Plant, Cell and Environment (2005) **28**, 813–821

Uptake and assimilation of nitrogen from solutions containing multiple N sources

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ABSTRACT

We assessed the extent to which plants can acquire amino acids when supplied as single N-sources or when plants have access to a mixture of amino- and inorganic N sources. Because the uptake of different N-sources is temperature-dependent, the effects of temperature on amino-N uptake were also tested. *Lolium perenne* (perennial rye-grass) was grown hydroponically at 11 °C or 21 °C. Uptake of N was determined using ^{15}N tracers at the growth temperature from solutions containing either nitrate, ammonium or glycine as single N sources and from a mixture containing all three N-forms. Estimates of the relative importance of amino acids such as glycine to the total N budget of plants will have been underestimated in studies where uptake was determined in single source solutions compared with those from solutions containing a mixture of N-forms. The proportion of total N acquired from the mixed N source as ammonium increased as temperature was reduced. Regarding the uptake and initial metabolism of glycine, uptake was probably the rate limiting step at 11 °C whilst it was the metabolism of glycine to serine at 21 °C. Although ^{15}N incorporation into the plant amino-N pool was generally in proportion to the abundance of individual amino acids, its incorporation into the glycine pool was sometimes significantly less than predicted.

Key-words: nitrate, ammonium, glycine, amino acids, temperature, uptake, mixed N solutions.

INTRODUCTION

Much information exists on the ability of plants to take up nitrogen (N) as nitrate and ammonium. However, as well as these inorganic forms, soil solutions contain soluble organic N, including amino acids (Schmidt & Stewart 1997; Henry & Jefferies 2002; Shand *et al*. 2002; Jones *et al*. 2005). Plants contain amino acid transporters, at least some of which are involved in amino acid acquisition by roots (Glass & Siddiqi 1995; Fischer *et al*. 1998; Williams & Miller 2001; Persson & Näsholm 2003). Additionally, a variety of techniques including supplying labelled (^{15}N and/or ^{13}C) amino acids and measuring their uptake using isotope ratio mass spectrometry (Näsholm *et al*. 1998; Streeter, Bol & Bardgett 2000; Näsholm, Huss-Danell & Högberg 2001), nuclear magnetic resonance (Hartung & Ratcliffe 2002) or gas chromatography-mass spectrometry (GC-MS) (Persson & Näsholm 2001a; Thornton 2001) all suggest that the uptake of intact amino acids can occur.

The contribution that the uptake of amino acids makes to the overall N budget of plants is unknown, especially when considered over long time periods such as an entire growing season. In many ecosystems, especially where rates of N mineralization are slow, the contribution of amino acid uptake could be significant (Kielland 1997; Näsholm, Huss-Danell & Högberg 2000; Streeter *et al*. 2000; Näsholm & Persson 2001; Näsholm *et al*. 2001; Persson & Näsholm 2001b; Henry & Jefferies 2003a). For example, amino acid uptake can account for at least 60% of N absorbed by the sedge *Eriophorum vaginatum* in arctic conditions (Chapin, Moilanen & Kielland 1993) and over 30% of N acquired by the grass *Puccinellia phryganodes* in salt marshes (Henry & Jefferies 2002). Even in other ecosystems where amino acid concentrations in soil solutions are relatively smaller, their potential to supply a significant amount of N to plants cannot be excluded. Amino acids in soil can have fast turnover rates, of the order of a few hours (Jones & Kielland 2001; Henry & Jefferies 2003b), hence the flux of amino acids into plants may be large despite low concentrations in the soil solution.

Some studies have compared the relative acquisition of nitrate, ammonium and amino acids by plants from solutions containing individual forms of N (Kielland 1997; Falkengren-Grerup, Månsson & Olsson 2000; Volder, Bliss & Lambers 2000; Miller & Bowman 2003) even though in soil solutions, all the various forms of N are present simultaneously and plants acquire N from mixtures. One form of N often interferes with the acquisition of other forms. Ammonium inhibits nitrate uptake (Lee & Drew 1989; Clarkson, Jones & Purves 1992) and the external application of amino acids to roots inhibits both nitrate and ammonium uptake (Lee *et al*. 1992; Causin & Barneix 1994; Muller, Tillard & Touraine 1995; Gessler *et al*. 1998; Thornton 2004). Such interactions may under-estimate the relative importance of amino acid uptake compared with that of nitrate and ammonium if uptake is determined from solutions containing single forms of N compared with from a solution containing a mixture. An additional problem in determining the relative uptake of amino acids compared

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with nitrate and ammonium in non-sterile systems is that of microbial transformation of N before uptake. For example, following the addition of a ^{15}N-labelled amino acid to soil, it cannot be assumed that appearance of ^{15}N in a plant is necessarily due to the uptake of the intact amino acid (McKane *et al*. 2002).

