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**Nitrogen retention  
patterns and  
controlling factors**

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# Nitrogen retention patterns and their controlling factors in an alpine meadow: implications for carbon sequestration

X. L. Xu<sup>1</sup>, H. Ouyang<sup>1</sup>, and G. M. Cao<sup>2</sup>

<sup>1</sup>Key Laboratory of Ecosystem Network Observation and Modelling, Institute of Geographic Sciences and Natural Research Resources, Chinese Academy of Sciences, Datun Road A 11, Chaoyang District, Beijing 100101, P. R. China

<sup>2</sup>Northwest Plateau Institute of Biology, Chinese Academy of Sciences, No. 59, Xiguan Street, Xining 810001, P.R. China

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Correspondence to: X. Xu (xuxingl@hotmail.com)

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## Abstract

We hypothesized that the patterns of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  retention are different over short-term scales while they are similar over long-term scales in alpine meadows and that abiotic and biotic factors might be responsible for their different patterns over short-term scales. In order to test the hypotheses, a  $^{15}\text{N}$ -labeled experiment was conducted in an alpine meadow in the Qinghai-Tibet Plateau over four years. Our results showed that  $^{15}\text{NO}_3^-$  and  $^{15}\text{NH}_4^+$  retention was distinctly different within two months, and even one year after tracer additions. The long-term retention of  $^{15}\text{N}$  at the whole-plot level did not differ significantly between  $^{15}\text{NH}_4^+$  and  $^{15}\text{NO}_3^-$  treatments, and averaged 50% after four years. Higher soil temperature or soil organic carbon concentration enhanced  $^{15}\text{NH}_4^+$  retention, but significantly reduced  $^{15}\text{NO}_3^-$  retention in the soil within two months following tracer additions. Soil moisture significantly affected  $^{15}\text{N}$  recovered in soil organic matter and microbial biomass as well as aboveground parts, but had no significant effects on  $^{15}\text{N}$  recovered in roots. These findings have important ecological implications with regard to the consequences of deposited nitrogen because of the possible difference in the fate of  $\text{NH}_4^+$  vs.  $\text{NO}_3^-$  in alpine meadow ecosystems.

## 1 Introduction

Nitrogen (N) is an element which is most affected by human activities on the earth. Up to date human activities have approximately doubled the annual N input to terrestrial ecosystems through a variety of mechanisms (Galloway et al., 1995; Vitousek et al., 1997; Galloway et al., 2004). A growing body of evidence shows that available N supplies often limit plant growth in a wide range of terrestrial ecosystems such as temperate forests (Flanagan and Van Cleve, 1983; Aber et al., 1995; Hedin et al., 1995; Perakis and Hedin, 2001; Perakis et al., 2005), temperate grasslands (Lauenroth et al., 1978; Harrison et al., 1994), and tundra (Shaver and Chapin, 1980; Nadelhoffer

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et al., 1992; Chapin et al., 1995) as well as alpine ecosystems (Bowman et al., 1993; Cao and Zhang, 1999; Makarov et al., 2001). Hence, N retention in a wild variety of terrestrial ecosystems has been extensively investigated to improve our understanding of the consequences resulting from anthropogenic N input (Wright and Tietema, 1995; Magill et al., 1997; Vitousek et al., 1997; Matson et al., 2002; Aber et al., 2003; Fenn et al., 2003; Townsend et al., 2003; Stevens et al., 2004; Zak et al., 2004).

Biogeochemical processes have been suggested to be mediated by interactions among physical and biological factors operating across a wide range of spatial and temporal scales. Accordingly, abiotic and biological factors have been considered as important mechanisms responsible for N retention in terrestrial ecosystems. For example, three possible mechanisms have been recognized to be responsible for abiotic N retention: ionic substitution of  $\text{NH}_4^+$  in soils (Young and Aldag, 1982; Stevenson, 1994), reduction of  $\text{NO}_2^-$  by humic substances at low pH and condensation of amino acids or  $\text{NH}_3$  with phenolic compounds (Nömmik, 1965, 1970; Nömmik and Vahtras, 1982; He et al., 1988; Thorn and Mikita, 1992). In contrast, microbial assimilation and plant uptake have been suggested as a critical pathway for biotic N retention (Vitousek and Reiners, 1975; Zak et al., 1990; Epstein et al., 1998; Hooper and Vitousek, 1998; Zogg et al., 2000; Epstein et al., 2001; Templer, 2001; Templer et al., 2005).

