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Interactions between uptake of amino acids and inorganic nitrogen in wheat plants

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Abstract

Soil-borne amino acids may constitute a nitrogen (N) source for plants in various terrestrial ecosystems but their importance for total N nutrition is unclear, particularly in nutrient-rich arable soils. One reason for this uncertainty is lack of information on how the absorption of amino acids by plant roots is affected by the simultaneous presence of inorganic N forms. The objective of the present study was to study absorption of glycine (Gly) and glutamine (Gln) by wheat roots and their interactions with nitrate (NO_3^-) and (NH_4^+) during uptake. The underlying hypothesis was that amino acids, when present in nutrient solution together with inorganic N, may lead to down-regulation of the inorganic N uptake. Amino acids were enriched with double-labelled ^{15}N and ^{13}C, while NO_3^- and NH_4^+ acquisition was determined by their rate of removal from the nutrient solution surrounding the roots. The uptake rates of NO_3^- and NH_4^+ did not differ from each other and were about twice as high as the uptake rate of organic N when the different N forms were supplied separately in concentrations of 2 mM. Nevertheless, replacement of 50 % of the inorganic N with organic N was able to restore the N uptake to the same level as that in the presence of only inorganic N. Co-provision of NO_3^- did not affect glycine uptake, while the presence of glycine down-regulated NO_3^- uptake. The ratio between ^{13}C and ^{15}N were lower in shoots than in roots and also lower than the theoretical values, reflecting higher C losses via respiratory processes compared to N losses. It is concluded that organic N can constitute a significant N-source for wheat plants and that there is an interaction between the uptake of inorganic and organic nitrogen.

1 Introduction

Amino acids are ubiquitously present in the soil solution and may constitute a significant source of N for plants in terrestrial ecosystems. The concentration of amino acids in the soil solution ranges from 0.1 to 60 µM and typically constitutes 10–40 % of the total soluble N (Hill et al., 2011; Jamtgard et al., 2008; Jones et al., 2002). The pool of dissolved amino acids is supplemented by a substantial pool of absorbed amino acids which can be up to 50 times higher than the amount of free amino acids and, in unfertilized soils, even higher than the content of ammonium and nitrate (Jamtgard et al., 2010). In cropping systems which rely on recycling and decomposition of organic N sources (e.g. animal manure based, low-input or organic agriculture), amino acids may represent a large N input (El-Naggar et al., 2009) and an important plant-available N pool (Jones et al., 2002). Under such circumstances, a significant proportion of total N in the soil solution consists of dissolved organic N, including both free amino acids and complex, humic-rich soluble organic matter which has relatively low bioavailability (Christou et al., 2005). The distribution of free amino acids in soil may show considerable spatial heterogeneity due to, e.g., degradation of sloughed-off root cell in the rhizosphere, leading to hotspots with greatly higher concentrations than in the bulk soil (Jones et al., 2002). The fast turnover of amino acids in soils implies that soil solution pool can be continuously renewed and, hence, provide the basis for significant plant uptake despite a low concentration in soil solution (Thornton and Robinson, 2005).

Amino acids may constitute a significant part of the N absorbed by plants in terrestrial ecosystems, especially under low N conditions (Harrison et al., 2000; Nasholm et al., 1998, 2000, 2009). Several amino acid transporters have been described in plants, conferring ability to absorb amino acids from the soil solution (Lipson and Nasholm, 2001). In *Arabidopsis* roots, the three amino acid transporters AAP1, AAP5 and LHT1 have been shown to play a role in amino acid uptake. They each have different specificity and affinity for amino acids (Hirner et al., 2006; Lee et al., 2007; Svennerstam et al., 2008). Amino acid transporters are less well characterised in other species but it is well documented that amino acid uptake occurs in a wide range of species including gymnosperms (Nasholm et al., 1998), dicots (Ge et al., 2009; Kielland, 1994; Nasholm et al., 1998, 2000; Streeter et al., 2000) and monocots (Biernath et al., 2008; El-Naggar et al., 2009; Henry and Jefferies, 2003; Jamtgard et al., 2008, 2010; Kielland, 1994; Lipson et al., 1999; Nasholm et al., 1998, 2000; Nasholm and Persson,

BGD
8, 11311–11335, 2011

2001; Schimel and Chapin, 1996; Streeter et al., 2000; Thornton, 2001; Thornton and Robinson, 2005; Yamagata and Ae, 1999). However, the importance and significance of organic N as a source of crop N under different management systems is still not well established.

One aspect causing uncertainty about the importance of organic N compounds as N source for plants is the possible interaction between organic and inorganic N forms during absorption by plant roots (Thornton and Robinson, 2005). The existing knowledge about these interactions is mainly based on Experimental approaches in which plants have been pre-treated with amino acids and subsequently exposed to nitrate or ammonium. In most cases, inorganic N absorption is down-regulated following pre-treatment with amino acids, although the actual responses are quite variable and may also depend on the N form used during the pre-treatment (Thornton, 2004). Less information is available from Experiments in which inorganic and organic N forms have been supplied together. Recently, Rasmussen and Kuzyakov (2009) and Rasmussen et al. (2010) showed – by use of $^{14}C/^{13}C/^{15}N$ triple-labelling instead of the double $^{15}N–^{13}C$ or $^{15}N–^{14}C$ labelling classically used for direct root uptake Experiments – that the simultaneous occurrence of inorganic C and N uptake interfered with root uptake of dual-labelled organic N. This shows that it is important to assess the interactions between organic and inorganic N forms during absorption by plant roots in order to establish the significance of organic N compounds for plant N nutrition.