Temperature is likely to be a key determinant of the forms of N acquired by plants at several levels. First, it will affect the forms of N available to plants through effects on the activity of microbes involved in soil N cycling. Nitrification rate increases with temperature, resulting in nitrate accounting for a larger proportion of the total dissolved N in soil water at 15 °C compared with 6.5 °C (Chapman, Williams & Hawkins 2001). Second, even if the concentrations of the various forms of N in soil solution remained constant, temperature could affect the N forms acquired through differential temperature sensitivity of N transporters. Such differential sensitivity has been observed in comparison of the H^+, Ca^{2+}, K^+, Na^+, NH_4^+ and Cl^- transporters recovering from chilling (Shabala & Shabala 2002). In solutions containing both nitrate and ammonium, the contribution of ammonium to total N acquisition by *Lolium perenne* increased as temperature was reduced (Clarkson, Hopper & Jones 1986). From solutions containing both nitrate and ammonium, the relative uptake of these two N-forms by a range of grass species alters diurnally (Ourry *et al*. 1996; Macduff, Bakken & Dhanoa 1997).

The hypotheses tested in the present study were that: (1) the contribution of the amino acid glycine to total N uptake from a mixture containing nitrate, ammonium and glycine is greater than its contribution predicted from the uptake of the individual forms of N; (2) the relative uptake of glycine from both single and mixed N sources increases as temperature is reduced; (3) the incorporation pattern of the ^{15}N tracers into plant amino acid pools is affected both by how N is supplied (single or mixed source) and by temperature.

MATERIALS AND METHODS

Uptake of nitrate, ammonium and glycine by *Lolium perenne* from single and mixed source solutions

Within a laminar airflow cabinet, seeds of *Lolium perenne* L. (Emorsgate Seeds, King's Lynn, UK) were surface-sterilized using 1% (v/v) peracetic acid but otherwise as described in Thornton (2004). Following the final water rinse the moist seeds were placed aseptically within glass Petri dishes sealed with Parafilm 'M' (American National Can, Chicago, IL, USA) and kept at 20 °C in the dark. After 5 d, when the seeds had germinated, they were transferred aseptically onto discs of Tygan mesh at a density of approximately 20 seeds per disc; individual discs were then placed over 1.0 L of deionized water in sterile culture vessels (Thornton 2001). Sixty culture vessels were placed, totally randomized, within a controlled environment room (Conviron, Winnipeg, Canada) at 20 °C in the dark. The following day a 16-h photoperiod of 290 ± 30 μmol m^{-2} s^{-1} photosynthetically active radiation at plant height was introduced. At the same time the water in the vessels was replaced by a complete nutrient solution, as described by Thornton & Bausenwein (2000) except that N was supplied as 1 mol m^{-3} NH_4NO_3 (2 mol m^{-3} N), sterilized by passing it through a 0.2-μm cellulose nitrate filter (Whatman, Maidstone, UK). The temperature of the controlled environment room was adjusted to maintain a constant 21 °C within the culture vessels.

Five days after germination, half the vessels were transferred to a second controlled environment room in which all conditions were identical to the first with the exception that the temperature of the room was adjusted to maintain a constant temperature of 11 °C within the culture vessels. The nutrient solution in all vessels was renewed aseptically within the laminar airflow 8 and 14 d after germination. Because N uptake varies diurnally (Ourry *et al*. 1996; Macduff *et al*. 1997), continuous light was introduced to all vessels 19 d after germination to minimize any effect of the timing of harvest (which took 5 h) on the uptake. Some roots, but especially in vessels at the lower temperature, developed a reddish purple colour. This observed pigmentation was most probably due to anthocyanin production consistent with its putative role in ameliorating cold-temperature stress (Chalker-Scott 1999). The vessels within each controlled environment room were subsequently arranged in five replicate blocks. Vessels containing plants with the whitest roots were designated to the first block and vessels containing roots of increasing redness allocated to subsequent blocks.

Twenty days after germination, the nutrient solutions used for growth of the plants were replaced by 'uptake' solutions; the growth and uptake solutions were identical to each other in all aspects except N. In three uptake solutions, N was supplied as a single source either: (1) 0.33 mol m^{-3} $(NH_4)_2SO_4$ with a ^{15}N abundance of 5.06 atom %; (2) 0.66 mol m^{-3} KNO_3 with a ^{15}N abundance of 5.19 atom %; or (3) 0.66 mol m^{-3} glycine with a ^{15}N abundance of 5.08 atom %. In a further three uptake solutions, N was supplied as a mixture containing 0.33 mol m^{-3} $(NH_4)_2SO_4$ (i.e. 0.66 mol m^{-3} NH_4^+) and 0.66 mol m^{-3} KNO_3 and 0.66 mol m^{-3} glycine (2 mol m^{-3} N) in which only one form of the N was labelled with ^{15}N, either: (1) NH_4^+ at 30.26 atom %; (2) NO_3^- at 30.44 atom %; or (3) glycine at 30.02 atom %. Plants remained at the temperature of their growth in the uptake solution for 24 h, after which they were harvested.

