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ORIGINAL PAPER

Significance of organic nitrogen acquisition for dominant plant species in an alpine meadow on the Tibet plateau, China

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Abstract Though the potential of plants to take up organic N (e.g., amino acids) is well established, the true significance of organic N acquisition to plant N nutrition has not yet been quantified under field conditions. Here we demonstrate that organic N contributes significantly to the annual N uptake of three dominant plant species (*Kobresia humilis*, *Saussurea superba* and *Stipa aliena*) of alpine meadows on the Tibet Plateau, China. This was achieved by using double-labelled (^{14}C and ^{15}N) algae as a source for slow and continuous release of amino acids, and tracing both labels in the above- and below-ground plant biomass. Four months after addition of algae, between 0.5% and 2.6% of ^{14}C and 5% and 14% of ^{15}N from added algae were recovered in the plants, which translate into an uptake of organic N between 0.3 mg N m^{-2} and 1.5 mg N m^{-2}. The calculated contribution of organic N to total N uptake was estimated to range between 21% and 35% for *K. humilis*, and between 13% and 21% for *S. aliena* and *S. superba*, respectively, implying that organic N uptake by grassland plants is quantitatively significant under field conditions in the studied alpine meadows. This finding has important ecological implications with regard to competition for organic N between microorganisms and plant roots.

Keywords Organic N uptake · ^{14}C · ^{15}N · Alpine grassland · N acquisition · *Spirulina*

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Introduction

The classical paradigm of the terrestrial N cycle, that organic N is mineralized into inorganic N (NO_3^- + NH_4^+) by soil microorganisms and only thereafter becomes available for plants, has been challenged recently (Schimel and Bennett 2004). Many plants in cold environments such as arctic (Chapin et al. 1993; Kielland 1994; Schimel and Chapin 1996), boreal (Näsholm et al. 1998;

Näsholm and Persson 2001) and alpine ecosystems (Lipson et al. 2001; Raab et al. 1996, 1999; Miller and Bowman 2003; Xu et al. 2004) showed the capacities to take up organic N from soils in the form of low molecular-weight substances, mainly amino acids. Laboratory studies using Michaelis–Menten kinetics have estimated that amino acids may contribute 60% and between 10% and 82% to the annual N acquisition of *E. vaginatum* (Chapin et al. 1993) and of 10 arctic tundra species (Kielland 1994), respectively, but the ecological significance of organic N to the total N uptake by plants has not been quantified under field conditions (Näsholm and Persson 2001; Merilä et al. 2002; Jones et al. 2004, 2005).

The lack of corresponding data in the field mainly results from the following problems: First, most studies in this respect have been conducted under artificial conditions (nutrient solution as growth medium), where the intact root environment with its physical, chemical and biological complexity has been strongly narrowed. Experiments that directly apply amino acid solutions instead of a soil environment neglect the importance of microorganisms as competitors for these substances and as main decomposers. It has been shown that the results obtained under such conditions are distinctly different from those obtained in the field (Jones et al. 2005) and hence cannot reveal the ecological significance of organic N acquisition. Second, classic ^{15}N-tracer studies alone, not in combination with ^{14}C or ^{13}C labels, cannot separate the uptake of intact amino acids from uptake of mineralized N. Recently, the acquisition of organic N as intact amino acids by plants has been demonstrated using dual-labelled (^{13}C and ^{15}N) amino acids (Näsholm and Persson 2001; Miller and Cramer 2004; Jones et al. 2005). In this regard, however, there still exists an obvious shortcoming that the amino acids were injected into the soil only once and in the form of aqueous solutions of one amino acid (Näsholm et al. 2000; Nordin et al. 2001; Taylor et al. 2004; Weigelt et al. 2005) or a mixture of several amino acids (Chapin et al. 1993; Persson and Näsholm 2003) while in soils free amino acids are continuously produced by microbial decomposition as a mixture of amino acids and therefore continuously contribute to plant organic N acquisition. Single injections of amino acids into soils in short-term studies are problematic because of fast decomposition and immobilization of free amino acids in soils (Schmidt et al. 1960; Kassim et al. 1981; Jones 1999; Jones and Kielland 2002).

