

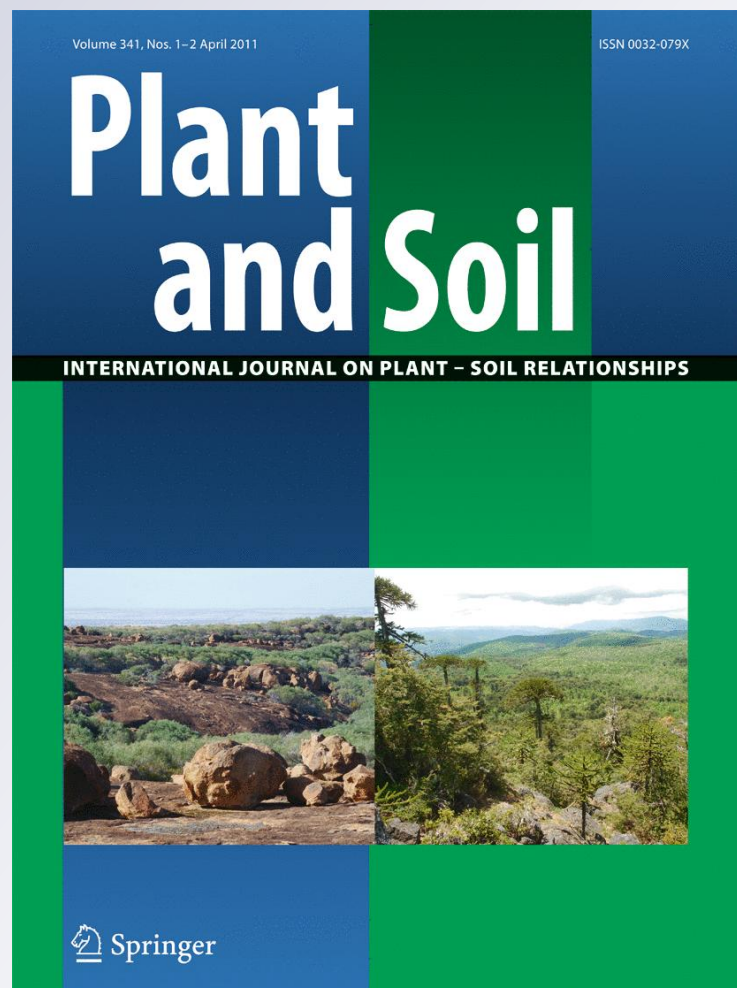
*Dominant plant species shift their nitrogen uptake patterns in response to nutrient enrichment caused by a fungal fairy in an alpine meadow*

**Plant and Soil**

An International Journal on  
Plant-Soil Relationships

ISSN 0032-079X  
Volume 341  
Combined 1-2

Plant Soil (2011) 341:495-504  
DOI 10.1007/  
s11104-010-0662-1



**Your article is protected by copyright and all rights are held exclusively by Springer Science+Business Media B.V.. This e-offprint is for personal use only and shall not be self-archived in electronic repositories. If you wish to self-archive your work, please use the accepted author's version for posting to your own website or your institution's repository. You may further deposit the accepted author's version on a funder's repository at a funder's request, provided it is not made publicly available until 12 months after publication.**

# Dominant plant species shift their nitrogen uptake patterns in response to nutrient enrichment caused by a fungal fairy in an alpine meadow

Xingliang Xu · Hua Ouyang · Guangmin Cao ·  
Andreas Richter · Wolfgang Wanek ·  
Yakov Kuzyakov

Received: 28 August 2010 / Accepted: 10 November 2010 / Published online: 4 December 2010  
© Springer Science+Business Media B.V. 2010