At harvest, the roots of the intact plants were dipped in fresh 1 mol m^{-3} $CaSO_4$ solution at 5 °C for 1 min and blotted dry. Plants were then separated into root and shoot material, the original seed being discarded. Samples were weighed fresh and then frozen and stored at –80 °C. The frozen samples were freeze-dried (Supermodulyo; Edwards High Vacuum International, Crawley, UK), reweighed then ball milled (Retsch MM2000; Haan, Germany). The total N and ^{15}N concentrations of weighed aliquots of the ball-milled plant material were determined using a TracerMAT

continuous flow mass spectrometer (Finnigan MAT, Hemel Hempstead, UK). The uptake of the ^{15}N labelled compounds was determined using the equations of Millard & Nielsen (1989). From the observed rates of uptake (see Results) it was estimated that depletion of any individual form of N from the uptake solution ranged from 7% (nitrate in the mixed nutrient solution at 11 °C) to 25% (ammonium in the single source solution at 21 °C).

Further 15 mg aliquots of the milled plant material were extracted with 3 cm^3 of 80% (v/v) ethanol for 1 h with occasional shaking. The solution was then centrifuged at 3500 *g* for 15 min. The supernatant was retained and the pellet re-suspended in 1.5 cm^3 of 80% ethanol for a further 1 h, then centrifuged at 3500 *g* for 15 min. The supernatants were combined, blow-dried in a stream of N_2 gas, then re-suspended once more in 1 cm^3 of 0.1 kmol m^{-3} HCl. Following a 10-min centrifugation at 10 000 *g*, the supernatant was poured onto cation exchange columns of 2 cm^3 bed volume of Dowex 50WX8-200 in the H^+ form (Sigma-Aldrich, St Louis, MO, USA). The columns were washed with 20 cm^3 of deionized water and the amino acids eluted with 20 cm^3 of 4 kmol m^{-3} NH_4OH. The eluate was blown overnight with a stream of N_2 gas to remove NH_3, then freeze-dried. Amino acids in the resultant extracts were converted to their t-butyldimethylsilyl derivatives and the concentration and ^{15}N abundance of the individual amino acids determined by gas chromatography mass spectrometry (GC-MS) as described by Millard *et al*. (1998).

The proportions of N taken up as different N-forms over the 24 h ^{15}N labelling period were calculated by assuming that plants in the various N source treatments were identical. This assumption is reasonable since all plants were raised on the same N source, NH_4NO_3, before the labelling period. Data from the three single source treatments were combined. For example, if 9.8, 23 and 4.5 mg N g^{-1} DW were taken up as nitrate, ammonium and glycine, respectively, when these were supplied to different plants as single N-sources, the corresponding proportional uptakes would be 0.26, 0.62 and 0.12. Similarly, data were combined across the three ^{15}N labelling schemes to calculate proportional uptake from the mixed N sources.

Differences between treatments were assessed by analysis of variance using GENSTAT 7th edition, Release 7.1 © Lawes Agricultural Trust (IACR-Rothamsted, Harpenden, UK). Results of the proportion of total uptake by a particular form of N were subject to angular arc-sine transformation before analysis. Since transformation did not alter the interpretation of results, untransformed data are presented for clarity.

RESULTS

There was a significant effect of the blocking structure on root ($F_{4,44} = 20.34$, $P < 0.001$) shoot ($F_{4,44} = 14.09$, $P < 0.001$) and whole plant ($F_{4,44} = 15.03$, $P < 0.001$) dry mass. This indicated that within a given treatment plants with the reddest roots were also the heaviest (data not shown).

Table 1. The dry mass (mg) and nitrogen content (mg) of the *L. perenne* plants grown at either 11 or 21 °C at the time of harvest

	Low temperature	High temperature
Total plant dry mass (mg)	12.4 (0.48)	26.8 (2.82)
Shoot dry mass (mg)	9.65 (0.39)	22.5 (2.45)
Root dry mass (mg)	2.70 (0.11)	4.30 (0.38)
Root:Shoot mass ratio	0.283 (0.006)	0.200 (0.004)
Total plant N content (mg)	0.66 (0.02)	1.47 (0.12)
Shoot N content (mg)	0.54 (0.02)	1.31 (0.11)
Root N content (mg)	0.12 (0.01)	0.16 (0.01)
Root : shoot N content ratio	0.220 (0.003)	0.120 (0.004)

Values are means (SE) of 30 replicates.

Both the dry mass and N content of plants grown at the higher temperature were over double those grown at the lower temperature (mass: $F_{1,44} = 46.40$, $P < 0.001$; N content: $F_{1,44} = 71.12$, $P < 0.001$; Table 1). These differences were caused by increases in mass and N content of both root (mass: $F_{1,44} = 36.33$, $P < 0.001$; N content: $F_{1,44} = 16.61$, $P < 0.001$) and shoot (mass: $F_{1,44} = 47.11$, $P < 0.001$; N content: $F_{1,44} = 77.22$, $P < 0.001$) with increased temperature (Table 1). Partitioning of both mass and N content were also affected by temperature, plants at the higher temperature had a smaller root : shoot ratios than those at the lower (mass: $F_{1,44} = 125.88$, $P < 0.001$; N content: $F_{1,44} = 399.21$, $P < 0.001$; Table 1).