Previous studies demonstrated that a huge amount of organic N is stored in soils in alpine meadows on the Qinghai–Tibet Plateau (Cao et al. 1999), and that plants of these ecosystems have the capacity to take up organic N from soils (Xu et al. 2004). This study therefore aimed at elucidating the contribution of organic nitrogen to total plant N uptake in alpine meadows. We used a double-labelled (^{14}C and ^{15}N) and protein-enriched algal preparation. Compared to direct application of amino acids, slow microbial decomposition of added algae continuously releases to the soil the full range of ^{15}N and ^{14}C labeled amino acids. The ratio of individual amino acids is similar to that produced by the decomposition of soil organic matter, and hence allows assessing the relative contribution of organic N and mineralized N to total plant uptake during a full vegetation period in the field.

Materials and methods

Study site

The experiment was conducted at the Haibei Alpine Meadow Ecosystem Station of the Chinese Academy of Sciences, Qinghai Province (37°36′60″ N, 101°19′14″ E, 3215 m asl). The experimental station has a typical alpine meadow climate. Annual temperature and annual precipitation averages −1.7°C and 600 mm, respectively. Dominant species are *Kobresia humilis* Serg., *Stipa aliena* Keng., *Poa* sp., *Festuca ovina* Linn., *Gentiana aristata* Maxim, *Gentiana straminea* Maxim., *Saussurea superba* Anth., and *Gueldenstaedtia diversifolia* Maxim. (Zhou 2001). The total plot area used for this study was 15 m × 15 m. The soil is classified as Mat Cryogelic Cambisol (Chinese Soil Taxonomy Research Group 1995) corresponding to Gelic Cambisol (WRB 1998), some characteristics of which were shown in Table 1.

Table 1 Characteristics of the upper 10 cm of soils at the study site, Haibei, Tibet Plateau. Data (means ± SE) are shown (n = 6–8)

pH	8.0 ± 0.1
Bulk density (g cm^{-3})	0.70 ± 0.05
C:N ratio	19.6 ± 0.3
SOC (kg m^{-2})	11.8 ± 0.3
Total soil N (kg m^{-2})	0.60 ± 0.04
Microbial biomass N (g m^{-2})	6.5 ± 0.3
DON (g m^{-2})	1.8 ± 0.1
Extractable inorganic N (g m^{-2})	1.4 ± 0.4

Preparation of dual-labelled algae

Spirulina was cultured in Zarouk's medium (Zarouk 1966) containing 0.5 g l^{-1} $NaNO_3$ and 0.25 g l^{-1} $CO(NH_2)_2$ at pH 8.5 in a culture vessel (glass box, 80 × 40 × 30 cm), that was continuously aerated by a small rotary air pump. When algae attained maximal growth rate, ^{15}N tracers (0.3 g $(^{15}NH_4)_2SO_4$, 98.27 atom% ^{15}N; 0.5 g $CO(^{15}NH_2)_2$, 98.37 atom% ^{15}N, Chemical Research Institute, Shanghai, China) were added to the medium. At the same time, the culture vessel was transferred to a closed chamber for ^{14}C labelling. A label of 10 mCi $Ba^{14}CO_3$ was put into a 500 ml glass flask and the flask was sealed. The flask was connected with two tubes: one was put into the medium, and the other was connected to a peristaltic pump. The flask was inserted into the chamber and the chamber was closed. Five millilitre of 0.5% H_2SO_4 was added into the flask through a Teflon tube and after starting the pump evolution of $^{14}CO_2$ was allowed. Algae were allowed to assimilate ^{14}C for 8 h per day under a light intensity of 800 μmol m^{-2} s^{-1} (PAR). In total ^{14}C labelling took place 5 times (each time 10 mCi) during the period of 5 days. The algae were then harvested, rinsed with distilled water, washed in an excess of 0.5 mmol l^{-1} $CaCl_2$ solution for 30 min, and again rinsed with distilled water to remove ^{14}C and ^{15}N tracers absorbed on their surface. Algae were then air-dried and ground to a fine powder using a ball mill (MM2, Fa Retsch). By this procedure we obtained ^{14}C and ^{15}N labelled algae with 22.60 μCi g^{-1} DW ^{14}C activity and 3.00 atom% ^{15}N. The C and N content of the algae was 51.6% and 8.5% dry weight, respectively, with a C/N ratio of 6.1.

