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Uptake and Recovery of Soil Nitrogen by Bryophytes and Vascular Plants in an Alpine Meadow

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Abstract: Due to their particular physiology and life history traits, bryophytes are critical in regulating biogeochemical cycles and functions in alpine ecosystem. Hence, it is crucial to investigate their nutrient utilization strategies in comparison with vascular plants and understand their responses to the variation of growing season caused by climate change. Firstly, this study testified whether or not bryophytes can absorb nitrogen (N) directly from soil through spiking three chemical forms of 15N stable isotope tracer. Secondly, with stronger ability of carbohydrates assimilation and photosynthesis, it is supposed that N utilization efficiency of vascular plants is significantly higher than that of bryophytes. However, the recovery of soil N by bryophytes can still compete with vascular plants due to their greater phytomass. Thirdly, resource acquisition may be varied from the change of growing season, during which N pulse can be manipulated with 15N tracer addition at different time. Both of bryophytes and vascular plants contain more N in a longer growing season, and prefer inorganic over organic N. Bryophytes assimilate more NH4+ than NO3− and amino acid, which can be indicated from the greater shoot excess 15N of bryophytes. However, vascular plants prefer to absorb NO3− for their developed root systems and vascular tissue. Concerning the uptake of three forms N by bryophytes, there is significant difference between two manipulated lengths of growing season. Furthermore, the capacity of bryophytes to tolerate N-pollution may be lower than currently appreciated, which indicates the effect of climate change on asynchronous variation of soil N pools with plant requirements.

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Introduction

The alpine ecosystem is strongly influenced by climate change (Thuiller 2007), and vegetation at high elevation is more sensitive to pronounced effects of climate change (Körner 1994). Therefore, it is expected that a range of alternatively phenological patterns may be disturbed by the responses of plant phenology to future climates, but they are species specific (Woodward and Cramer 1996; Chapin et al. 2000; Chmielewski and Rotzer 2001; Penuelas and Filella 2001; Badeck et al. 2004; Cleland et al. 2006; Sherry et al. 2007). A longer growing season has been indicated at the community and biome levels across most areas of the Northern Hemisphere (Steltzer and Post 2009). Moreover, shifts in resource availability caused by climate change can also disturb plant community structure and stability (Kelly and Goulden 2008; Tylianakis et al. 2008), which may induce difficulties to study intensively and precisely due to ecosystems' complexity. Because of reducing the complex and uncharted characteristics of species diversity and being connected with complicated community structure and compositions, plant functional groups can be a general device to project plants' responses to global perturbation (Woodward and Cramer 1996; Diaz and Cabido 1997). For their different N utilization strategies and aboveground biomass proportions, plants in alpine meadows can be divided into two functional groups, vascular plants and bryophytes. The former is best known as assimilating nutrient primarily from soil with developed root systems (Chapin 1980; vonWiren et al. 1997; Miller and Cramer 2005), while the latter is thought to access its major nutrient source from atmospheric deposition due to lack of developed root and vascular systems (Vantooren et al. 1990; Ayres et al. 2006). Although bryophytes generally appear to rely mainly on atmospheric deposition, the previous research of *Polytrichum alpinum* and *Racomitrium lanuginosum* through ^{15}N tracer applications demonstrated that bryophytes can directly derive N from the soil (Ayres et al. 2006).