Less nitrate ($F_{1,12} = 38.63$, $P < 0.001$), ammonium ($F_{1,12} = 8.23$, $P < 0.05$) and glycine ($F_{1,12} = 10.06$, $P < 0.01$) were taken up from the mixed N source than from the respective single sources. For nitrate, this difference applied only at the higher temperature since there was an interaction between N source and temperature ($F_{1,12} = 11.74$, $P < 0.05$; Fig. 1). Glycine uptake as a proportion of total N uptake increased ($F_{1,12} = 14.38$, $P < 0.01$) from the mixed compared with the single N source (Table 2). In contrast, the proportion of total uptake as ammonium was similar from the mixed and single source ($F_{1,12} = 0.08$, $P > 0.05$), and the proportion of total N uptake as nitrate was less ($F_{1,12} = 13.46$, $P < 0.01$; Table 2).

Less nitrate ($F_{1,12} = 20.93$, $P < 0.001$) and glycine ($F_{1,12} = 160.11$, $P < 0.001$) were taken up at the lower temperature, although for nitrate this occurred only when nitrate was supplied as a single source ($F_{1,12} = 11.74$, $P < 0.05$; Fig. 1). In contrast, ammonium uptake was unaffected by temperature ($F_{1,12} = 1.51$, $P > 0.05$; Fig. 1). Consequently, the proportion of total N uptake as ammonium increased ($F_{1,12} = 6.37$, $P < 0.05$) at the lower temperature (Table 2). The proportion of the total N uptake as glycine was unaffected by temperature ($F_{1,12} = 2.77$, $P > 0.05$) and the proportion taken up as nitrate decreased at the lower temperature ($F_{1,12} = 7.23$, $P < 0.05$, Table 2).

When grown at the higher temperature, each plant on average contained 40 μg of amino acid N. This more than doubled to 86 μg in plants grown at the lower temperature

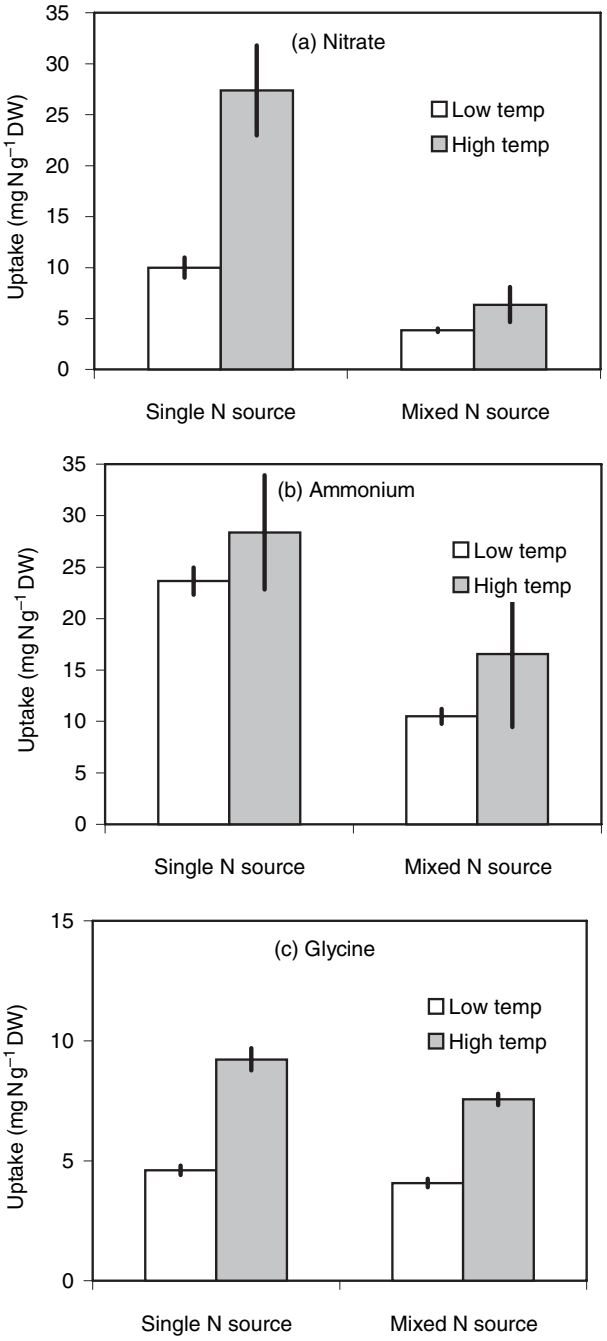

Figure 1. Total uptake of N (mg N g^{-1} DW root) in the form of (a) nitrate, (b) ammonium and (c) glycine by *L. perenne* over a 24-h period. Plants were grown and uptake measured at either 11 °C (low temperature) or 21 °C (high temperature) from either a single or mixed source of N. Values are mean ± standard error of five replicates.

