Patterns of Plant Biomass Partitioning Depend on Nitrogen Source

Camila Agetoni Cambui¹, Henrik Svennerstam¹, Linda Gruffman², Annika Nordin¹, Ulrika Ganeteg¹, Torgny Näsholm²*

1 Department of Forest Genetics and Plant Physiology Swedish University of Agricultural Sciences, Umeå, Sweden, 2 Department of Forest Ecology and Management, Swedish University of Agricultural Sciences, Umeå, Sweden

Abstract

Nitrogen (N) availability is a strong determinant of plant biomass partitioning, but the role of different N sources in this process is unknown. Plants inhabiting low productivity ecosystems typically partition a large share of total biomass to belowground structures. In these systems, organic N may often dominate plant available N. With increasing productivity, plant biomass partitioning shifts to aboveground structures, along with a shift in available N to inorganic forms of N. We tested the hypothesis that the form of N taken up by plants is an important determinant of plant biomass partitioning by cultivating Arabidopsis thaliana on different N source mixtures. Plants grown on different N mixtures were similar in size, but those supplied with organic N displayed a significantly greater root fraction. ¹⁵N labelling suggested that, in this case, a larger share of absorbed organic N was retained in roots and split-root experiments suggested this may depend on a direct incorporation of absorbed amino acid N into roots. These results suggest the form of N acquired affects plant biomass partitioning and adds new information on the interaction between N and biomass partitioning in plants.

Introduction

Plants adjust to variations in resource availabilities by variable partitioning to root and shoot growth [1,2]. Among various edaphic factors that affect plant biomass partitioning, nitrogen (N) is particularly important; higher rates of available N shift partitioning from roots to shoots [3–7]. Studies of partitioning responses to shifts in N supply have revealed a positive linear relationship between shoot-root ratios and the internal N status of plants [8–10]. Plants growing on infertile, low N soils are also reported to have a higher root mass fraction than plants growing on more fertile and N rich soils [11–12]. Thus, in natural ecosystems, soil N availability and plant biomass partitioning exhibit strong co-variation.

However, there is quantitative and qualitative variation in soil N availability in most ecosystems [13]. In poor soils of natural ecosystems of the arctic, boreal [14–18] and alpine [19,20] regions, organic N predominates. In contrast, an increasing share of mineral N, particularly NO₃⁻, is found in soils of temperate [21] and subtropical [22–23] regions, particularly in agricultural soils [24,25]. The well-documented effect of soil N availability on regulating plant biomass partitioning may thus, in natural ecosystems, be confounded by changes in the forms of N in soil solution.

Optimisation of biomass partitioning is thought to minimise the stress imposed by the limiting resource, in this case N. Arguably, such optima must depend on the actual chemical form(s) of N present for uptake, because plant N acquisition is mainly limited by processes through which N sources come into contact with root surfaces: mass flow induced by transpiration, diffusion and interception [26]. Nitrate can be efficiently acquired through mass flow because of its high mobility in soils. Organic N compounds are less mobile in soil solution [25] and hence may mainly be acquired through diffusion. This implies that, for similar rates of N uptake, plants would need to partition a larger share of biomass to roots (or mycorrhizal hyphae) when N is available as organic N, compared with conditions when NO₃⁻ is the dominant N source. Thus, for a given total N availability, an optimum for biomass partitioning in a NO₃⁻-dominated environment should be different and with a higher shoot:root ratio, compared with the corresponding optimum with organic N as the dominant N source.

In a study of Pinus sylvestris seedlings [27], it was shown that the short-term distribution of N varied for different N sources. Thus, in this study, N absorbed as NO₃⁻ was to a higher extent allocated to shoots compared to N absorbed as organic forms. Whether such short-term differences in N allocation between different N forms are relevant for the long-term distribution of N is, however, not known. As discussed above, plants in natural environments usually encounter a range of organic and inorganic N compounds simultaneously and may thus absorb and utilize several different forms of N. Under such conditions, mechanisms for tuning biomass partitioning to both soil N availability and soil N chemical composition would be of significant value. One possible mechanism would be if the different chemical forms of absorbed N were...
distributed within the plant in line with the optimal partitioning of biomass for that particular form of N. Thus, N absorbed as NO$_3^-$ would be partitioned to a larger extent to shoots while N absorbed in organic forms to a larger extent to roots and this would offer a mechanism through which plants could optimise biomass partitioning according to the chemical composition of soil N.

We hypothesised that the chemical forms of N that plants take up would affect biomass partitioning between shoots and roots. Furthermore, we hypothesised that the internal distribution of absorbed N would depend on which form of N is absorbed. So, for example, absorbed organic N would be retained in roots to a greater extent than absorbed inorganic N. We used a sterile growth system with agar as a root medium and cultivated Arabidopsis thaliana with mixtures of different N sources, in order to study how N forms affected plant biomass partitioning. We traced the origin of total plant N, as well as the origin of root and shoot N, from individual N sources using $^{15}$N-labelling. We also investigated the movement of different N sources between plant parts.

