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The uptake diversity of soil nitrogen nutrients by main plant species in *Kobresia humilis* alpine meadow on the Qinghai-Tibet Plateau

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We studied the uptake of ammonium, nitrate, and a variety of amino acids by alpine plant species in the *Kobresia humilis* alpine meadow ecosystem *in situ*. We examined the extent of niche separation in uptake of N source by different plant species in alpine communities, and investigated the contribution of symbiotically fixed N to the total N in alpine meadow. The results are (1) $\delta^{15}N$ natural abundance values of 13 plant species lie between −2.680‰ and 5.169‰, and the scope is 7.849‰. (2) Leguminous plants, such as *Trigonella ruthenica*, *Gueldenstaedtia diversiffolia,* and *Oxytyopis ochrocephala,* and non-leguminous plant *Gentiana straminea* uptake low amounts of ^{15}N labeled ammonium, nitrate, glycine or aspartate in soil. (3) As far as the plant uptake of organic N is concerned, *Kobresia humilis, Poa pratensis*, and *Gentiuna spathutifolta* can effectively uptake organic nitrogen, and about 37%–40% of the nitrogen of these species comes from soil organic nitrogen sources (such as glycine and aspartate). *Stipa aliena* can effectively uptake nitrate, and 60% of its nitrogen comes from soil nitrate. *Potentilla anserina*, *Poa pratensis,* and *Thalictrum alpinum* can effectively absorb ammonium in comparason to other plant species in the meadow, and about 25%–27% of the nitrogen in these plants comes from soil ammonium. (4) The contribution of leguminous fixed N to total N is 7.48%–9.26% in *Kobresia humilis* alpine meadow. (5) These data show many plant species of alpine meadow may effectively utilize dissolved organic nitrogen such as amino acids, and these plants have diverse ways to uptake soil nitrogen in alpine meadows. Based on the results we can partly explain why there are abundant biodiversities and how plants at alpine habitat utilize the limited soil N sources.

Kobresia humilis **alpine meadow, ^{15}N tracer technique, plant organic nutrition, soil nitrogen**

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Since doctrine of mineral nutrition was established in 1840 by Liebig, people have always held the view that plants could just absorb inorganic nitrogen instead of organic nitrogen, and organic nitrogen could be absorbed only when it is mineralized by soil microorganisms. So a large number of studies have focused on absorption of inorganic nitrogen by plants. However, after years of study, we have observed that the annual absorbed nitrogen by plants in the alpine meadow, tundra, and boreal forest ecosystem is much higher than that of net mineralized nitrogen by soil microorganisms [1–4]. This implies that other forms of nitrogen besides in-

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organic N make a significant part in plant nutrition in this area. Chapin et al. [5] proved for the first time that non-mycorrhizal vascular plants preferentially absorbed and used organic nitrogen, and the tundra plant *Eriophorum vaginatum* of Cyperaceae could swiftly absorb free amino acids and these amino acids accounted for 60% of the total nitrogen, while the grains (control) growing in mineral soil showed a poor growth in amino acids culture. Along with the improved research methods and refined research, there are more reports about the uptake of soil organic nitrogen in arctic tundra, alpine meadow, and boreal forest ecosystem [6–19]. The importance of organic nitrogen in plant nutrients has been proved by some ecosystems, and many non-mycorrhizal vascular plants could directly and effectively absorb dissolved organic nitrogen, particularly amino acids. Such phenomenon is challenging our understanding of current terrestrial nitrogen circulation [12, 18].

Nutrition and energy are two backbones of life. Nutrition, acting as resources of interspecific competition, dominates population behavior to a large extent. On the other hand, plants absorb nutrition from surroundings in different ways, and some species have special ways to uptake soil nutrients. Nutrition cannot be regarded simply as equally effective resources when we study nutrition competition between populations. As pointed by McKane et al. [19], species in arctic tundra communities limited by the N nutrient coexist through differentiated absorbing times, depths, and types. The most productive species use the most abundant nitrogen forms and less productive species use less abundant forms due to reduced competition resulted from niche differentiation. This is the first documentation that the composition of a plant community is related to partitioning of differentially available forms of a single limiting resource. Nadelhoffer et al. [20] studied natural abundance of ^{15}N of tundra plants in Alaska, and the results show that the variance value of ^{15}N in tundra plant leaves could amount for 10%. They held the view that different plants have different ways for competing and obtaining limited nitrogen in the limited habitat, which makes species coexist sequentially and with abundant biodiversity in tundra ecosystem. Also, coexisting species occupy different niches in the community, which improves the utilization efficiency of limited resources such as nitrogen and phosphorus in soil. Now, scientists have begun to pay attention to plant organic nutrition and the diversity of plant nutrition uptake.