As one non-vascular cryptogam and the second most diverse group of plants on the earth, bryophytes play important roles in ecosystems where environmental stress limits the distribution of vascular plants (Lindo and Gonzalez 2010). Thick layers of live and dead bryophytes control the hydrology of vast peatland areas (Beringer et al. 2001; Blok et al. 2011), and preserve permafrost through their temperature-insulating capacity (Gornall et al. 2007; Blok et al. 2011). N (N) is the most limiting nutrient to net primary productivity in many terrestrial ecosystems, particularly in temperate and boreal regions (Chapin 1980; Vitousek and Howarth 1991; Elser et al. 2007; LeBauer and Treseder 2008). Alpine ecosystems are also characterized by nutrient limitation, low temperature and long winter (Atkin et al. 1996; Körner 2003). Bryophytes often serve as effective traps for water and nutrients, and they are especially efficient in assimilating N (Turetsky 2003; Krab et al. 2008). The N fixation capacity of some bryophytes is crucial in boreal ecosystems where N (N) availability is low (Solheim et al. 1996; Turetsky 2003). It is also notable for *Polytrichum commune* with uptake of glycine and aspartic acid in the lab, but negligible or no uptake in the field (Krab et al. 2008). Furthermore, amino acid as one kind of organic N forms has been found to represent another important N source for plants in 'cold biomes' (Kielland 1995; Raab et al. 1996, 1999; Persson et al. 2003; Krab et al. 2008). It is proposed that differences between species to utilize particular N sources can be an important feature of plant community composition (Kielland 1994; Schimel and Bennett 2004; Cambui et al. 2011). Therefore, further studies are required to test how N utilization of different plants vary, which would be another significant issue to explore the differentiation of N utilization, especially at functional groups level (Ayres et al. 2006; Krab et al. 2008).

Resources in alpine ecosystems exhibit seasonal variability in availability (Körner 2003). Dynamics of soil N availability have synchronism with plant and soil microbial acquisition capacity for their growth and development, which can make coupling of N budgets and contribution to plant community stability (Jaeger et al. 1999; Bardgett et al. 2007; Xu et al. 2011). Nevertheless, climate change may lead to asynchronous variation of soil N pools with plant requirements, which is the potential effect of altered plant phenology on the plant's acquisition of soil resources (Nord and Lynch 2009; Smith et al. 2009). As a result of longer growing season, resource acquisition can be raised. Plant community might be disturbed, and its N strategy would also be altered. However, there is no empirical evidence so far for both bryophytes and vascular plants in alpine belts to show the same trend in resource acquisition under the influence of different durations of growing season.

In the current study, we aimed to examine N utilization strategies of bryophytes in comparison with vascular plants, and understand their responses to the variation of growing season caused by climate change. Stable isotopic techniques (^{15}N) were adapted *in situ* at different times to quantify the uptake and recovery of N by bryophytes and vascular plants. The duration of growing season was manipulated utilizing ^{15}N tracer spiking at different time and then test whether the uptake and recovery of N by bryophytes and vascular plants could be disturbed by “time mismatch” N pulse. The specific objectives were as follows: (1) this study tested whether or not bryophytes can absorb N directly from soil utilizing an ^{15}N stable isotope tracer approach; (2) the study compared N utilization efficiency and the recovery of soil N between vascular plants and bryophytes; (3) the study tested, with ^{15}N tracer addition at different times to manipulated N pulse, the variation of resource acquisition triggered by the change of growing season.

1 Material and Methods

1.1 Site description

The field site is located in KAKA Valley (32° 59' N, 103°40' E, 3,950 m a.s.l), which belongs to the middle section of Minshan Mountain, eastern Tibetan Plateau. The area has a mean annual precipitation of 720 mm and seasonally- varied precipitation distribution. More than 70% precipitation falls in summer from June to August. The annual average temperature is 5.7°C. The average temperature in January and July is -7.6°C and 9.7°C respectively. There is no absolute frost-free period. Snowfall usually occurs from the end of September to early May. Vegetation presents a typical alpine meadow with numerous and unique alpine plants. The bryophyte layer is dominated by *Polytrichum swartzii* and *Trematodon acutus c. mull.* Vascular plants include species mainly belonging to *Kobresia* and *Carex*. Other common species are grasses and forbs in *Festuca, Gentiana* and *Leontopodium*. The main soil type is Mat Crygelic Cambisols (Chinese Soil Taxonomy Research Group 1995).