Materials and Methods

Experiment 1. Growth and biomass partitioning on different N source mixtures

Wild type (ecotype Col-0) Arabidopsis thaliana (Arabidopsis) were grown on sterile agar plates containing the equivalent of N-free half-strength Murashige and Skoog (MS) medium [28], with 0.65% w/v agar (plant agar, Duchefa Biochemie), 0.5% w/v sucrose and the pH set to 5.8 with MES buffer. Nitrogen was administered as one of the following N source mixtures: (1) 6 mM NO$_3^-$; (2) 3 mM NH$_4$NO$_3$; (3) 1 mM glutamine+4 mM NO$_3^-$; (4) 1.5 mM glutamine+3 mM NO$_3^-$ and (5) 2 mM glutamine+2 mM NO$_3^-$. Thus, all treatments included 6 mM N and mixtures of glutamine and NO$_3^-$ were in the ratios 1:2; 1:1 and 2:1. Sterile filtered glutamine was added to the agar mixture after autoclaving, to ensure that it was intact in the medium. Plates were filled with 40 ml of agar and five seeds were sown onto each plate. Agar plates were incubated in a cold room for two days to synchronize germination and then transferred to a growth cabinet with a 16/8 h light/dark (200 μmol photons m$^{-2}$ s$^{-1}$) and 23/ 18°C (day/night) regime. Plants were transferred to a split-root experiment system. This system consisted of Petri dishes with two separate root-growth compartments.

Two treatments were conducted with this setup. In the first, both cells of the plate contained the equivalent of N-free half-strength MS medium and N was supplied as a mixture of 3 mM NO$_3^-+1.5$ mM glutamine. In one of the halves, one of the N sources (either NH$_4$NO$_3$ or glutamine) was $^{15}$N labelled at a rate of 1.0 atom % excess. Transferred plants (n = 6 for plants grown on mixtures where the glutamine was labelled and n = 7 for plants grown on mixtures where the NO$_3^-$ was labelled) were placed on the mid rib and their root systems divided into two similar fractions that were positioned on either side of the rib. After two weeks of growth in this system, plants were harvested and shoot and root biomass for each treatment was determined.

In a separate experiment, Arabidopsis plants were grown as described above, but with N supplied as 3 mM NO$_3^-+30$ μM U$^{15}$N$_2$, U$^{15}$C$_2$L-Arg (>90% $^{15}$N; corresponding to 120 μM L-Arg-N). The $^{15}$N-labelling enabled analysis of the distribution of absorbed arginine-N. $^{13}$C labelling was included to allow analysis of distribution of absorbed arginine-C but this part of the study was later discontinued since it was realized that the risk for fixation of respired arginine-$^{13}$C through photosynthesis would lead to non-conclusive results. Harvests and analysis of total N and $^{15}$N content of shoots and roots were performed as described above. Five plants were grown on each plate and four plates were harvested (n = 4) for each measurement.

Experiment 3. Split root experiment

Arabidopsis thaliana (ecotype Col-0) seeds were surface-sterilized, exposed to 4°C during 48 h (to synchronize germination) and cultivated for 11–14 days on vertical plates containing half-strength N-free Murashige and Skoog (MS) medium [28], with 5 mM KNO$_3$, 0.5% w/v sucrose, 1% w/v agar (plant agar, Duchefa Biochemie), buffered to pH 5.6 with 7.7 mM MES. Plants were grown in climate chamber under short-day conditions (light/dark period of 8/16 hours), temperature regime of 22/18°C degrees and light intensity of 200 μmol photons m$^{-2}$ s$^{-1}$ in order to avoid early flowering. Flowering causes internal re-distribution of N, which would complicate any assessment of the internal N fluxes absorbed as different sources. After 2 weeks of growth, the primary roots of plants were removed to stimulate lateral root development and, one week afterwards, when the new roots were approximately 2–4 cm long, the plants were transferred to a split-root experiment system. This system consisted of Petri dishes with two separate root-growth compartments.

Two trials were conducted with this setup. In the first, both cells of the plate contained the equivalent of N-free half-strength MS medium and N was administered as a mixture of 3 mM NO$_3^-+1.5$ mM glutamine. In one of the halves, one of the N sources (either NH$_4$NO$_3$ or glutamine) was $^{15}$N labelled at a rate of 1.0 atom % excess. Transferred plants (n = 6 for plants grown on mixtures where the glutamine was labelled and n = 7 for plants grown on mixtures where the NO$_3^-$ was labelled) were placed on the mid rib and their root systems divided into two similar fractions that were positioned on either side of the rib. After two weeks of growth in this system, plants were harvested and shoot and root biomass from the two compartments were separated.

In the second trial, the two compartments were filled with half-strength N-free MS medium and N was supplied as 3 mM NO$_3^-$ on one of the root compartments while on the other half, N was supplied as 1.5 mM glutamine. Potassium was compensated in the glutamine treatment with the equivalent addition of KCl. For each plate, one of the N sources was labelled with 1 atom % $^{15}$N excess (five plates containing 1% $^{15}$NO$_3^-$ and five plates with 1 atom % excess $^{15}$N-glutamine). Plants were grown for 14 days before harvest.