($F_{1,43} = 52.55$, $P < 0.001$). Taking into account the smaller biomass of plants at the lower temperature (see above), this resulted in a 4.6-fold increase in plant amino acid N concentration at the lower temperature. At the lower temperature, a greater proportion of ^{15}N was measured in the plant's amino acid pools compared with at the higher temperature (Table 3). Over all three forms of N and in both single and mixed solutions this increase was 3.5-fold greater at the lower temperature ($F_{1,40} = 113.08$, $P < 0.001$). Proportionally less nitrate-derived ^{15}N was subsequently detected in the plant's amino acid pools at harvest compared with ^{15}N derived from ammonium or glycine ($F_{2,40} = 20.71$, $P < 0.001$, Table 3.

When the mixed nutrient solution at 21 °C contained ^{15}N-glycine along with unenriched nitrate and ammonium, most ^{15}N was subsequently incorporated in the root glycine pool with progressively smaller amounts as serine, glutamine and asparagine (Fig. 2a). When either nitrate or ammonium was ^{15}N-labelled in the mixed solution at 21 °C, the label was primarily found as glutamine with smaller amounts as asparagine, glycine, glutamic acid, γ-aminobutyric acid and serine (Fig. 2b & c). Similar patterns of ^{15}N incorporation were observed for all three forms of N from the corresponding single source N solutions at 21 °C (data not shown). However, when the mixed N source contained ^{15}N-glycine at the lower temperature, the amount of ^{15}N-glycine detected in the plant's root amino acid pool was extremely small (Fig. 2d), the ^{15}N being primarily incorporated into glutamine and serine. The incorporation of ^{15}N into the root pool of glycine was similarly small from the ^{15}N-glycine single source solution at 11 °C (data not shown), ^{15}N being incorporated into serine and glutamine as in the corresponding mixed solution at 11 °C and additionally into asparagine. At 11 °C the patterns of incorporation of ^{15}N-ammonium from both the mixed and single source solutions and of ^{15}N-nitrate from the single source solution followed those described for the 21 °C treatment (data not shown). Incorporation of ^{15}N-nitrate from the mixed solution at 11 °C was primarily into glutamic acid; otherwise it had a similar pattern to its incorporation at 21 °C into glutamine (data not shown).

The ^{15}N was generally incorporated into amino acids in proportion to their abundance in the tissues, almost irrespective of the amino acid in question. Combining data from all treatments, and treating roots and shoots as independent data ($n = 24$), the equation describing this general pattern was: ^{15}N-amino N = 0.19 amino-N$^{0.99}$, where amino-N and ^{15}N are measured in mg N g^{-1} DW; the standard error of both the coefficient and exponent was 0.03. This indicates that, on average, about one-fifth of the amino-N in the plant was derived from the ^{15}N label. However, significantly less ^{15}N was sometimes incorporated into glycine than the general trend would predict. This occurred in the shoots of *L. perenne* in four high temperature treatments (single ammonium and glycine sources, and when these sources were ^{15}N-labelled in mixtures). An example of this effect is shown in Fig. 3a and b for *L. perenne* grown at 21 °C in a mixed N solution containing ^{15}N-ammonium.

DISCUSSION

Interactions of one form of N on the acquisition by roots of another form are assumed to occur in the field (Thornton 2004). Therefore, although the equimolar concentrations of

Table 2. The proportions of the total N uptake acquired as nitrate, ammonium and glycine by *L. perenne* from either the single or mixed N sources at either 11 or 21 °C

	Proportion of total N uptake		
Treatment	Nitrate	Ammonium	Glycine
Single N source, low temperature	0.26 (0.01)	0.62 (0.01)	0.12 (0.01)
Single N source, high temperature	0.42 (0.03)	0.42 (0.04)	0.15 (0.03)
Mixed N source, low temperature	0.21 (0.01)	0.57 (0.02)	0.22 (0.02)
Mixed N source, high temperature	0.24 (0.05)	0.45 (0.11)	0.32 (0.07)

Values are mean (SE) of five replicates.

Table 3. The proportion of the total ^{15}N uptake present in the amino acid pools of *L. perenne* at harvest

	Proportion of total plant ^{15}N uptake in the plants' amino acid pools at harvest		
Treatment	^{15}N -Nitrate	^{15}N-Ammonium	^{15}N-Glycine
Single N source, low temperature	0.24 (0.05)	0.50 (0.13)	0.56 (0.04)
Single N source, high temperature	0.04 (0.01)	0.11 (0.02)	0.22 (0.05)
Mixed N source, low temperature	0.28 (0.05)	0.56 (0.07)	0.44 (0.07)
Mixed N source, high temperature	0.06 (0.01)	0.14 (0.04)	0.17 (0.01)

Plants were supplied ^{15}N as either nitrate, ammonium or glycine in single or mixed N sources at either 11 or 21 °C. Values are mean (SE) of five replicates.

nitrate, ammonium and glycine used in the mixed N solution cannot be considered representative of actual soil solutions, they illustrate potential interactions that are applicable to the physiology of field-grown plants.