1.2 Experimental design

In the mid-May 2011, 24 plots were set up at the fenced alpine meadows, which were approximately uniform in coverage and species composition. Intact soil was encircled by PVC (polyvinyl chloride) pipes as circular micro-plots, with 18 cm diameter and 15 cm height, whose top edges were at ground level. 24 plots were randomly located and separated from each other no less than 1 m. With 2 ^{15}N isotope spiking times and 2 harvest time being considered to manipulate 2 growing season lengths, 12 PVC plots as one group were divided into 3 chemical forms of ^{15}N isotope labeled treatments and control treatment. Each treatment had three replicates. In this model simulation, two different growing seasons were artificially created at different time (i.e. September 24th and October 4th), and three forms of ^{15}N stable isotope solution were injected at different time, which could generate simplified ‘time mismatch’ of N pulse. Plants in each plot were separated as two functional groups (bryophytes and vascular plants). To trace how bryophytes and vascular plants differing in uptake of different soil N sources, ^{15}N (98.42-99.19 atom% ^{15}N) in solution (11 m mol L^{-1}) was injected as ammonium chloride, potassium nitrate and glycine carefully to avoid ^{15}N contamination.

For the majority (70%-80%) of plant roots distributed above 10 cm depth (Xu et al. 2011), ^{15}N-labeled N were injected into the soil layer within 4 cm depth on May 12th and May 22nd 2011 separately. The aboveground biomasses were harvested on September 24th (the first group, 12 plots) and October 4th (the second group, the other 12 plots) separately. A syringe with a four-side port needle was applied to deliver 4 ml to the desired depth (4 cm) at each of 16 injection sites per plot (McKane et al. 2002). The injection sites were spaced in a 2 cm by 2 cm cell (the diffusion radius were 1 cm to 1.2 cm around injection points), with alternate rows offset to spread ^{15}N evenly within the soil. ^{15}N atom% excess was calculated as atom% of ^{15}N difference between injected treatments and control treatments. Bryophytes’ growing part during the experiment period was determined by marking the tips of bryophytes with red paint after the ^{15}N tracer added immediately, i.e. paint mark (Pouliot et al. 2010). Bryophytes above the marked symbols and vascular plants were cut with scissors to measure ^{15}N content in aboveground tissues. Meanwhile, quadrat survey of alpine meadow community was carried out in the study site, and at least 10 quadrates (1/4 m^2) were placed in each transect. The total phytomass was investigated and then divided into four components including litter, bryophytes, lichens and vascular plants respectively.

The mean seasonal concentration of dissolvable inorganic N (DIN) in soil is about 16.88 mg N/g dry soil, with approximately equal quantities of nitrate and ammonium, whereas the seasonal average for total dissolved organic N concentration for this alpine meadow is 16.74 mg N/g soil. The exchangeable pool was extracted with 2 M KCl while soil solutions were extracted by centrifugal drainage (Jones et al. 2004).

Soil temperature was logged per hour by using iButton-thermometer (Dallas Semiconductor Company, USA) in the middle plot of each treatment at soil depth of 5 cm. After the soil was sieved with 2 mm × 2 mm mesh and dried in an oven, soil water content was measured. N and carbon concentrations of the dried and ground plant samples were determined using an elemental analyzer (Vario MICRO cube, ELEMENTAR, Germany). N concentrations and atom percent of ^{15}N were determined in separate runs for N using an K-05-Automatic kieldahl apparatus (Hongshen, Shanghai, China), coupled with a continuous-flow isotope ratio mass spectrometer (Delta Plus, ThermoQuest Finnigan, Bremen, Germany).

1.3 Data analysis

^{15}N atom% excess was calculated as the atom% ^{15}N difference between the treated and control plants. ^{15}N recovery by plants was calculated by multiplying the N content in the pool by its mass per square meter and ^{15}N atom% excess, divided by total added ^{15}N per square meter. In the figures and tables, information is presented as the means and the standard errors of means. One-way ANOVA (Analysis of Variance) was applied to compare N content of bryophytes and vascular plants during two mismatched periods, phytomass, N and carbon concentration, and ^{15}N abundance in bryophytes injected with different ^{15}N forms. Post hoc multiple comparisons were adopted when there were no less than three groups. Multivariate ANOVA was calculated to estimate the effects of N forms, period (the time from injection till sampling), plant functional groups (PFGs, i.e. bryophytes and vascular plants) and their interactions on ^{15}N atom% excess and ^{15}N recovery by bryophytes and vascular plants using the SPSS 16.0 software package (SPSS Inc., Chicago, IL, USA). The normality of data tested with One-Sample K-S test and Q-Q plot, or else log-transformation was adopted to meet the normality requirement. Homogeneity of variance test was also utilized during the analysis process. All differences were tested at P=0.05 level.