At harvest, all samples were washed 3 times with 0.5 mM CaCl$_2$ to remove N compounds adhering to root surfaces, dried at 60°C and homogenized for determination of total N and $^{15}$N content. Analyses were conducted using a Europa Scientific Isotope Ratio Mass Spectrometer.

Statistics

Significant differences between N treatments and between plant parts were tested using ANOVA followed by Tukey's post hoc test.
Results

In the first experiment, we tested how various mixtures of glutamine and NO$_3^-$ affected growth and biomass partitioning in Arabidopsis, as compared with pure NH$_4$ and NO$_3^-$. The rationale behind this experiment was to test whether an increasing fraction of organic N in the growth media would correspond to an increased fraction of plant biomass partitioned to roots. Plants grown on NO$_3^-$ were significantly smaller than the other N treatments. A significant effect of N source on the root mass fraction was found; the root fractions of plants grown on any of the glutamine mixtures were in all cases significantly higher than those of plants grown on NH$_4$NO$_3$ or NO$_3^-$. (Fig. 1). The root mass fraction did, however, not display a significant increase with an increasing share of glutamine in the growth media.

In the second experiment, we used $^{15}$N-labelled N sources to assess plant uptake and internal distribution of individual N compounds in the mixtures. This enabled us to test if an effect of a specific N source on biomass partitioning was paralleled by a specific pattern of distribution of N from that source within the plant.

Plants supplied with mixtures of glutamine and NO$_3^-$ ($^{15}$Ngln-NO$_3$ and gln-$^{15}$NO$_3$) had higher biomass, root biomass and a higher root mass fraction than plants supplied with NH$_4$NO$_3$ (Table 1). No statistical difference in N concentration of plants from the different treatments was found. The abundance of $^{15}$N in plant parts was clearly different for plants grown on glutamine and NO$_3^-$ mixtures, but this difference was smaller for plants grown on NH$_4$NO$_3$ (Table 2). $^{15}$N of shoots of plants supplied $^{15}$Ngln-NO$_3$ was lower than any of the other three treatments (gln-$^{15}$NO$_3$, $^{15}$NNO$_3$ and NH$_4$$^{15}$NO$_3$). These differences suggested that root N was derived more from uptake of glutamine than from NO$_3^-$, while the opposite was true for shoot N. Values of $^{15}$N abundances were therefore used to calculate fractions of plant, root and shoot N that were derived from each individual N source (Fig. 2). Arabidopsis plants grown on NH$_4$NO$_3$ had 50% N content derived from NH$_4^+$ and 46% derived from NO$_3^-$. Thus, 96% of N in the biomass was accounted for by uptake of the two N sources in these plants. For root N, 55% was derived from NH$_4^+$ and 39% from NO$_3^-$. For shoot N, 49% was derived from NH$_4^+$ and 47% from NO$_3^-$, i.e. compared with total plant N, N derived from NO$_3^-$ was slightly more abundant in shoots (Fig. 2a). The ratio between N derived from NH$_4^+$ and that derived from NO$_3^-$ was thus 1.4, 1.0 and 1.1 for root, shoot N and total plant N, respectively. The biomass of roots were always much smaller than that of shoots (cf. Table 1) and hence a high abundance of N derived from organic N in roots still had a limited effect on the fraction of total plant N derived from organic N.

The N content of Arabidopsis plants grown on mixtures of NO$_3^-$ and glutamine also reflected the different N sources. N derived from glutamine accounted for 52% and N derived from NO$_3^-$ accounted for 43% of plant N. Thus, in these plants, 95% of total plant N was accounted for by uptake of the two N sources. Nitrogen not accounted for in these measurements would to some extent result from the N contained in seeds but could also result from a somewhat lower $^{15}$N abundance of tracers compared to those given by the manufacturer. Small variations in the $^{15}$N abundance of the non-labelled compounds could also be part of the explanation of the N not accounted for in the $^{15}$N mass-balance calculations. Significantly more root N was derived from glutamine (76%) and significantly less from NO$_3^-$ (24%), compared with total plant N. Shoot N was derived equally much from glutamine (47%) and from NO$_3^-$ (47%), but compared with total plant N, N derived from glutamine was less represented in shoots (Fig. 2b). The ratio between N derived from glutamine and N derived from NO$_3^-$ was thus 3.1, 1.0 and 1.2 for root N, shoot N and total plant N, respectively.

We then asked if the observed preferential distribution of N derived from uptake of glutamine was specific for this particular N source or if N derived from other amino acids would show similar patterns of distribution between shoot and roots. Thus, in an additional experiment, we grew plants as described above but with N supplied as 3 mM NO$_3^-+30$ μM of labelled L-arginine. This mixture enabled tracing of absorbed arginine-N but avoided the problem of growth inhibition of L-arginine that occurs at higher rates [29]. $^{15}$N-values of roots and shoots from plants grown on the 3 mM NO$_3^-+30$ μM arginine mixture were (average ± SE) 5.2±0.08 and 2.7±0.08 atom % respectively. Thus, N derived from L-arginine uptake was more than twice as abundant in roots as in shoots, suggesting N derived from uptake of arginine displays a similar pattern of preferential allocation to roots as does glutamine.