The contribution of glycine to N uptake from a mixture of N sources

Glycine uptake was least, and nitrate uptake most, affected by the presence of other forms of N (Table 2). Depending on temperature, the proportion of N taken up as glycine approximately doubled from 12 to 15% when supplied singly to 22 to 32% when supplied with equimolar concentrations of nitrate and ammonium. This increased proportion was not brought about by increased glycine uptake *per se* but rather by decreased uptake of ammonium and especially nitrate in the mixed compared with the single nutrient solution (Fig. 1). This indicates that the potential contribution of amino acids such as glycine to a plant's total N budget could be underestimated if uptake is determined only from single N source solutions (e.g. Kielland 1997; Falkengren-Grerup *et al*. 2000; Volder *et al*. 2000; Miller & Bowman 2003).

In the field, however, the actual contribution of amino acids such as glycine to plant N uptake will depend on their soil solution concentrations, production and transport in the soil, and root uptake kinetics, compared with the corresponding concentrations, rates and kinetics for nitrate and ammonium, and on environmental conditions. Concentrations of amino acids in some soils can rival those of inorganic N (Jones *et al*. 2004), but that in itself does not indicate that amino-N is necessarily a significant N source for plants. Although our results show that plants have greater physiological potential to acquire amino-N than previously thought from single N-source experiments, we agree with Jones *et al*. (2005) that under field conditions 'evidence demonstrating this as a major plant N acquisition pathway is still lacking'.

The temperature dependence of glycine uptake from single and mixed N sources

Temperature had a large effect on glycine uptake irrespective of whether it was supplied singly or in a mixture (Fig. 1). That temperature response contrasted with those of nitrate or ammonium uptake. Nitrate uptake was unaffected by temperature when in a mixture, but was limited by low temperature when supplied as a single N source. Ammonium as a proportion of total N uptake, both in single and mixed source solutions, increased as temperature was reduced. In the single source solutions, nitrate uptake was severely inhibited as the temperature was reduced from 21 to 11 °C (Fig. 1). In contrast, nitrate uptake was not affected significantly by low temperature in the inhibitory presence of ammonium and glycine. Therefore, how nitrate uptake responded to temperature depended on the presence of other N-forms. Ammonium uptake is less sensitive to reduced temperature in comparison with that of glycine (Henry & Jefferies 2003a) and in *L. perenne* supplied with ammonium nitrate, the proportion of N acquired as ammonium increased as temperature was reduced (Clarkson *et al*. 1986). Our results agree with these findings.