In order to obtain a clear insight into the mechanisms above, a large number of studies have recently been conducted to understand the role of abiotic and biotic factors in N retention in different terrestrial ecosystems. Fisk et al. (1998) have showed that the topographic soil moisture gradient controls N retention patterns in alpine tundra. As one of the most important abiotic factors, temperature has been shown to control N cycling in terrestrial ecosystems (Van Cleve, 1983; Hill and Shackleton, 1989; Tietema and Verstraten, 1992), but little is known about the role of soil temperature in N retention in terrestrial ecosystems. In contrast, soil organic matter has won more concerns as one of the most important soil properties. It shows that SOC has a great influence on abiotic N immobilization in grassland soils (Barrett and Burke, 2000; Barrett et al., 2002) and plays an important role in stabilizing different inorganic N forms

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in both grassland (Barrett and Burke, 2000; Barrett et al., 2002; Kaye et al., 2002) and forest soils (Johnson et al., 2000; Dail et al., 2001). Besides abiotic factors above, plants have also been shown to have an influence on N retention in both forests (Templer, 2001; Templer et al., 2005) and steppes (Epstein et al., 1998, 2001). Moreover, Johnson et al. (2000) compared abiotic and biotic N retention in a variety of forest soils and showed that abiotic N immobilization can be a significant process in these forest soils. However, most studies in this regard have focused on  $^{15}\text{NH}_4^+$  or  $^{15}\text{NO}_3^-$  retention in grasslands (Hart et al., 1993; Barrett et al., 2002) and forests (Hart et al., 1993; Johnson et al., 2000; Zogg et al., 2000; Dail et al., 2001; Perakis and Hedin, 2001; Zak et al., 2004). Only a few have been conducted to compare the patterns of  $^{15}\text{NO}_3^-$  and  $^{15}\text{NH}_4^+$  retention in studied ecosystems, except in a number of forest ecosystems (Bengtsson and Bergwall, 2000; Perakis and Hedin, 2001; Fitzhugh et al., 2003). By comparison, very few have been conducted to explore the difference between short-term and long-term N retention patterns of  $^{15}\text{NO}_3^-$  and  $^{15}\text{NH}_4^+$  in grasslands, especially in alpine meadows in the Tibet Plateau.

Alpine meadows occupy more than 35% of the Tibet Plateau which extends over 2.5 million  $\text{km}^2$ . These meadows are very fragile and sensitive to environmental changes due to the huge altitude of the Plateau. Previous studies showed that plant growth is limited by available N supplies with an output of  $159 \text{ kg hm}^{-2} \text{ yr}^{-1}$  and an input of  $85 \text{ kg hm}^{-2} \text{ yr}^{-1}$  (Cao and Zhang, 1999) while this region is experiencing N input of  $7.2\text{--}10 \text{ kg N hm}^{-2} \text{ yr}^{-1}$  through rain (Zuo et al., 1986). We have investigated the patterns of  $^{15}\text{NO}_3^-$  and  $^{15}\text{NH}_4^+$  retention over 13 months in this kind of ecosystems (Xu et al., 2003, 2004b). Meanwhile, we measured soil temperature at 5 cm depth as well as soil moisture on each sampling day when gas samples were processed within two months following tracer additions. Subsequently, we sampled soil and plant materials again over four years after tracer additions. This permits us to test the following hypotheses: (1)  $^{15}\text{NO}_3^-$  and  $^{15}\text{NH}_4^+$  retention patterns are different over short-term scales while they are similar over longer term scales in alpine meadows, and (2) abiotic and biotic factors are responsible for the difference between  $\text{NO}_3^-$  and  $\text{NH}_4^+$  retention patterns over short-

term scales.

## 2 Materials and methods

### 2.1 Site description

This study was carried out in a typical *Kobresia pygaea* meadow around Haibei Research Station of Alpine Meadow Ecosystem, the Chinese Academy of Sciences, located in the northeast of the Tibetan Plateau. This area is characterized by a typical alpine meadow climate. During the past 30 years annual precipitation averaged 560 mm, 85% of which was concentrated in the growing season (from May to September). Annual temperature averaged  $-1.7^{\circ}\text{C}$ . Dominant species in this kind of meadows are *Kobresia pygaea*, *Ptilagrostis concinna*, *Saussurea superba*, *Potentilla nivea*, *Potentilla bifurca*, *Gentiana straminea*, *Leonto podium nanum* and *Thalictrum alpinum* (Zhou and Li, 1982). The soil is classified as Mat Cryo-gelic Cambisol (Bao et al., 1995; Chinese Soil Taxonomy Research Group, 1995) corresponding to Gelic Cambisol (WRB, 1998).

Three sites were selected near Haibei Research Station of Alpine Meadow Ecosystem in July, 2000. Site I is located at Haibei Research Station of Alpine Meadow Ecosystem of the Chinese Academy of Sciences, Qinghai Province with an altitude of 3215 m above sea level ( $37^{\circ} 36' \text{N}$ ,  $101^{\circ} 19' \text{E}$ ), site II located 16 km northwest of the station with an altitude of 3515 m above sea level ( $37^{\circ} 52' \text{N}$ ,  $101^{\circ} 02' \text{E}$ ), whereas site III located at the south slope ( $<30^{\circ}$ ) of Oboling, 24 km northwest of the station with an altitude of 3715 m above sea level ( $38^{\circ} 00' \text{N}$ ,  $100^{\circ} 55' \text{E}$ ).