The *Kobresia humilis* alpine meadow is dominated by short rhizome perennial *Kobresia* plant species. There are distinct differences in the composition of animals and plant species. The microbial decomposition, energy flow, and material circulation are also different compared to those in grassland ecosystems at the low altitude. Transfer process of N nutrition in alpine meadow has a close connection with development of vegetation and soil, and it significantly affects the structure and the function of alpine meadow ecosystem. The soil's total N in alpine meadow is 10.63 t hm^{-2} and more than 95% of which appears as organic N while only about 1% of N appears as inorganic N, such as NO_2–N, NO_3–N, NH_4–N [21]. Soil organic N appears as acidic N that is composed mainly of macromolecular organic compounds, proteins and polypeptides and these chemicals account for 69.90%–82.10% of the total N in soil, Among these, amino acid-N shares 30.00%, ammonium- nitrogen shares 19.00%, and unknown N forms account for about 29.00%, respectively. Organic N decreases with deepening of the soil [22]. Currently, much work has been done on alpine meadow production and soil inorganic N, but little is known about the fore-process and mechanism by which NH_4^+ is produced in N circulation. Also the mechanism and the utilization preference of soil organic N by alpine plants are unknown although this is an indispensable part in evaluating N circulation in alpine meadow ecosystem.

So, we conducted this research in the *Kobresia humilis* alpine meadow ecosystem on the Qinghai-Tibet Plateau. Using ^{15}N tracer technique, we studied the uptake of soil organic (amino acids) and inorganic N (ammonium N, nitrate N) by alpine plant species *in situ*, and examined the extent of niche separation in N source uptake by different plant species in alpine communities. The research aimed to reveal organic nutrition traits of alpine plants and improve our understanding about N circulation in terrestrial alpine meadow ecosystem. The research can provide scientific information for maintaining of biodiversity and sustainable development on the Qinghai-Tibet Plateau.

1 Sketch of the research area

We conducted the research at Haibei Open Research Station of Chinese Academy of Sciences. The site lies between 37°29′–37°45′N in latitude and 101°12′–101°23′E in longitude with an average altitude of 3200 m. Annual average temperature is −1.7°C, annual precipitation ranges from 426 to 860 from 1957–2000, and 80% of the precipitation is concentrated in the growing seasons. Vegetation types mainly are *Kobresia humilis* alpine meadow dominated by *Kobresia humilis,* which is mainly distributed in sunny slope and bottomland, alpine shrub dominated by *Potentilla fruticosa* in shady slope, piedmont and valley lowland, and swampy meadow dominated by *Kobresia tibetica* in flood land. The corresponding soil types are alpine meadow soil, alpine shrubby meadow soil and swampy soil, respectively. The soil is thin and has a short history. Also, 10–15 cm of herby layer is full of high content of soil organic matter and has a relatively high content of soil total N, P and K. At the same time, there are little available nutrients, especially nitrogen and phosphorus and thus the N mineralization is weak in this area.

2 Materials and methods

2.1 Sampling plot settings

We chose *K. humilis* alpine meadow as our research object.

Eight treatment sampling plots were randomly set in alpine *K. humilis* meadow. We had four types of ^{15}N labeled chemicals including $^{15}NH_4Cl$, $K^{15}NO_3$, ^{15}N labeled glycine and aspartate (abundance of 98%). The ^{15}N concentration of all the chemicals was the same at 11 m mol L^{-1}. The eight sampling plots all contained two reduplications and each replication was set as 96 cm× 96 cm and at 2 m intervals. There were 289 injecting sites within each sampling plot, which was separated by a 6 cm× 6 cm grid. Two ml of solution was injected by an injector into soil 5cm under the ground at the crossing sites of each grid, and thus the concentration could reach 103.455 mg m^{-2} in each sampling plot (Figure 1).