The purpose of the present work was to evaluate interactions between the amino acids glycine and glutamine and the inorganic N forms nitrate and ammonium during uptake by plant roots. The underlying hypothesis was that amino acids, when co-present in nutrient solution with inorganic nitrogen, may lead to a down-regulation of inorganic N uptake or vice-versa. A series of experiments was conducted in which double labelled ($^{15}N–^{13}C$) amino acids applied to wheat plants were used to trace amino acid absorption, while the net-uptake rate of inorganic N was determined by regular sampling and analysis of the nutrient solution.

BGD
8, 11311–11335, 2011

2 Materials and methods

Three independent Experiments were carried out as detailed below. The purpose of Experiment I was to test the hypothesis that partial substitution of an inorganic N source (NO_3^- or NH_4^+) with an organic form (glycine or glutamine) would be able to restore the N uptake to the same level as that in the presence of only inorganic N. In Experiment II the objective was to test how glycine affected nitrate uptake, and *vice-versa*, when the concentration of each of the two sources was the same whether supplied alone or in mixture (i.e. increasing total N concentration when the two sources were applied together). Finally, the purpose of Experiment III was to test the hypothesis that plants were able to sustain growth and N acquisition when inorganic N (NO_3^-) was completely replaced by an organic N source (glycine, glutamine, arginine or asparagine).

2.1 Plant cultivation and harvest

2.1.1 Experiment I

Spring wheat (*Triticum aestivum* L. cv. Amaretto) was germinated in vermiculite for 10 days at 27 °C. Uniform seedlings were transferred to 40 opaque 4 l cultivation units each holding 4 plants. The units were filled with aerated basic nutrient solution having the following composition: 0.2 mM KH_2PO_4, 0.2 mM K_2SO_4, 0.3 mM $MgSO_4 \cdot 7H_2O$, 0.1 mM NaCl, 0.1 mM $Mg(NO_3)_2 \cdot 6H_2O$, 0.7 mM $Ca(NO_3)_2 \cdot 4H_2O$, 0.4 mM KNO_3, 0.8 µM $Na_2MoO_4 \cdot 2H_2O$, 0.7 µM $ZnCl_2$, 0.8 µM $CuSO_4 \cdot 5H_2O$, 2 µM H_2BO_3, 1 µM $NiSO_4 \cdot 6H_2O$, 50 µM $Fe(NO_3)_3 \cdot 9H_2O$, and 10 mM EDTA. The plants were grown in a greenhouse with controlled environment at 250 to 280 µmol m^{-2} s^{-1} photon flux density and a 18 °C/16 °C day/night (16/8 h) temperature regime. The nutrient solution was replaced every week and the position of the cultivation units shifted to minimize differences in growth due to micro-environmental differences.

After 30 days in the basic nutrient solution, plants from 4 cultivation units (16 individual plants) were harvested while the remaining 36 units were allocated to nine different nitrogen treatments. The nitrogen treatments consisted of (i) 2 mM glycine (Gly), (ii) 2 mM glutamine (Gln), (iii) 1 mM Gly + 1 mM NO_3^-, (iv) 1 mM Gln + 1 mM NO_3^-, (v) 1 mM Gly + 1 mM NH_4^+, (vi) 1 mM Gln + 1 mM NH_4^+; (vii) 2 mM NO_3^-; (viii) 2 mM NH_4^+; (ix) no nitrogen (N_0) with four replicates. Amino acids were enriched by 1.37 atom% with double-labelled ^{15}N and ^{13}C (Icon Services Inc., New York, USA). Ammonium was supplied as $(NH_4)_2SO_4$ and nitrate as KNO_3. The other plant nutrients were provided as in the basic nutrient solution except for Ca^{2+} and SO_4^{2-} which were used to obtain cation-anion balance. The pH in the nutrient solution was maintained around 5.5 by use of calcium hydroxide or sulphuric acid every 12 h. Twenty ml samples of the nutrient solution were taken from each cultivation unit at 4 time points, viz. 0, 22, 45 and 63 h after beginning of the treatment period.

Experiment II

Wheat plants were pre-grown for 30 days under the same conditions as above. Plants were then subjected to addition of 1 mM glycine and 3 mM NO_3^- or 1 mM glycine alone (4 replicates). N uptake from glycine was then measured over 4 days. The glycine was 10 % enriched with double-labelled ^{15}N and ^{13}C.

Experiment III

Under the same light and temperature conditions as in Experiments I and II, wheat plants were grown for 7 days in complete nutrient solutions with NO_3^- as the sole N source followed by a 17-day period in which 1 mM unlabelled glycine, glutamine, arginine or asparagine were provided as the only N source (4 replicates).