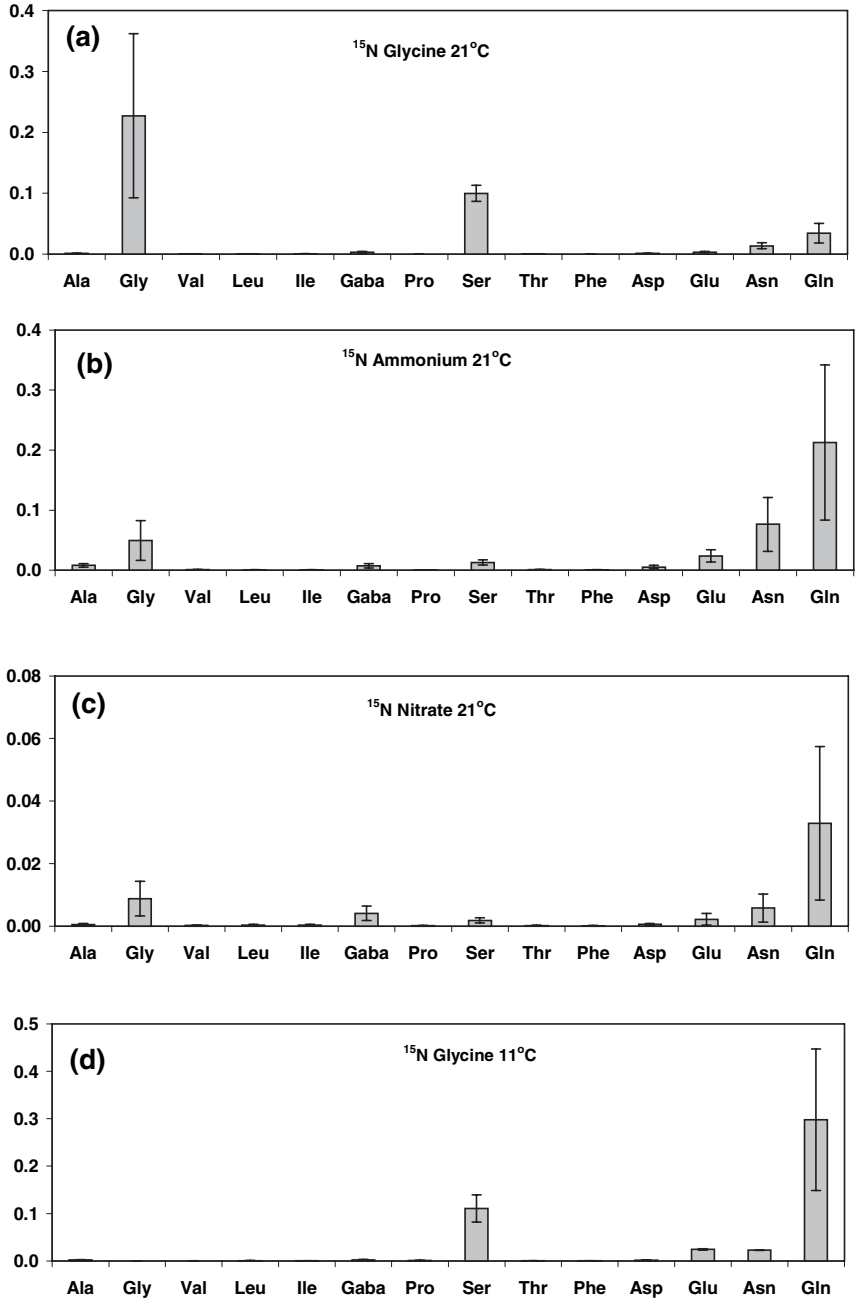

Figure 2. The ^{15}N labelled amino acid contents (mg N g^{-1} DW) of roots of *L. perenne* supplied with a mixed N solution containing nitrate, ammonium and glycine in which only one form of N was ^{15}N labelled at a time over a 24-h period. (a) Glycine ^{15}N labelled, at 21 °C; (b) ammonium^{15}N labelled, at 21 °C; (c) nitrate^{15}N labelled, at 21 °C; (d) glycine^{15}N labelled, at 11 °C. Values are mean ± standard error of five replicates.

It is interesting to note that at the high temperature the total N uptake in the mixed solution (nitrate + ammonium + glycine = 30.5 ± 9.0 mg N g^{-1} DW) equalled the uptake of nitrate (27.4 ± 4.4 mg N g^{-1} DW) and ammonium (28.4 ± 5.6 mg N g^{-1} DW) in the single source solution (Fig. 1). Furthermore, at the lower temperature the total N uptake in the mixed solution (18.4 ± 1.0 mg N g^{-1} DW, Fig. 1) roughly equalled ammonium uptake (23.6 ± 1.3 mg N g^{-1} DW) in the respective single source solution (Fig. 1). This suggests that the maximum total N uptake, on a per unit root mass basis, was regulated to approximately 21 mg N g^{-1} DW at the low temperature and 29 mg N g^{-1} DW at the high temperature irrespective of whether N was supplied as a single or mixed form.

N incorporation patterns in amino acids as affected by N source and temperature

Amino acid concentrations in *L. perenne* increase as temperature is reduced (Draper 1975). The pattern of ^{15}N appearance in the root amino acid pool following ^{15}N-labelling of nitrate or ammonium (Fig. 2b & c) was consistent with their assimilation into amino acids occurring via the GOGAT cycle. The combined action of glutamine syn-