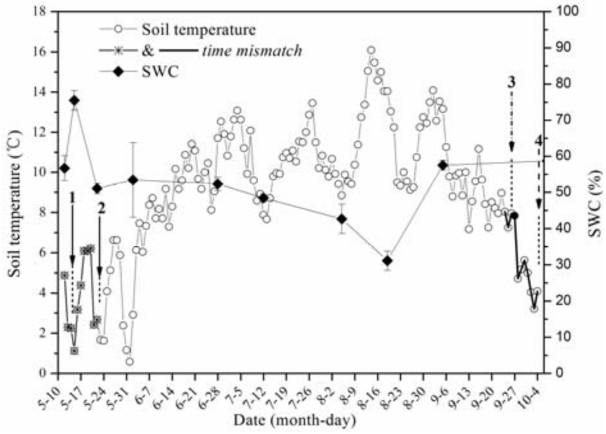

Figure 1 Soil temperature, soil water content (SWC) and two different durations after stable isotope ^{15}N injection. “1” and “2” indicate “time mismatch” of N pulse in manipulated shorter and longer growing season, while “3” and “4” indicate sampling at different dates to generate two growing season lengths, respectively.

2 Results

2.1 Soil temperature, soil water content and ^{15}N tracer spiking dates

The experiment was conducted from May 12th till the early October (October 4th). Soil temperature and soil moisture content were presented in Figure 1, which also included two different manipulated lengths of growing season and stable isotope ^{15}N injection dates. Dry season and the maximum soil temperature appeared in August synchronously. There were two wet seasons during experiment period including an early wet season from early May to mid-June due to snow melting, and a late wet season from early September till the end of experiment period for rainfall. Soil temperature increased at the early stage of growing season, reached its climax in August, and then dropped (Figure 1).

2.2 Phytomass and chemical properties of bryophytes and vascular plants

Bryophytes and vascular plants were accounted for 77.83% and 21.28% of total biomass (litter not included) respectively. Shoot biomass of higher plants was significantly less than that of bryophytes (Table 1; *F*=119.75, *P*<0.0001). *Carex atrofusca, Kobresia humilis* and *Kobresia kansuensis* were the co-dominant species in term of shoot biomass. For bryophytes, *Polytrichum swartzii* and *Trematodon acutus c. mull.* were two co-dominant species, and *Entodon concinunnus (De Not.)* was common but not dominant species.

Table 1 Quadrat survey about the aboveground biomass of different fractions

Variable	Aboveground biomass (g/m^2)
Total phytomass	637.49±22.21
Litter	165.88±14.77*b*
Mosses	367.06±23.73*a*
Lichens	4.18±1.16*d*
Vascular plants	100.36±5.57*c*

Table 2 ^{15}N (*μ*g/g), N concentration, carbon concentration (% dry wt.), TC and TN per unit area, C/N in the shoot of bryophytes and aboveground biomass of vascular plants. Different lowercase letters mean significantly difference between bryophytes and vascular plants at *p*=0.05 level (the same below)

Contents	Bryophytes	Vascular plants	n	*F*	*P*
^{15}N (*μ*g/g)	56.82±0.76	54.24±1.42	3	2.56	0.18
N (%)	1.55±0.04	1.52±0.04	4	0.56	0.48
C (%)	38.56±0.71*b*	42.10±0.23*a*	4	22.79	0.00
C/N	24.84±0.23*a*	27.89±0.57*b*	4	24.49	0.00
TN (g/m^2)	2.19±0.14*a*	0.64±0.04*b*	10	113.25	0.00
TC (g/m^2)	141.54±9.15*a*	42.25±2.35*b*	10	110.46	0.00

There was no significant difference of N content (N%) (*F*=0.56, *P*=0.48, Table 2) and isotopic ^{15}N abundance (*F*=2.56, *P*=0.18, Table 2) between bryophytes and vascular plants. Carbon content (C%) was significantly different between bryophytes and vascular plants (*F*=22.79, *P*<0.01, Table 2), and bryophytes had less carbon content than vascular plants. C/N ratio of vascular plants was significantly higher than bryophytes (*F*=24.49, *P*<0.01, Table 2). Although there was no significant difference of N concentration in 'per dry weight' biomass, total carbon (TC) and total N (TN) per unit area were different significantly (TC: *F*=110.46, *P*<0.001; TN: *F*=113.25, *P*<0.001) between bryophytes and vascular plants for their distinct biomass difference.