2.2 Analysis of plants and nutrient solution

At harvest, plants in each cultivation unit were quickly separated into roots and shoots which were weighed, frozen in liquid N and stored at −80 °C. Prior to analysis all plant

BGD
8, 11311–11335, 2011

material was freeze-dried until constant weight and milled to < 0.5 mm. The dried samples were subsequently used for analyzing total C, $^{13}C/^{12}C$, total N and $^{15}N/^{14}N$ by mass spectrometry in a system consisting of an ANCA-SL elemental analyzer coupled to a 20-20 tracermass mass spectrometer (SerCon Ltd., Crewe, UK).

All samples of nutrient solutions were analysed for NO_3^- and NH_4^+ using flow injection analysis (Lachat 8000 series, Hach, Loveland, Colorado). $^{15}NH_4^+$, $^{15}NO_3^-$ and total N were in selected cases measured by mass spectrometry following the micro-diffusion technique (Brooks et al., 1989). In order to double check the recovery of amino acids in solution, CF-IRMS analyses of the nutrient solutions sampled at the completion of the experiments were performed after oven-drying at 50 °C.

2.3 Calculations

N recoveries and N uptake rates were calculated on the basis of excess ^{15}N and ^{13}C in the harvested plant material combined with the concentrations of N left in the nutrient solution.

N and C atom% excess was calculated as excess compared to the mean atom% of the pre-treatment plants, according to the equation:

$$APE = AP_p - AP_{ptp} \tag{1}$$

where APE = atom percent excess, AP_p = atom percent in treated plant, and AP_{ptp} = atom percent in pre-treatment plants (average).

The amount of amino-acid derived ^{15}N or ^{13}C taken up by the plant was calculated by use of Eq. (2):

$$AA - {}^{15}N = DW \times N\,\% \times APE(N) \tag{2}$$

where $AA - {}^{15}N$ = amino acid-derived ^{15}N in plant tissue (mg), DW = plant dry weight, N % = plant nitrogen concentration (%), and APE(N) = nitrogen atom percentage excess.

BGD
8, 11311–11335, 2011
Organic and inorganic N interactions in wheat
E. Gioseffi et al.

Recovery of the added amino acids in plant material was calculated by use of Eq. (3):

$$R_N = \frac{AA-{}^{15}N}{AA-{}^{15}N_{add}} \times 100 \qquad (3)$$

where R_N = N recovery in plant (%) and $AA-{}^{15}N_{add}$ = added amino acid-^{15}N (mg).

For inorganic N, the uptake rate was calculated based on the N left in solution according to Eq. (4), and for organic N based on the ^{15}N excess in plant material after harvest, according to Eq. (5):

$$UR_{inorgN} = \frac{\left(N_{left.T_2} - N_{left.T_1}\right)}{DW_{root} \times (T_2 - T_1)} \qquad (4)$$

$$UR_{orgN} = \frac{AA-{}^{15}N}{DW_{root} \times T_2} \qquad (5)$$

where UR = Uptake rate and T_2, T_1 = sampling times (hours after beginning of treatments).

2.4 Statistical analysis

Statistical analysis was carried out using R version 2.12.2 (www.r-project.org) through mixed linear models and analysis of variance followed by Tukey's and Duncan's test.

3 Results

3.1 N content and ^{13}C : ^{15}N ratio

^{15}N and ^{13}C excess increased in plants supplied with amino acids (Table 1). The excess of the two stable isotopes was almost twice as high in plants receiving organic

BGD
8, 11311–11335, 2011

N (2 mM Gly or Gln) as the sole N supply compared to plants receiving only half the concentration of organic N (1 mM Gly or Gln) in combination with inorganic N (1 mM NH_4^+ or NO_3^-). The N concentration in the plant dry matter did not differ ($P > 0.05$) among N-fed plants irrespective of in which form the N was supplied (Table 1). The total N content (g plant^{-1}) was 10 % higher in plants receiving either glutamine or nitrate compared to the other treatments which did not differ from each other (Table 1).

The ratio between ^{13}C and ^{15}N recovered in the plant tissues was lower than the theoretical values of 2 : 1 for glutamine and 2.5 : 1 for glycine in all plants receiving organic N (Fig. 1). The ^{13}C : ^{15}N ratio was in all cases lower in shoots than in roots ($P \leq 0.05$). Plants receiving glutamine as the sole N supply had higher ^{13}C : ^{15}N ratio in the roots than plants supplied with glycine, reflecting the higher C : N ratio of glutamine. However, the opposite was the case for shoots, showing higher ^{13}C : ^{15}N ratio for plants receiving glycine relative to glutamine (Fig. 1). Irrespective of the amino acid supplied, shoot ^{13}C : ^{15}N ratios were higher in plants receiving NH_4^+ compared to NO_3^- ($P \leq 0.05$).

3.2 N uptake rates

The uptake rates of NO_3^- and NH_4^+ did not differ from each other and were about twice as high as the uptake rate of organic N when the different N forms were supplied separately in concentrations of 2 mM (Fig. 2). Nevertheless, replacement of 50 % of the inorganic N with organic N was able to restore the N uptake to the same level as that in the presence of only inorganic N (Fig. 2).

Significantly different amino acid uptake rates were obtained depending on whether ^{13}C or ^{15}N were used for the calculations (Fig. 3). In all cases, ^{13}C-based uptake rates were much lower than those based on ^{15}N.