In the third experiment, we aimed at studying the movement of absorbed glutamine-N and NO$_3^-N$ between plant parts. We therefore established a split-root system and used this for two different trials. In the first trial, roots on both sides of a rib were supplied with identical mixtures of glutamine and NO$_3^-$, but one of these N sources was $^{15}$N-labelled on just one side of the rib. This experiment tested if the overrepresentation of glutamine-N in roots (Fig. 2b) was primarily a result of it being incorporated at the site of uptake or if absorbed glutamine-N was primarily allocated to growth of roots, irrespective of the site of absorption. Overrep-
respectively while root N on the glutamine side was to 87% for glutamine and NO3 fraction of root N residing at the site of uptake was 57% and 21% was higher than that of labelled glutamine (38%; Fig. 3). The from NO3 shoots. A clear difference was observed in the partitioning of N sources: only 6% and 4% (Fig. 3).

In the second split-root trial, the two root compartments contained different N sources; NO3 on one side and glutamine on the other. This enabled assessment of how different N sources are allocated between the two parts of the root system and to the shoot. A clear difference was observed in the partitioning of N from NO3 and from glutamine by the plant (Fig. 4). Thus, shoot N was to 58% and 33% derived from NO3 and glutamine respectively while root N on the glutamine side was to 87% derived from glutamine and 9% from NO3. Root N on the NO3 side was to 70% derived from NO3 and to 25% from glutamine. Thus, N derived from glutamine was less abundant in shoots but more abundant in roots and also showed a greater tendency for translocation to parts of the root system that grew in a NO3 medium (Fig. 4).

### Discussion

Numerous studies have shown how soil N availability affects plant biomass partitioning [3–5, 8–10]. These reports have, however, largely neglected any potential role of the chemical species of available N in the soil but see [6]. We hypothesised that plant biomass partitioning is linked to the actual source(s) of N available for uptake. The rationale underpinning this hypothesis is that plants growing on soils where N is mainly present in the form of ammonium and/or organic forms should at similar rates of N supply should exhibit similar patterns of biomass partitioning [8]. The interdependency of internal N status, relative growth rate and biomass partitioning has been reported [9]. Thus, comparisons of biomass partitioning are only valid for plants displaying similar growth rates and internal N status. In the current study, plant N was not significantly different availabilities, theoretically require a larger root (or mycorrhizal) surface area for N acquisition compared to when a fraction of soil N is present in the form of NO3 simply because of the difference in mobility displayed by different N sources [24]. Our results show that inclusion of an amino acid in the growth medium resulted in higher (Table 1) total biomass accumulation and that biomass partitioning to roots in all cases was significantly enhanced (Fig. 1). This result is clearly in contradiction to the general idea that plants at similar rates of N supply should exhibit similar patterns of biomass partitioning [8].

### Table 1. Biomass (mg plant^−1), N concentrations (mg g^−1) and root mass fraction of plants grown in sterile agar culture and with N supplied as either a mixture of 1.5 mM glutamine and 3 mM NO3 or 3 mM NH4NO3.

| N-source   | Root biomass | Root N conc | Shoot biomass | Shoot N conc | Total biomass | Root mass fraction |
|------------|--------------|-------------|---------------|--------------|---------------|--------------------|
| Gln-NO3   | 1.2±0.1 (a)  | 49±2 (a)    | 5.1±0.3 (a)   | 57±2 (a)     | 6.3±0.3 (a)   | 0.19±0.0 (a)       |
| NH4NO3    | 0.8±0.05 (b) | 48±1 (a)    | 4.7±0.1 (a)   | 59±1 (a)     | 5.5±0.1 (b)   | 0.14±0.0 (b)       |

Average values ± SE, n = 5. Different letters indicate differences at p<0.05 between N treatments.

[doi:10.1371/journal.pone.0019211.t001]

### Table 2. 15N of Arabidopsis roots and shoots of plants grown in sterile agar culture and with N supplied as either a mixture of 1.5 mM glutamine and 3 mM NO3 or 3 mM NH4NO3.

| N-treatment   | Root δ15N | Shoot δ15N |
|---------------|-----------|------------|
| 15Gln-NO3     | 1034±53 (a, A) | 639±40 (a, B) |
| Gln-15NO3     | 657±53 (b, A)  | 1297±32 (b, B) |
| 15NH4NO3      | 1526±54 (c, A)  | 1351±28 (b, B) |
| NH4-15NO3     | 1066±56 (a, A)  | 1305±21 (b, B) |

In these mixtures, one of the added N sources was labelled with 15N at a rate of 1 atom %. Average values ± SE, n = 5. Different lower-case and capital letters indicate differences at p<0.05 between N treatments, and between plant parts, respectively.

