# 人工草地氮素分流:青藏高原野外$^{15}N$示踪实验研究

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**摘要**:本论文应用$^{15}N$示踪技术,野外原位研究三种高寒牧草垂穗披碱草(*Elymus nutans*)、中华羊茅(*Festuca sinensis*)和冷地早熟禾(*Poa crymophila*)对$^{15}NH_4^+$—N和$^{15}NO_3^-$—N的吸收,检测$^{15}N$示踪剂在施肥和不施肥人工草地在地上部分茎叶、根系和土壤中的分布.旨在揭示高寒牧草氮草吸收特征,加深对高寒人工草地氮素循环的理解,为高寒人工草地可持续管理提供科学依据.研究结果表明(1)在不施肥人工草地,三种高寒牧草茎叶$\delta^{15}N$天然丰度差异为3.10‰(0.32‰—3.42‰),在施肥人工草地三种高寒牧草茎叶$\delta^{15}N$天然丰度差异为4.18‰(—3.55‰—0.63‰).人工草地四年的化肥施入显著降低了牧草茎叶、根系和土壤的$\delta^{15}N$天然丰度值;(2)在$^{15}N$示踪实验中,不施肥样地垂穗披碱草对$^{15}NO_3^-$—N和$^{15}NH_4^+$—N的吸收分别占76.58%和23.42%,施肥样地垂穗披碱草对两种氮素的吸收分别占63.88%和36.12%.施肥措施显著降低了垂穗披碱草对硝态氮的吸收;不施肥样地中华羊茅对两种氮素的吸收分别占56.32%和43.67%,施肥样地对两种氮素的吸收分别占42.74%和57.26%;冷地早熟禾氮吸收与垂穗披碱草相似;(3)平均而言,在不施肥样地和施肥样地,$^{15}NO_3^-$—N在牧草茎叶的回收率分别为9.75%和6.61%,$^{15}NH_4^+$—N在牧草茎叶的回收率为3.25%和4.15%;$^{15}N$在牧草根系真的回收率为2.67%—3.54%;在不施肥样地和施肥样地,$^{15}NO_3^-$—N在土壤中的回收率分别为16.80%和24.88%,$^{15}NH_4^+$—N在土壤中的回收率为40.75%和34.19%.

**关键词**:人工草地;$^{15}N$示踪技术;植物氮吸收;$^{15}N$回收

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## Introduction

Qinghai Tibet Plateau is one of the major animal husbandry base of China. Grassland area is about 150 million hm$^2$, accounting for 37.64% of the total area of the grassland in China. The degraded grassland is 42.51 million hm$^2$, occupying 33% of the available grassland area on the Tibetan Plateau [1]. It is now widely accepted that long—term overgrazing is the major reason causing grassland degradation on the Tibetan Plateau. Therefore, maintaining a balance between forage and livestock number is critical to arresting grassland degradation. It is necessary to build artificial grasslands on severely degraded grassland. The artificial grassland can provide a large number of forage for Tibet sheep and yak, and reduce the grazing intensity of natural meadow. Studies have shown that it is feasible to establish high—yield artificial grasslands on heavily degraded alpine meadows or ecotones with good water—heat resources on the Tibetan Plateau, followed by proper management [2,3]. There are two key points for artificial grassland establishment, (i) the selection of grass species adapted to local ecological environment and (ii) proper agronomic practices, grassland use and management. Based on standards of forage yield, winter survival rate and seed maturation rate, about 20 forage species have been cultivated and 5 species, viz. *Elymus natans*, *Elymus breviaristatum*, *Poa crymophila*, *Poa pratensis* and *Festuca sinensis*, have been used widely in artificial grasslands because of their good adaptation, forage quality and high availability [4]. About 160000 hm$^2$ artificial grasslands were established during 2000～2010 in Qinghai province to support a series of demonstration projects. However, N output of grasslands is increasing with forage removal from the artificial grassland ecosystems. Nutrition balance between plants and soils is becoming a key limiting factor in the

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grassland ecosystems. Input of nitrogen fertilizers to artificial grasslands is essential to maintain grassland productivity. Although land degradation and artificial grassland rehabilitation are widespread and sometimes in large scale, there is little information on N partitioning and recovery in plants and soils of artificial grassland ecosystems on the Tibetan Plateau.