2.2 Sampling and analyzing

Forty-eight hours after the injection, we randomly collected aboveground tissues within a range of 90 cm × 90 cm from the plot center in alpine *K. humilis* meadow from labeled *Poa pratensis*, *Elymus nutans*, *Stipa aliena*, *Kobresia humilis*, *Potentilla saundersiana*, *Potentilla anserine*, *Gueldenstaedtia diversiffolia*, *Oxytyopis ochrocephala*, *Trigonella ruthenica*, *Gentiana straminea*, and *Gentiuna aristata.* In addition, we measured N content and ^{15}N natural abundance of each species from the controlled plants the day before injection. Plant samples were dried at 80℃, grinded, and afterwards analyzed with controlled and labeled δ^{15}N of aboveground tissues using a Flash EA 1112HT and a Finnigan MAT Delta V advantage (accomplished by Isotope Lab of Research Institute of Forestry, Chinese Academy of Forestry). At the same time we selected 5 plots with 20 cm×20 cm to measure the aboveground biomass of each species. The fixed N by leguminous plants was determined by means of isotopic dilution method.

2.3 Calculations and statistical analyses

In this study, we used stable N isotope tracer method to determine the plant uptake value to different nitrogen forms. Common tracer methods were ^{15}N natural abundance and ^{15}N dilution methods. The stable N isotope tracer was the use of ^{15}N natural abundance variations to reflect the evolution of the natural environment, or the use of *in situ* label method to trace N movement in the ecosystem. The former is usually the atmospheric nitrogen isotope composition as the standard, and the values are expressed as variation coefficient of tracer atoms (δ):

$$\delta^{15}\text{N}(‰,\ \ \text{air})=(R_\text{p}/R_\text{a}-1)\times1000, \tag{1}$$

where R_p and R_a represent the ratio of ^{15}N and ^{14}N atom number in the sample and air respectively; ^{15}N dilution method is expressed as ^{15}N atom%−excess in the sample (A):

$$A = 100\,[n_{15}/(n_{15}+n_{14})]=100[R_\text{p}/(R_\text{p}+1)], \tag{2}$$

where n_{14} and n_{15} represent the atom percent of ^{14}N and ^{15}N in the sample respectively.

$$\text{The uptake value of } ^{15}\text{N} = [T(A_S-A_B)]/A_F, \tag{3}$$

where T is N concentration of sample, A_S ^{15}N atom%−excess in the sample, A_B ^{15}N atom%−excess in the natural plant, A_F ^{15}N atom%−excess in tracer.

In this study, ^{15}N isotope dilution method was used to determine the amount of fixed nitrogen by leguminous plants. When ^{15}N labeled nitrogen compounds with certain abundance were applied to the grassland in certain areas, there would be some differences for ^{15}N uptake in N fixing plants (legumes) and none-nitrogen fixing plants (the reference plants) because the former would fix certain amount N from atmospheric N_2. Thus we can calculate the contribution of biological nitrogen fixation to plant total nitrogen content (%N_{ysm}).

$$\%\text{N}_\text{ysm}=(1-{}^{15}\text{N atom\%−excess in legume }/{}^{15}\text{N atom\%−excess in reference plant})\times100, \tag{4}$$

$$\text{N}_\text{fixed}=\text{N}_\text{t}\times\%\text{N}_\text{ysm}, \tag{5}$$

Figure 1 ^{15}N injection plot in *Kobresia humilis* alpine meadow.

where %N ysm is percentage of legume fixed nitrogen; N_t plant N content of unit area (mg N m^{-2}); N_{fixed} amount of legume fixed N in unit area.

We tested the significance of difference of all the parameters derived from different treatments using one-way ANOVA, LSD and *t*-test depending on the data. All statistical analyses were conducted using Excel 2003 and SPSS 11.0.

3 Results and analyses

3.1 The nitrogen concentration and δ^{15}N natural abundance in plant aboveground tissues in *Kobresia humilis* alpine meadow

N concentration and δ^{15}N natural abundance in aboveground tissues of 13 primary plant species were analyzed during the growth season (in middle July) in the *K. humilis* alpine meadow. The results show N concentration in the aboveground tissues of cyperaceae and graminaceous plants is the lowest. For instance, the N concentrations in *K. humilis*, *E. nutans*, *S. aliena and P. pratensis* are 17.82, 17.16, 18.59, 17.19 g kg^{-1}, respectively (Table 1). But the N concentrations in leguminous plants are significantly higher than that in other plants such as *O. ochrocephala*, *T. ruthenica*, and *G. diversiffolia.* The values in these plants are 38.65, 45.09, and 42.00 g kg^{-1}, respectively, which are twice as much as that in Cyperaceae and graminaceous plants. The N concentrations in other dicotyledons lie in the middle of the values among all measured plants with the value in aboveground tissues varying from 17.38 to 27.13 g kg^{-1}.