2.3 Comparison of ^{15}N abundance in bryophytes with injection of different ^{15}N forms

There were significant differences among the ^{15}N abundances in bryophytes with forms of $^{15}NO_3^-$, $^{15}NH_4^+$, ^{15}N-glycine injected respectively and control treatment (CK) (*F*=10.60, *P*<0.0001; Figure 2). The ^{15}N abundance labeled with nitrate-N in shoot of bryophytes was higher than treatments labeled with ammonia-N (*P*=0.830) and glycine-N (*P*=0.294). For the different preferential uptake of ^{15}N forms by two PFGs, there were variations of ^{15}N abundance values for each treatment, and ^{15}N-glycine was absorbed least although there is no significant difference. To sum up, bryophytes can take N in different forms from soil, and the ^{15}N abundance is 54.24±1.42 μg/g of no labeling treatment. All of the treatments with ^{15}N tracer injected are significantly different to control treatments whose ^{15}N abundances are 235.62±30.27 μg/g (*P*<0.001), 211.91±18.22 μg/g (*P*<0.01) and 160.86±8.60 μg/g (*P*<0.05) respectively.

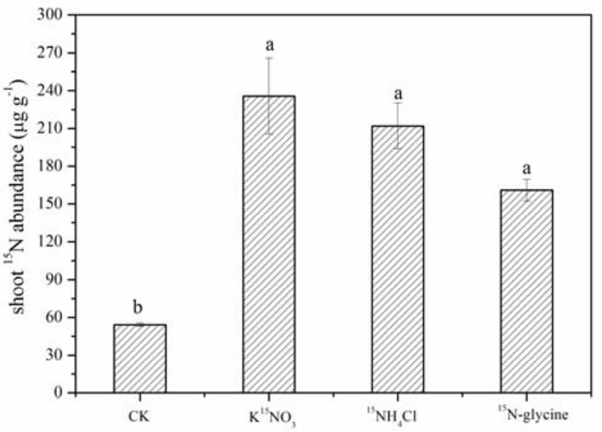

Figure 2 The variations of ^{15}N abundance in bryophytes shoots labeled with different chemical forms ^{15}N (Mean±SE, *n*=6).

2.4 N content, shoot excess ^{15}N, and ^{15}N recovered by bryophytes and vascular plants after growing season prolonged

Bryophytes and vascular plants had a different N content significantly (*F*=6.91, *P*=0.001) between two different lengths of growing season (Figure 3). They were evidenced by the greater N contents in a longer growing season (F_{12}=1.046, *P*=0.314 for interaction of two functional groups and 2 growing season lengths). With respect to different ability in uptake of N forms, there were also significant differences (*F*=4.26, *P*=0.047) in N content between two different functional groups in each kind of growing season length. Although three forms of ^{15}N added at different times conducted a "time mismatch" of N pulse, there were neither significant differences of bryophytes nor vascular plants between shorter growing season and longer growing season (Figure 4).

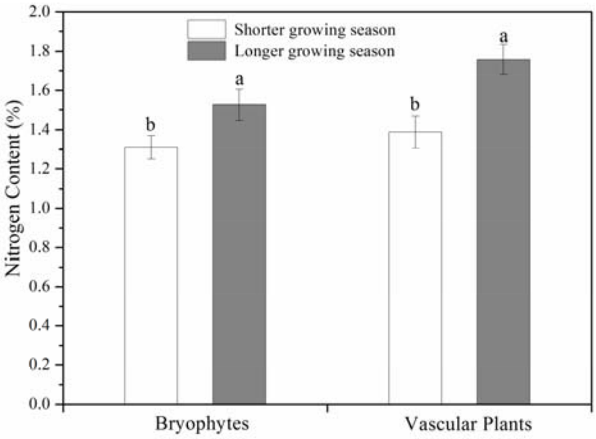

Figure 3 N content of bryophytes and vascular plants in two different growing season length (*n*=3). Different lowercase letters indicated significantly different between two growing season lengths (the same below).