### 2.2 $^{15}\text{N}$ addition

In July, 2000, a block ( $15 \times 15 \text{ m}$ ) uniform in species composition and cover was selected in this type of meadow at each site. Three plots ( $3 \times 3 \text{ m}$ ) were established with 2-m wide buffer zones between them. On 26 July, 2000,  $\text{Na}^{15}\text{NO}_3$  (99.26 atom%) and

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( $^{15}\text{NH}_4$ ) $_2\text{SO}_4$  (99.40 atom%) were dissolved in  $\text{H}_2\text{O}$  and sprayed on two of the three plots at rates of 4.4 and 5.6 kg N  $\text{hm}^{-2}$ , respectively. Another amount of  $\text{H}_2\text{O}$  was sprayed on each plot in order to prevent more  $^{15}\text{N}$  from being absorbed on the leaves. The total amount of  $\text{H}_2\text{O}$  was equivalent to 2 mm of rain. At the same time, equivalent  $\text{H}_2\text{O}$  was sprayed on the third plot as the control.  $^{15}\text{N}$  tracers were added to alpine meadows only for one growth season.

### 2.3 Sampling and analyzing

Soils and plants were collected 2, 4, 6 and 8 weeks after  $^{15}\text{N}$  addition at per site. Four years after  $^{15}\text{N}$  additions we collected the samples at site I and III again, but did not collect samples at site II because the plots were destroyed. Detailed description about sampling and analyzing are available elsewhere (Xu et al., 2004a). Briefly, we collected soils using a soil corer (2.7 cm in diameter, 15 cm in depth). Live roots carefully removed from the soil cores were used to estimate root biomass. Aboveground parts were estimated by harvesting a 25×25 cm square (n=3). Dried soil and plant materials were used to measure total N,  $^{15}\text{N}/^{14}\text{N}$  ratio and organic C.

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 Kalem-basa and Jenkinson (1973). Microbial biomass N (MBN) was estimated by a chloroform fumigation-direct extraction method (Brookes et al., 1985).  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N in  $\text{K}_2\text{SO}_4$  extracts were measured by stream distillation with  $\text{MgO}$ , using Dewarda's alloy to reduce  $\text{NO}_3^-$  to  $\text{NH}_4^+$  (Bremner, 1965). All the samples for  $^{15}\text{N}$  analysis followed the methods described by Buresh et al. (1982) and Pruden et al. (1985), except  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N samples, using a Finnigan MAT-251 mass spectrometer. Soil temperature at 5 cm depth was measured by geothermometers when gas samples were being processed with a two-week intervals during two months following tracer additions (Xu et al., 2004a).

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## 2.4 $^{15}\text{N}$ recovery calculations

We calculated  $^{15}\text{N}$  recovery in plant and inorganic pools using the N mass, the amount of added  $^{15}\text{N}$  and the atom% excess  $^{15}\text{N}$  of the corresponding pools.  $^{15}\text{N}$  recovery of MBN and SOC pools were calculated following the description by Zogg et al. (2000).  $^{15}\text{N}$  recovered in the soil was calculated as the sum of  $^{15}\text{N}$  recovered in MBN, SOC and inorganic pools. We used  $^{15}\text{N}$  recovery as an estimate of net N retention in a given pool at a given time.

## 2.5 Statistics

Repeated measures analysis of variance was used to test for effects of N species, time, and N species x time interactions as well as soil moisture on  $^{15}\text{N}$  recovery of different components within two months following tracer addition. All results were considered significant at the  $P < 0.05$  level. Statistical calculations were run using a SPSS 11.5 statistical package for windows (SPSS Inc., Chicago, IL). Standard errors of the means were presented in the tables and on the figures as a variability parameter.

# 3 Results

## 3.1 $^{15}\text{N}$ recovery in different ecosystem components

Results from repeated measures analysis of variance calculations within two months following tracer addition indicated that the recovery of  $^{15}\text{NH}_4$  and  $^{15}\text{NO}_3$  were significant different in SOC, roots and green as well as inorganic N pools except MBN (Table 3). Detailed values of the recovery of  $^{15}\text{NH}_4$  and  $^{15}\text{NO}_3$  of different components at per site within two months following tracer addition were presented in Table 1. The concentration of inorganic N was considerably low and varies between 0.5 to 1.8 g m<sup>-2</sup> during growing seasons in this kind of alpine meadows, resulting in a remarkable low