Experimental design and treatments

Ten circular collars with 10 cm in diameter were inserted into the soil so that all the three target plant species (*K. humilis*, *S. aliena* and *S. superba*) were present within each collar. On 16 May 2004, each 0.4 g algae powder was carefully injected as a water suspension into the soil of six plots at a depth of 5 cm using the method described by Schimel and Chapin (1996) for injecting amino acid solution. Four plots were used as control, by injecting the same amount of water. N and C inputs corresponded to 54 μg N g^{-1} soil and 328 μg C g^{-1}.

Sampling and analyses

On 10 September 2004, plants and soil within the collars were completely collected up to 10 cm depth since over 80% of the roots were concentrated within this horizon (Wang and Shi 2001) and immediately transferred to the laboratory. Roots were carefully separated from soil cores so that ''intact'' plant individuals were collected. Plants were classified to species level, rinsed shortly with water, following by 0.5 mmol l^{-1} $CaCl_2$ solution for 30 min, and rinsed again with distilled water. Plant material was dried at 60°C for 48 h, weighed for total dry biomass and ground for measurement of total C and N content, ^{14}C activities, and $^{15}N/^{14}N$ ratios. Soil samples were sieved to 2 mm to remove coarse fragments for measuring pH, microbial biomass N and dissolved organic N (DON) as well as exchangeable inorganic N. Dried subsamples were ground to a fine powder and after removal of carbonates by diluted HCl used to measure soil organic C (SOC) and total N.

Soil pH values were measured using a glass electrode using a 1:2 soil-to-water ratio. Total N was measured by Kjeldahl digestion with a salicylic acid modification (Pruden et al. 1985), and SOC was measured following the method described by Kalembasa and Jenkinson (1973). Microbial biomass N was estimated by a chloroform fumigation-direct extraction technique (Brookes et al. 1985; Davidson et al. 1989). Total N in 0.5 M K_2SO_4 extracts (1:4 soil:extractant) was also determined by Kjeldahl digestion of a

salicylic acid modification (Pruden et al. 1985), whereas NH_4^+-N and NO_3^--N were measured by steam distillation with MgO, using Devarda's alloy to reduce NO_3 to NH_4 (Bremner 1965). DON was calculated as the difference between total N and exchangeable inorganic N in extracts.

To measure ^{14}C activity in plant samples, 100 mg were combusted in an oxidizer (Model 307, Canberra Packard Ltd., USA) and released $^{14}CO_2$ was trapped in CARBO-SORB E (Perkin Elmer Inc., USA) and the scintillation cocktail Permafluor E^+ (Perkin Elmer Inc., USA). ^{14}C activities in these samples were measured by liquid scintillation counting (Rackbeta, 1419 LKB). Aliquots of plant material (2 mg) were weighed into tin capsules for analyzing total N, C and atom% ^{15}N by continuous-flow gas isotope ratio mass spectrometry (CF-IRMS). The CF-IRMS system consists of an elemental analyser (EA 1110, CE Instruments, Milan, Italy), a ConFlo II device (Finnigan MAT, Bremen, Germany) and a gas isotope ratio mass spectrometer (Delta^{PLUS}, Finnigan MAT).

Calculations and statistics

Atom% ^{15}N was calculated as the following:

$$\text{at\%}^{15}\text{N}[\%] = {}^{15}\text{N} \div ({}^{14}\text{N} + {}^{15}\text{N}) \times 100 \quad (1)$$

Atom% excess ^{15}N (APE) was calculated as the atom% ^{15}N difference between plants from algae treated and from control plots.

$$\text{APE}[\%] = \text{at\%}^{15}\text{N}_{\text{algae-treated}} - \text{at\%}^{15}\text{N}_{\text{control}} \quad (2)$$

^{15}N recovery (F_{15N}) and ^{14}C recovery (F_{14C}) were calculated as the amount of ^{15}N and ^{14}C taken up by per plant species of per collar divided by the amount injected into the collars.

$$F_{15N}[\%\ \text{added}] = {}^{15}\text{N}_{\text{uptake}} \div {}^{15}\text{N}_{\text{added}} \times 100; \quad (3)$$

$$F_{14C}[\%\ \text{added}] = {}^{14}\text{C}_{\text{uptake}} \div {}^{14}\text{C}_{\text{added}} \times 100 \quad (4)$$

The fraction of crude protein was estimated by multiplying the N content of algae by a conversion factor of 6.25 (Maynard and Loosli 1969).

