Plasticity in nitrogen form uptake and preference in response to long-term nitrogen fertilization

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Abstract

Background and aims Niche complementarity arising from divergence in resource use is an important mechanism underlying species coexistence. We hypothesized fertilization with different N forms would generate plastic divergence among species with regard to their N form uptake and preference.

Methods In the eighth year of a long-term N fertilization experiment in an alpine meadow on the Tibetan plateau, we labeled 11 common plant species with ammonium-¹⁵N or nitrate-¹⁵N in subplots without fertilization (control) or fertilized with 7.5 g N m⁻² yr⁻¹ in the form of ammonium, nitrate, or ammonium nitrate to trace N form uptake.

Results Depending on species, fertilization with nitrate or ammonium nitrate had positive, negative or neutral effects on NO₃-N uptake rate, although ammonium fertilization showed little impact. By contrast, fertilization with any N form had little impact on NH₄-N uptake rate. Consequently, effects of nitrate fertilization and ammonium nitrate fertilization on relative N form preference diverged among the species and the functional groups (grasses, sedges, legumes and forbs).

Conclusions Alpine plant species can diverge in N form uptake and preference in response to long-term N fertilization, and such divergence may contribute to species coexistence after long-term fertilization.

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Introduction

Competitive interactions drive species to partition resources (Hutchinson 1959; MacArthur and Levins 1967; Silvertown 2004), and such resource-based niche partitioning is regarded as a mechanism underlying species coexistence and diversity effects on ecosystem function (McKane et al. 2002; Chase and Leibold 2003; Silvertown 2004). When resource availability changes, plant species may respond differentially by shifting resource usage (Ashton et al. 2010) in a way that may potentially reduce interspecific overlap and increase niche differentiation (Manthey et al. 2011), thereby contributing to species coexistence and ecosystem function

(Kahmen et al. 2006; Ashton et al. 2010). Therefore, understanding how resource use of plant species responds to changing resource availability is critical for predicting ecosystem function in the face of environmental changes.

Nitrogen (N) is often a limiting resource in terrestrial ecosystems (McKane et al. 2002; Lebauer and Treseder 2008), and available in different chemical forms (e.g. ammonium, nitrate, organic N forms, etc.; McKane et al. 2002; Martens-Habbena et al. 2009). Atmospheric deposition enriched with certain N forms and long-term addition of agricultural fertilizers with different N forms can disproportionally increase the availability of ammonium or nitrate in soil (Fowler et al. 1998; Galloway et al. 2008; Stevens et al. 2011), and alter the original proportion of soil available N forms (Krupa 2003; Wathes et al. 2003). In response to changes in the availability of an N chemical form, plant species may show plasticity in the uptake of the N form and/or shift preference from one N form to another to maintain or even increase their overall N uptake (McKane et al. 1990; Martens-Habbena et al. 2009). Furthermore, such responses may depend on which N form is altered, and also diverge greatly among plant species. However, there is little information on how changes in the availability of different N forms affect the uptake and preference of different N forms in plant species or functional groups.

There may be different ways for plant species to deal with changes in N form availability (McKane et al. 2002; Ashton et al. 2008; Houle et al. 2014). They may shift relative N form preference by increasing the uptake of the more abundant N form more than the less abundant one. By contrast, species characterized by niche conservatism (Diekmann and Lawesson 1999) may be less flexible and may continue to rely on a specific N form regardless of changes in availability of alternative forms. Niche differentiation due to the divergence in plasticity of N form uptake and preference may be important to the maintenance of species coexistence and ecosystem functioning under fluctuating resource availability (Ashton et al. 2010; Houle et al. 2014).

Our long-term experiment in an alpine meadow on the Tibetan Plateau showed that species richness did not differ when it was fertilized with different N chemical forms, while biomass showed idiosyncratically annually varying responses (Song et al. 2012). Here we tested the hypothesis that fertilization with different N forms can generate plastic divergence among plant species with

regard to their N form uptake and preference, which may contribute to niche differentiation and thus species coexistence. We tested this hypothesis by conducting a ^{15}N labelling experiment to trace the ability of different species to take up N of different forms in the eighth year of the N fertilization experiment.