The δ^{15}N natural abundance values of aboveground tissues of 13 species in *K. humilis* alpine meadow are shown in Table 1. The value of δ^{15}N varies from −2.680‰ to 5.169‰ and the variability scope is 7.849‰. The current research results show that there is a large difference among ^{15}N natural abundance values of different plant species. The plant leaf δ^{15}N value of tropical rain forest characterized by positive value can reach to 10‰–15‰ whereas that of soil N limited ecosystem characterized by negative value does not exceed −2‰ to −3‰. In addition, there is a difference for nitrogen isotope composition between plant and soil where plants grow and the value in plants is lower than that in soil. However, at the moment there are no sufficient experimental data to explain it. Currently, it is known that the plant nitrogen isotope composition (^{15}N/^{14}N ratio) is affected by three main factors: (1) the isotope composition differences of plant uptake N compounds (atmosphere N_2, soil ammonium, soil nitrate, soil organic nitrogen); (2) the fractionation during plant uptaking and assimilating N compounds; and (3) plant mycorrhizal species [23].

3.2 The plant uptake to different soil N forms in *Kobresia humilis* alpine meadow

Based on nitrogen concentration and ^{15}N atom% excess of aboveground tissues, we calculated the uptake values of ^{15}N marked glycine, potassium nitrate, ammonium chloride and aspartic acid and tested the capacity of uptaking different N forms by plants in *K. humilis* alpine meadow. As shown in Table 2, there is a significant difference among absorbing values of four forms of N by plants. *P. pratensis* has the value of 2.520, 2.753 and 1.952 μmol ^{15}N g^{-1} DW (dry weight) respectively for absorbing ^{15}N-Gly, ^{15}N-NO_3^-, ^{15}N-NH_4^+. On the other hand, the uptake of ^{15}N-Gly, ^{15}N-NO_3^-, ^{15}N-NH_4^+, ^{15}N-Asp by legumes such as *G. diversiffolia*, *O. ochrocephala*, and *T. ruthenica* is very low, varying from 0.081 to 0.375 μmol ^{15}N g^{-1} DW and the uptake is not significantly different. Besides, non-leguminous plant *G. straminea* has similar traits as the leguminous plants and the uptake value varies from 0.052 to 0.316 μmol ^{15}N g^{-1} DW.

Furthermore, other plants can be divided into three cate-

Table 1 The aboveground tissue nitrogen concentration and δ^{15}N natural abundance value of dominant species in *Kobresia humilis* alpine meadow

Plant species	Nitrogen concentration in aboveground tissues (g kg^{-1})	δ^{15}N natural abundance δ^{15}N (‰)
Kobresia humilis	17.82	0.289
Elymus nutans	17.16	−0.938
Stipa aliena	18.59	−0.921
Poa pratensis	17.91	5.169
Trigonella ruthenica	45.09	−2.466
Gueldenstaedtia diversiffolia	42.00	−1.837
Oxytyopis ochrocephala	38.65	0.660
Gentiuna aristata	23.95	1.383
Thalictrum alpinum	27.13	−2.680
Potentilla anserine	17.38	0.496
Potentilla saundersiana	21.87	−2.026
Gentiana straminea	19.77	3.898
Saussurea superba	21.04	2.636

Table 2 The mean uptake value of different plant species to labeled nitrogen compounds in *Kobresia humilis* alpine meadow (SD) (μmol ^{15}N g^{-1} DW)[a)]

	Gly	Nitrate	Ammonium	Asp
P. pratensis	2.520 (0.177)ab 1	2.753 (0.272)a 1	1.952 (0.187)b 1	0.558 (0.124)c1
E. nutans	0.517 (0.107)b 2, 3, 4	1.493 (0.195)a 2, 3	0.690 (0.093)b 2, 3	0.426 (0.095)b1
S. aliena	0.363 (0.053)b 3, 4	1.259 (0.209)a 2, 3	0.304 (0.016)b 3	0.188 (0.037)b2
K. humilis	0.905 (0.359)a 2	1.344 (0.371)a 2, 3	0.734 (0.009)a 2, 3	0.334 (0.068)b1, 2
P. saundersiana	0.633 (0.006)b 2, 3	1.888 (0.181)a 2	0.722 (0.238)b2, 3	0.660 (0.094)b1
P. anserine	0.669 (0.154)b 2, 3	1.448 (0.202)a 2, 3	0.921 (0.222)ab 2	0.423 (0.125)b1
G. aristata	0.512 (0.146)a 2, 3, 4	1.068 (0.275)a 3, 4	0.534 (0.000)a 3	0.445 (0.109)a1
T. alpinum	0.682 (0.062)b 2, 3	1.876 (0.642)a 2	1.097 (0.245)b 2	0.644 (0.143)b1
S. superba	0.803 (0.153)b 2	1.511 (0.091)a 2, 3	1.306 (0.625)ab 1, 2	–
G. diversiffolia (Leguminosae)	0.222 (0.001)a 4	0.373 (0.168)a 4	0.344 (0.197)a 3	0.081 (0.013)b2
O. ochrocephala (Leguminosae)	0.239 (0.171)a 4	0.169 (0.027)a 4	0.103 (0.027)a 3	0.257 (0.117)a2
T. ruthenica (Leguminosae)	0.177 (0.011)a 4	0.375 (0.078)a 4	0.235 (0.030)a 3	0.150 (0.018)a2
G. straminea	0.144 (0.024)a 4	0.316 (0.055)a 4	0.167 (0.006)a 3	0.052 (0.016)a2