The amount of C uptake by each plant species from the organic N source derived from the added algae was calculated using added activity ($Alg_{activity}$, DPM) and C content (C_{added}, mg C m^{-2}) of algae and the total activity ($S_{activity}$, DPM) of each sample on a dry weight basis per collar (Wallenda and Read 1999).

$$C_{uptake}[\text{mg C m}^{-2}] = (S_{activity} \div Alg_{activity}) \times C_{added} \quad (5)$$

The amount of N uptake by plants derived from the added algae was estimated using the 15N recovery (F_{15N}, %) and N content (N_{added}, mg N m^{-2}) of added algae

$$N_{uptake}[\text{mg N m}^{-2}] = F_{15N} \times N_{added} \quad (6)$$

The relative contribution of organic N to plant N acquisition (N_{ctr}) was estimated by two independent methods. (A) One was based on the ratio of the F_{14C} to the F_{15N}, considering the fraction of ^{14}C lost through plant respiration

$$N_{ctr}[\%] = F_{14C} \div F_{15N} \div (1 - {}^{14}C_{lost}) \times 100 \quad (7)$$

which present the minimal portion of N taken up by plants in organic form. Several studies have indicated respiratory losses of the C taken up by plant roots (e.g., Schimel and Chapin 1996; Lipson and Näsholm 2001), but data are still scarce. More recently, Heidi-Jayne et al. (2005) found that respiratory losses of $^{13}CO_2$ were very small in a dual-labelled (^{13}C and ^{15}N) glycine study. Kuzyakov and Jones (2006) showed that utilization of glucose acquired from soil solution and transported to the shoot was used by up to 1% for shoot respiration during the initial 48 h and approximately 5% during the last 48 h after glucose injection. This implies that the respiratory losses of the C label could be related to plant species and applied substrates as well as the experimental period. Considering the ^{14}C loss via root respiration and the experimental period, here we assumed the maximal respiratory loss of the ^{14}C uptake by alpine plants was 15% in the current study (Kuzyakov et al. 2001; Kuzyakov and Jones 2006). (B) The other method was based on estimations using the C uptake by plants

derived from algae and assumed average C:N ratios of the multiple amino acids released by decomposition of added algae

$$N_{ctr} = (C_{uptake} \div 12 \times 14 \div R_{C/N}) \div N_{uptake}) \times 100 \quad (8)$$

Because plant roots can also take up N-free compounds such as soluble carbohydrates, although at a lower rate (Hendrix 1984; Kuzyakov and Jones 2006), we subtracted this part from the total C uptake by plants from the algal source. A simultaneous appearance of algal-derived C and N in plant tissues suggests that amino acids are taken up in an intact form. To this point, the ratio of absorbed C to N is proportional to that of the multiple amino acid species released by the decomposition of added algae. *Spirulina* normally contains 60–70% protein, 5–9% lipids and 25–35% carbohydrates by dry weight (Wang and Shan 2003). In our study the fraction of crude protein in *Spirulina* was 53%. The algae contained not more than 30% carbohydrates and 10% lipids. Considering that lipids normally contain about 70% and carbohydrates 40% C, the crude protein fraction comprised at least 63% of total C in algae. Here we assume that the plants have a similar capacity to take up amino acids and sugars (Kuzyakov and Jones 2006), and thus sugars contributed a maximum of 37% to total C uptake by plant roots. In other words, 63% of the C uptake by plants is protein-derived from the algae source. Therefore, considering (i) the dominant amino acids in *Spirulina* (Narasimha et al. 1982; Campanella et al. 1999), (ii) the C:N ratio of 3.8 for the crude protein fraction in the algae, and (iii) CO_2 losses by decomposition, we assumed that the average C:N ratio of algae-derived amino acids ranged between 3 and 5.

The significance of differences in ^{13}C and ^{15}N uptake as well as C and N contents between plant species was examined using one-way analysis of variance (ANOVA). Critical LSD values for 5% error probability were calculated. The standard errors of means are presented on the figures and in the table as a variability parameter.