Materials and methods

Long-term N fertilization experiment

The long-term N fertilization experiment was described in detailed in Song et al. (2012). In short, the experiment was carried out in an alpine meadow co-dominated by a perennial tussock sedge (*Kobresia humilis* Serg.) and grasses (e.g. *Stipa aliena* Keng. and *Elymus nutans* Griseb). The meadow is located in the northeastern Tibetan Plateau in Qinghai Province, China. This area has a mean annual temperature of $-1.7\text{ }^{\circ}\text{C}$ and annual precipitation of 560 mm. The meadow is N limited, and the net N mineralization rate is $-10.3 \pm 4.1\text{ mg kg}^{-1}\text{ day}^{-1}$ during August when plant growth peaks (Zhou 2001). Vegetation cover is nearly 100 % (Song et al. 2012).

The experiment had nine N addition treatments and an unfertilized control, with three replicate plots ($2 \times 2\text{ m}^2$) each. In the control treatment no N was added, and the other nine treatments were a factorial combination of three N chemical forms and three N supply rates. The three N forms were (1) ammonium N ($\text{NH}_4\text{-N}$), (2) nitrate N ($\text{NO}_3\text{-N}$), and (3) both $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ ($\text{NH}_4\text{-N} + \text{NO}_3\text{-N}$). The three N rates were 0.375, 1.500 and $7.500\text{ g N m}^{-2}\text{ yr}^{-1}$. The experiment was started in 2005.

Isotope labeling experiment

The ^{15}N labeling experiment was conducted only within the control plots and the plots with the highest N rate ($7.5\text{ g N m}^{-2}\text{ yr}^{-1}$) of all three N forms. In this study, the treatments with the addition of $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$ and both $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ were coded as Am, Ni, AN, respectively, and the control was coded as C. There were 12 plots in total. In each plot we selected three $30 \times 30\text{ cm}^2$ quadrats outside the central $1 \times 1\text{ m}^2$ area used for long-term monitoring of species richness (Song et al. 2012). Of the three quadrats, one was used for $^{15}\text{NH}_4\text{NO}_3$ labeling, one for $\text{NH}_4^{15}\text{NO}_3$ labeling, and one for

measuring natural ^{15}N abundance (without ^{15}N labeling). Within each plot any two quadrats were spaced at least 50 cm apart.

The labeling treatments were conducted under sunny conditions on 2 August 2012. We evenly sprayed 500 ml N solution containing 7.68 mmol l^{-1} $^{15}\text{NH}_4\text{NO}_3$, $\text{NH}_4^{15}\text{NO}_3$ or NH_4NO_3 to each of the three quadrats. We sprayed the solution close to the ground, and also care was taken to spray the solution mostly on the soil. The atom percentage of ^{15}N in the labeled moiety was 98.4 % in $^{15}\text{NH}_4\text{NO}_3$ and 98.2 % in $\text{NH}_4^{15}\text{NO}_3$. A pilot test with water showed a movement distance of about 2.5 cm around the spray area. The solution sprayed in each plot was equivalent to 5 mm rainfall. We delivered $0.32 \text{ g } ^{15}\text{N m}^{-2}$ in each quadrat, which was less than 1/20th of the amount of N added to the plot with fertilization and thus unlikely to significantly influence the effect of N fertilization.

Sampling and measurements

We harvested plants 48 h after labeling. In each quadrat we harvested only plants within the central $25 \times 25 \text{ cm}^2$ area to avoid potential edge effects. Plants were clipped close to the ground, and sorted into species. All plant materials were rinsed with deionized water before being dried in oven at 60°C for 48 h, and weighed to obtain biomass. We selected 11 common plant species of four functional groups (grasses, sedges, legumes and forbs), which had cover of over 2.0 % (Table 1). We milled their dry shoots to measure ^{15}N abundance (atom percentage ^{15}N) and N content at species level. The remaining material was sorted into four functional groups, i.e.

grasses, sedges, legumes and forbs, and plant species of each functional group were mixed homogeneously and ground using a ball mill (MM2, Fa. Retsch, Haan, Germany) for measuring ^{15}N abundance and N concentration. Atom percentage ^{15}N and N content in plant materials were determined using a continuous-flow gas isotope ratio mass spectrometer (MAT253, Finnigan MAT, Bremen, Germany).