**Abstract** Niche partitioning by time, space and chemical forms has been suggested as an important mechanism to maintain species coexistence. Climate warming is assumed to increase soil nutrient availability through enhancing mineralization of soil organic matter in a variety of terrestrial ecosystems. However, few studies have yet examined how dominant plant species contribute to species coexistence when nutrient enrichment occurs in native

ecosystems. We studied a single fairy ring (5 m diameter) in a *Kobresia* meadow in the Tibetan Plateau. This kind of rings is caused by a basidiomycete fungus *Agaricus campestris*, and is evidenced by dark-green vegetation boundaries. Nutrient enrichment occurs due to enhanced decomposition of soil organic matter (SOM) in the fungus growth zone of these rings. We conducted a short-term  $^{15}\text{N}$  labelling experiment and found that dominant plant species shifted their N uptake patterns and preferred N form ( $\text{NO}_3^-$ ,  $\text{NH}_4^+$ , and amino acid N) in response to nutrient enrichment in an N-limited alpine meadow. The legume *Gueldenstaedtia diversifolia* had the lowest aboveground biomass among the five plant species studied at low available N level, although it mainly utilized ammonium (the most abundant N form). The two graminoids (*Elymus nutans* and *Stipa aliena*) demonstrated similar aboveground biomass at low and high available N levels, showing a similar pattern switching from  $\text{NH}_4^+/\text{NO}_3^-$  uptake outside the ring to glycine uptake in the annulus zone of the ring. The biomass of the forb *Gentiana straminea* differed significantly at low and high available N levels, but its N uptake pattern almost remained unchanged. Species therefore differed in their response to nutrient enrichment, most species showing chemical niche shifts instead of niche conservatism. This finding has important implications with regard to understanding the mechanisms responsible for species coexistence when natural nutrient enrichment is induced by climate warming in terrestrial ecosystems.

Responsible Editor: Herbert J. Kronzucker.

X. Xu (✉) · H. Ouyang  
Key Laboratory of Ecosystem Network Observation and Modelling, Institute of Geographic Sciences and Natural Resources Research, the Chinese Academy of Sciences, 11A Datun Road, Chaoyang District, Beijing 100101, China  
e-mail: xuxingl@hotmail.com

G. Cao  
Northwest Institute of Plateau Biology,  
the Chinese Academy of Sciences,  
23 Xinning Street,  
Xining 810008, China

A. Richter · W. Wanek  
Department of Chemical Ecology and Ecosystem Research,  
Vienna Ecology Centre, University of Vienna,  
Althanstrasse 14,  
1090 Wien, Austria

Y. Kuzyakov  
Department of Agroecosystem Research,  
University of Bayreuth,  
95440 Bayreuth, Germany

**Keywords** Chemical niche shift · Inorganic nitrogen · Nitrogen uptake pattern · Nutrient enrichment · Organic nitrogen

## Introduction

The control of how plant diversity is maintained is a central topic in ecology, mainly focusing on species coexistence in ecosystems (Chesson 2000; Silvertown 2004). Several mechanisms have been invoked to explain plant species coexistence in N-limited ecosystems, including variation in resource requirements of individual species and partitioning of N acquisition in time, space and chemical forms (Schoener 1974; Tilman 1982; Chesson 2000; McKane et al. 2002; Miller and Bowman 2002; Silvertown 2004). Recent fertilization experiments showed a tendency for species loss with increasing soil nutrient availability (Gough et al. 2000; Stevens et al. 2004; Harpole and Tilman 2007; Clark and Tilman 2008; Manning et al. 2008). Experiments showed that N enrichment can modify plant community composition through altering plant-soil feedbacks (Manning et al. 2008). Moreover, numerous field and laboratory experiments showed that soil nutrient availability can be enhanced by soil warming through stimulating decomposition of SOM (Lükewille and Wright 1997; Rustad et al. 2001; Sardans et al. 2006), and thereby may result in species losses (Klein et al. 2004). A possible mechanistic explanation of grassland species loss caused by nutrient enrichment was put forward by Harpole and Tilman (2007), who suggested that species loss resulted from decreasing their niche dimension. However, few studies have been conducted to examine how dominant plant species coexist when nutrient enrichment occurs in native ecosystems.