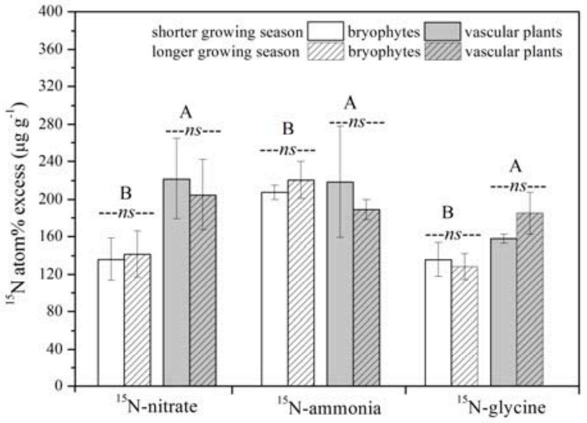

Figure 4 Shoot excess ^{15}N concentration of bryophytes and vascular plants, applied with labeled three chemical forms of dissolved ^{15}N in soil. Vertical bars represent standard error (*n*=3), and *ns* meant not significantly different between two growing season lengths while different capital letters indicated significant difference between bryophytes and vascular plants at *P*=0.05 level tested with Turkey's test (the same below).

The evidence of variation in preference for N forms found that both of bryophytes and vascular plants preferred the inorganic over organic N form (Table 3; *F*=0.168, *P*=0.685 for plant functional groups and 2 different length of growing season interaction. The same result was found for plant functional groups and 3 ^{15}N form treatments interactions, *F*=0.15, *P*=0.86). There was significant difference of variation in preference for N forms across growing season length (*F*=7.549, *P*=0.011) and N forms (*F*=9.946, *P*=0.001), respectively. There was no significant difference in shoot ^{15}N content at plant functional groups level (*F*=0.018, *P*=0.895). Bryophytes assimilated NH_4^+ more preferentially over NO_3^- and glycine (*F*=4.129, *P*=0.021). In addition there was no significant difference about the interaction of PFGs and 3 treatments (*F*=0.150, *P*=0.862) However, a marginally significant difference of ^{15}N content was detected in vascular plants between treatments (*F*=2.635, *P*=0.078), demonstrating that they assimilated more ^{15}N from NO_3^- in shoot material than that from NH_4^+ and glycine.

Table 3 Multifactorial analysis of variance for the effects of ^{15}N spiking date, PFGs, ^{15}N forms and their interactions on ^{15}N recovery by plants. *P* values for significant effects and interactions are in bold.

Source of variation	^{15}N atom% excess		^{15}N recovery by vascular plants and bryophytes	
	F value	*P* value	*F* value	*P* value
^{15}N spiking date	7.549	**0.011**	5.523	0.027
PFGs	0.018	0.895	137.778	**<0.001**
^{15}N forms	9.946	**0.001**	7.019	**0.004**
^{15}N spiking date *PFGs	0.168	0.685	2.481	0.128
^{15}N spiking date *^{15}N forms	4.152	**0.028**	2.592	0.096
PFGs*^{15}N forms	0.150	0.862	2.982	0.070
^{15}N spiking date *PFGs*^{15}N forms	0.598	0.558	1.128	0.340

In addition, considering their different phytomass (Table 3), ^{15}N recovery by bryophytes and vascular plants from three forms of ^{15}N in soil at two spiking time was calculated and presented in Figure 5, and the effects of ^{15}N spiking date, PFGs, ^{15}N forms and their interactions on ^{15}N recovery were concluded in Table 3. Although there was no significant difference in three forms of ^{15}N between bryophytes and vascular plants being in different lengths of growing season, ^{15}N recovery by bryophytes showed a different pattern to vascular plants with ^{15}N injection at different dates (Figure 5). Because of more phytomass per unit area, ^{15}N recovery by bryophytes of three forms ^{15}N was significantly greater than vascular plants (*F*=137.778, *P*<0.001). The values of ^{15}N recovery by bryophytes and vascular plants were significantly different between three different forms of N with spiking date (*F*=7.019, *P*=0.004). There were no interactions of spiking date, PFGs and ^{15}N forms except a marginally significant difference of interaction from PFGs and ^{15}N forms (*F*=2.982, *P*=0.070).