McKane's $^{15}N$—tracer experiment showed that plant species growing in arctic tundra communities were differentiated in chemical form, time and depth of nitrogen uptake. Species dominance was strongly correlated with uptake of the most available soil nitrogen forms [5]. More importantly, plant N uptake may change with environment [6—8]. Such phenomenon is challenging our understanding of terrestrial N circulation.

This research was conducted in the artificial grassland ecosystems on the Qinghai—Tibet Plateau. Using $^{15}N$ tracer technique, the uptake rate of $^{15}NH_4^+$—N and $^{15}NO_3^-$—N by three plant species in two days, and the fate of two forms of $^{15}N$ into plant aboveground tissues, plant roots and surface soil N pools were investigated in unfertilized and fertilized plots. In addition, $^{15}N$ natural abundances in aboveground tissues and roots in the three plant species and in soils in different depths were measured before the $^{15}N$ tracer experiment. The research aimed to reveal the N uptake traits of three dominant alpine forages, improve the understanding of N cycle of artificial grassland ecosystems, and provide scientific information for N fertilizer application and sustainable management of the alpine grassland ecosystems.

## Materials and Methods

### Study site

The experiment was conducted at the Tongde County of Qinghai Province, China. The name of the authority that issued the permit for each location is Qinghai Forage and Seed Breeding Factory (Contact: WANG Xin—chuan; E—mail: wangxinchuan6 @163. com). Location of research site is E100°39′, N35°09′ with an average altitude of 3307 m. The annual average temperature is 0. 2℃, and annual average precipitation is 429. 8 mm, 80% of which falls in the growing seasons. The main natural vegetation is mountain steppe dominated by *Stipa krylovii*, *Orinus kokonorica* and *Carex rigescens*. Companion species are *Poa pratensis*, *Aneurolepidium dasystachys*, *Kobresia humilis*, and *Oxytropis spp*. The field studies did not involve endangered or protected species. The total vegetation cover is 50～65%. The soil is classified as dark Castanozems. Some general soil properties are presented in Table 1.

Table 1 Characteristics of the upper 10 cm of soils at the study site. Means (SD) are shown (n=8—10)

| | |
|---|---|
| pH ($H_2O$) | 8. 20 (0. 10) |
| Soil total organic carbon (mg $g^{-1}$) | 17. 49 (1. 38) |
| Soil total nitrogen (mg $g^{-1}$) | 1. 77 (0. 23) |
| $NH_4^+$—N($\mu$g $g^{-1}$) | 6. 25 (1. 85) |
| $NO_3^-$—N ($\mu$g $g^{-1}$) | 13. 45(6. 24) |

## Experimental design

The artificial grasslands (a mix of *E. nutans*, *F. sinensis*, *P. crymophila* in a ratio of 5∶4∶1)were established in late May 2007 with two treatment plots(unfertilized and fertilized). Fertilized plots received 150 kg urea $hm^{-2}$ in early July every year since 2007. Rehabilitation measures of artificial grasslands were adopted as follows: severely degraded land was plowed and harrowed, mixed grass seeds were planted, then the soil land was again harrowed lightly and rolled solid to prevent erosion. Seeds were sown to a depth of 2～3 cm at a rate of 20 kg $hm^2$. Reseeded artificial grasslands have not been grazed or mowed in the first year of establishment and were mowed in winter of the second year after establishment.

**$^{15}N$ microplot**

$^{15}N$ tracer experiment was carried out late July in 2010. The eight 0.54 m×0.54 m $^{15}N$ treatment microplots (2 N forms ($^{15}NH_4Cl$ and $K^{15}NO_3$;98% atom percent $^{15}N$; 11 mmol $L^{-1}$) × 2 replicates) were randomly allocated in unfertilized or fertilized plots(2 plots). There were eight $^{15}N$ treatment microplots in total. There were 100 injecting sites within each microplot, which were divided into 6 cm×6 cm grids. Two ml of solution was injected using syringe into 0～5 cm soil depth at the intersection of each grid. before injection, inserting a stick into the soil about 5 cm septh. Thus the concentration was 33 mg $^{15}N$ $m^{-2}$ in each microplot.

**Sampling and analyzing**

Prior to $^{15}N$ tracer experiment, 4～6 plants of *E. nutans* ,*F. sinensis* and *P. crymophila* were collected from each corresponding plot, separated into aboveground parts and roots. Three soil cores were collected at 0～10 cm and 10～20 cm depths from each plot. Aboveground parts and roots were dried at 80℃ for 48h. Soil samples were air－dried. Subsequently samples were ground to powder. Analysis of $^{15}N$ natural abundance and N concentration of aboveground parts, roots and soils was done with an isotope ratio mass spectrometer (Finnigan MAT Delta V advantage) coupled with an elemental analyzer (Flash EA 1112HT).