On August 4, 2012, five soil cores of 5 cm in diameter and 10 cm in depth were collected in each quadrat sprayed with NH_4NO_3 , and they were homogeneously mixed into one composite soil sample. After removing thick roots and stones by passing through a 2 mm mesh sieve, soil samples were stored in iceboxes and transferred to the laboratory. The fresh samples were extracted with 60 mL of 1 M KCl, and concentrations of available nitrate (NO_3^-) and ammonium (NH_4^+) were analyzed with a FIASTAR 5000 (Dual Channel Soil Nitrogen Analyzer System, FOSS, Hoana, Sweden).

N uptake rate and relative N form preference

We calculated $\text{NH}_4\text{-N}$ uptake rate and $\text{NO}_3\text{-N}$ uptake rate, respectively, in aboveground plant parts in each of the 11 species using an isotopic mixing model (McKane et al. 2002) as $U_{\text{unlabelled}} = U_{\text{labelled}} (m_{\text{unlabelled}}/m_{\text{labelled}})$, where m_{labelled} is total mass (g m^{-2}) of ^{15}N -labelled N spray per treatment, $m_{\text{unlabelled}}$ is the mass of the “target” pool of available nitrogen estimated to be within the diffusion zone of the sprayed ^{15}N , U_{labelled} is the uptake (g m^{-2}) of ^{15}N from the source m_{labelled} , and $U_{\text{unlabelled}}$ is the uptake of available N from the source $m_{\text{unlabelled}}$.

Table 1 The 11 selected common plant species and their four functional groups in the alpine meadow community. Mean and S.E. were given ($n=3$)

Species	Family	Functional group	Cover (%)
<i>Kobresia humilis</i> Serg.	Cyperaceae	sedge	2.6 ± 0.1
<i>Thalictrum alpinum</i> L.	Ranunculaceae	forb	5.9 ± 0.8
<i>Euphrasia pectinata</i> Ten.	Scrophulariaceae	grass	5.2 ± 1.9
<i>Ranunculus pulchellus</i> C.A.Mey.	Ranunculaceae	forb	5.4 ± 0.9
<i>Deschampsia caespitosa</i> (Linn.) Beauv.	Gramineae	forb	2.9 ± 0.7
<i>Oxytropis ochrocephala</i> Bunge.	Fabaceae	legume	4.9 ± 0.4
<i>Stipa aliena</i> Keng.	Gramineae	grass	38.4 ± 7.1
<i>Elymus nutans</i> Griseb.	Gramineae	grass	4.3 ± 0.9
<i>Scirpus distigmaticus</i> Tang et Wang.	Cyperaceae	sedge	2.3 ± 0.1
<i>Gentiana algida</i> Pall.	Gentianaceae	forb	2.8 ± 0.2
<i>Taraxacum lugubre</i> Dahlst.	Asteraceae	forb	2.2 ± 0.1

Note: Cover data are for 2012, the eighth year of the N fertilization experiment

We calculated $\ln(\text{NO}_3\text{-N uptake rate}/\text{NH}_4\text{-N uptake rate})$ in each of the 11 species and the four functional groups as a measure of relative preference for different N chemical forms. Values greater than zero suggest that species or functional groups prefer to take up nitrate, and values smaller than zero indicate that they prefer ammonium. Increasing values suggest a relative increase in nitrate preference and a relative decrease in ammonium preference.