Results

Four months after addition of dual-labelled algae APE ^{15}N and ^{14}C activities of *S. aliena* were significantly different from those of *K. humilis* and *S. superba* (Fig. 1). Among the three dominant species, the highest values of APE ^{15}N and ^{14}C activity were found in *S. superba*, and the lowest in *S. aliena* (Fig. 1A), while *K. humilis* and *S. superba* did not differ significantly (Fig. 1B).

The three dominant species showed a significant difference in ^{14}C recovery (F_{14C}), with the highest value for *K. humilis* and the lowest for *S. aliena* (Fig. 2A). Concordantly to F_{14C}, *K. humilis* also acquired more ^{15}N than the other two species, but there was no significant difference between *S. aliena* and *S. superba* (Fig. 2C). Among the three species, the ratio of F_{14C} to F_{15N} was much higher in *K. humilis* than in the other two species (Fig. 2B). This F_{14C} to F_{15N} ratio represents the minimal fraction of N taken up by plants in organic form (Fig. 2) as a part of ^{14}C taken up by plants might be lost through shoot and root respiration. Here we assumed that the maximal respiratory loss of ^{14}C taken up by plants was 15% through plant respiration (Kuzyakov et al. 2001; Kuzyakov and Jones 2006). Considering this losses we can estimate the contribution of organic N derived from decomposition of the added algal protein to F_{15N}. Based on these calculations we estimate organic N to contribute 25% to *K. humilis* N uptake and 15% each to the uptake of the other two species (Fig. 2C).

Among the three species, plant biomass at the time of sampling was remarkably higher in *K. humilis* compared to the two other species, while plant biomass of *S. aliena* and *S. superba* was similar (Fig. 3A). Based on plant biomass, N content, ^{15}N and ^{14}C uptake, we calculated C and N uptake by plant roots and estimated the contribution of organic N to plant N acquisition, which are presented in Fig. 3 and Table 2. Consistent with the high plant biomass of *K. humilis*, this species took up more algae-derived C and N than *S. aliena* and *S. superba*. Although *S. aliena* and *S. superba* had a similar final biomass, *S. superba* took up more algal-derived C and N presumably because of its higher capacity to acquire organic N compared to *S. aliena* (Fig. 3B, C).

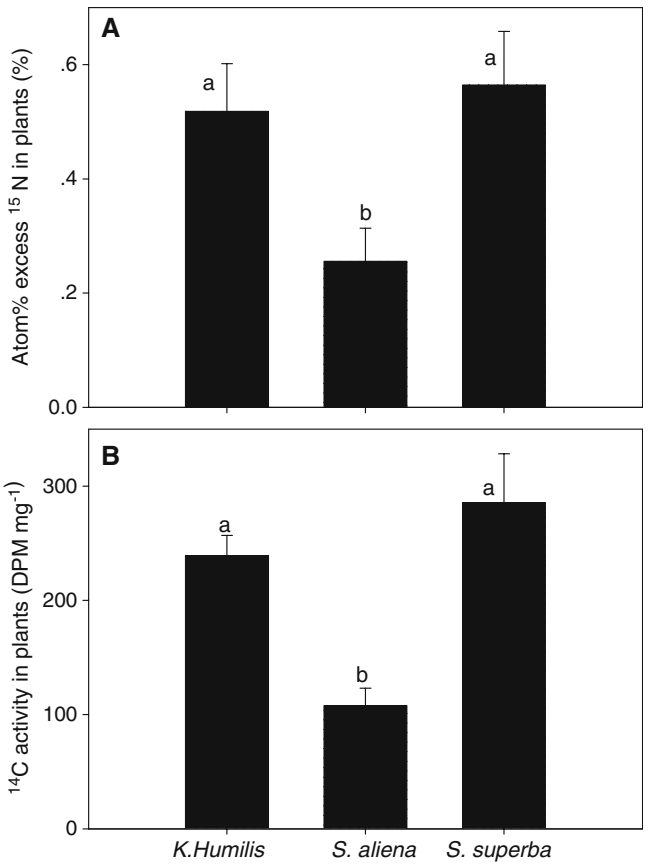

Fig. 1 Atom% excess ^{15}N and ^{14}C activity of alpine plant species 4 months after addition of double-labelled algae. Values are means (±SE) of six replicates, corrected by those in the control plots. Letters indicate significant differences at 0.05 error probability level