Forty－eight hours after the injection of $^{15}N$, aboveground parts, roots and soils were collected from each microplot. About 15～20 individual shoots and leaves per species were randomly collected within an area of 45 cm×45 cm at the center of the subplot. Six cores (3.8 cm diameter) of soil samples (0～10 cm depth) were collected from each microplot. We sampled the upper 10 cm soil layer because over 80% of the roots are concentrated in this layer in the grasslands [3]. Soil samples were mixed well and sieved to ＜2 mm. Live roots, after removal from the soil cores, were rinsed with destilled water after tap water and put into 0.5 mmol $L^{-1}$ $CaCl_2$ solution for 30 min, then rinsed with distilled water to remove tracers attached on their surface. All labeled plant material was dried at 80℃ for 48 h, and labeled soils were air－dried. Ground plants and sieved soils were analyzed for total N concentration and $\delta^{15}N$ enrichment as described before.

After $^{15}N$ labeled plant materials were collected, aboveground plant parts were clipped at 3 cm height from the whole area of each microplot, and sorted by species. Root biomass per unit area was extrapolated from root biomass obtained in soil cores. Aboveground plant biomass and roots were dried at 80℃ for 48 h, and weighed.

**Calculations and statistical analyses**

Since $\delta^{15}N$ values refer to $^{15}N$ enrichment relative to standard atmospheric air $N_2$, we used $^{15}N_{uptake}$ ($\mu$mol $g^{-1}$ dry mass) based on N concentration, fractional abundance in the sample, in the reference sample (samples before $^{15}N$ injection) and in the tracer;

$$^{15}N_{uptake} = [T\ (F_{sample} - F_{ref})]/F_{tracer}$$

where$T$ is the N concentration (in g $kg^{-1}$) of the sample; $F_{sample}$, $F_{ref}$ and $F_{tracer}$is the fractional abundance in the sample, in the non－labeled reference sample and in the tracer, respectively.

We used$^{15}N$ tracer recoveries based on N mass, amount of $^{15}N$ applied, and $^{15}N$ enrichments of different pools. We calculated the proportion of $^{15}N$ tracer recovered ($^{15}N_{rec}$) within different pools as (Templer et al. 2012):

$$^{15}N_{rec} = \frac{F_{sample} - F_{ref}}{F_{tracer} - F_{ref}} \times \frac{N_{pool}}{N_{tracer}}$$

where F is thefractional abundance in the sample, in the non－labeled reference sample and in the tracer, respectively; $N_{pool}$ and $N_{tracer}$ are the masses of N in the different pools and in the tracer (in $\mu$mol/$m^2$) applied to that microplot, respectively.

We tested the significance of difference of all the parameters derived from different treatments using two－way ANOVA, LSD and $t$－test depending on the data. All statistical analyses were conducted using

Excel 2003 and SPSS 11.0. The standard errors of means are presented in figures and tables. All differences were tested at $P<0.05$.

## Results

### $^{15}N$ natural abundance and N concentration of plant and soil

Foliar $^{15}N$ natural abundance of three different plant species ranged from 0.32 to 3.42 ‰ in unfertilized plots and from −3.55 to 0.63 ‰ in fertilized plots (Table 2). Root $^{15}N$ natural abundance of the three species varied from 1.17 to 2.38 ‰ in unfertilized plots and −0.71 to 2.47 ‰ in fertilized plots. The $^{15}N$ natural abundance values of soil at 0～10 cm and 10～20 cm depth were 5.11 ‰ and 5.26 ‰, respectively for unfertilized plots. The corresponding values for the fertilized plots were 4.54 ‰ and 4.79 ‰. This suggested that addition of mineral fertilizer N led to an significant decrease in $^{15}N$ natural abundance of plant and soil in artificial grasslands. Fertilizer application lowered the $\delta^{15}N$ values by 2.16～3.87 ‰ for foliages, 0.09～1.88 ‰ for roots and 0.47～0.57 ‰ for soils. In addition, the root $\delta^{15}N$ values were higher than that of the foliages. Variation in foliar $\delta^{15}N$ natural abundance across the three species was 3.10 ‰ (ranging from 0.32 to 3.42 ‰) in unfertilized plot and 4.18 ‰(ranging from −3.55 to 0.63 ‰) in fertilized plot. Soil $\delta^{15}N$ value did not differ between 0～10 cm and 10～20 cm layers regardless of fertilizer plots.