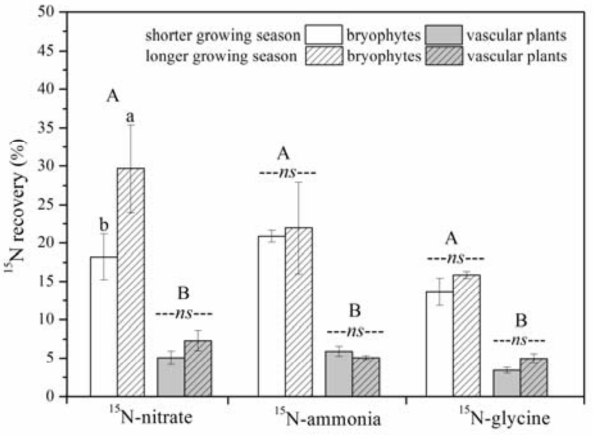

Figure 5 ^{15}N recovery by bryophytes and vascular plants (% of added ^{15}N) from $^{15}NH_4^+$, $^{15}NO_3^-$ and ^{15}N-glycine) of two spiking dates from May to October.

3 Discussion

Uptake of N by bryophytes from soil directly was testified by spiking three forms of stable isotope ^{15}N tracers in this study. In boreal ecosystem Ayres et al. (2006) also demonstrated uptake of N by bryophytes from soil not just from atmosphere deposition. However, compared with no ^{15}N tracer added control treatment, there are significant differences among the uptake of different chemical forms of N by bryophytes (*F*=10.60, *P*<0.0001). Rather, no significant difference of N uptake across three chemical forms by bryophytes might indicate that bryophytes adapted to scarcity of available N in alpine ecosystem. Furthermore, since many ecosystems are experiencing increased anthropogenic atmospheric N deposition (Vitousek et al. 1997), absorption of soil N by widespread bryophytes may have consequences for their ability to tolerate N pollution and potentially species specific negative impacts (Potter et al. 1995; Nilsson et al. 2002; Zvereva and Kozlov 2011), which will have some cascade effects upon alpine ecosystems.

N utilization efficiency of vascular plants is significantly higher than bryophytes (Table 2, *F*=24.49, *P*<0.01) due to their stronger capacity in assimilating carbohydrates and photosynthesis. Bryophytes have less carbon content than vascular plants, because bryophytes synthesize greater concentrations of phenolics and nonpolar compounds in tissues rather than lignin (Turetsky 2003; Eskelinen et al. 2009). Moreover, N utilization efficiency of bryophytes is at least partly related to N recycling during senescence, while vascular plants differ from bryophytes in their N assimilation strategy, mainly relying on N mineralized from litter or soils, and absorbed through roots and vascular tissue (Malmer et al. 1994). Thus, vascular plants can acquire nutrients during the growing season with developed vascular tissue and larger root volume enabling their uptake of resources from deeper soil layer. Besides, soil structure of alpine meadow is loose, whose leaching will have a great effect on the soil N distribution. Consequently, during a longer growing season, N can be supplied to vascular plants from subsoil. Instead, without “regular” developed root system, bryophytes just distributed their rhizoid in shallow soil.