The Tibetan Plateau has been regarded as “the third pole of the Earth”, covering over 2.5 million km<sup>2</sup> with an average altitude of more than 4000 m above sea level. Approximately 35% of its area is alpine meadows (Zheng 2000). The high OM status of these alpine meadow soils is due to harsh climatic conditions. This leads to the trapping of N in forms unavailable to plants in alpine *Kobresia humilis* meadows (Song et al. 2007). As a result, plant growth is strongly limited by soil-available N in this type of meadow (Zhou 2001). Several lines of evidence suggest that the Tibetan Plateau is experiencing

climatic warming (Thompson et al. 1993; French and Wang 1994) and the Plateau has been predicted to undergo greater than average increases in temperatures in the future (Giorgi et al. 2001). This indicates that climate warming might enhance mineralization of SOM and give rise to nutrient enrichment in alpine meadows. Therefore, an important question arises: how do dominant plant species acclimate to nutrient enrichment and how does this contribute to species coexistence in alpine meadows? It was shown that plant species occupy distinct niches with regard to their relative N uptake (Kahmen et al. 2006). We here advance a hypothesis that dominant plant species shift their N uptake patterns (here referred as chemical niche) in response to nutrient enrichment and contribute to species coexistence in these alpine meadows.

Fairy rings in grasslands are manifestations of basidiomycete activities, and are often observed due to close-cropped and homogeneous vegetation. They are often classified as three types according to whether vegetation is killed at the ring margin, grows more vigorously or is unaffected (Griffith and Roderick 2008). In typical alpine *Kobresia* meadows, the basidiomycete fungus *Agaricus campestris* develops fairy rings. The vegetation in the fungus growth zone grows vigorously, which is evidenced by dark-green vegetation boundaries (Fig. 1) and circular fruit body distributions. Basidiomycete fungi grow on dead organic matter (Cooke and Rayner 1984) and return nutrients to the soil, leading to higher N availability in annular areas of rings than outside the rings (Kaiser 1998; Edwards 1988; Gramss et al. 2005; Griffith and Roderick 2008). This provides a unique environment for testing how dominant plant species acclimate to nutrient enrichment in alpine meadows, circumventing manipulations by fertilizer addition. To test the hypothesis above, a short-term <sup>15</sup>N experiment was conducted in the fungus growth zone and outside the fairy ring, focusing on chemical niches (i.e. N uptake preferences) of five common dominant grassland species.

## Materials and methods

### Study site

The experiment was conducted in an alpine meadow at the Haibei Alpine Meadow Ecosystem Station of



**Fig. 1** The basidiomycete fungus develops a fairy ring as evidenced by a dark-green vegetation boundary **a**, showing three zones: (*a*) outside the fairy ring not yet colonized by the fungus, (*b*) in the annulus zone with fungus growth, and (*x*) the inner zone where the *Agaricus* has already grown through. Sampling points within the fairy ring were positioned in the centre of the fungal growth zone **b**: white rectangles were injected with water as the control, diagonal rectangles with  $^{15}\text{N}$ -glycine, dotted rectangles with  $^{15}\text{N}\text{-NO}_3^-$ , and hatched rectangles with  $^{15}\text{N}\text{-NH}_4^+$

the Chinese Academy of Sciences, Qinghai Province (37° 36' 60" N, 101° 19' 14" E, 3215 m asl). 25-year means for temperature and rainfall were  $-1.7\text{ }^\circ\text{C}$  and 600 mm, respectively. Dominant species are *Kobresia humilis* Serg., *Elymus nutans* Griseb., *Stipa aliena* Keng., *Poa sp.*, *Festuca ovina* Linn., *Gentiana aristata* Maxim., *Gentiana straminea* Maxim., *Saussurea superba* Anth., and *Gueldenstaedtia diversifolia* Maxim. (Zhou 2001). The soil is classified as Mat Cryo-gelic Cambisol (Chinese Soil Taxonomy Research Group 1995) corresponding to Gelic Cambisol (WRB 1998). There were on average 28 plant species within 25 cm $\times$ 25 cm quadrats outside the fairy ring and 23 species in the fungus growth zone. The five selected dominant species accounted for 69% of total aboveground biomass in the outer zone not yet colonized by the fungus and 56% in the annulus zone of the ring. The height of herbaceous plants averaged about 20 cm in the outer zone and 26 cm in the fungus growth zone of the fairy ring.