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$^{15}\text{N}$  recovery at all three sites within two months following  $^{15}\text{N}$  additions (Table 1).  $^{15}\text{N}$  was mainly recovered in the four major pools: roots, green, SOC and MBN. Plants including roots and green exhibited a strong retention of  $^{15}\text{NO}_3^-$  and recovered more than 40% of added  $^{15}\text{NO}_3^-$  over three sites within two months following  $^{15}\text{N}$  addition. Though alpine plants retained nearly one third of added  $^{15}\text{NH}_4^+$ , more  $^{15}\text{NH}_4^+$  than  $^{15}\text{NO}_3^-$  was recovered in SOC on each sampling day. Time also exhibited significant effects on the short-term recovery of  $^{15}\text{NH}_4^+$  and  $^{15}\text{NO}_3^-$  of each of the five components, whereas there were no effects of interactions of N species by time within two months following tracer additions (Table 3). Results from repeated measures analysis of variance calculations within two months following tracer additions also indicated that there were significant effects of N species and time on the short-term total recovery of  $^{15}\text{N}$ , and no effects of interactions of  $^{15}\text{N}$  species by time (Table 3). More  $^{15}\text{NO}_3^-$  than  $^{15}\text{NH}_4^+$  was recovered in alpine meadows on short-term scales.

About one year after  $^{15}\text{N}$  addition a significant difference was still found between the recovery of  $^{15}\text{NO}_3^-$  and  $^{15}\text{NH}_4^+$  of the major three pools: plants, SOC and MBN at site I (Xu et al., 2004b). However, over four years after tracer additions the recovery of  $^{15}\text{NO}_3^-$  and  $^{15}\text{NH}_4^+$  were similar in roots, green, MBN and SOC pools at site I and site III (Table 2). About 50% of added N was still retained in alpine meadows over four years after tracer addition.

### 3.2 Effects of abiotic factors on $^{15}\text{N}$ recovery

Soil moisture varied wildly at site I and increased from 19% in early August to 29% in early September and decreased to 26% thereafter in late September. Those at both site II and site III varied slightly, but higher at site II than at site III (Fig. 1). Soil moisture had a significant effect on the  $^{15}\text{N}$  recovery of different N pools except roots at a significance level of 0.05 (Table 3).

Soil temperature at depth of 5 cm increased with increasing altitude. It was a bit higher at site I than at site II, with similar values at site II and site III (Fig. 2). Soil



temperature at depth of 5 cm correlated negatively with  $^{15}\text{N}$  recovered in the soil in the  $\text{NO}_3^-$  plots two weeks after  $^{15}\text{N}$  additions (Fig. 2a;  $R^2=0.64$ ,  $P=0.006$ ). In contrast, a weak positive correlation ( $R^2=0.12$ ,  $P<0.5$ ) was shown between soil temperature at depth of 5 cm  $^{15}\text{N}$  recovered in the soil in the  $\text{NH}_4^+$  plots two weeks after  $^{15}\text{N}$  additions (data not shown).

SOC increased with increasing altitude from 8.7% at site I to 9.6% at site III. The concentration of SOC was negative related to  $\text{NO}_3^-$   $^{15}\text{N}$  recovered in the soil (Fig. 3A;  $R^2=0.55$ ,  $P=0.47$ ), but positively correlated to  $\text{NH}_4^+$   $^{15}\text{N}$  recovered in the soil (Fig. 3b;  $R^2=0.70$ ,  $P=0.37$ ).

## 4 Discussion

We measured N retention at scales of weeks to years in a unique and relatively unexplored alpine meadow using pulse-chase tracer methods, which permits us to clarify short-term (within two months) and long-term (up to four years)  $^{15}\text{NO}_3^-$  and  $^{15}\text{NH}_4^+$  retention patterns in these alpine meadow ecosystems. Besides, we determined abiotic factors such as soil moisture, temperature and SOC when we investigated short-term N retention. This can allow us to demonstrate how abiotic factors affect short-term N retention patterns in alpine meadows.

### 4.1 Difference of $^{15}\text{N}$ retention of $^{15}\text{NO}_3^-$ and $^{15}\text{NH}_4^+$ in alpine meadows

Perakis and Hedin (2001) have demonstrated a very similar N pattern of  $^{15}\text{NO}_3^-$  and  $^{15}\text{NH}_4^+$  in an unpolluted temperate forest over short-term (days to weeks) and long-term (up to two years) time scales. In our study a significant different  $^{15}\text{NO}_3^-$  and  $^{15}\text{NH}_4^+$  retention pattern was shown within two months following tracer addition (Table 3), even one year after  $^{15}\text{N}$  addition at site I (Xu et al., 2004b). However,  $^{15}\text{NO}_3^-$  and  $^{15}\text{NH}_4^+$  exhibited a very similar N retention pattern four years after tracer additions.