[doi:10.1371/journal.pone.0019211.t002]

**Figure 2.** Origin of root N, shoot N and plant N, in Arabidopsis thaliana plants grown on 3 mM NH4NO3 (a) or a mixture of 1.5 mM glutamine+3 mM NO3 (b). Fractions of N derived from individual N sources in the mixtures were calculated from N contents and rates of 15N abundance in plant parts. Plants were grown on sterile agar plates for 21 days. Bars represent average values ± SE, n = 5. Different lower-case and capital letters indicate differences at p<0.05 between plant parts and between N sources, respectively. [doi:10.1371/journal.pone.0019211.g002]
Figure 3. Split-root experiment with Arabidopsis thaliana. Plants were grown on agar plates that were divided into two identical compartments by a plastic rib. The growth medium was identical on both sides of the rib and with N supplied as a mixture of 1.5 mM glutamine+3 mM NO₃⁻ but on one side, one of the N sources (either glutamine or NO₃⁻) was ¹⁵N-labelled. Bars indicate the fraction of N derived from each source and represent average ± SE, n = 6–7. Different lower-case and capital letters indicate differences at p≤0.05 between plants parts and between N sources, respectively. doi:10.1371/journal.pone.0019211.g003

Figure 4. Split-root experiment with Arabidopsis thaliana. Plants were grown on agar plates that were divided into two identical compartments by a plastic rib. The two compartments contained either 1.5 mM glutamine or 3 mM NO₃⁻ as N sources. For each plate, one of the N sources (either glutamine or NO₃⁻) was ¹⁵N-labelled. Bars indicate the fraction of N derived from each source for the shoot and for roots growing in the NO₃⁻ compartment and the glutamine compartment. Bars represent average ± SE, n = 5. Different lower-case and capital letters indicate differences at p≤0.05 between plant parts, and between N sources, respectively. doi:10.1371/journal.pone.0019211.g004

for plants grown on different N source mixtures (Table 1) and thus, we are able to compare how individual N forms affect biomass partitioning of plants. Earlier studies of the effects of different N forms on biomass partitioning have mostly compared NH₄⁺ and NO₃⁻ [6] and references therein. However, root growth is often inhibited by high concentrations of NH₄⁺ but not of NO₃⁻ [6], which complicates comparison between these N sources. Some earlier studies have also investigated the effects of amino acids on biomass partitioning. In a study of Catasatum fimbriatum (Orchidaceae), glutamine was included as a N source [30] but in that study, plants supplied with glutamine grew nearly twice as fast as those with the other tested N sources (urea, NH₄⁺ and NO₃⁻), precluding direct comparison of how biomass partitioning was affected by N source. Comparisons of the effects of glutamine, NH₄⁺ and NO₃⁻ on biomass partitioning have also been made for Phaseolus vulgaris, but no significant differences were detected [31,32]. However, in all these studies, plants were not grown in sterile culture and thus the actual contribution of glutamine to plant N uptake was unknown. In a recent article, Paungfoo-Lonhienne et al. [33] showed that Arabidopsis and Habia adicis can use protein as a source of N. Interestingly, they reported that root, but not shoot growth was stimulated when protein was supplied as the sole N source to plants (cf. Fig. 1 of [33]).

We also hypothesized that the distribution of absorbed N between shoots and roots would differ for different N compounds. We therefore employed a stable isotope labelling approach to trace the fate of different N sources. For plants grown on mixtures of NH₄⁺ and NO₃⁻, a slight over-representation of N derived from NH₄⁺ was detected in roots (Fig. 2a). However, for plants grown on mixtures of glutamine and NO₃⁻, we found a significant over-representation of N derived from the organic source in roots: as much as 76% of root N was derived from absorbed organic N (Fig. 2b). Thus, the increased root mass fraction of plants supplied with glutamine in the growth media was paralleled by a large share of root N derived from the uptake of glutamine. Results of tests with Arabidopsis grown on 3 mM NO₃⁻ and supplied with 30 mM ¹⁵N [96–98 atom %] labelled arginine showed over-representation of N derived from arginine in roots (5.2 and 2.7 atom % excess for roots and shoots, respectively). This supports the hypothesis that N derived from uptake of organic sources may be over-represented in roots.

The primary site of assimilation differs for NO₃⁻ and glutamine, so that a significant share of absorbed NO₃⁻ may be directly transported to the shoot [31], while absorbed amino acids may be preferentially metabolised in roots [27]. An over-representation of N derived from glutamine in roots may, therefore, simply reflect the difference in the site of assimilation. However, several studies have reported that absorbed N may cycle between the roots and shoots through xylem and phloem transport [34,35]. Such N cycling is probably an important trait in plant plasticity and may, for example, enable roots to grow through patches of soil with low N availabilities [2,36] and enable N partitioning to be controlled by developmental cues [37,38]. Nevertheless, our results (Fig. 2–4) suggest a significant fraction of absorbed amino acid N resides, and is incorporated, at the site of primary assimilation. This would lead to the observed over-representation of N derived from glutamine in roots and the concomitant overrepresentation of NO₃⁻-N in shoots. Over-representation of N derived from glutamine in roots may also be related to the energetic differences between the two N sources (NO₃⁻ and glutamine). If root growth was limited by carbohydrate supply, utilization of glutamine as a N source for the growing root would lead to appreciable energy savings [32,37,39]. However, Zerihun et al. [32] suggested that the importance of differences in energy requirements for utilisation of various N forms was negligible, in comparison with the costs of protein turnover. Nevertheless, this does not preclude energy savings for specific cell types or tissues, e.g. in root meristems. This is supported by studies that show a strong effect of sucrose added to growth media on root elongation rates, in particular for plants growing under low light conditions [40]. Following absorption by roots, inorganic N is assimilated into glutamine. High glutamine concentrations either resulting from high rates of synthesis or from uptake from the root medium are known to stimulate expression of the enzyme PEP-carboxylase, possibly as a means to supply 2-oxo acids, drawn from the TCA-cycle through amino acid synthesis [41]. Thus, in
our experiment, PEP-carboxylase activities may be expected to be up-regulated in response to uptake of N, irrespective of in which form this N was absorbed but the input of C via uptake of glutamine would eventually counteract the depletion of oxo-acids in root cells.