Tab. 2 Mean $\delta^{15}N$ natural abundance and N concentration (±SE) in foliage, root and soil in unfertilized and fertilized plots

| | | $\delta^{15}N$ natural abundance (‰) | | N concentration (%) | |
|---|---|---|---|---|---|
| | | Unfertilized plot | Fertilized plot | Unfertilized plot | Fertilized plot |
| Foliage | *E. nutans* | 1.42 (0.03) a2 | −0.74 (0.16) b2 | 1.15 (0.04)A1 | 1.07 (0.12)A2 |
| | *F. sinensis* | 0.32 (0.09) a3 | −3.55 (0.30) b3 | 0.67 (0.04)B2 | 1.04 (0.04)A2 |
| | *P. crymophila* | 3.42 (0.27) a1 | 0.63 (0.10) b1 | 1.11 (0.07)B1 | 1.66 (0.08)A1 |
| Root | *E. nutans* | 1.78 (0.06) a2 | 0.53 (0.11) b2 | 0.84 (0.05) B2 | 1.14 (0.03)A2 |
| | *F. sinensis* | 1.17 (0.04) a3 | −0.71 (0.04) b3 | 0.98 (0.06) A1 | 1.15 (0.08) A2 |
| | *P. crymophila* | 2.38 (0.01) a1 | 2.47 (0.12) a1 | 0.97 (0.04) B1 | 1.66 (0.11) A1 |
| Soil | 0～10cm | 5.11(0.12)a1 | 4.54(0.17)b1 | 0.25(0.04)A1 | 0.21(0.01)A1 |
| | 10～20cm | 5.26(0.05)a1 | 4.79(0.16)b1 | 0.21(0.00)A1 | 0.20(0.01)A1 |

Data in the same row followed by the same letter do not differ, at $P=0.05$; data for foliage, root or soil in the same column followed by the same number do not differ, at $P=0.05$.

Plant N concentrations of the three plant species were 0.67～1.15% in unfertilized plots and 1.04～1.66% in fertilized plots. This suggests that fertilizer N application increased N concentration in plants. Soil N concentration was 0.25% (0～10cm) and 0.21%(10～20cm) in unfertilized plots, and 0.21% and 0.22% in fertilized plots. Fertilizer application had no significant effecton soil N concentration.

### $^{15}N$ uptake

The $^{15}N$ tracer experiment showed that *E. nutans* absorbed significantly more $^{15}N$ from the $NO_3^-$ source (76.58% of absorbed total $^{15}N$) than from $NH_4^+$ (23.42%) in unfertilized plots (Table 3). There was no significant difference for $^{15}N$ uptake by *E. nutans* between $NO_3^-$ (63.88%) and $NH_4^+$ (36.12%) source in fertilized plots. Fertilizer N application decreased significantly $^{15}NO_3^-$ uptake by *E. nutans*, but had no effect on its $^{15}NH_4^+$ uptake.

Tab. 3 The $^{15}NH_4-N$, $^{15}NO_3-N$ uptake values of the three plant species and their percentage of total N uptake. Values are means (SE)

| | $^{15}NO_3-N$ (umol $^{15}N\ g^{-1}$) | $^{15}NH_4-N$ (umol $^{15}N\ g^{-1}$) | $^{15}NO_3-N$ percentage (%) | $^{15}NH_4-N$ percentage (%) |
|---|---|---|---|---|
| A−$N_0$ | 3.247 (0.577)1a | 0.993 (0.372)1,2b | 76.58 | 23.42 |
| A−$N_{added}$ | 1.675 (0.080)2,3a | 0.947 (0.560)1,2a | 63.88 | 36.12 |
| B−$N_0$ | 2.792 (0.348)1,2a | 2.165 (0.988)1a | 56.32 | 43.67 |
| B−$N_{added}$ | 1.056 (0.325)3a | 1.415 (0.267)1,2a | 42.74 | 57.26 |
| C−$N_0$ | 2.960 (0.739)1,2a | 1.193 (0.301)1,2b | 71.27 | 28.73 |
| C−$N_{added}$ | 1.304 (0.442)3a | 0.575 (0.163)2a | 69.40 | 30.60 |

Note: A is *Elymus* nutans, B is *Festuca* sinensis; C is *Poa crymophila*; $N_0$: unfertilized plots; $N_{added}$: fertilized plots; 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 $P=0.05$.