Statistical analysis

Data were tested for normality and homogeneity of variance before analysis. Data were transformed to square root when needed. We used two-way ANOVA to test the effects of species identity or functional group and N fertilization (C, Am, Ni and AN) or N form (Am, Ni and AN) on $\text{NO}_3\text{-N uptake rate}$, $\text{NH}_4\text{-N uptake rate}$ and relative N form preference. We then used Tukey multiple comparison to test the differences in $\text{NO}_3\text{-N uptake rate}$, $\text{NH}_4\text{-N uptake rate}$ and relative N form preference of each species and functional group between the four N fertilization treatments (C, Am, Ni and AN). Data that did not satisfy assumptions of normality or homogeneity of variance (e.g. $\text{NO}_3\text{-N uptake ratios}$ of *Deschampsia caespitosa* and *Scirpus distigmaticus*) were analyzed by non-parametric tests. All analyses were done using SPSS version 13.0 (SPSS Inc. Chicago, Illinois, USA).

Results

Effects of N fertilization on N uptake rate

Effects of N fertilization on both $\text{NO}_3\text{-N uptake rate}$ and $\text{NH}_4\text{-N uptake rate}$ were highly significant (Table 2: main effect of N fertilization), and such effects also varied among plant species (Table 2: highly significant interaction effect). Furthermore, N form also had highly significant effects on both $\text{NO}_3\text{-N uptake rate}$ ($F_{2, 66}=73.8$, $P<0.001$) and $\text{NH}_4\text{-N uptake rate}$ ($F_{2, 66}=17.0$, $P<0.001$), and such effects varied among plant species (interaction effect for $\text{NO}_3\text{-N uptake rate}$: $F_{20, 66}=4.0$, $P<0.001$; for $\text{NH}_4\text{-N uptake rate}$: $F_{20, 66}=4.8$, $P<0.001$).

Fertilization with ammonium (Am) had little impact on $\text{NO}_3\text{-N uptake rate}$: compared to the control, Am significantly decreased $\text{NO}_3\text{-N uptake rate}$ of only one species (*Kobresia humilis*; Fig. 1a), but had no significant impact on that of the other ten species (Fig. 1b–k). By

contrast, both fertilization with nitrate (Ni) and fertilization with ammonium nitrate (AN) had larger impacts on $\text{NO}_3\text{-N uptake rate}$, and the impacts were also divergent among the species (Fig. 1). Compared to the control, Ni increased $\text{NO}_3\text{-N uptake rate}$ of seven species (Fig. 1b–d, f, g, i, k), decreased that of one species (Fig. 1a), but had no impact on the other three species (Fig. 1e, h, j). Compared to the control, AN significantly increased $\text{NO}_3\text{-N uptake rate}$ in eight species (Fig. 1b, c, e–j), but had no effect in the other three species (Fig. 1a, d, k). At the functional group level, Am did not significantly affect $\text{NO}_3\text{-N uptake}$ compared to the control (Fig. 1l–o). Ni increased $\text{NO}_3\text{-N uptake}$ in legumes, but did not affect it in the other three functional groups. AN increased $\text{NO}_3\text{-N uptake}$ in grasses and forbs, but did not affect $\text{NO}_3\text{-N uptake}$ in the other two functional groups.

Compared to the impact of N fertilization on $\text{NO}_3\text{-N uptake rate}$, the impact on $\text{NH}_4\text{-N uptake rate}$ was small and also less divergent among plant species. Compared to the control, both Am and Ni significantly affected $\text{NH}_4\text{-N uptake rate}$ of only one species each (Am: *Deschampsia caespitosa*, Fig. 2e; Ni: *O. ochrocephala*, Fig. 2f), while AN affected $\text{NH}_4\text{-N uptake}$ in three species (*Ranunculus pulchellus*, *D. caespitosa* and *Gentiana algida*; Fig. 2d, e, j). Compared to the control, Am, Ni or AN had no effect on $\text{NH}_4\text{-N uptake}$ in any of the four functional groups (Fig. 2l–o).

Effects of N fertilization on relative N form preference

In the control, all the 11 plant species preferred ammonium [$\ln(\text{N form uptake ratio}) < 0$; Fig. 3]. Effects of N fertilization on relative N form preference were highly significant (Table 2: main effect of N fertilization), and such effects varied greatly among plant species (Table 2: highly significant interaction effect). N form also had highly significant effects on relative N form preference ($F_{2, 66}=21.2$, $P<0.001$), and such effects varied among plant species (interaction effect: $F_{20, 66}=2.4$, $P=0.005$).