3.3 Interactions between N forms

The statistical analysis of the data in Fig. 2 showed a significant interaction between the uptake of organic and inorganic N ($P \leq 0.05$). Such interaction was also evident in Experiment II, where co-provision of NO_3^- did not affect glycine uptake, while the presence of glycine down-regulated NO_3^- uptake (Fig. 4). Pre-starvation of plants did not lead to increased uptake of glycine (Fig. 4).

Plants exposed to glycine as the sole N form after pre-cultivation on nitrate had lower NO_3- concentrations in roots as well as shoots compared to plants continuously receiving NO_3^- (Fig. 5). Pre-starvation also led to a reduction in tissue NO_3^- (Fig. 5). Tissue NH_4^+concentrations were similar across the different N treatments (Fig. 5).

3.4 N and C recovery in wheat plants

About 70–80 % of the added amino-acid-N was recovered in the plants. In all cases, at plant harvest there was still a small fraction of N left in the solutions containing amino acids (Fig. 6). Labelled $^{15}NH_4^+$ was detected in some of the originally pure amino acid solutions, indicating possible deamination by microorganisms in solution or efflux of $^{15}NH_4^+$ from the roots. However, deamination prior to uptake did not seem prominent as evidenced by the fact that when plants in Experiment III were grown for an extended period with amino acids as the sole N source, plant growth was reduced by 50 % or more compared to plants grown in NO_3^- (Fig. 7).

4 Discussion

4.1 Interaction between organic and inorganic N forms

Inorganic nitrogen is considered to be the preferred N form taken up by higher plants. This was also the case in the present study, as shown by twice as high uptake rates of NO_3^- and NH_4^+ compared to amino acids when the total N concentration was the same (i.e. 2 mM; Fig. 2). Compared across Experiments, the uptake rate of glycine in wheat roots seemed only to respond marginally to an increase in external concentration, being similar at 1 mM (Fig. 4) and 2 mM (Fig. 2) when glycine was supplied

BGD
8, 11311–11335, 2011

as the sole N source. In contrast, the uptake of NO_3^- was higher at 3 mM (around 28 µmol g^{-1} root DW h^{-1}; Fig. 4) compared to 2 mM (around 23 µmol g^{-1} root DW h^{-1}; Fig. 2). Assuming that NO_3^- uptake responds linearly to changes in the external NO_3^- concentration in the low-affinity range (Glass, 2009) this would imply a net uptake rate of around 18 µmol g^{-1} root DW h^{-1} at 1 mM external NO_3^-. The actual observed uptake rate of NO_3^- in solutions containing 1 mM NO_3^- in the presence of 1 mM glycine was only around 13 µmol g^{-1} root DW h^{-1} (Fig. 2) indicating that NO_3^- uptake was down-regulated. This conclusion based on data from Experiment I is corroborated by the data obtained in Experiment II, showing that co-provision of 1 mM glycine and 3 mM NO_3^- resulted in a down-regulation of NO_3^- uptake while that of glycine was unaffected (Fig. 4). Since glycine uptake is not down-regulated in the presence of NO_3^-, plants may be able to maintain a similar total N uptake as when NO_3^- constitutes their sole N source, as shown by the fact that replacement of 50 % of the inorganic N with organic N in Experiment I was able to restore the N uptake rate to the same level as that in the presence of only inorganic N (Fig. 2). Plants may thus be able to fulfil their N requirement when organic N is provided as an N source in mixture with NO_3^-. In contrast, NH_4^+ seems to cause a certain down-regulation of amino acid uptake and vice-versa (Henry and Jefferies, 2003; Thornton and Robinson, 2005) although in some cases this may not appear (Rodgers and Barneix, 1993).

The interaction between root uptake of inorganic and organic N forms reflects a feed-back repression in which the amino acid glutamine represents an important signal for the shoot to communicate N status to the roots, thereby enabling plants to regulate their rate of N uptake to accommodate for N demand during plant growth (Girin et al., 2007; Nazoa et al., 2003). Nitrate starvation may relieve this feed-back repression (Krapp et al., 1998) but did not lead to increased uptake of glycine or nitrate in the present work (Fig. 4). The feed-back repression is primarily targeted at transport proteins involved in NO_3^- and NH_4^+ uptake, explaining why glycine uptake may not be affected to the same extent.

BGD
8, 11311–11335, 2011

Despite being taken up at a high rate, amino acids can not constitute the sole N supply without leading to growth depression (Fig. 7). This may reflect bottlenecks in the rate at which nitrogen is transferred to other essential amino acids via deamination and or transamination. In addition, signalling pathways controlling phytohormone balance may be impeded causing growth inhibition (Krouk et al., 2011).