a) Data in the same row followed by the same letter do not differ, at P=0.05; data in the same column followed by the same number do not differ, at P=0.05.

gories: one is represented by *E. nutans*, *S. aliena*, *P. saundersiana*, and *T. alpinum*, which preferably absorb ^{15}N-NO$_3$ and no significant difference in absorption of ^{15}N-Gly, ^{15}N-NH$_4^+$, ^{15}N-Asp is found. The second is represented by *K. humilis* and *G. aristat,* and there is no significant difference in absorption of ^{15}N-Gly, ^{15}N-NO$_3^-$ and ^{15}N-NH$_4^+$ by these plants. However, the uptake value of *K. humilis* and *G. aristat* to ^{15}N-Asp is lower. The third is represented by *P. anserine* and *S. superba* with a remarkable high capacity in absorption of ^{15}N-NO$_3^-$ and ^{15}N-NH$_4^+$ compared to that of absorbing organic nitrogen. From the absolute quantity of absorbed nitrogen compounds by all plants we can see that nitrate is absorbed most by all plants except *G. straminea* and leguminous plants, ammonium nitrogen and glycine lie in the second place, and aspartic acid is the least N compound absorbed by plants.

Overall, the leguminous plants and *G. straminea* absorb less ^{15}N labeled four types of compounds in *K. humilis* alpine meadow. *P. pratensis, K. humilis* and *G. aristata* absorb nitrate nitrogen as well as organic nitrogen such as glycine. Secondly, most plants mainly absorb nitrate nitrogen, which show obvious preferences in obtaining nitrogen nutrition. These results suggest there are differences and diversities in absorption of nitrogen nutrition by plants in *K. humilis* alpine meadow. Some of the plants effectively absorb soluble organic nitrogen, especially amino acids in soil.

3.3 Estimation on annual N fixing capacity by leguminous plants in *Kobresia humilis* alpine meadow

Dominant species in alpine *K. humilis* meadow such as *S. aliena*, *E. nutans* or *K. humilis* were chosen as the reference plants to estimate annual N fixing capacity by leguminous plants on the basis of ^{15}N isotope-dilution analysis when K^{15}NO$_3^-$ or ^{15}NH$_4^+$Cl is used (Table 3). The results show that leguminous plants have a similar N fixing capacity no matter which reference plant is used in the situation of absorption of K^{15}NO$_3^-$. For instance, the N fixing capacity per unit area of *G. diversiffolia* varies from 70.51 to 72.49 mg N m^{-2} and the N fixing capacity of *O. ochrocephala* is 287.07–291.38 mg N m^{-2} and for *T. ruthenica* the value is from 164.3 to 169.14 mg N m^{-2}. Based on the data presented above we can calculate that the total fixed N amount is 521.88–533.41 mg N m^{-2} from atmosphere in *K. humilis* alpine meadow.

However, if we use ^{15}NH$_4^+$Cl, the results are different on N fixing capacity when a different reference plant is selected. For instance, the per unit area N fixing capacity of *G. diversiffolia* is 33.44 mg N m^{-2} (*S. aliena* as the reference plant), 54.03 mg N m^{-2} (*E. nutans* as the reference plant), and 59.30 mg N m^{-2} (*K. humilis* as the reference plant) respectively. Thus we can calculate that when ^{15}NH$_4^+$Cl is used, the total fixed N from air by all leguminous plants is 444.73–550.76 mg N per square meters in *K. humilis* alpine meadow.