#### Experimental layout

Only a single ring (5 m diameter) was investigated in this study. Twenty-four 10 $\times$ 20 cm plots were set up

in a *Kobresia humilis* meadow in July, 2007. Twelve plots were positioned in the centre of the 50 cm wide annulus area (Fig. 1b) developed by *Agaricus campestris*, the other twelve plots were located more than 25 m distant from the fairy ring within the alpine meadow. Five dominant species in the annulus area and outside the fairy ring were selected as target species: one sedge (*Kobresia humilis*), two graminoids (*Elymus nutans* and *Stipa aliena*), and two forbs (*Gueldenstaedtia diversifolia* and *Gentiana straminea*).

A mixture of glycine,  $\text{NH}_4^+$ , and  $\text{NO}_3^-$  (1:1:1 glycine-N/ $\text{NH}_4^+$ -N/ $\text{NO}_3^-$ -N) was injected into 5 cm soil depth. Before N was injected, each 10 $\times$ 20 cm plot was divided into eight 5 $\times$ 5 cm subplots. One milliliter solution was injected at the center of each subplot, yielding  $19\text{ }\mu\text{g N g}^{-1}$  d.w. soil for each 10 $\times$ 20 cm plot. The amount and forms of N added in the three treatments were identical, but only one of the three N forms was labeled with  $^{15}\text{N}$  in each case (98.2 at%  $^{15}\text{N}$  enrichment for  $\text{NO}_3^-$ , 98.4 at%  $^{15}\text{N}$  enrichment for  $\text{NH}_4^+$ , and 95.0 at%  $^{15}\text{N}$  enrichment for glycine). Three replicate plots received each  $^{15}\text{N}$  form treatment. Six untreated plots (three in the fungus growth zone and three in the outer zone of the ring) which were not injected with  $^{15}\text{N}$  tracer were supplied with equivalent amounts of water and were taken as controls. When  $^{15}\text{N}$  tracers were injected into soils, great attention was given to an even distribution of the  $^{15}\text{N}$  labeled solution in the soil.

Because of rapid turnover of amino acids in soil (Jones and Kielland 2002), plants and soil were collected three hours after  $^{15}\text{N}$  tracer injection. The plots were completely sampled to 10 cm depth because over 80% of the roots are concentrated within this horizon (Zhou 2001) and immediately transferred to the laboratory. The whole 10 $\times$ 20 cm plot was excavated, from which roots were carefully separated so that 'intact' plant individuals were collected. The mycelium was invisible to the naked eye when these plots were excavated and broken up. Plants were sorted to species level. They were rinsed shortly after with water, then for 30 min with  $0.5\text{ mmol L}^{-1}$   $\text{CaCl}_2$  solution, and again with distilled water to remove  $^{15}\text{N}$  absorbed on the surface of plants. Plant material was dried at  $60\text{ }^\circ\text{C}$  for 48 h, weighed for total dry mass, N content, and  $^{15}\text{N}/^{14}\text{N}$  ratio measurements. After roots were carefully removed from soil cores, the remaining soils were sieved to through 2 mm and stored at  $4\text{ }^\circ\text{C}$  until measurements of available N.

## Sample analysis

Soil  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N were determined by autoanalyser in 0.5 M  $\text{K}_2\text{SO}_4$  extracts. Soil glycine concentrations were measured by high-performance liquid chromatography (Waters 515) on the same extracts (Nasholm et al. 1987).

Dried intact plants including roots and shoots were ground to a fine powder using a ball mill (MM200, Retsch). Aliquots (2 mg) of ground plant material were weighed into tin capsules for analyzing total N, C and atom%  $^{15}\text{N}$  by a isotope ratio mass spectrometry (Mat253, Finnigan MAT). Atom% excess  $^{15}\text{N}$  (APE) was calculated as the atom%  $^{15}\text{N}$  difference between plants from  $^{15}\text{N}$  treated and from control plots.

## N uptake calculation

Uptake of  $^{15}\text{N}$  ( $\text{mg } ^{15}\text{N m}^{-2}$ ) of individual plant species was calculated by multiplying N content ( $\text{mg N g}^{-1}$  d.w.), APE, and biomass ( $\text{g m}^{-2}$ ). Uptake of available N species corresponding to the  $^{15}\text{N}$  treatment was calculated following McKane et al. (2002):

$$U_{\text{unlabelled}} = U_{\text{labelled}}(m_{\text{unlabelled}}/m_{\text{labelled}})$$

where  $m_{\text{labelled}}$  is the total mass ( $\text{g m}^{-2}$ ) of  $^{15}\text{N}$ -labelled N injected per plot,  $m_{\text{unlabelled}}$  is the mass of available N species measured in soils.  $U_{\text{labelled}}$  is uptake ( $\text{g m}^{-2}$ ) of  $^{15}\text{N}$  from the source  $m_{\text{labelled}}$ , and  $U_{\text{unlabelled}}$  is uptake of available N from the source  $m_{\text{unlabelled}}$ .