Am had no significant effect on relative N form preference in any of the 11 species compared to the control (Fig. 3a–k). Effects of AN on relative N form preference were less divergent; compared to the control AN significantly increased relative nitrate preference (or decreased relative ammonium preference) in nine species (Fig. 3a–h, j) and had no impact on that of the other two species (Fig. 3i, k). Effects of Ni were more divergent; Ni significantly increased relative nitrate preference in five species (Fig. 3b–d, f, k), but had no impact

Table 2 Effects of species or functional group and N fertilization and their interaction on $\text{NH}_4\text{-N}$ uptake rate, $\text{NO}_3\text{-N}$ uptake rate and relative N form preference

Effect	DF	NH ₄ -N uptake ratio		NO ₃ -N uptake ratio		N form preference	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
a) Species level							
Species	10, 88	25.0	<0.001	29.1	<0.001	3.9	<0.001
N fertilization	3, 88	8.9	<0.001	103.6	<0.001	31.9	<0.001
Interaction	30, 88	2.6	<0.001	5.4	<0.001	2.4	<0.001
b) Functional group level							
Functional group	3, 32	1.62	0.203	1.14	0.346	3.26	0.034
N fertilization	3, 32	2.97	0.046	15.05	<0.001	16.27	<0.001
Interaction	9, 32	1.29	0.283	1.35	0.252	2.77	0.016

Fig. 1 Uptake rate of $\text{NO}_3\text{-N}$ of the 11 common species (a–k) and the four functional groups (l–o) in the alpine meadow under the four nitrogen treatments. C, Am, Ni and AN stand for the control (no added N) and addition of 7.5 g $\text{NH}_4\text{-N}$, 7.5 g $\text{NO}_3\text{-N}$ and

3.75 g of both $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ m⁻² yr⁻¹, respectively. Means and SE are given. For $\text{NO}_3\text{-N}$ uptake rate and $\text{NH}_4\text{-N}$ uptake rate, respectively, bars sharing the same letters are not different at $P=0.05$

Fig. 2 Uptake rate of $\text{NH}_4\text{-N}$ of the 11 common species (a–k) and the four functional groups (l–o) in the alpine meadow under the four nitrogen treatments. C, Am, Ni and AN stand for the control (no added N) and addition of 7.5 g $\text{NH}_4\text{-N}$, 7.5 g $\text{NO}_3\text{-N}$ and

3.75 g of both $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ $\text{m}^{-2} \text{ yr}^{-1}$, respectively. Means and SE are given. For $\text{NO}_3\text{-N}$ uptake rate and $\text{NH}_4\text{-N}$ uptake rate, respectively, bars sharing the same letters are not different at $P = 0.05$

on the other six species (Fig. 3a, e, g–j). At the functional group level, Am had no significant effect on relative N form preference in any of the four functional groups (Fig. 3l–o). Ni increased relative nitrate preference in forbs (Fig. 3o), but did not affect that in the other three functional groups (Fig. 3l–n). AN significantly increased relative nitrate preference in grasses, legumes and forbs, but not in sedges (Fig. 3l–o).

Discussion

Consistent with our hypothesis, we found that plant species and functional groups in the alpine meadow

diverged in their N chemical form uptake and preference in response to long-term fertilization with different N forms. Such divergence may contribute to niche differentiation and thus species coexistence in the alpine meadow after long-term fertilization.

There is evidence that some grassland species prefer nitrate over ammonium because nitrate can diffuse more rapidly to roots through soil water and is less toxic when stored in plant tissue (Lambers et al. 1998; Miller et al. 2007; von Felten et al. 2009; Ashton et al. 2010). However, in some alpine ecosystems where nitrate is less available compared to ammonium, plant species tend to prefer ammonium over nitrate (McKane et al. 2002; Ashton et al. 2010). Our results confirm this view.