The $NO_3^-N$ and $NH_4-N$ uptake by *F. sinensis* accounts for 56.32 and 43.67% respectively in unfertilized plots, and 42.74% and 57.26% in fertilized plots. There was no preferential uptake of $^{15}N$ by *F. sinensis* from the $NH_4^+$ or $NO_3^-$ sources irrespective of fertilizer plots (Table 3). Compared to *E. nutans*, *F. sinensis* absorbed more soil $NH_4^+-N$ but less soil $NO_3^--N$ for both plots.

*P. crymophila* absorbed more $NO_3^-$ (69.40～71.27%) than $NH_4^+$ (28.73～30.60%) regardless of fertilizer plots. *P. crymophila* and *E. nutans* had the similar uptake mode for soil $NH_4^+-N$ and $NO_3^--N$ sources.

So, N uptake of the alpine plants is related with soil N forms and fertilization. Addition of fertilizer N decreased $NO_3^-N$ uptake and not significantly effect for $^{15}NH_4-N$ uptake by the alpine plant species, especially *E. nutans*. There is difference in absorption of soil nitrogen forms among the three plant species in artificial grasslands on the Tibetan Plateau, which show obvious different preferences in obtaining soil N.

## $^{15}N$ recovery in plant foliage, roots and soils

When $^{15}N$ tracer was applied as $^{15}NO_3^-$, total plant foliage $^{15}N$ recovery was 9.75% and 6.61% in unfertilized and fertilized plots, respectively. When $^{15}N$ tracer was applied as $^{15}NH_4^+$, the corresponding values of the recovery were significantly lower, and were 3.25% and 4.15% (Table 4). Fertilizer application did not affect the recovery of $^{15}NO_3^-$ or $^{15}NH_4^+$.

Tab. 4 $^{15}N$ recovery in plant foliage, roots and soils

| | $^{15}NO_3^-$ recovery (%) | | $^{15}NH_4^+$ recovery (%) | |
|---|---|---|---|---|
| | Unfertilized Plots | Fertilized Plots | Unfertilized Plots | Fertilized Plots |
| Foliage | 9.75 (1.84)a | 6.61 (0.28)a | 3.25 (1.26)b | 4.15 (1.66)b |
| Root | 3.17 (0.72)a | 3.33 (0.79)a | 2.67 (1.60)a | 3.54 (1.41)a |
| Soil | 16.80 (3.55)b | 24.88 (5.93)ab | 40.75 (7.92)a | 34.19 (5.99)a |
| Total | 29.72 (6.12)a | 34.82 (11.98)a | 46.66 (16.26)a | 41.88 (14.09)a |

Data in the same row followed by the same letter do not differ, at $P=0.05$.

Root $^{15}N$ recovery was in the range of 2.67～3.54%. The recovery was not significantly affected by $^{15}N$ form or fertilizer N application. When $^{15}N$ tracer was applied as $^{15}NO_3^-$, the recovery of N in soil was 16.80% and 24.88% in unfertilized and fertilized plots, respectively. The corresponding values (40.75% and 34.19%) were significantly higher when $^{15}N$ tracer was applied as $^{15}NH_4^+$ (Table 4). This suggested that most $^{15}NH_4^+$ was retained in soil, and relative more $^{15}NO_3^-$ was retained in plants, especially in unfertilized plots.

When $^{15}N$ tracer was applied as $^{15}NO_3^-$, the total $^{15}N$ recovery in plant and surface soil was 29.72% and 34.82% in unfertilized and fertilized plots, respectively. The corresponding values were 46.66% and 41.88% when $^{15}N$ tracer was applied as $^{15}NH_4^+$. This suggests that the added $^{15}N$ loss is high in this artificial grasslands. It accounted for 53%～71% among different treatments.