**Table 1** Characteristics of topsoils (0–10 cm) outside the fairy ring and in the annulus zone of the ring in a *Kobresia humilis* meadow. Means $\pm$ 1SE are presented ( $n=12$ ). Asterisks indicate

	The outer zone	The fungus growth zone	Increase (%)
Organic carbon (%)	7.36 $\pm$ 0.42*	10.60 $\pm$ 0.54	44.02
Total N (%)	0.58 $\pm$ 0.08*	0.76 $\pm$ 0.12	30.26
C/N	12.7 $\pm$ 0.3	14.0 $\pm$ 0.2	10.57
pH (H <sub>2</sub> O)	8.0 $\pm$ 0.2	7.6 $\pm$ 0.3	–
Ammonium ( $\mu\text{g N g}^{-1}$ d.w. soil)	5.16 $\pm$ 0.32*	8.03 $\pm$ 0.12	55.62
Nitrate ( $\mu\text{g N g}^{-1}$ d.w. soil)	2.20 $\pm$ 0.17*	2.95 $\pm$ 0.09	34.09
Glycine ( $\mu\text{g N g}^{-1}$ d.w. soil)	2.86 $\pm$ 0.56*	6.47 $\pm$ 0.75	126.22
TFAA <sup>a</sup> ( $\mu\text{g N g}^{-1}$ d.w. soil)	30.59 $\pm$ 2.64	40.79 $\pm$ 3.00	33.35

<sup>a</sup>Total free amino acid concentration (sum of the 17 amino acids)

## Quantification of mycorrhizal colonization

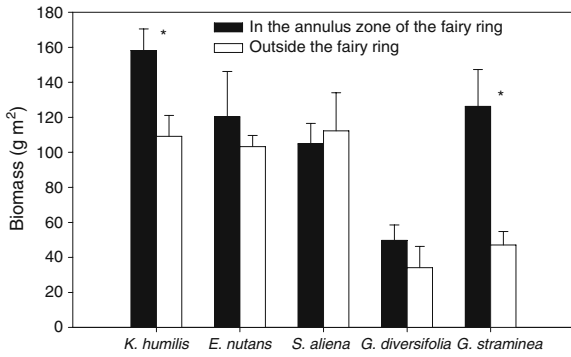
To test whether arbuscular mycorrhizal fungi (AMF) contribute to shifts in N uptake pattern, mycorrhizal colonization was determined for the five dominant plant species. We collected fresh roots of the observed five plant species close to our  $^{15}\text{N}$  treatment plots. The roots after gentle washing were stored in 50% ethanol until measurements of AMF colonization. When measuring mycorrhizal colonization, roots were rinsed with tap water and then cleared in 10% KOH solutions for 45 min at 90 °C. After clearing, roots were boiled (95 °C) for at least 3 min in the staining solution consisting of 5% ink diluted in 5% acetic acid. A gridline-intersection method was used to quantify extent of mycorrhizal colonization (Vierheilg et al. 2005).

## Results

N availability was higher in the annular area than outside the fairy ring (Table 1). Soil total N and available N such as ammonium, nitrate, and glycine in the annulus area were significantly higher than outside the fairy ring. Compared with the outer zone of the ring, C/N increased about 11% in the fungus growth zone while soil pH showed no significant difference.