The hypotheses mentioned above relate to the association between glutamine uptake and C and N use for root growth. An alternative to this model is that absorbed glutamine-N preferentially targets the growth of roots, irrespective of site of uptake. We tested this possibility in two split-root trials, the first aiming at studying patterns of N partitioning between roots growing in identical N environments, the second assessing N partitioning between roots growing on different N sources. A relatively low rate of labelling of roots in the non-labelled compartment was found for both 15NO3

-4% of root N) and 15N-glutamine (6% of root N; Fig. 3) when roots were growing in identical N environments. This suggests that translocation of N from either N source from one side of the root system to the other is relatively small. The large accumulation of translocation of N from either N source from one side of the root system to the other is relatively small. The large accumulation of translocation of N from either N source, glutamine on one side and NO3

- over to the NO3

- side while the movement of 15NO3

- over to the glutamine side was small. Thus, root-N on the NO3

- side was to 25% derived from uptake of glutamine while root-N on the glutamine side was to only 9% derived from uptake of NO3

- . Recalculated, a breakdown of total 15N label of plants supplied 15NO3

- showed that 84%, 15% and 1% of detected 15N excess was found in shoots, roots growing on the 15NO3

- side and roots growing on the glutamine side respectively. Corresponding figures for plants supplied 15N-glutamine were 72%, 20% and 8%, clearly showing the smaller contribution of glutamine-N to shoot N and the larger contribution to root N. Thus, the use of the split-root system allowed us to verify N fluxes in planta and the preferences of specific organs for the N sources supplied. A clear difference was observed in the use of nitrate and glutamine by plants, as both sources were not equally distributed in the organs. Thus, these two trials corroborate that N absorbed in organic form is to a larger extent used for growth of roots than of shoots compared to N absorbed as NO3

- . They also show that translocation of N from different parts may be substantial from roots absorbing organic N to roots absorbing NO3

- .

Our data thus show that absorbed organic N is preferentially used for root growth and that partitioning of biomass to roots is enhanced in the presence of organic N in the root medium. What remains to be explained is if, and how the two are connected, i.e. if and how the preferential allocation of absorbed organic N to root growth promotes an increase in root biomass. We have speculated that organic N would entail significant savings in terms of C for roots compared to that of inorganic N [31] and that this would enable a higher rate of root growth, all other things being equal. Such a mechanism would imply that the rate of root growth is at least partially a function of local soil conditions [2] and/or that the C:N status of roots is part of a signalling network that regulates biomass partitioning in plants [7].

Nitrogen availability exerts strong control of plant biomass partitioning and this response has been interpreted as plants maximising resource capture through allocating resources to the tissue in which the limiting resource is acquired [3]. More recent studies [42,43] and reviews [7] have explored the mechanisms by which biomass partitioning is tuned by N availability. Using mutants with impaired capacity for NO3

-reduction, Scheible et al. [44] showed that NO3

- concentrations of leaves exerted a strong impact on biomass partitioning, as well as on carbohydrate metabolism. However, the proposed role of NO3

- as a signal for shoot-root partitioning has been challenged [43], partly on the basis that partitioning responses to shifts in N supply are similar for both NO3

- and NH4

+. Experiments in which root systems are exposed to a spatially heterogeneous supply of N using split-root set-ups or with localised supply of N [36,37,43], show that root growth is stimulated in areas of high N. Zhang & Forde [46] described how localised supply of NO3

- stimulated initiation and growth of lateral roots. Thus, root growth is dependent on internal N status of plants but is also, to some degree, directly affected by the spatial distribution of soil N.

Different N forms exhibit highly variable diffusion coefficients (D), Owen & Jones [24] estimated D, for some different N forms in agricultural soils; values for NO3

-, NH4

+ and glucose were 0.3, 0.02 and 0.08 cm2 d−1, respectively. If N movement toward root surfaces is mainly through diffusion, these differences imply that plants need a smaller root surface area to acquire the same N uptake with NO3

- as a source compared with NH4

+ and/or organic N [47]. Movement of N towards root surfaces may also occur via mass flow induced by transpiration. This mechanism would be especially important for NO3

- acquisition [48,49], but less for less mobile ions, such as phosphate [50]. Thus, optimisation of the acquisition of mobile ions such as NO3

- could be functionally linked to preferential partitioning of biomass growth to above-ground tissues, while the opposite holds true for less mobile ions, including organic N compounds. There are also other potential links between NO3

- utilisation and shoot growth: reduction of NO3

- in the shoot is functionally linked to photosynthesis and may hence be assimilated and used for growth in above-ground tissues.