## Discussion

### $^{15}N$ natural abundance in plant and soil

Mean $^{15}N$ natural abundance of three plant species are 3～4‰ lower than that of soil total－N. This suggests that isotopic fractionations during soil N transformation and possibly during plant N uptake could lead to the observed differences in $^{15}N$ natural abundance in plant parts and soil layers. In addition, except for *P. crymophila* grown in unfertilized plots, root $\delta^{15}N$ values were higher than that of foliage. This was more obvious for plants grown in fertilized plots. It has been widely reported that the lighter $^{14}N$ isotope reacts more rapidly than the heavier $^{15}N$ due to lower bond strength, and hence accumulates in the products, whilst the residual (source) becomes enriched in $^{15}N$[9－10]. This study suggests that there is a significant N isotope fractionation in the process of inorganic N transport from root to shoot or protein synthesis, especially in fertilized soil and plants had a lower $\delta^{15}N$ than soil total N in the artificial grassland ecosystems. Soil $\delta^{15}N$ values do not vary with soil depth in this study. This is consistent with Hogberg's point that in grasslands and agricultural systems plants are depleted in $^{15}N$ relative to soil $^{15}N$, but aboveground parts are taken away at harvests or mixed into deeper soil layers by ploughing [11]. So there is not a $^{15}N$－depleted surface layers.

In our study, fertilizer N application lowered the $\delta^{15}N$ values of plant foliage, roots and soils by 2.94‰, 1‰, 0.47－0.57‰ respectively. Bremer [12] also concluded that plant $\delta^{15}N$ decreased with increasing contribution of fertilizer－N to total plant－N contents. This could be attributed to the application of isotopically different N, where the $\delta^{15}N$ of mineral fertilizer N is lower than that of the total soil－N [13]. Application of fertilizer N decreased the $\delta^{15}N$ of inorganic－N pools, which resulted in depletion of plants in $^{15}N$ under fertilization in the present study.

As described in detail previously by Nadelhofer et al. (1996), the $\delta^{15}N$ values of plants are affected by various factors, such as the source of N, the depth in soil from which N is taken up, the form of N used (e.g. $NH_4^+$, $NO_3^-$, organic N sources), concentrations of inorganic N, influence of mycorrhizal symbiosis, and fractionations during and after N uptake by plants [14]. our results show that the source of N (e.g. soil N, fertilizer N), the form of N and fractionations of N uptake by plants can lead to variations in $\delta^{15}N$ of alpine plant species of the artificial grassland ecosystem.

### The uptake of different N forms by plant species

In our study, *E. nutans* and *P. crymophila* absorbed much more soil $NO_3^-$－N than $NH_4^+$－N while *F. sinensis* showed no preferential uptake for the form of inorganic N in unfertilized or fertilized plots. Meanwhile, foliar $^{15}N$ value of *E. nutans* and *P. crymophila* was higher than that of *F. sinensis* in unfertilized or fertilized plots. As described in detail previously by Nadelhofer et al(1996), generally fractionations during soil N transformations can also influence the $^{15}N$ contents of plant－available N pools, and $NH_4^+$－N and $NO_3^-$－N pools at any given soil depth could have different $^{15}N$ content[14]. Nadelhofer et al. (1996) suggest that when denitrification is not substantial, $\delta^{15}N$ values of soil $NO_3^-$－N would likely to be lower than $NH_4^+$－N values. In contrast, where denitrification rates are high, $\delta^{15}N$ value of residual $NO_3^-$－N could equal or exceed that of $NH_{4+}$－N [14]. So, the higher $^{15}N$ enriched nitrate uptake by *E. nutans* and *P. crymophila* may suggest that denitrification rate was high in the present study. But we do not have in-

formation on the $^{15}N$ values of soil inorganic nitrogen. Measurement of $^{15}N$ contents of $NH_4^+$ −N and $NO_3^-$ −N in this soil under various conditions are required for a better understanding of how the form of uptake of inorganic N influence these plant $^{15}N$ signature.

Recently more studies on alpine native grassland ecosystems have shown that plants living within the same community have different uptake preference for soil $NH_4^+$ − N, $NO_3^-$ − N and organic N sources [15−17]. The authors believe that plants could obtain the limited N sources with different ways, which makes species coexist in the small habitat and maintain biodiversity in these ecosystems. In addition, coexisting species occupy different niches in the community, which improves the utilization efficiency of limited resources in soils [17]. In our study the ratios of $NO_3^-$ −N and $NH_4^+$ −N uptake were about 3:1 in *E. nutans* and *P. crymophila* species, and about 1:1 in *F. sinensis* species. However fertilizer application decreased significantly the uptake of soil $NO_3^-$ −N. These suggest that it is important to understand the preference of N demand of different plant species used in rehabilitated artificial grasslands, and that planting species with different N uptake preference will help reduce inter−species competition and improve soil N nutrient use efficiency in grassland production.