*K. humilis* and *G. straminea* showed significantly higher total biomass in the annulus area than outside the ring, whereas no significant difference in total biomass was observed for *E. nutans*, *S. aliena* and *G. diversifolia* (Fig. 2).

significant differences between outside the fairy ring and in the annulus zone at 0.05 error probability level



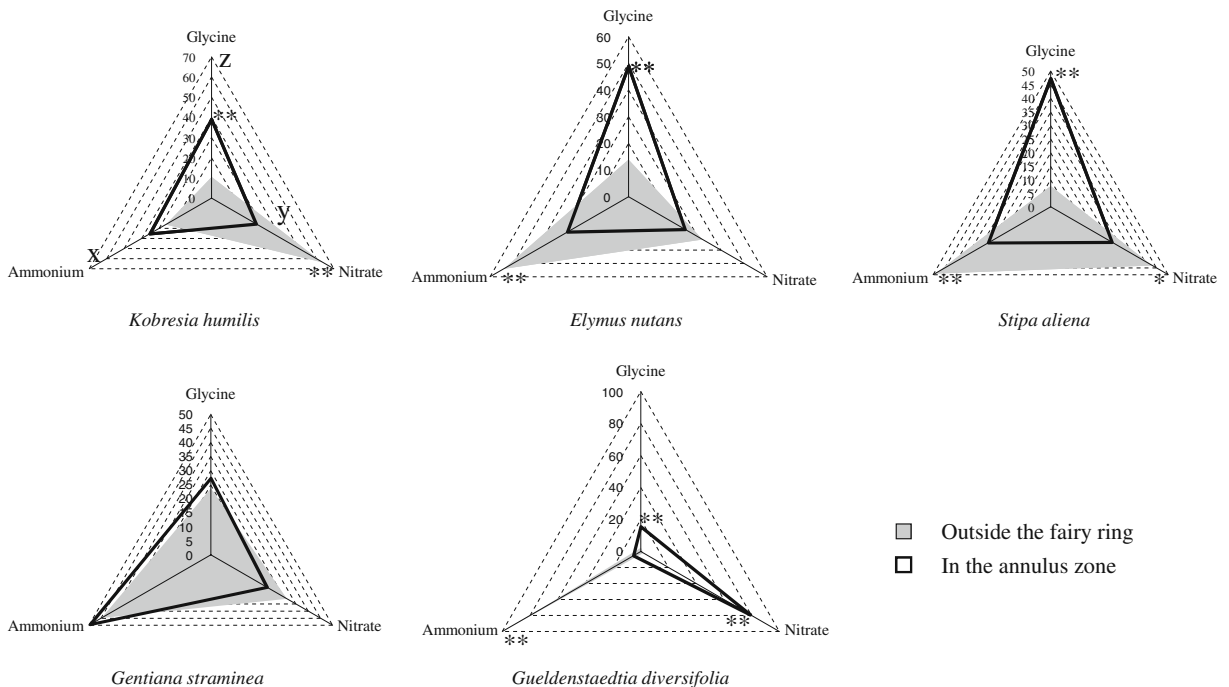
**Fig. 2** Total biomass of dominant plant species both in the annulus zone and outside the fairy ring in an alpine meadow in the Tibetan Plateau. Values are means ( $\pm 1$  SE) of 12 replicates. Asterisks indicate significant differences between in the annulus area and outside the fairy ring at 0.05 error probability level

The sedge *K. humilis* used mainly nitrate (the least abundant N-form in the soil) outside the fairy ring, but in the annulus zone there was a switch to uptake of glycine and ammonium (Table 2, Fig. 3). The legume *G. diversifolia* utilized the most abundant N form (ammonium) outside the ring but the least

abundant N form (nitrate) under high N availability. The graminoids *E. nutans* and *S. aliena* both showed a similar pattern switching from  $\text{NH}_4^+/\text{NO}_3^-$  uptake outside to glycine uptake in the annulus zone (Table 2, Fig. 3). They mainly acquired N from both the most and the least abundant N forms outside the ring while utilizing preferentially the second most abundant N form at higher N availability. Uptake patterns of the forb *G. straminea* were similar in both areas (Table 2, Fig. 3).

Outside the fairy ring, inorganic N made a major contribution to total N uptake for all five plant species (Fig. 4a). Compared to outside the ring, the five dominant plant species acquired more amino acid N in the annulus zone of the ring (Fig. 4b). The sedge (*K. humilis*) and graminoids (*E. nutans* and *S. aliena*) showed a significant difference in N uptake between in the annulus zone and outside the ring, whereas the forbs (*G. diversifolia* and *G. straminea*) didn't show significant differences (Table 2).