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Perakis and Hedin (2001) ascribe the similarity in the retention of  $^{15}\text{NO}_3^-$  and  $^{15}\text{NH}_4^+$  to a lack of preferential retention of either form of inorganic N, and further suggest that it is the result of similar mechanisms of uptake and redistribution in the plant-microorganism-soil system. In our study the difference in retention of  $^{15}\text{NO}_3^-$  and  $^{15}\text{NH}_4^+$  over short-term scales can be explained by the properties of the two ions. In the soil solution  $\text{NO}_3^-$  ions are more mobile than  $\text{NH}_4^+$  ions (Owen and Jones, 2001) whereas  $\text{NH}_4^+$  ions are easily bonded by soil colloids. Additionally, higher SOC concentration (more than 8.0%) occurs in this kind of soils. Therefore, it is easier for plants to acquire  $\text{NO}_3^-$  than  $\text{NH}_4^+$  from soils, which is confirmed by the correlations between SOC and  $^{15}\text{N}$  recovered in the soil from  $^{15}\text{NO}_3^-$  and  $^{15}\text{NH}_4^+$  treatments over short time scales (Fig. 3). Over two months following tracer additions, alpine plants always took up more  $^{15}\text{N}$  from  $\text{NO}_3^-$  than from  $\text{NH}_4^+$  (Table 1). Because  $\text{NO}_3^-$  and  $\text{NH}_4^+$  can be quickly transformed into each other in soil solutions (Zak et al., 1990; Zogg et al., 2000), our data didn't confirm whether alpine plants preferentially take up  $\text{NO}_3^-$  in a two-week interval. However, the results from our another experiment show that alpine plants take up more  $^{15}\text{N}$  from  $\text{NO}_3^-$  than from  $\text{NH}_4^+$  over a two-day scale (Song et al., 2007). This indicates that preferential uptake of  $^{15}\text{NO}_3^-$  by alpine plants might be a second explanation for the difference in retention of  $^{15}\text{NO}_3^-$  and  $^{15}\text{NH}_4^+$  in alpine meadows over short-term scales. Stronger stimulation of  $\text{NO}_3^-$  on root biomass than  $\text{NH}_4^+$  (Xu et al., 2004a) also seems to confirm our speculation above.

$^{15}\text{N}$  recovered in MBN varied significantly with time in spite of similar recovery of  $^{15}\text{NO}_3^-$  and  $^{15}\text{NH}_4^+$  from two weeks to two months after tracer addition (Tables 1 and 3). This clearly implies that MBN is a temporary N pool and rapid N replacement might occur in the MBN pool through internal cycling and that the difference in microbial assimilation of  $^{15}\text{NO}_3^-$  and  $^{15}\text{NH}_4^+$  in the soil might occur over days' scales. No statistical difference was observed between microbial  $^{15}\text{N}$  sink from  $\text{NH}_4^+$  and  $\text{NO}_3^-$  (Table 3), indicating that soil microorganisms didn't show a preferential uptake for either of inorganic N although rapid replacement occurred. The similarity in the retention of  $^{15}\text{NO}_3^-$  and

$^{15}\text{NH}_4^+$  over 4 years implies that added N has been stabilized in the two major pools (SOC and vegetation) and that the two N forms have the same destiny in the long run in alpine meadows.

Regarding the capacity to retain N, alpine meadows is compared to shortgrass prairie for  $^{15}\text{NO}_3^-$  (Clark, 1977) and annual grasslands for  $^{15}\text{NH}_4^+$  (Hart et al., 1993) within one month following tracer additions. However, four years after tracer addition the capacity to retain  $^{15}\text{NO}_3^-$  is much weaker in alpine meadows than in a shortgrass prairie (Clark, 1977).

#### 4.2 Effects of abiotic factors on N retention in alpine meadows

Over short-term scales  $^{15}\text{NH}_4^+$  retention in the soil increased with SOC content in alpine meadows (Fig. 3), as observed by Barret and Burke (2000) that potential N immobilization increases with increasing SOC content in grassland soils. By comparison, only a limited number of studies have involved  $\text{NO}_3^-$  retention in SOC. Dail et al. (2001) suggest that rapid abiotic transformation plays an important role in  $\text{NO}_3^-$  assimilation in an acid forest soil. Perakis and Hedin (2001) show that SOC possesses a slightly higher affinity for added  $^{15}\text{NO}_3^-$  than  $^{15}\text{NH}_4^+$  over one day scale. Our study shows that  $^{15}\text{NO}_3^-$  retention decreased with increasing SOC concentration in alpine meadow soils over short-term scales under field conditions (Fig. 3). This implies that abiotic processes might play less important role in  $\text{NO}_3^-$  assimilation in alpine meadow soils than in forest soils (Dail et al., 2001). In other words, biotic immobilization such as microbial assimilation and plant uptake might be important pathway for  $\text{NO}_3^-$  retention in alpine meadows.