According to plant aboveground biomass and their N concentration we can calculate that the total N content of aboveground tissues per unit area is 5.949 g N m^{-2} and the capacity of symbiotic nitrogen fixation shares 7.48%–9.26% of the total aboveground nitrogen in *K. humilis* alpine meadow.

4 Discussions

4.1 Types of organic nitrogen in the soil

The soil organic nitrogen mainly includes proteins, nucleic acids, peptides, chitosan peptides, chitin and water-soluble amino acids, amino sugar, and urea. Most of these compounds belong to insoluble organic nitrogen, which cannot

Table 3 Comparison of N fixing capacity by aboveground tissues of leguminous plants using different reference plants and different ^{15}N labeled nitrogen compounds (mg N m^{-2})

Leguminous plants	$K^{15}NO_3^-$			$^{15}NH_4^+Cl$		
	Reference plants					
	S. aliena	*E. nutans*	*K. humilis*	*S. aliena*	*E. nutans*	*K. humilis*
G. diversiffolia	70.51	72.49	70.74	33.44	54.03	59.30
O. ochrocephala	287.07	291.78	287.6	284.89	315.6	323.46
T. ruthenica	164.3	169.14	164.85	126.40	159.53	168.01
Total fixed nitrogen capacity	521.88	533.41	523.19	444.73	529.16	550.76

be absorbed by plants directly. Only soluble organic nitrogen with small molecular weights can be absorbed by plant roots (such as urea, amino acids, amino, etc.) with amino acids being the main part on the list. Therefore, soil amino acid bank is generally compared with that of inorganic nitrogen when evaluation of available nitrogen by plants [18].

Only a small part of soil amino acids that are dissolved in soil solution is called free amino acids. According to the results of our determination, phenylalanine, aspartic acid, leucine, arginine, proline, glutamic acid, and glycine are the dominant ingredients in soil extract in *Kobresia humilis* alpine meadow, which account for 70%–90% of the total free amino acids. The average total soil free amino acid-nitrogen concentration in growing season amounts for 7.44 mg N kg^{-1} (dry soil) and the value varies from 4.50 to 10.82 mg N kg^{-1}. The concentration changes significantly with time; the concentration is the highest (10.82 mg kg^{-1}) at the end of May, and then slowly declines to the lowest (4.50 mg kg^{-1}) by early October [24]. Weintraub [25] reported that free amino acid concentration and inorganic nitrogen concentration are 2–8 mg g^{-1} and 0.5–1.1 mg g^{-1} dry soil in soil extract of wet tundra. Lipson and Nasholm [12] reported that the concentration of amino nitrogen in soil extract is 0.04–24 μg N g^{-1} (dry soil) and can be as high as 158 μmol L^{-1} in soil pore space water. Raab et al. [26] reported that the concentration of amino acids in soil pore space water from alpine meadow is 13–158 μmol L^{-1} with glycine (neutral) being the main amino acid. The concentration of amino acids from subalpine swamp is 13–158 μmol L^{-1} and main form is aspartic acid., High concentrations of amino acids in these soils are mainly resulted from hydrolysis of soil proteins by soil protease. Glycine, aspartic acid and glutamic acid are the most common amino acids in soil solution although there are many kinds of amino acids in soil solution and in soil pore space water [27]. Thus, soil glycine and aspartic acid play an important role in plant organic nutrition.

4.2 Uptake of soil organic and inorganic nitrogen by different plants

Chapin et al. [5] reported for the first time that tundra plant, Cyperaceae plant and *Eriophorum vaginatum*, effectively absorb free amino acids, which amount for 60% of the total nitrogen. Kielland [7] reported that vascular plants grown in arctic dry heath shrubs, grass tundra, shrub tundra and wet meadow directly absorb amino acids, which amount for 10%–82% of total nitrogen, while the ability of absorption varies in species and negatively correlates to amino acid molecular weight. Raab et al. [26] carried out an experiment on absorption of organic-(glycine) and inorganic nitrogen (NO_3^-, NH_4^+) by thirteen non-mycorrhizal sedges grown in five different ecosystems. The results show that all sedge species absorb glycine except for one species grown in dry tropic forest. One alpine sedge cannot absorb NH_4^+ and its absorption rate is lower than that of absorbing amino acids. Furthermore, the absorption rate of glycine is higher than that of absorbing NH_4^+-N and NO_3^--N by alpine sedges and subalpine sedges. Lipson [28] suggested that absorption ability of glycine by tundra *Kobersia myosuroides* exceeds the utilization ability of glycine by microorganisms and glycine might be the specific organic nitrogen nutrition for tundra sedges. From above analyses we may say that the absorbed quantity of amino acid amounts for 10%–100% of the total nitrogen with variability deriving from the differences of plant communities and different compositions of species. Moreover, the uptake value of plants in N solution can better reflect the fundamental niche because this has neglected the competition between plants and microorganisms. This stands for the upper bound of absorbed amino acids in a year based on dynamic model of plant uptake. The in situ experiment that measures the quantity of nitrogen uptake in a competitive environment, however, may better reflect actual niche of uptake nitrogen by plants.