The roots of *K. humilis* and *G. diversifolia* were not colonized by AMF, while the other three species were colonized by AMF. *G. straminea* had a very low



**Fig. 3** Chemical niche shifts in terms of N acquisition of dominant plant species between in the annulus zone and outside the fairy ring in an alpine meadow in the Tibetan Plateau. The axes of x, y and z represent the contribution of ammonium, nitrate and glycine to total N uptake (as %). Gray areas refer to chemical niches of different dominant plant species outside the

fairy ring (low N availability), whereas areas enclosed by dark lines indicate the same species in the fungus growth zone of the fairy ring (enhanced N availability). Values are means ( $\pm 1$  SE) of 3 replicates. Asterisks indicate significant difference between in the fungus growth zone and outside the fairy ring at 0.01 (\*) and 0.001 (\*\*)

**Table 2** Percent of N taken up by plants from three N forms both in the fungus growth zone and outside the fairy ring. Means±1SE are presented ( $n=3$ ). Asterisks indicate significant

differences for the same N form between outside the fairy ring and in the fungus growth zone at 0.05 error probability level

Plant species	Outside the fairy ring			The fungus growth zone		
	NH <sub>4</sub> <sup>+</sup>	NO <sub>3</sub> <sup>-</sup>	Glycine	NH <sub>4</sub> <sup>+</sup>	NO <sub>3</sub> <sup>-</sup>	Glycine
<i>K. humilis</i>	26.1±6.9	63.3±6.7*	10.6±1.1*	35.3±2.1	25.6±1.8	39.1±0.4
<i>E. nutans</i>	54.1±0.7*	32.0±0.7	14.0±0.1*	26.5±3.0	24.5±2.1	49.0±3.0
<i>S. aliena</i>	49.1±1.1*	43.1±1.7*	7.7±1.5*	26.5±3.8	26.1±3.4	47.3±1.0
<i>G. diversifolia</i>	95.7±0.7*	1.8±0.3*	2.5±0.4*	5.3±2.2	79.2±10.2	15.5±8.0
<i>G. straminea</i>	45.0±4.5	31.3±4.1	23.8±2.2	49.6±10.2	23.1±3.4	27.3±7.6

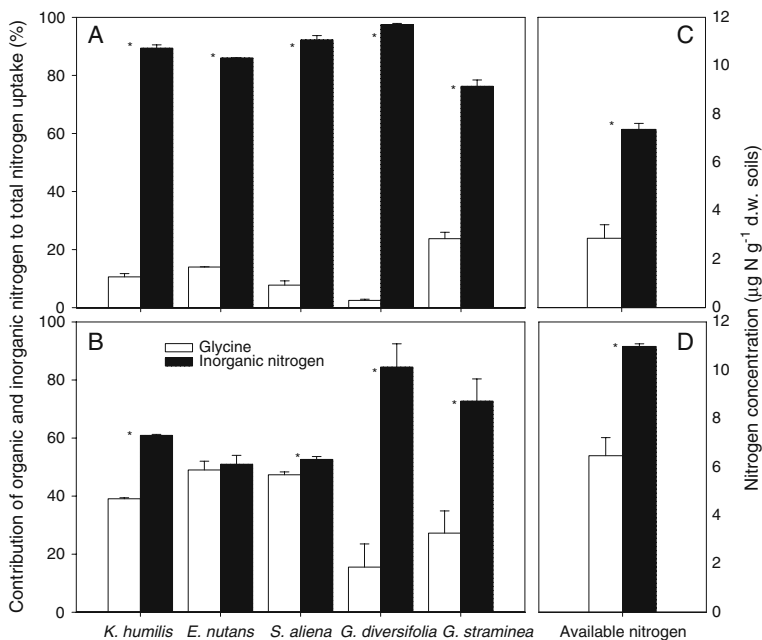
mycorrhizal colonization, ranging between 5% (in the annulus zone of the fairy ring) and 8% (outside the fairy ring). *S. aliena* (in vs. outside, 65% vs. 70%) and *E. nutans* (in vs. outside, 70% vs. 56%) showed higher rates of mycorrhizal colonization.