Under natural conditions, soil solution N concentrations co-vary with the chemical composition of soluble N. Hence; low N availabilities usually correspond to a large share of organic N in the soil solution while at increasing soil solution N concentrations, increasing shares of inorganic N and in particular NO3

- are found. Earlier studies have shown that plants are capable of competing with microbes for organic N substrates also under field conditions. Although the extent to which organic N is a significant N source for plants is still a matter of controversy, it is clear that plants do access such N forms in the field when available. A potential role of organic N in promoting root growth of plants under field conditions cannot, therefore, be dismissed. From the data presented in this study, we may speculate that the high values of root mass fraction of plants growing on poor soils may, to some extent result from an abundance of organic N in such soils while the gradual increase in above-ground biomass of plants inhabiting rich soils may to some extent be promoted by higher rates of NO3

- availabilities on these sites. Unravelling the dependence of plant biomass partitioning on the abundance of organic and inorganic N sources under natural conditions will be a challenge for future studies.

Author Contributions

Conceived and designed the experiments: TN HS CC. Performed the experiments: HS LG CC. Analyzed the data: HS TN LG CC. Wrote the paper: TN CC UG AN HS LG.
References

1. Marschner H (1995) Mineral Nutrition of Higher Plants (2nd edn). Academic Press.
2. Hodge A (2009) Root decisions. Plant Cell Environ 32: 629–640.
3. Brouwer R (1962) Nutritive influences on the distribution of dry matter in the plant. Neth J Agr Sci 10: 399–408.
4. Chapin FS, III (1980) The mineral nutrition of wild plants. Ann Rev Ecol Syst 11: 253–260.
5. Levin SA, Mooney HA, Field C (1989) The dependence of plant root:shoot ratios on internal nitrogen concentration. Ann Bot 64: 71–75.
6. Sattelmacher B, Gerendas J, Thoms K, Bruck H, Bagdady NH (1993) Interaction between root growth and mineral nutrition. Environ Exp Bot 33: 63–73.
7. Hermans C, Hammond JP, White PJ, Verbruggen N (2006) How do plants respond to nutrient shortage by biomass allocation? TIPS 11: 610–617.
8. Ågren GI, Ingestad T (1987) Root:shoot ratio as a balance between nitrogen productivity and photosynthesis. Plant Cell Environ 10: 579–596.
9. Ingestad T, Ågren GI (1991) The influence of plant nutrition on biomass allocation. Ecol Appl 1: 168–174.
10. Tan W, Hogan GD (1990) Dry weight and N partitioning in relation to substrate N supply, internal N status and developmental stage in Jack Pine (Pinus banksiana Lamb.) seedlings: implications for modeling. Ann Bot 81: 195–201.
11. Shaver GR, Chapin FS, III (1991) Production: Biomass relationships and element cycling in contrasting arctic vegetation types. Ecol Monog 61: 1–31.
12. Tilman D, Wedin D (1991) Plant traits and resource reduction for five grasses growing on a nitrogen gradient. Ecology 72: 695–700.
13. Nasholm T, Kielland K, Ganeteg U (2009) Uptake of organic nitrogen by plants. New Physiol 102: 31–40.
14. Kieland K (1994) Amino-acid-absorption by arctic plants - implications for plant nutrition and nitrogen cycling. Ecology 75: 2373–2383.
15. Kieland K (1995) Landscape patterns of free amino acids in arctic tundra soils. Biogeochem 31: 85–98.
16. Kieland K, McFarland J, Olson K (2006) Amino acid uptake in deciduous and coniferous taiga ecosystems. Plant & Soil 288: 297–307.
17. Nordin A, Hogberg P, Nasholm T (2001) Soil nitrogen form and plant nitrogen uptake along a boreal forest productivity gradient. Oecologia 129: 125–132.
18. Nordin A, Schmidt IK, Shaver GR (2004) Nitrogen uptake by arctic soil microbes and plants in relation to soil nitrogen supply. Ecology 85: 955–962.
19. Lipson DA, Schmidt SK, Monson RK (1999) Links between microbial populations dynamics and nitrogen availability in an alpine ecosystem. Ecology 80: 1623–1631.
20. Raab TK, Lipson DA, Monson RK (1996) Non-mycorrhizal uptake of amino acids by roots of the alpine sedges Kobresia myosuroides: Implications for the alpine nitrogen cycle. Oecologia 108: 489–494.
21. Berthrong ST, Finzi AC (2006) Amino acid cycling in three cold-temperate coniferous taiga ecosystems. Plant & Soil 288: 297–307.
22. Schmidt SK, Stewart GR (1999) Glycine metabolism by plant roots and its occurrence in Australian plant communities. Aust J Pl Phys 26: 251–264.
23. Warren CR (2006) Potential organic and inorganic N uptake by six Eucalyptus species. Funct Plant Biol 33: 653–660.