4.2 Fate of absorbed C and N in the plant

The ratio between ^{13}C and ^{15}N excess in roots and shoots was significantly lower than the theoretical values of 2.5 and 2 in glutamine and glycine (Fig. 1). It has been argued that if the enrichment of ^{13}C and ^{15}N follows a linear relationship upon exposure to double-labelled amino acids, then the amino acids have been absorbed as intact molecules (Jones et al., 2005). However, some of the absorbed amino acid C will always be lost in the form of CO_2 produced during deamination and breakdown of the C skeleton in the TCA cycle (Nasholm and Persson, 2001) or in processes related to photorespiration (Bauwe et al., 2010). In wheat plants this may amount to 60–80 % of the absorbed amino acid C (Hill et al., 2011), agreeing with the losses reported in our study (Fig. 6). N can be lost by N volatilization from the leaves (Schjoerring et al., 2002; Sommer et al., 2004) but these losses are typically highest towards plant maturation and only amount to 1–5 % of the N, i.e. being considerably lower than C losses due to respiration. A lower ^{13}C : ^{15}N excess ratio does therefore not necessarily imply that amino acids were not taken up in intact form as also concluded by Schimel and Chapin (1996).

Lower ^{13}C : ^{15}N excess ratios in plant tissues relative to applied amino acids could also result from deamination of amino acids before uptake, followed by uptake of inorganic ^{15}N. It does not seem likely that this process contributed significantly in our Experiments, because only a few percentages of the ^{13}C were recovered in solution at the end of the Experiment (Fig. 6). If the amino acids had been decomposed by microorganisms and the N released in inorganic form to the solution and subsequently taken up by the roots, a significant portion of the labelled C would have remained in the

Discussion Paper | Discussion Paper | Discussion Paper | Discussion Paper |

BGD

8, 11311–11335, 2011

Organic and inorganic N interactions in wheat

E. Gioseffi et al.

bacteria without being lost by respiration during the 3 day Experimental period. The utilization efficiency of easily decomposable substrates typically varies between 0.4–0.7 (Parton et al., 1987; Steinweg et al., 2008; Thiet et al., 2006), hence loss by microbial decomposition can only explain a few percent of the unrecovered ^{13}C in most of the treatments. Our assumption that the majority of the ^{13}C loss occurred after uptake of intact amino acids is further corroborated by the fact that the growth of plants exposed to amino acids as the sole N source was more than 50 % reduced (Fig. 7) which would not have been the case if a substantial deamination in the nutrient solution had made NH_4^+ available as N source. Finally, amino acids are chemically very stable in water and would not deaminate spontaneously under the Experimental conditions we applied.

The transport of amino acids from roots to shoots in plants is dominated by the amides glutamine and asparagine (Finnemann and Schjoerring, 1999; Harrison et al., 2000). Before being utilized as a N-source, a substantial part of the glycine absorbed by roots is therefore probably deaminated and channeled into amide synthesis, a process accompanied by release and re-assimilation of NH_4^+. Glutamine may on the other hand be transported directly to shoots and utilized directly as an N source in the glutamate synthase-glutamine synthetase cycle. Alternatively, a glutamine transaminase may transfer the amino nitrogen to glycine or other amino acids (Joy, 1988). These amino acids may act as substrates for aminotransferases transferring the amino group to glyoxalate, thereby forming glycine which enters the photorespiratory pathway, resulting in release of CO_2 and NH_4^+ (Bauwe et al., 2010). The fact that the ^{13}C : ^{15}N excess ratio was much lower in shoots compared to roots (Fig. 2) suggest that photorespiration may have played an important role in the assimilation of the N derived from the absorbed amino acids. Despite the release of NH_4^+ in the various metabolic processes involved in processing the absorbed amino acids, plants fed with glycine or glutamine did not contain elevated tissue NH_4^+ levels in neither roots not shoots (Fig. 5). The capacity for re-assimilation of the generated NH_4^+ was thus not exceeded.

Discussion Paper | Discussion Paper | Discussion Paper | Discussion Paper |

BGD

8, 11311–11335, 2011

Organic and inorganic N interactions in wheat

E. Gioseffi et al.

Title Page
Abstract Introduction
Conclusions References
Tables Figures
◂ ▸
Back Close
Full Screen / Esc
Printer-friendly Version
Interactive Discussion

5 Conclusions

It is concluded that amino acids can constitute a significant N source for wheat plants and that there is an interaction between the uptake of inorganic and organic N. Glycine uptake is not down-regulated in the presence of NO_3^-, while NO_3^- uptake is reduced in the presence of glycine. In contrast, NH_4^+ causes a down-regulation of amino acid uptake and vice-versa. Nitrogen pre-starvation appears to counteract the down regulation of NO_3^- uptake in the presence of glycine. The $^{13}C:^{15}N$ excess ratio is lower in shoots than in roots and is also lower than in the provided source of double-labelled amino acid, reflecting higher C losses via respiratory processes compared to N losses. The observed interactions between root absorption of inorganic and organic N forms have implications for further assessment of the contribution of organic N compounds to plant N nutrition.

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BGD

8, 11311–11335, 2011

Title Page

Abstract | Introduction

Conclusions | References

Tables | Figures

◀| |▶

◀ ▶

Back | Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

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BGD
8, 11311–11335, 2011
Organic and inorganic N interactions in wheat
E. Gioseffi et al.