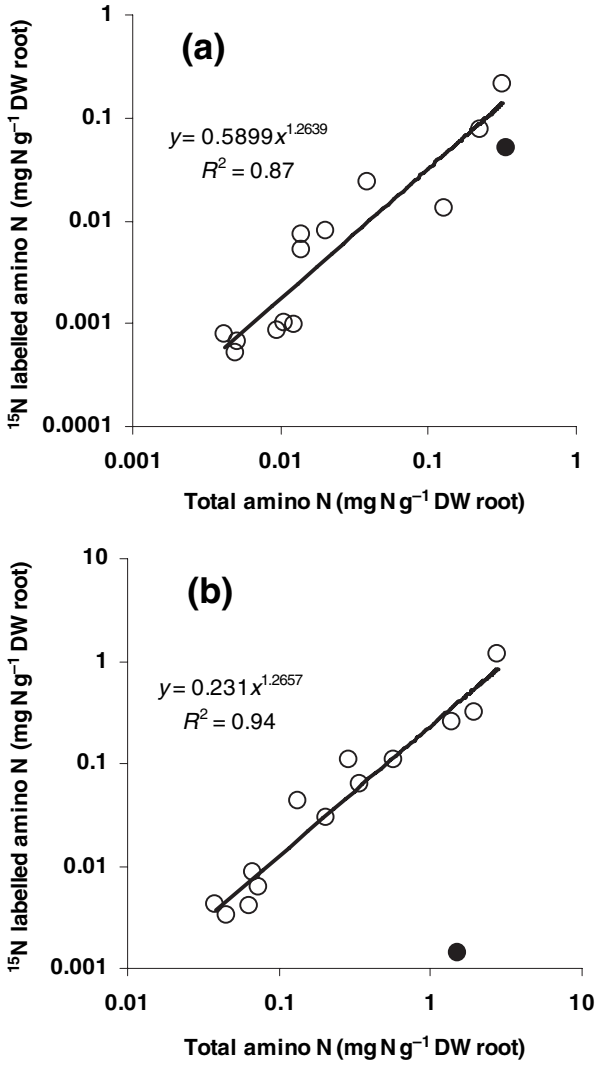

Figure 3. The ^{15}N labelled amino acid N contents (mg N g^{-1} DW root) versus total amino acid N contents (mg N g^{-1} DW root) of (a) root and (b) shoots of *L. perenne* grown at 21 °C in a mixed N solution containing nitrate, ammonium and glycine in which ammonium was ^{15}N-labelled for 24 h. Each symbol represent the mean of five replicates for an individual amino acid. Filled symbol = glycine, open symbols = amino acids other than glycine. The regressions were fitted to the open symbols only.

thetase and glutamate synthase on ^{15}N-ammonium explains the appearance of ^{15}N in glutamine and glutamic acid; the subsequent action of asparagine synthetase on glutamine would transfer some of the ^{15}N to asparagine (Lea & Ireland 1999). The smaller percentage of total N uptake found in the amino acid pools at harvest when N was supplied as nitrate compared with ammonium (Table 2) probably reflects a relatively low assimilation rate by nitrate reductase (Arndt *et al*. 2002); indeed, vacuolar nitrate can act as an N storage compound (Van der Leij, Smith & Miller 1998).

When glycine was labelled in the mixed N solution at 21 °C, the appearance of ^{15}N in the root amino acid pool primarily as glycine with smaller amounts as serine and glutamine (Fig. 2a) is consistent with the incorporation pattern of ^{15}N-glycine from single source solutions by *L. perenne* at 20 °C (Thornton 2001) and by the tropical C_3-CAM tree *Clusia minor* (Arndt *et al*. 2002). The different pattern of ^{15}N incorporation of glycine compared with ammonium proves that glycine was not deaminated to ammonium before its uptake. The appearance of ^{15}N-glycine in roots provides strong evidence that glycine was taken up intact. The accumulation of ^{15}N-glycine in the root amino acid pool at 21 °C, but not in the equivalent treatment at 11 °C (Fig. 2d), suggests that the rate-limiting step at 11 °C was glycine uptake, but at 21 °C it was probably the metabolism of glycine to serine. In the presence of glycine, the activity of the glycine decarboxylase/serine hydroxymethyltransferase complex (GDC/SHMT) is stable at 40 °C (Lenne, Neuburger & Douce 1993). The relative increase in glycine uptake from 11 to 21 °C (Fig. 1) must therefore exceed any relative increase in GDC/SHMT activity over the same temperature range.

Our results suggest that the assimilation pattern of glycine-derived N into root amino acid pools depends on temperature. In turn, the different accumulation patterns of root amino acids could differentially inhibit inorganic N transport across the root surface (Muller & Touraine 1992). This then provides a mechanism for the temperature-dependence of the inhibition of inorganic N acquisition even with a constant external pool of amino acids.

The remarkable instances where glycine contained far less ^{15}N than would have been predicted from its total content (Fig. 3) could be explained if a pool of unlabelled glycine was being synthesized in the tissues. Photorespiration is one potential mechanism for this and is consistent with the effect occurring only in leaves at the higher temperature. At that temperature, the ratio of the rate of the oxygenase reaction of Rubisco to that of the carboxylase reaction would have been greatest (Berry & Björkman 1980). Additionally, an increased photorespiration rate increases leaf glycine concentrations (Di Martino *et al*. 1999). However, other facts undermine this explanation. An increased photorespiration rate also increases tissue concentrations of amino acids other than glycine, such as serine and alanine (Di Martino *et al*. 1999), yet only glycine deviated in its ^{15}N incorporation. This deviation occurred only when either ammonium or glycine itself was ^{15}N-labelled, but photorespiration would have increased in all plants at the higher temperature, including those grown on nitrate. Alternatively, the pattern shown in Fig. 3b is consistent with a specific down-regulation of glycine synthesis in leaves. That seems unlikely because there is no known mechanism by which it could occur and it would conflict with evidence for a general co-ordinated synthesis of amino acids (Noctor *et al*. 2002). At present, we have no convincing explanation for the phenomenon.

ACKNOWLEDGMENTS

The Scottish Executive Environment and Rural Affairs Department funded this work. We thank M. Tyler and M.

Leitch for skilled technical assistance, the Analytical group of the Macaulay Institute for IR-MS and GC-MS analysis and E.I. Duff of Biomathematics and Statistics Scotland (BioSS) for statistical advice. We also thank anonymous referees for helpful comments on an earlier version of the manuscript.

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Received 5 October 2004; received in revised form 6 January 2005; accepted for publication 13 January 2005