Discussion

This study represents the first to estimate the contribution of organic N to plant N nutrition in the medium-term (one vegetation period) and under field conditions. We used an approach, which quantified the contribution of algae-derived organic N to total plant N uptake via the ratio of F_{14C} to F_{15N} from algae, but this does not directly estimate plant uptake of organic and mineral N from soil sources. Despite this limitation, we propose that plant utilization of organic N from algae represents an approximation for uptake of organic N from soil sources, and argue that alpine plants under field conditions indeed depend on organic N sources. There is no reason to believe that the processes and pathways of depolymerization of macromolecular matter, of N mineralization and of nitrification should be different for organic matter derived from different sources, i.e. soil, litter or added algae. Whatever the source material is, amino acids, ammonium and nitrate produced are liberated into the soil solution from which they are taken up by the plants in an identical way. This new (^{15}N–^{14}C) approach to quantify the significance of organic N uptake by plants offers greater sensitivity than commonly applied ^{15}N–^{13}C techniques, since the detection of ^{14}C as opposed to ^{13}C is not hindered by the large background of ^{12}C (Näsholm and Persson 2001). Moreover, the slow-release kinetics of soluble organic N from macromolecular N (algae, isolated protein) enable the assessment of organic N nutrition over extended periods as opposed to the inherent problems when injecting double-labelled amino acids that turnover within minutes to hours (Jones 1999; Jones and Kielland 2002). Two points should be considered that potentially interfere with the technique. First, a fraction of ^{14}C may have been taken up by plant roots in the form of N-free compounds such as soluble carbohydrates (e.g., Hendrix 1984;