Although soil temperature at 5 cm depth was similar at site II and site III, temperature at site I was significant higher than those at the other two sites. These data at least show that higher soil temperature reduced  $^{15}\text{NO}_3^-$  retention in the alpine meadow soils (Fig. 2a). Possible explanation is that higher soil temperature simulates  $^{15}\text{NO}_3^-$  uptake by plants and thus reduces  $^{15}\text{NO}_3^-$  recovered in the soil, e.g. numerous studies show

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that higher temperature enhances  $\text{NO}_3^-$  uptake by plants using solution culture (Clarkson and Warner, 1979; Macduff and Jackson, 1991; Cruz et al., 1993). A strong positive correlation between soil temperature and  $^{15}\text{NO}_3^-$  uptake by alpine plants (Fig. 2b) further confirms this. In contrast, higher soil temperature slightly reduced  $^{15}\text{NH}_4^+$  retention in the soil with a weaker correlation (data not shown). Several studies show that uptake of  $\text{NH}_4^+$  increases with root temperature in solution culture and often exceeds uptake of  $\text{NO}_3^-$  (Clarkson and Warner, 1979; Macduff and Jackson, 1991). However, in this study only a weak negative correlation was presented between soil temperature and  $^{15}\text{NH}_4^+$  uptake by plants (data not shown). This implies that different mechanisms are responsible for uptake of  $^{15}\text{NO}_3^-$  and  $^{15}\text{NH}_4^+$  and their redistribution in alpine meadows. The behavior of  $\text{NH}_4^+$  ions in solution culture is different from that in soil solution because of the complicated nature of soil organic matter. Higher soil temperature might strengthen the condensation of  $\text{NH}_3$  with phenolic compounds as well as the combination of  $\text{NH}_4^+$  with soil colloids, but few data are available in this respect. Hence, a real mechanism behind them needs to be investigated in future.

Fisk et al. (1998) have shown that the topographic soil moisture gradient controls fundamentally the patterns of N turnover among communities. In this study soil moisture significantly affected  $^{15}\text{N}$  recovered in both SOC and MBN ( $P < 0.05$ ). The reason is that microbial processes are strongly related to soil moisture (Fisk et al., 1998), which results in the variation of  $^{15}\text{N}$  recovered in both MBN and SOC.  $^{15}\text{N}$  recovered by green was also significantly influenced by soil moisture ( $P < 0.005$ ) while  $^{15}\text{N}$  recovered by roots was not affected by soil moisture ( $P < 0.5$ ). Potential explanation is that N transport from roots to shoots might be controlled by soil water conditions.

## 5 Conclusions

These results confirmed the hypothesis that the patterns of  $^{15}\text{NO}_3^-$  and  $^{15}\text{NH}_4^+$  retention are significantly different over short-term scales in alpine meadows, lasting over

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one year after tracer additions, whereas they are very similar four years after tracer additions. Abiotic and biotic factors account for a part of the difference between  $\text{NO}_3^-$  and  $\text{NH}_4^+$  retention in alpine meadows over short-term scales. SOC controls  $^{15}\text{NH}_4^+$  retention whereas plants play more important role in  $^{15}\text{NO}_3^-$  retention over a short-term scale. This finding provides a clear implication that N forms should be taken into account when the consequences of deposited N are assessed in alpine ecosystems over at least about one year scale. It is because there is no reason to believe that  $\text{NO}_3^-$  has less impact on species composition than  $\text{NH}_4^+$  when  $\text{NO}_3^-$  enhances root biomass more than  $\text{NH}_4^+$  in alpine meadows (Xu et al., 2004a).

Moreover, most of N is often bonded with organic C in terrestrial ecosystems. In this study half of added N is still retained in alpine meadows four years after N additions (Table 2). We here assume that an average C to N ratio is 15 for the organic substances responsible for N retention. The minimal C sequestration derived from this part N is estimated to be about  $75 \text{ kg hm}^{-2}$  per year because this estimation is only based the fate of added N and without considering the effects of added N on plant biomass. Plant growth is limited available N supplied, which is mainly caused by low temperature in this kind of meadows. At the same time, it is experiencing N deposition through rain. More than 50% of added N can be retained in the plant-microorganism-soil system for about four years. This indicates that N deposition can make a great contribution to C sequestration in alpine meadows in the Qinghai-Tibet Plateau.

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**Table 1.** <sup>15</sup>N recovery in plant and soil components 2 wk, 4 wk, 6 wk and 8 wk after <sup>15</sup>N addition in alpine meadows at the three sites. Means ±SE of three replicates at each site are shown.