We calculated the absorption percentage of nitrate nitrogen, ammonium nitrogen and organic nitrogen (glycine and aspartic acid) using eight species in alpine meadow except for leguminous plants and *G. straminea*. We can see from Figure 2 that the quantity of absorbed organic N (glycine and aspartic acid) amounts for 26%–40% of the total absorbed nitrogen, nitrate nitrogen amounts for 35%–56%, and ammonium nitrogen makes up 14%–27%, respectively.

The most powerful species to uptake organic nitrogen in alpine meadow are *P. pratensis*, *K. humilis*, and *G. aristata*, which uptake 37%–40% of total absorbed organic nitrogen. *S. aliena* is the most powerful species to uptake nitrate nitrogen, which shares 60% of the total N. The second important group of species in absorption of nitrate nitrogen is *E. nutans* and *P. saundersiana* and the uptake of nitrate N

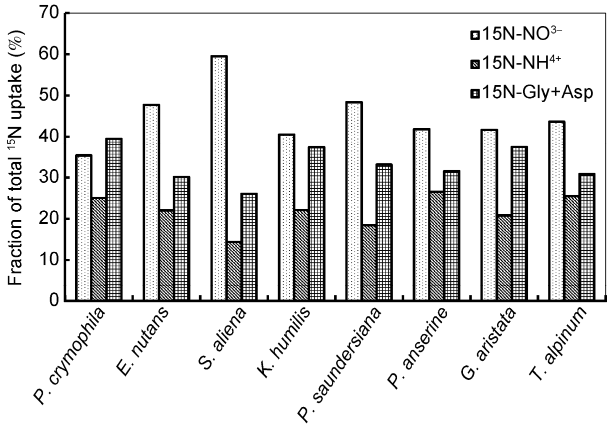

Figure 2 The absorption percentage of nitrate nitrogen, ammonium nitrogen, and organic nitrogen by plants in alpine meadow.

by these species account for 48% of the total N. In contrast, the quantity of ammonium nitrogen absorbed by *P. anserine*, *P. pratensis,* and *T. alpinum* is relatively higher than that of other forms of N (e.g. ammonium nitrogen amounts for 25%–27% of the total N). These results show that plants in alpine meadow can absorb amino acid instead of mineralized nitrogen by microorganisms, and that absorption of organic nitrogen is of importance in the nitrogen budget in alpine meadow ecosystem. Many plants in alpine meadow effectively absorb soil soluble organic nitrogen, and they also have diversity in absorption of nitrogen nutrition. Thus so-existing species reduce interspecific competition and improve utilization of limited soil resources such as nitrogen and phosphorus through holding differentiated niches. Therefore we can explain why so many biodiversities and limited resources are efficiently utilized by plants in an alpine environment limited by available nitrogen.

4.3 Estimation on annual plant nitrogen fixing capacity in alpine *Kobresia humilis* meadow

When ^{15}N labeled nitrogen compounds are applied to grassland within certain areas using isotope-dilution analysis, there are some differences in absorption of ^{15}N by nitrogen fixing plants and non-nitrogen fixing plants. As the former will fix certain amount of atmospheric nitrogen, the amount of fixed nitrogen from atmospheric N can be calculated according to this. But a question remains unanswered: how the nitrogen fixation by leguminous plants is affected by different types of ^{15}N labeled compounds and how the selection of reference plants affects the calculation of N fixation. Our data show that the amounts of fixed nitrogen by leguminous plants are basically the same no matter which kind of reference plant is selected when $K^{15}NO_3^-$ is used. However, there is some influence on calculation of nitrogen fixing capacity using different reference plants when $^{15}NH_4^+Cl$ is used. For instance, the per unit area nitrogen fixing capacity by *G. diversiffolia* ranges from 33.44 to 59.30 mg N m^{-2}, N fixing capacity by *O. ochrocephala* is from 284.89 to 323.46 mg N m^{-2}, and *T. ruthenica* is from 126.40 to 168.01 mg N m^{-2}, respectively.