24. Owen AG, Jones DL (2001) Competition for amino acids between wheat roots and the rhizosphere microorganisms and the role of amino acids in plant acquisition. Soil Biol Biochem 33: 651–657.
25. Jamtgård S, Nasholm T, Huss-Danell K (2008) Uptake of organic nitrogen by Barley. Plant & Soil 302: 221–231.
26. Tinker PB, Nye PH (2008) Solute Movement in the Rhizosphere. Oxford: Univ. Press.
27. Persson J, Gardstrom P, Nasholm T (2006) Uptake, metabolism and distribution of organic and inorganic nitrogen sources by Plantago media. J Exp Bot 57: 2653–2659.
28. Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15: 473–497.
29. Forum O, Svennerstam H, Ganeteg U, Nasholm T (2008) Capacities and constraints of amino acid utilization in Arabidopsis. New Phytol 179: 1056–1069.
30. Majewska NA, Hatcher PS, III (2002) Effects of nitrogen forms on dry matter partitioning and ad nitrogen metabolism in two contrasting genotypes of Cirsium arvense (Asteraceae). Env Exp Bot 47: 249–258.
31. Andrews M (1986) The partitioning of nitrate assimilation between root and shoot of higher plants. Plant Cell Environ 9: 511–519.
32. Zerahn A, McKenzie BA, Morton JD (1998) Photosynthesize costs associated with the utilization of different nitrogen-forms: influence on the carbon balance of plants and shoot-root biomass partitioning. New Phytol 138: 1–11.
33. Paungfoo-Lonhienne C, Lonhienne TGA, Rentsch D, Robinson N, Christie M, et al. (2000) Plants can use protein as a nitrogen source without assistance from other organisms. Proc Natl Acad Sci U S A 105: 4524–4529.
34. Cooper HD, Clarkson DT (1989) Cycling of amino nitrogen and other nutrients between shoots and roots in cereals: a possible mechanism integrating shoot and root in the regulation of nutrient uptake. J Exp Bot 40: 753–762.
35. Larsson CM, Larsson M, Purves JV, Clarkson DT (1991) Translocation and cycling through roots of recently absorbed nitrogen and sulfur in wheat (Triticum aestivum) during vegetative and generative growth. Physiol Plant 82: 343–362.
36. Robinson D (1994) The response of plants to non-uniform supplies of nutrients. New Phytol 127: 635–674.
37. Walch-Liu P, Filleur S, Gan Y, Forde BG (2003) Signaling mechanisms integrating root and shoot responses to changes in the nitrogen supply. Photosynth Res 83: 239–250.
38. Walch-Liu P, Liu L-H, Remans T, Tester M, Forde BG (2006) Evidence that L-Glutamate can act as an exogenous signal to modulate root growth and branching in Arabidopsis thaliana. Plant Cell Physiol 47: 1043–1057.
39. Bloom AJ, Meyerhoff PA, Taylor AR, Root TL (2003) Root development and the absorption of ammonium and nitrate from the rhizosphere. J Plant Growth Regul 21: 416–431.
40. Frexex S, Thalabard MC, Taride F, Muller B (2002) Root elongation and branching is related to local hexose concentration in Arabidopsis thaliana seedlings. Plant Cell Environ 25: 1357–1366.
41. Britto DV, Kronzucker HJ (2005) Nitrogen acquisition, PEP carboxylase, and cellular pH homeostasis: new views on old paradigms. Plant Cell Environ 28: 1396–1409.
42. Stum M, Feil R (1999) Lateral root frequency decreases when nitrate accumulates in tobacco transformants with low nitrate reductase activity: consequences for the regulation of biomass partitioning between shoots and root. Plant & Soil 215: 143–153.
43. Andrews M, Raven JA, Lea P, Sprent JI (2006) A role for shoot protein in shoot root dry matter allocation in higher plants. Ann Bot 97: 3–10.
44. Schible W-R, Lauerer M, Schulze E-D, Calocho M, Stitt M (1997) Accumulation of nitrate in the shoot acts as a signal to regulate shoot-root allocation in tobacco. Plant J 13: 671–691.
45. Drew MC, Saker LR (1975) Nutrient supply and the growth of the seminal root system in barley. II. Localized compensatory increases in lateral root growth and rates of nitrate uptake when nitrate supply is restricted to only part of the root system. J Exp Bot 26: 79–90.
46. Zhang H, Forde BG (1998) An Arabidopsis MADS box gene that controls nutrient-induced changes in root architecture. Science 279: 407–409.
47. Raven JA, Wolfeübwer B, Handley L (1992) A comparison of ammonium and nitrate as nitrogen sources for photolithoautotrophs. New Phytol 121: 19–32.
48. Cramer MD, Hawkins H-J, Verboom GA (2009) The importance of nutritional regulation of plant water flux. Oecologia 161: 15–24.
49. Gorska A, Holfbrook NM, Ye Q, Zwieniecki MA (2008) Nitrate control of root hydraulic properties in plants: translating local information to whole plant response. Plant Physiol 148: 1159–1167.
50. Yanai RD (1994) A steady-state model of nutrient uptake accounting for newly grown roots. Soil Sci Soc Am J 58: 1562–1571.