Sites	Components	2wk		4wk		6wk		8wk	
		<sup>15</sup> NO <sub>3</sub> <sup>-</sup>	<sup>15</sup> NH <sub>4</sub> <sup>+</sup>	<sup>15</sup> NO <sub>3</sub> <sup>-</sup>	<sup>15</sup> NH <sub>4</sub> <sup>+</sup>	<sup>15</sup> NO <sub>3</sub> <sup>-</sup>	<sup>15</sup> NH <sub>4</sub> <sup>+</sup>	<sup>15</sup> NO <sub>3</sub> <sup>-</sup>	<sup>15</sup> NH <sub>4</sub> <sup>+</sup>
I	Roots	43.2±4.4	18.5±2.0	22.6±2.0	13.3±1.5	30.4±1.6	10.4±1.5	36.6±1.4	22.5±1.4
	Green	16.8±1.4	9.0±1.5	17.8±1.5	9.2±1.2	17.6±1.4	5.3±1.3	12.8±1.3	8.8±0.5
	SOC	10.9±3.7	33.8±3.5	6.9±1.0	11.8±0.7	10.2±1.1	22.5±0.4	20.7±1.4	29.5±5.2
	MBN	13.7±0.8	13.7±0.5	16.4±1.0	20.5±0.8	13.0±0.4	8.7±0.2	9.3±1.8	11.6±1.3
	Inorganic	0.21±0.02	0.02±0.00	0.20±0.02	0.02±0.00	0.31±0.04	0.16±0.02	0.27±0.03	0.04±0.00
II	Roots	43.6±0.8	27.9±0.6	37.8±2.1	20.2±0.9	41.3±1.1	28.2±2.2	45.9±2.6	34.1±0.2
	Green	6.6±0.5	6.8±0.3	12.1±1.4	11.2±0.8	6.9±0.7	3.9±1.0	4.7±1.0	2.4±0.4
	SOC	22.2±0.7	25.4±3.2	8.0±1.2	8.0±1.9	21.8±1.7	19.0±1.7	24.5±2.2	25.5±4.2
	MBN	10.9±0.5	12.7±0.1	16.5±1.6	16.7±0.3	9.9±0.6	7.5±0.3	12.4±1.5	8.4±1.7
	Inorganic	0.54±0.03	0.44±0.02	0.27±0.01	0.20±0.02	0.26±0.01	0.25±0.01	0.07±0.00	0.24±0.07
III	Roots	24.7±1.4	22.7±1.5	26.0±2.6	19.4±2.3	23.4±3.0	17.0±2.1	34.7±0.8	21.3±1.3
	Green	11.3±1.5	7.5±1.4	9.3±1.7	8.3±0.7	9.1±1.1	7.2±2.3	5.7±0.9	2.3±0.6
	SOC	22.7±1.9	26.4±0.5	6.0±1.0	7.8±1.4	18.1±1.2	13.8±1.2	18.0±1.9	19.5±0.7
	MBN	13.8±0.6	20.8±0.3	29.0±2.2	21.6±0.9	7.6±2.2	5.0±0.3	8.5±0.3	5.9±0.3
	Inorganic	1.09±0.13	0.59±0.01	0.31±0.03	0.23±0.02	0.28±0.04	0.11±0.01	0.14±0.02	0.09±0.00

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**Table 2.** <sup>15</sup>N recovery in plant and soil components 4 years after tracer additions at site I and III. Means ±SE of three replicates at per site are shown.

Sites	Components	4 years after <sup>15</sup> N addition	
		<sup>15</sup> NO <sub>3</sub> <sup>-</sup>	<sup>15</sup> NH <sub>4</sub> <sup>+</sup>
I	Roots	20.2±3.8	17.2±1.9
	Green	3.5±0.7	1.8±0.3
	SOC	23.4±1.3	26.2±3.7
	MBN	3.3±0.2	3.7±0.2
III	Roots	22.5±4.6	14.8±0.9
	Green	2.5±0.6	2.6±0.3
	SOC	22.5±2.3	27.0±4.3
	MBN	3.4±0.4	3.2±0.8

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**Table 3.** Results of repeated-measures ANOVA for the effects of  $^{15}\text{N}$  species added, time, and  $^{15}\text{N}$  species x time as well as soil moisture on  $^{15}\text{N}$  recovery of different components within two months following tracer additions.

Nitrogen pools	$^{15}\text{N}$ species	Time	<i>P</i>	
			$^{15}\text{N}$ species x time	Soil moisture
SOC	<0.001	<0.001	0.07	0.020
MBN	0.340	<0.001	0.17	0.020
Roots	<0.001	0.001	0.95	0.130
Green	<0.001	0.004	0.81	0.004
Inorganic	0.002	<0.001	0.20	0.006
Whole-plot total	<0.001	<0.001	0.24	0.040

