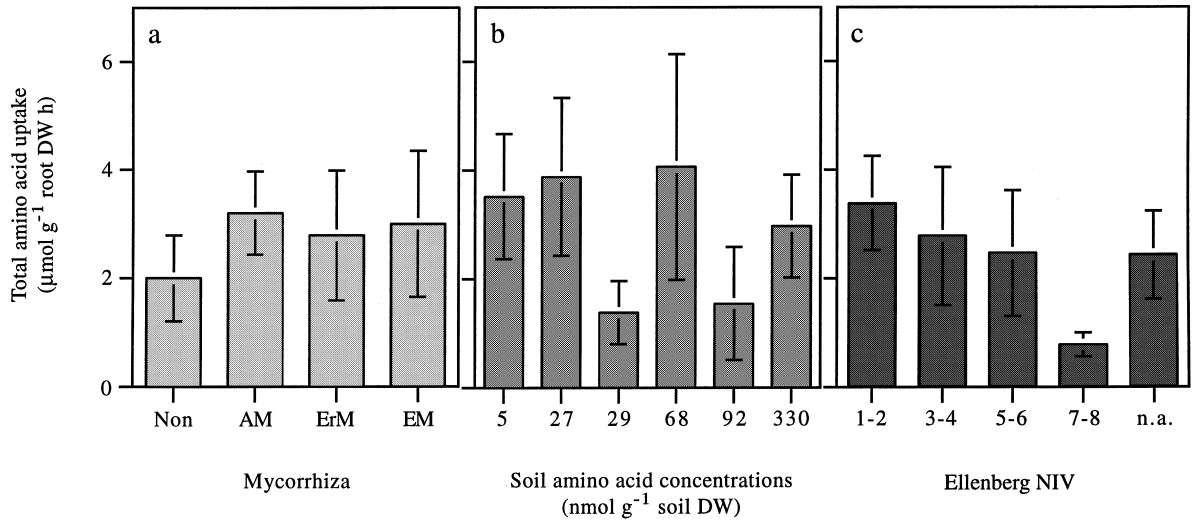

Figure 1 Amino acid uptake rates (µmol g^{-1} root DW h) related to: (a) plant-associated mycorrhizal type; Non denotes nonmycorrhizal; AM, ErM and EM denote arbuscular mycorrhizal, ericoid and ectomycorrhizal, respectively. (b) Habitat soil amino acid concentrations in nmol g^{-1} soil DW. (c) Ellenberg nitrogen indicator values, n.a. denotes not applicable (plant species having no Ellenberg NIV). Grouping of species can be deduced from Table 1. Values presented are means ± SE.

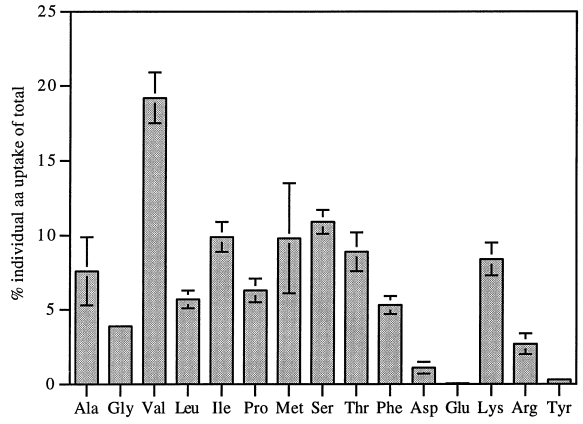

Figure 2 Relative uptake of individual amino acids. Values presented are means ± SE of all species. Uptake rates have been corrected for presence in the incubation solution and are expressed as percentage uptake of individual amino acid of total amino acid uptake.

also been deduced from studies of plant nitrogen availability, uptake and competition (Leadley *et al.* 1997; Hodge *et al.* 2000; Näsholm & Persson 2001; Owen & Jones 2001).

Although our study did not take into account seasonal variation in soil amino acid concentrations and plant uptake, the uptake rates give an indication of the potential role of amino acids for plant nitrogen nutrition. The present study also clearly points out that the need for long-term studies of plant acquisition of amino acids in the field is compelling in order to learn more of the importance of these compounds for plant nitrogen nutrition.

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BIOSKETCH

Jörgen Persson is a member of a research group concerned with plant-ecosystem nitrogen dynamics, where he is committed to elucidating the role of organic nitrogen for plant nitrogen nutrition in boreal forest ecosystems.

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