Generally, bryophytes are very efficient in assimilating N, which are competitive scavengers of N and can reduce N availability of vascular plants, because bryophytes can not only absorb N from soil directly but can also fix atmospheric N_2 by forming facultative symbioses with cyanobionts (DeLuca et al. 2002; Turetsky 2003; Ayres et al. 2006). In addition, bryophytes absorb more ammonium-N than nitrate-N and glycine-N. It was had been demonstrated that nitrate must be converted to ammonium before it is combined with carbon compounds (Haynes and Goh 1978; Margeson et al. 1980). After the exposure of bryophytes to both ammonium and nitrate, nitrate reductase activity can be lowered by inhibiting NR-enzyme induction or by enhancing degradation of the induced enzyme because of the ammonium accumulation in tissues (Jauhiainen et al. 1998). Therefore, vascular plants absorbmore nitrate-N which indicates that for the nutrient competition ability from soil, vascular plants may be favored over non-vascular plants (Aerts et al. 2006; Nord and Lynch 2009). As some previous work illustrates, nitrate-N is highly soluble and mobile in soil, acquired in the transpiration-driven bulk flow of soil solution (Rengel 1993; Cardon and Gage 2006). On the contrary, ammonium-N is a low-mobility resource (Nord and Lynch 2009), whose acquisition is primarily influenced by root length duration, and the lower use efficiency of glycine-N can be mainly for its larger molecular size. However, by being relatively independent of ammonium or nitrate for their N nutrition, bryophytes also have the ability to short-cut the N cycle, which would give them a great ecological advantage in soils with low available N.

^{15}N recovery of three forms ^{15}N by bryophytes was significantly greater than vascular plants for their different aboveground phytomass. Furthermore, this study supports the view that widespread bryophytes species in “cold biomes” may derive a significant proportion of their N demand from free amino acids. It might give them a competitive advantage for not merely depending on inorganic N sources. Niche differentiation reduces competition for soil N, and its signature should be evident in patterns of species dominance (e.g., productivity). That is, dominant species should use the most available forms of soil N and subordinate species less available forms (McKane et al. 2002). Different functional groups follow different N use strategies to meet their N demands (Kahmen et al. 2006), and different responses by different functional groups in a community may buffer changes in community-level resource acquisition and net primary productivity (Cole et al. 2008; Rich et al. 2008).

N acquisition is altered due to seasonal resource dynamics. Plants responded to the asynchrony of resources availability between bryophytes and vascular plants. Plant productivity and patterns of N retention can be altered by a prolonged growing season for the variation of soil available N. In spring, plant activity in alpine ecosystems is primarily initiated by temperature, while the end time of a growing season it can be triggered by temperature, photoperiod and other issues (Shaver and Kummerow 1992; Oberbauer et al. 1998). Thus, early spring is critical to plant development and even a short period of ‘time mismatch’, e.g. 10 days can trigger the alteration of N uptake. In addition, mineralization rates generally increase with temperature, which would enhance nutrient availability (Rustad et al. 2001). However, nutrient availability may also be indirectly affected by temperature for the variation of soil moisture or the input of organic matter to soil (Cassman and Munns 1980; Zaman and Chang 2004). Therefore, prediction of the changes in N availability and plant community structure is complicated and difficult.

4 Conclusion

This study aims to verify the direct uptake of soil N by bryophytes at plant functional group levels, explores the potential effects of manipulated alteration of growing season length, and compares the acquisition of soil N between bryophytes and vascular plants. Based on soil N pulse in different time, all of the objectives were conducted through injection of stable isotope ^{15}N with three different chemical forms. The conclusions are as follows:

a) In alpine meadow habitats, bryophytes can directly derive not only inorganic N but also small molecule organic N (i.e. amino acids) from soil, which may indicate their capacity to tolerate N-pollution to be lower than that currently appreciated. Thus, bryophytes can be used as one of indicators monitoring not only environmental pollution, but also the alteration of soil N availability triggered by prolonged growing season.

b) Vascular plants may be favored over non-vascular plants in seasonal resource dynamics and nutrient cycling, but the recovery of soil N by bryophytes is greater than vascular plants owing to greater phytomass of bryophytes. Therefore, bryophytes are also crucial to soil N dynamic or pulse caused by the variation of growing season duration. Besides, the trophic niche complementarity, i.e., the differences of preferential N absorption between bryophytes and vascular plants or diversified N forms, could contribute to the formation of community structure and stability in alpine ecosystems.

This study does not conduct all the possible cases in which resource acquisition might respond to duration variation of growing season, because shifts of plant phenological range can alter the availability of water and N in complicated ways. Instead, it aims to explore some potential effects of prolonged growing season induced by global change on the nutrients acquisition of plants.

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