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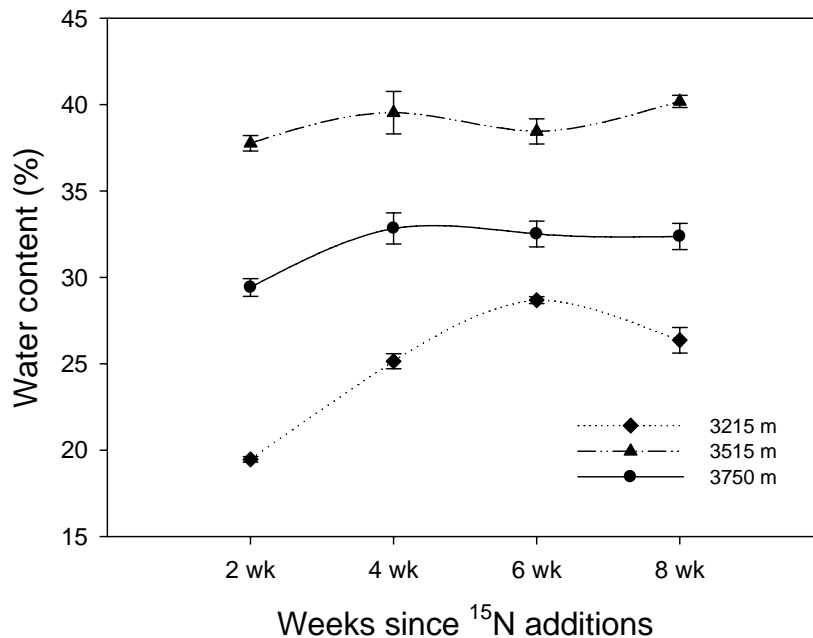
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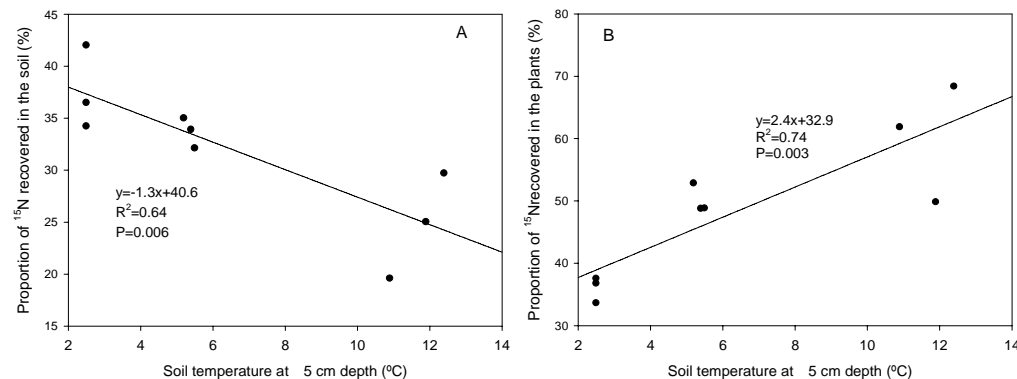


**Fig. 1.** Changes in soil moisture at the three sites 2 wk, 4 wk, 6 wk and 8 wk following tracer additions to alpine meadows. Values are means ( $\pm$ SE) of six replicates.

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**Fig. 2.** Relationship between soil temperature at depth of 5 cm and  $^{15}\text{N}$  retention in soils (A) and plants (B) of the  $^{15}\text{NO}_3^-$  plots two weeks after tracer additions.

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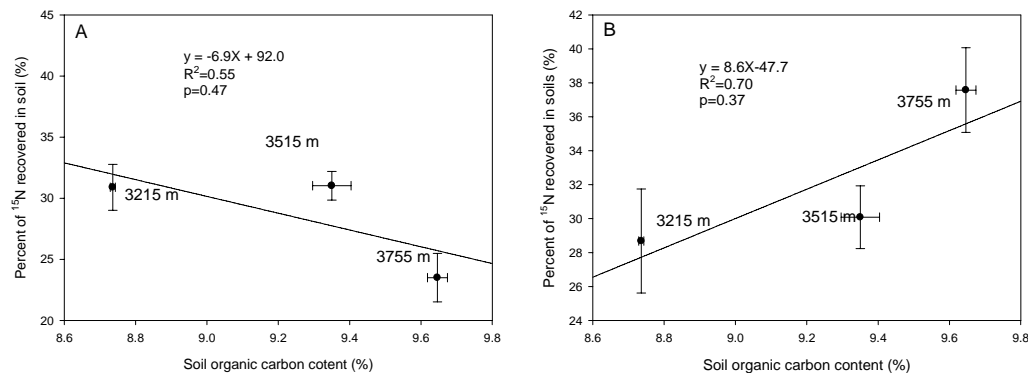
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Interactive Discussion



## Nitrogen retention patterns and controlling factors

X. Xu et al.



**Fig. 3.** Relationship between SOC content and  $^{15}\text{N}$  retention in the soil of  $^{15}\text{NO}_3^-$  plots (A) and  $^{15}\text{NH}_4^+$  plots (B) two weeks after  $^{15}\text{N}$  additions. Values are means ( $\pm$ SE) of 9–12 replicates.

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