**$^{15}N$ recovery in plants and soils**

In agreement with Inselsbacher [18], we conclude that the rate of plant $^{15}N$ recovery was closely related with form of applied N, and higher recovery rates with $NO_3^-$ addition. A multitude of studies, including molecular studies using bacterial and fungal model organisms, showed that soil microbes prefer $NH_4^+$ over $NO_3^-$ as N source [19−21]. In various ecosystems plants gain most of the added N only at a later stage, as they often acquire N which has initially been immobilized by soil microbes and thereafter gradually released into the soil during microbial turnover [22−24].

According to results of a meta−analysis of $^{15}N$ tracer field studies, the average total ecosystem $^{15}N$ recovery was 59.6% for <1 week, 80.1% for 1 week to 1 month, 50.7% for 1 to 3 months, 69.4% for 3 to 18 months, and 61.6% for > 18 months [25]. In our study, mean $^{15}N$ recovery in plants and surface soil was 38% (30−47%) 48 h after application of $^{15}N$ tracer in artificial grassland ecosystems. This indicates that on average 62% of the $^{15}N$ applied was not recovered in plant parts and surface soil. In our study, $^{15}N$ tracers were injected to 5 cm soil depth, so part of the labeled compounds may have leached to the soil layers beyond our sampling depth. Besides, $NO_3^-$ −N could be lost to the environment as $N_2O$ and $N_2$ via denitrification whereas $NH_4^+$ −N via ammonia volatilization in this alkaline soil. This suggest that reducing gaseous and perhaps leaching losses from the artificial grassland systems remains a major challenge. More intensive interdisciplinary research will be needed to gain insights into mechanisms involved in the N cycle of artificial grasslands and to increase N fertilizer use efficiency of forages.

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# Nitrogen Partitioning in Artificial Grasslands: A $^{15}N$ Tracer Field Study on the Tibetan Plateau

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**Abstract**: Using $^{15}N$ tracer techniques, this study examined $^{15}NH_4^+$—N and $^{15}NO_3^-$—N tracer uptake of three plant species, viz. *Elymus nutans*, *Festuca sinensis* and *Poa crymophila* as well as the fate of two $^{15}N$ form into plant aboveground tissues, plant roots and surface soil N pools within 48 hours on unfertilized or fertilized artificial grassland of Tibetan Plateau. The research aimed to reveal the N uptake traits of dominant alpine forages, improve the understanding of N cycle of artificial grassland ecosystems, and provide scientific information for N fertilizer application and sustainable management of the alpine grassland ecosystems. The results showed (1) variation in foliar $\delta^{15}N$ natural abundance across the three species was 3.10‰ (ranging from 0.32 to 3.42‰) in unfertilized plot and 4.18‰ (ranging from −3.55 to 0.63‰) in fertilized plot. Fertilizer application lowered the $\delta^{15}N$ natural abundance values by 2.16—3.87‰ for plant foliage, 0.09—1.88‰ for roots and 0.47—0.57 ‰ for soils after 4 year's application. (2) In the $^{15}N$ tracer experiment, the $NO_3^-$ N and $NH_4$—N uptake by *E. nutans* accounted for 76.58 and 23.42% of the applied N, respectively, in unfertilized plots, and 63.88% and 36.12%, respectively, in fertilized plots. Fertilizer N application significantly decreased $^{15}NO_3^-$ uptake by *E. nutans*. The recovery of $NO_3^-$ N and $NH_4$—N by *F. sinensis* was 56.32 and 43.67%, respectively, in unfertilized plots, and 42.74% and 57.26% in fertilized plots. The N uptake pattern of *P. crymophila* was similar to that of *E. nutans*. (3) The plant foliage $^{15}N$ recovery was 9.75% and 6.61% when $^{15}N$ tracer was applied as $^{15}NO_{3-}$ to unfertilized and fertilized plots, respectively, and was 3.25% and 4.15% when $^{15}N$ tracer was as $^{15}NH_4^+$. Root $^{15}N$ recovery was in the range of 2.67—3.54%. The soil $^{15}N$ recovery was 16.80% and 24.88% when $^{15}N$ tracer as $^{15}NO_3^-$ on unfertilized and fertilized plots, respectively. The corresponding values was 40.75% and 34.19% when $^{15}N$ tracer as $^{15}NH_4^+$.

**Key words**: artificial grassland; $^{15}N$ tracer; plant N uptake; $^{15}N$ recovery

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