Fig. 3 Relative N form preference [$\ln(\text{NO}_3\text{-N uptake rate} / \text{NH}_4\text{-N uptake rate})$] of the 11 common species (a–k) and the four functional groups (l–o) in the alpine meadow under the four nitrogen treatments. Values greater than zero suggest that $\text{NO}_3\text{-N}$ uptake rate exceeds $\text{NH}_4\text{-N}$ uptake rate. C, Am, Ni and AN stand for the

control (no added N) and addition of 7.5 g $\text{NH}_4\text{-N}$, 7.5 g $\text{NO}_3\text{-N}$ and 3.75 g of both $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ $\text{m}^{-2} \text{yr}^{-1}$, respectively. Means and SE are given. Bars sharing with the same letters are not different at $P=0.05$

We found that without N fertilization all the 11 plant species in the alpine meadow preferred ammonium over nitrate, likely because soil available ammonium was much higher than soil available nitrate during the entire growing season (Zhang and Cao 1999).

More interestingly, we found that plant species in the alpine meadow differed greatly in their N form uptake and preference in response to N fertilization, and such responses also differed when different N forms were added. For instance, depending on the species,

fertilization with nitrate could have a positive, negative or neutral effect on $\text{NO}_3\text{-N}$ uptake rate, and fertilization with ammonium nitrate could also have a positive or neutral effect. Also, fertilization with nitrate increased relative nitrate preference in five species and had no impact in the other six species. Plasticity in resource use can be an important strategy for species coexistence (Ashton et al. 2010), and has been found to be directly associated with species fitness to changing environments (Sultan 2004; Miner et al. 2005) and indirectly

with species interactions (Werner and Peacor 2003). For instance, in the absence of competition competitively superior species from a dry alpine meadow at Niwot Ridge, Colorado, USA preferred $\text{NO}_3\text{-N}$ to $\text{NH}_4\text{-N}$, but in competition with competitively inferior species they shifted to increased $\text{NH}_4\text{-N}$ uptake (Ashton et al. 2010). Interspecific competition can force species to reduce niche overlap with coexisting species and decrease the strength of interspecific competition, which may be realized by niche contraction or niche shifts (Morse 1974; Chesson 2000). Niche complementarity arising from plasticity in resource use may be one of the important mechanisms in promoting species coexistence in diverse plant communities (Ashton et al. 2010; Zuppinger-Dingley et al. 2014). Our findings suggest that plant species in the alpine meadow could alter N form uptake and shift N form preference in response to fertilization of different N forms, which may provide additional niche differentiation for species coexistence in the alpine meadow, at least for the species that we considered in this study. At the functional-group level, fertilization with both nitrate and ammonium nitrate could have a positive or neutral effect on $\text{NO}_3\text{-N}$ uptake rate. Fertilization with ammonium alone, however, did not affect $\text{NO}_3\text{-N}$ uptake rate of the four functional groups. These results suggest that divergence in N form uptake was much smaller at the functional-group level than at the species level.

The impact of fertilization on N form uptake and relative N form preference also depended on N chemical forms. While addition of nitrate or ammonium nitrate affected $\text{NO}_3\text{-N}$ uptake rate and relative N form preference in many of the 11 common species, addition of ammonium significantly affected $\text{NO}_3\text{-N}$ uptake rate of only *K. humilis*, and affected relative N form preferences in none of the 11 species. Under strong competition or in stressful habitat, plant species, especially abundant ones, generally show plasticity and shift to predominantly use the most abundant N chemical form available, which may not be their favored N form (McKane et al. 1990, 2002; Ashton et al. 2010). However, they can also shift back to preferentially use their favored N chemical form when the availability of that N form increases. Consequently, N form utilization of a plant species can be closer to their fundamental niche after the release of the N form limitation. Although previous studies showed that addition of ammonium decreased $\text{NO}_3\text{-N}$ uptake in trees due to a reduction of the membrane potential, leading to a

break-down of NO_3/H^+ co-transport (Ullrich 1987) or the inhibition of the nitrate carrier (Boxman and Roelofs 1988; Marschner et al. 1991), it had little effect on $\text{NO}_3\text{-N}$ uptake of the alpine plants in our study. An explanation may be that nitrate uptake of alpine plants is not sensitive to ammonium because the availability of ammonium is much higher than that of nitrate in their native soil (Kamminga-van and Prins 1993).