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BGD
8, 11311–11335, 2011

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BGD
8, 11311–11335, 2011

Table 1. N concentration and total N content of plants in Experiment I. Numbers are mean values ($n = 4$). Letters indicate significant differences at $P = 0.05$ (Tukey's test). Within columns, values followed by the same letter are not significantly different. Excess ^{15}N % and ^{13}C ‰ values were calculated taking the N_0 treatment as reference (^{15}N % = 0.3706 for roots and 0.3681 for shoots; ^{13}C %=1.0786 for roots and 0.0773 for shoots).

Treatment	N conc.	N content	^{15}N excess		^{13}C excess	
			Root	Shoot	Root	Shoot
	%	g	‰	‰	‰	‰
Pre-treated	4.50 a	0.37 a	NA	NA	NA	NA
NH_4^+	4.35 a	0.46 ab	−0.002 c	0.000 c	0.000 d	0.000 d
NO_3^-	4.10 a	0.52 b	−0.002 c	0.000 c	0.002 d	0.001 d
Gln	3.89 ab	0.50 b	0.413 a	0.184 a	0.408 a	0.055 b
Gly	4.02 a	0.47 ab	0.425 a	0.210 a	0.272 bc	0.100 a
Gln + NH_4^+	4.11 a	0.47 ab	0.208 b	0.104 b	0.227 bc	0.054 b
Gly + NH_4^+	3.70 ab	0.44 ab	0.173 b	0.101 b	0.169 c	0.058 b
Gln + NO_3^-	3.94 ab	0.48 ab	0.229 b	0.097 b	0.304 ab	0.026 c
Gly+ NO_3^-	4.06 a	0.44 ab	0.242 b	0.117 b	0.183 c	0.048 b
N_0	3.19 b	0.41 ab	0.000 c	0.000 c	0.000 d	0.000 d

BGD
8, 11311–11335, 2011

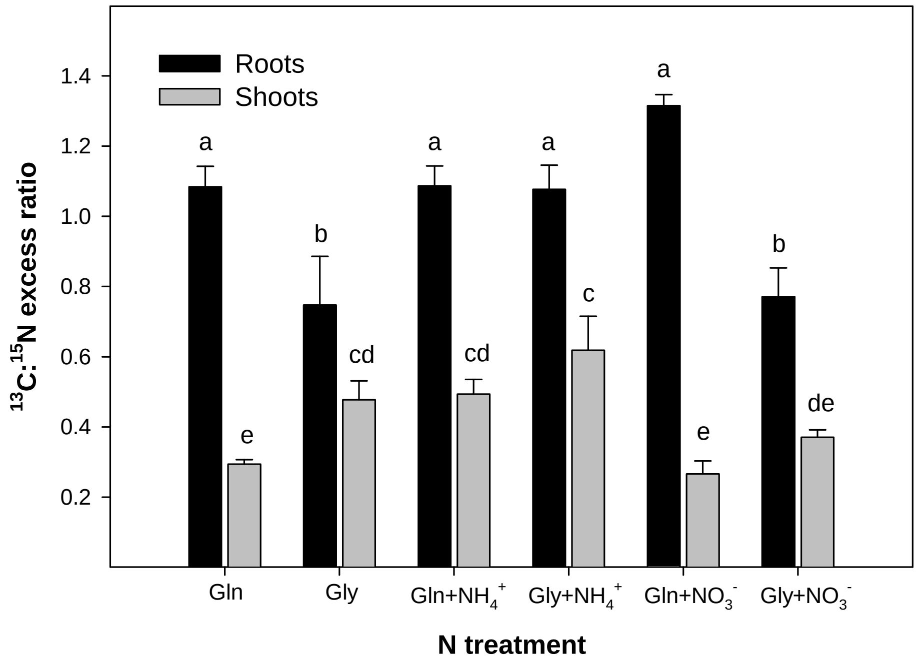


Fig. 1. Ratio between ^{13}C : ^{15}N excess in roots and shoots of wheat plants supplied with double-labelled amino acids, either alone or mixed with inorganic N. Values are means ($n = 4$) obtained in Experiment I and letters indicate significant differences at $P = 0.05$ (Duncan test).