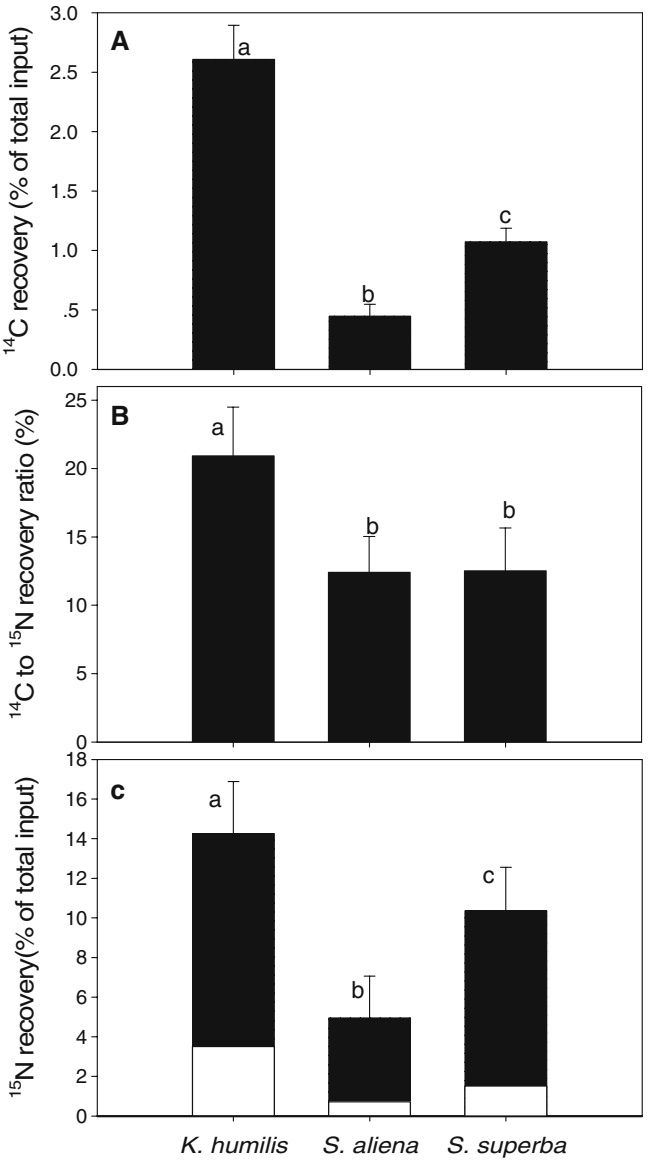

Fig. 2 Fractional recovery (as % of total input) of ^{14}C and ^{15}N derived from algae in three different alpine plant species. (**A**) ^{14}C recovery, (**B**) ^{14}C to ^{15}N recovery ratio (as % of ^{15}N recovery), and (**C**) ^{15}N recovery in plants 4 months after addition of double-labelled algae. Values are means (±SE) of six replicates. The white proportion of the bars in (**C**) shows the minimal contribution of organic N derived from algae to plant N acquisition, calculated according to the assumption that at most 15% of ^{14}C uptake by plant roots was lost via shoot and root respiration. Different letters indicate significant differences at 0.05 error probability level

Kuzyakov and Jones 2006), although this may only introduce a small error. Second, we have to consider respiratory losses of C taken up by plant roots in the form of amino acids (Schimel and Chapin 1996; Lipson and Näsholm 2001). We assumed that the maximal respiratory loss of the ^{14}C taken up by plants was 15% (Kuzyakov et al. 2001; Kuzyakov and Jones 2006). Due to such respiratory losses of ^{14}C the calculated contribution of organic N to plant N uptake represents a conservative estimate. Therefore, more extensive and rigorous measurements of respiratory ^{14}C losses have to be undertaken to better constrain the contribution of organic N to plant N nutrition.

The three tested alpine plants species exhibited species-specific differences in the uptake of algal-derived C and N (Fig. 3B, C). This indicates that alpine plant species possess different capacities to acquire organic N in alpine ecosystems, as has also been shown in laboratory experiments by Miller and Bowman (2003) and Xu et al. (2004). A previous study with ^{15}N-labelled glycine showed that *K. humilis*, a key species in this type of alpine grassland, had a greater capacity to