The impact of N fertilization on $\text{NH}_4\text{-N}$ uptake was much smaller than that on $\text{NO}_3\text{-N}$ uptake. N fertilization, no matter which chemical form was added, had little impact on rates of $\text{NH}_4\text{-N}$ uptake, suggesting that plant species in the alpine meadow showed little plasticity of $\text{NH}_4\text{-N}$ uptake in response to N fertilization. One likely explanation is that $\text{NH}_4\text{-N}$ uptake rates in these alpine plants were already high due to the greater availability of ammonium in the soil. Another explanation may be that excessive $\text{NH}_4\text{-N}$ uptake is restricted in these alpine plants to avoid ammonium toxicity (Lambers et al. 1998). Indeed, fertilization with ammonium even decreased $\text{NH}_4\text{-N}$ uptake rate of *D. caespitosa* (Fig. 2e). Therefore, in the main, shifts in relative N form preference arose from an increase in $\text{NO}_3\text{-N}$ uptake rate in response to fertilization with nitrate or ammonium nitrate, rather than a decrease in $\text{NH}_4\text{-N}$ uptake rate. However, the response of $\text{NO}_3\text{-N}$ uptake rate to fertilization with nitrate differed from the response to fertilization with ammonium nitrate. For instance, three species (*O. ochrocephala*, *T. lugubre* and *E. pectinata*) took up more $\text{NO}_3\text{-N}$ when fertilized with nitrate than when fertilized with ammonium nitrate, but four species (*K. humilis*, *D. caespitosa*, *S. aliena* and *E. nutans*) took up less (Fig. 1). In addition, five species shifted their preference from $\text{NH}_4\text{-N}$ to $\text{NO}_3\text{-N}$ when fertilized with ammonium nitrate, and one shifted when fertilized with nitrate. The shift of the relative preference from $\text{NH}_4\text{-N}$ to $\text{NO}_3\text{-N}$ in response to fertilization with nitrate or ammonium nitrate suggests that the balance in the availability of ammonium and nitrate in soil is beneficial to the utilization of $\text{NO}_3\text{-N}$ in the alpine meadow plants.

One should note that the nitrification process can transform $\text{NH}_4\text{-N}$ into $\text{NO}_3\text{-N}$, which may result in an overestimation of the N uptake when fertilized with ammonium. We tried to reduce such an impact by conducting the experiment on a clear sunny day and by harvesting plant materials within a relatively short period (48 h). Moreover, the fertilization treatments might influence abiotic factors that could further affect

N uptake. For example, data on soil properties and N mineralization measured in the sixth year of the N fertilization treatments showed that N addition decreased pH and the extent of the effect depended on N chemical form (Table S1). Also, compared to the control, soil water content increased by 9.6 % when fertilized with ammonium nitrate, and soil N mineralization at 5 °C increased no matter what N form was added (Table S1). However, it is unclear what roles these changes played in the plasticity of N form uptake and preference, and future studies should be conducted to explicitly address such questions.

We conclude that some plant species in this alpine meadow can track changes in availability of different N forms by plasticity in N form uptake and by shifts in N form preference, while others cannot. Such divergence may be a strategy for resource portioning under increased N availability. This is especially true with regard to plasticity in $\text{NO}_3\text{-N}$ uptake and N form preference in response to fertilization with nitrate. However, the alpine plant species showed little plasticity in $\text{NH}_4\text{-N}$ uptake rate, especially in response to ammonium fertilization. The exact mechanism underlying such divergence in N form uptake and preference among these alpine plant species is unclear. It is possible that differences in nutrient use strategy and life-history traits among species result in such divergence. Future studies could be designed to test these hypotheses. To our knowledge, our study is the first to show that long-term fertilization with different N forms can generate divergence in N form uptake and preference among plant species, which may contribute to niche differentiation and species coexistence in this alpine meadow.

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