BGD
8, 11311–11335, 2011

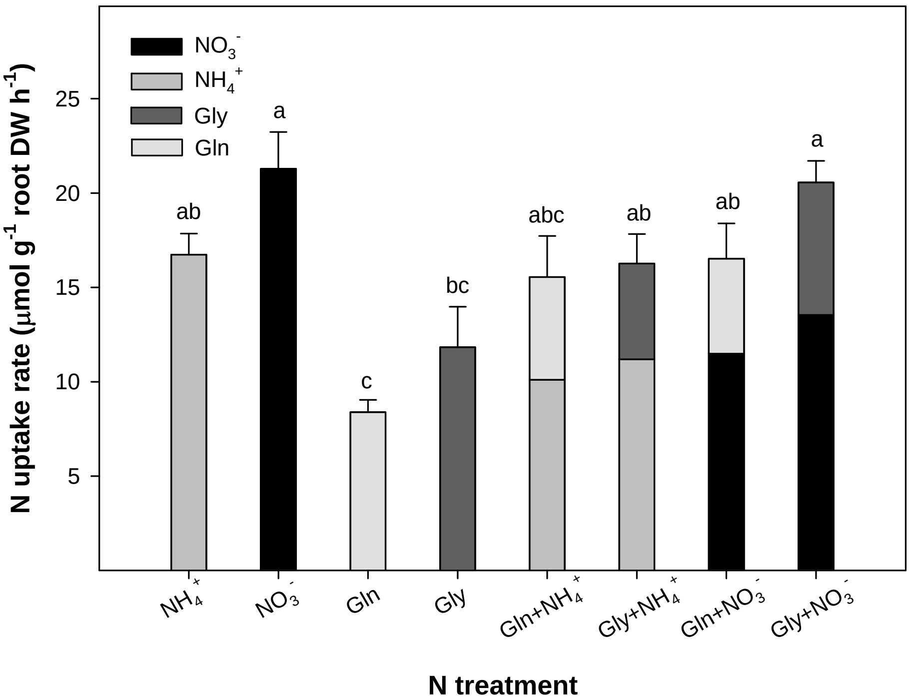


Fig. 2. Nitrogen uptake rates in wheat plants supplied with different combinations of amino acids and inorganic N forms in Experiment I. For inorganic-N, values were calculated on the basis of the N remaining in solution, while for amino acid-N values were calculated in terms of ^{15}N content in plants. All treatments had a total N concentration of 2 mM. Bars indicate mean values ($n = 4$), and letters indicate significant differences at $P = 0.05$ (Tukey's test).

BGD
8, 11311–11335, 2011

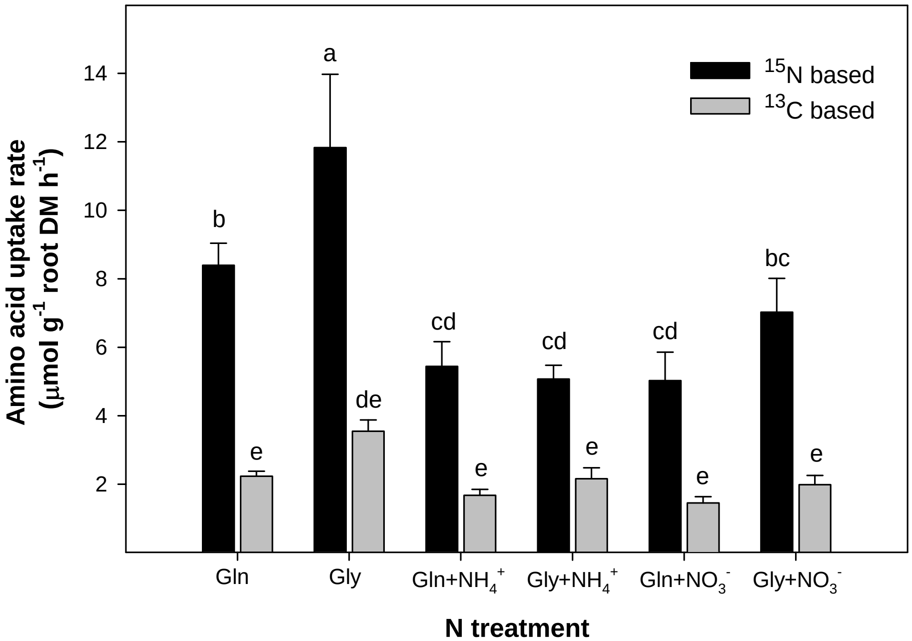


Fig. 3. Amino acid uptake rates calculated on the basis of either ^{15}N or ^{13}C data in Experiment I. Error bars indicate standard error ($n = 4$).

BGD
8, 11311–11335, 2011

Organic and inorganic N interactions in wheat

E. Gioseffi et al.

Title Page
Abstract | Introduction
Conclusions | References
Tables | Figures
◀| |▶
◀ ▶
Back | Close
Full Screen / Esc
Printer-friendly Version
Interactive Discussion

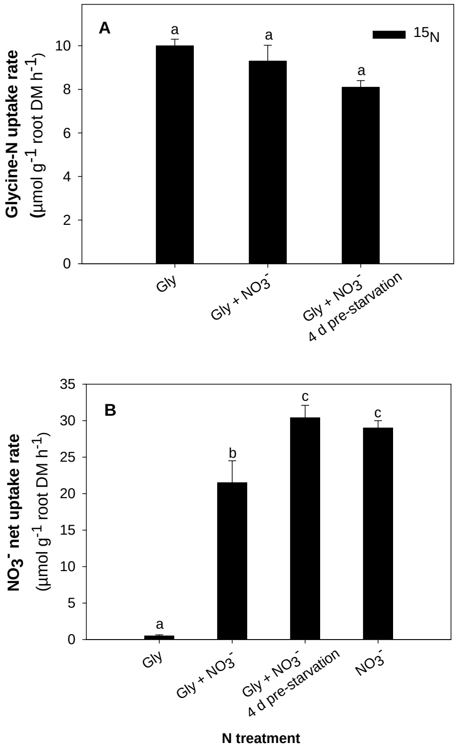


Fig. 4. Glycine **(A)** and nitrate uptake **(B)** in wheat plants. ^{15}N, ^{13}C double-labelled glycine (1 mM) was applied during a 3-day period with or without 3 mM NO_3^- to hydroponically grown wheat plants pre-cultivated on NO_3^-. Data are means ±SE ($n = 4$) obtained in Experiment II and letters indicate significant differences at $P = 0.05$.