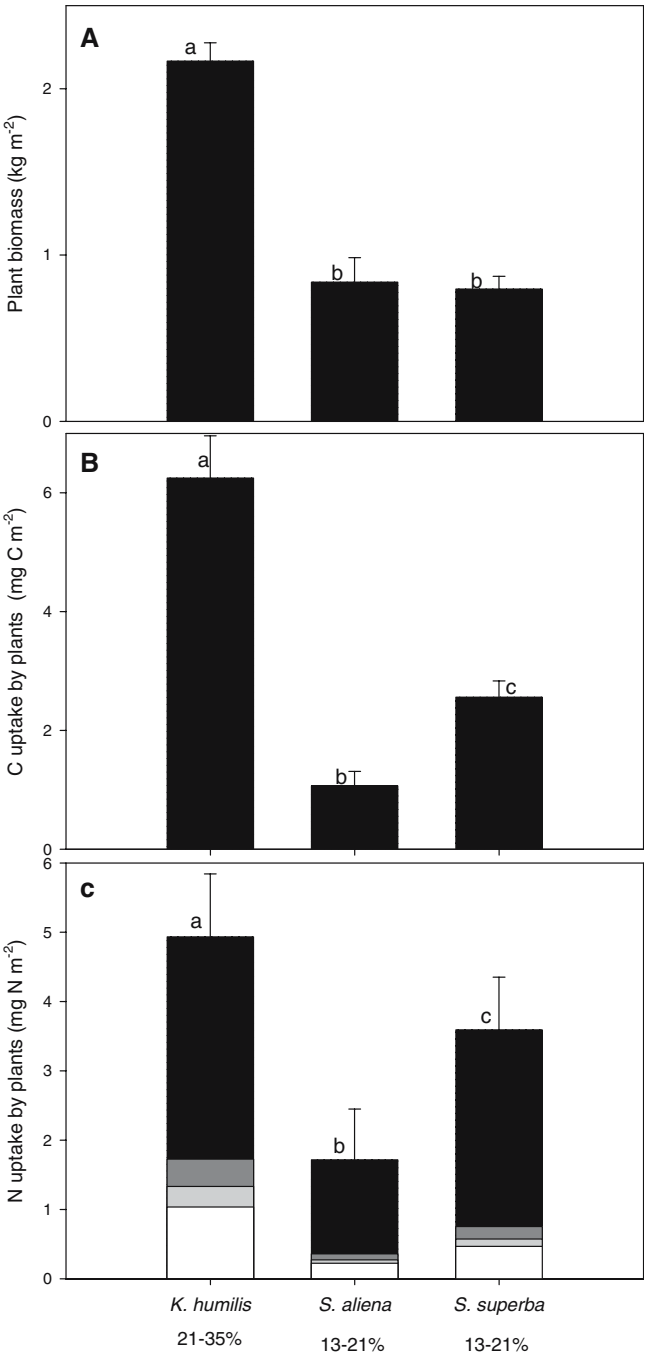

Fig. 3 Plant biomass and C and N uptake (on an area basis) by three alpine plant species 4 months after addition of labelled algae. Harvested plant biomass includes a fraction from last year as the plant species studied are perennial. The number under each column of the bottom graph (**C**) shows the range of contributions of organic nitrogen to plant N acquisition. The white, light-grey, and dark-grey bars in each column of (**C**) represent the contribution of organic N assuming the average of C:N ratio of algae-derived amino acids to be 5, 4 and 3. Values are means (±SE) of six replicates. Different letters indicate significant differences at 0.05 error probability level

acquire organic N than several sub-dominant species (Xu et al. 2004). In the present study *K. humilis* acquired more C and N by their root systems on an area basis than the other two species (Fig. 3). However, *S. aliena* showed a lower uptake capacity than *K. humilis* and *S. superba* on a dry matter basis (Fig. 1). Two mechanisms can be responsible for the discrepancy in organic N uptake between the three species. First, it was shown that organic N uptake by plants depends on physiological and morphological properties of the roots (Chapin et al. 1993). Distinct

Table 2 Estimated contribution[A] of organic N based on ^{14}C and ^{15}N uptake by plants from algae-derived $^{14}C/^{15}N$-labelled amino acids

Species	Average C:N ratio of amino acids derived from the added algae		
	3	4	5
K. humilis	35 ± 6[a]	27 ± 4[a]	21 ± 3[a]
S. aliena	21 ± 4[b]	16 ± 3[b]	13 ± 3[b]
S. superba	21 ± 3[b]	16 ± 2[b]	13 ± 2[b]

[A]The contribution of organic N to plant N acquisition is estimated based on the data of ^{14}C and ^{15}N uptake by plants from algae-derived amino acids, assuming different C:N ratios of the multiple amino acids originating from the decomposition of added algae, ranging from 3 to 5. Values are means ± SE, $n = 6$. Different letters in a column show significant differences at 0.05 error probability level

differences in rooting morphology and depth in this type of alpine meadows may contribute to the observed differences, e.g., *S. superba* possesses well-developed taproots while *K. humilis* develops phalanx-type rhizomes and *S. aliena* produces shallow fibre roots. Second, a strong niche overlap between *S. aliena* and *K. humilis* in this meadow type (Chen and Zhou 1995) gives rise to intense competition and thus may result in smaller amounts of N acquired by *S. aliena*.

Although the three dominant species showed different capacities to acquire organic N from soils (Fig. 1), the contribution of organic N to plant N acquisition in all cases was important for plant N nutrition (Table 2, Fig. 2). Two different approaches were followed to calculate the contribution of organic N to plant N uptake. The first approach, based on the ratio of F_{14C} to F_{15N} from algae, showed that organic N contributed more to N acquisition of *K. humilis* (25%), than to that of *S. aliena* (15%) and *S. superba* (15%, Fig. 2C). The second approach, based on ^{14}C uptake and mean C/N ratios of amino acids released from the algae, yielded slightly higher values for organic N contribution, ranging between 21–35% (*K. humilis*) and 13–21% (*S. aliena* and *S. superba*), respectively (Table 2 and Fig. 3C).

Several studies suggest strong competition between roots and soil microbes for inorganic N as well as for organic N (Kaye and Hart 1997; Bardgett et al. 2003), especially in alpine meadows, where mineralization rates are usually slow due to low temperatures. In our experiment added algae were depolymerized by soil enzymes and further mineralized by microbes to inorganic N. Thus, labelled organic and inorganic N species derived from added algae coexisted in soils, and plants and microbes may have strongly competed for them. Despite this competition, alpine plants acquired more than 13% of their total N in the form of organic N. This clearly demonstrates that organic N is an important N source for dominant plant species of alpine meadows. These results provide further evidence for the importance of organic N to alpine plant nutrition.

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