Furthermore, our data show that *G. straminea* absorbs N at the same levels as leguminosae plants and this plant uptakes low amounts of all four types of N compounds. That implies that *G. straminea*, in addition to its symbiotic nitrogen fixation as leguminous plants in alpine *K. humilis* meadow, might belong to non-leguminous nitrogen-fixing plants for its peculiar way of absorption of nitrogen nutrition. If this hypothesis is correct, we may get the per area unit nitrogen fixing capacity by *G. straminea* at 801.40–839.68 mg N m^{-2} when $K^{15}NO_3^-$ is used and 568.62–844.28 mg N m^{-2} when $^{15}NH_4^+Cl$ is used (Table 4). The total fixed atmospheric nitrogen in alpine *K. humilis* meadow approximates 1013.35–1395.04 mg N m^{-2}. The capacity of symbiotic nitrogen fixation shares 17.03%–23.45% of the total aboveground nitrogen in alpine *K. humilis* meadow. Generally, *G. straminea* has a large size, dark leaf color, dominant growth rate, and high competition capability [21], which may explain its peculiarity and efficiency in use of nitrogen nutrition. The mechanism and process of nitrogen fixation by this plant need to be further studied.

Plant fixing nitrogen quantity from atmospheric N_2 varies significantly in different ecosystems according to current studies. Jacot et al. [29] suggested that biological nitrogen fixation capacity varies from below 1 g N m^{-2} a^{-1} in tundra ecosystem to 30 g N m^{-2} a^{-1} in agro-ecosystem. Solheim et al. [30] reported that the nitrogen fixation rate is 19–255 mg m^{-2} a^{-1} with an average value of 127 mg m^{-2} a^{-1} in arctic and alpine ecosystems. Jacot et al. [29] studied nitrogen fixation rate by leguminous plants grown under different altitudes in Alps and the results showed that the quantity of nitrogen fixation from the atmosphere is averaged 0.1 g N m^{-2} by leguminous plants that grow between 2100 and 2300 m above the sea level and 1.8 g N m^{-2} when plants grow between 900 and 1380 m above the sea level, respectively.

Table 4 Nitrogen fixation capacity by leguminous plants and *G. straminea* using different reference plants and ^{15}N labeled compounds (mg N m^{-2})

Leguminous plant	$K^{15}NO_3^-$			$^{15}NH_4^+Cl$		
	Reference plant					
	S. aliena	*E. nutans*	*K. humilis*	*S. aliena*	*E. nutans*	*K. humilis*
N fixed capacity by *G. straminea*	801.40	839.68	805.78	568.62	788.18	844.28
N fixed capacity by leguminous plant	521.88	533.41	523.19	444.73	529.16	550.76
Total N fixed capacity	1323.28	1373.09	1328.97	1013.35	1317.34	1395.04

The contribution to nitrogen content in aboveground tissues of leguminous plants decreases from 16% to 9% along with the elevation increase.

5 Conclusions

The plants in alpine meadow can absorb soil soluble organic N such as amino acid besides mineralized nitrogen by microorganisms. The uptake of soil organic nitrogen is important for the nitrogen cycle in alpine meadow ecosystem. Many plants in alpine meadow can effectively absorb soil soluble organic nitrogen such as amino acid and these plants also have the diverse nature in absorption of soil N of different forms. The most powerful species to uptake organic nitrogen in alpine meadow are *P. pratensis*, *K. humilis*, and *G. aristata*, and 37%–40% of total absorbed nitrogen by these plants belongs to organic nitrogen. *S. aliena* is the most powerful species to uptake nitrate nitrogen, which shares 60% of the total N. The second group of species in preferable absorption of nitrate nitrogen is *E. nutans* and *P. saundersiana* (e.g. nitrate nitrogen shares 48% to the total N). But the quantity of ammonium nitrogen absorbed by *P. anserine*, *P. pratensis* and *T. alpinum* is relatively higher than that of other forms of N (i.e. ammonium nitrogen amounts to 25%–27% of the total N). The amount of symbiotic nitrogen fixation by leguminous plants shares 7.48%–9.26% of the total aboveground nitrogen in *K. humilis* alpine meadow. Our research has shown that many plants in alpine meadow effectively absorb soil soluble organic nitrogen such as amino acid. Thus co-existing species reduce inter-specific competition and improve utilization of limited soil resources such as nitrogen and phosphorus through niche differentiation. Therefore we can explain how biodiversity forms and how limited resources are efficiently utilized by plants in an alpine environment limited by available nitrogen.

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