BGD
8, 11311–11335, 2011

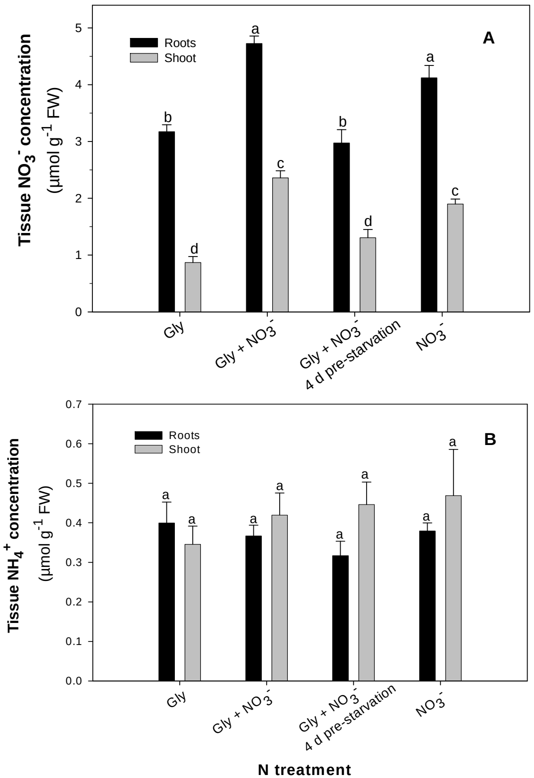
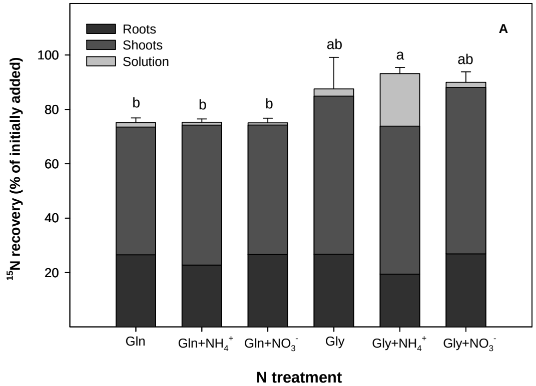


Fig. 5. Tissue NO_3^- **(A)** and NH_4^+ **(B)** in wheat plants pre-cultivated on NO_3^- and subsequently applied glycine (1 mM) during a 3-day period with or without 3 mM NO_3^-. Data are means ±SE ($n = 4$) obtained in Experiment II and letters indicate significant differences at $P = 0.05$.

BGD
8, 11311–11335, 2011



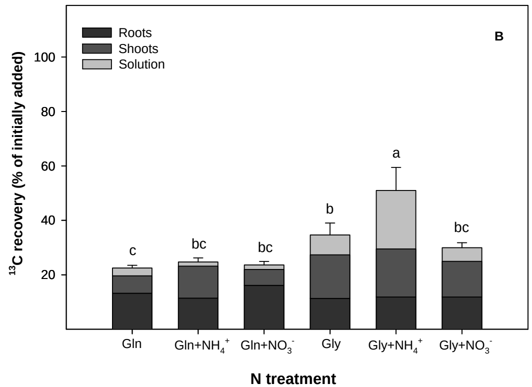


Fig. 6. Recoveries in plant roots, plant shoots and solution based on plant and solution analyses: **(A)** ^{15}N; **(B)** ^{13}C in Experiment I. Error bars indicate total standard error ($n = 4$) and letters significant differences at $P = 0.05$.

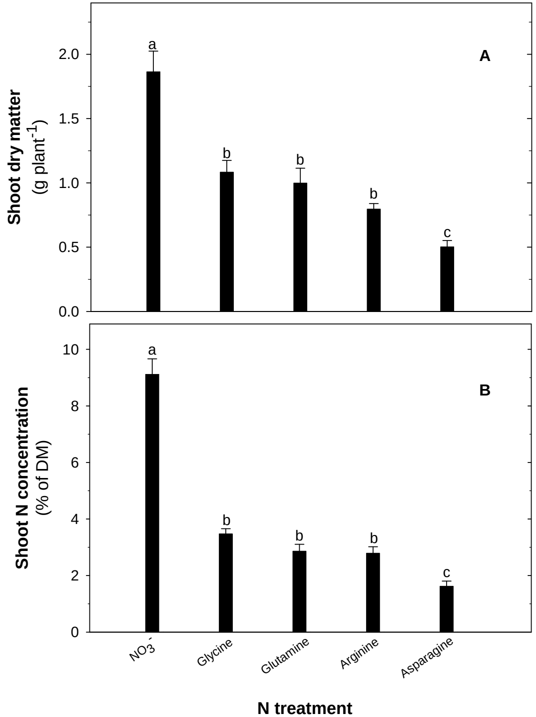


Fig. 7. Growth **(A)** and N concentration **(B)** of wheat plants supplied different amino acids as sole N source during a 17-day period in Experiment III. Data are mean values ±SE ($n = 4$) and letters indicate significant differences at $P = 0.05$.

BGD
8, 11311–11335, 2011