**Discussion**

We observed different patterns than those reported by McKane et al. (2002) for an arctic tundra ecosystem, i.e. that the most productive species preferentially utilized the most abundant N form while less productive species used less abundant N forms in an arctic tundra ecosystem. In our study *G. diversifolia* had the lowest

total biomass among the five plant species studied at low available N level (Fig. 2), although it utilized the most abundant N form (ammonium) (Fig. 3). The two graminoids demonstrated similar total biomass at low and high available N levels (Fig. 2), and showed a similar pattern switching from NH<sub>4</sub><sup>+</sup>/NO<sub>3</sub><sup>-</sup> uptake outside the ring to glycine uptake in the fungus growth zone of the ring (Fig. 3). In contrast, total biomass of *G. straminea* differed significantly at low and high available N levels, but its N uptake pattern almost remained unchanged (Fig. 3). Only one out of five dominant plant species showed niche conservatism (*G. straminea*), indicating that chemical niche shifts or niche conservatism vary in a species-specific manner. Nonetheless, different patterns of N uptake by dominant

**Fig. 4** Contribution of N from glycine or inorganic N (NO<sub>3</sub><sup>-</sup>+NH<sub>4</sub><sup>+</sup>) to total N uptake by dominant plant species in an alpine meadow in the Tibetan Plateau **a** outside the fairy ring and **b** in the fungus growth zone of the fairy ring. The two extra plots to the right present concentrations of available soil inorganic and organic N **c** outside the fairy ring and **d** in the fungus growth zone of the fairy ring. Asterisks indicate significant differences between inorganic N and glycine uptake at the same site at 0.05 error probability level





plant species under different available N levels mean that dominant plant species can switch their chemical niches for different N forms to acclimate to nutrient enrichment in alpine meadows (Fig. 3).

AMF are mainly associated with P uptake, but numerous studies suggest that they can participate in N acquisition by plants from OM (e.g., Hodge et al. 2001; He et al. 2003; Govindarajulu et al. 2005; Lambers et al. 2008; Hodge and Fitter 2010). This can not fully explain the chemical niche shifts observed here. The roots of *K. humilis* and *G. diversifolia* were not colonized by AMF either in the annulus zone or outside the fairy ring, though both species showed strong and opposite N uptake patterns. At high available N level *K. humilis* shifted to more abundant N forms while *G. diversifolia* shifted to the least abundant N form. *G. diversifolia* had the lowest biomass among the five dominant plant species. A possible explanation for these differences in niche trajectory therefore is that *G. diversifolia* preferentially acquired  $\text{NO}_3^-$ , the least preferred N form at low available N levels, to reduce competition with the other dominant plant species. Several lines of evidence indicate that some grassland species preferentially utilize  $\text{NO}_3^-$  (Miller et al. 2007; von Felten et al. 2009). *K. humilis* has been suggested as a keystone species in this type of meadow, but this is mainly caused by grazing stress because of its stronger tolerance of over-grazing by yaks and sheep and nutrient-poor environments (Kaiser et al. 2008). Field observations have shown that graminoids are gradually becoming dominant when grazing is stopped. Under such conditions *K. humilis* preferentially acquired N from the least available form (nitrate) although it had the highest total biomass (Fig. 2). When N enrichment occurred, *K. humilis* shifted towards using the more abundant N forms. Compared with *K. humilis* and *G. diversifolia*, the roots of the other three species were colonized by AMF. *G. straminea* had a very low mycorrhizal colonization, whereas both *S. aliena* and *E. nutans* exhibited higher rates of mycorrhizal colonization. However, the lack of large differences in mycorrhizal colonization between in the annulus zone and outside the fairy ring, suggests that the shifts in N uptake patterns of these three species were not caused by altered AMF colonization. Overall, colonization of AMF clearly did not object our major finding that the dominant plant species in

these alpine meadows showed chemical niche shifts and differed in their strategy to acquire available soil N under different nutrient levels.

To our knowledge, this is the first report showing shifts in N uptake patterns of dominant plant species in response to nutrient enrichment induced by basidiomycete fungi in native ecosystems, completely different from previous studies. First, most studies about the effects of N enrichment on plant diversity were manipulated by adding extraneous N (Gough et al. 2000; Stevens et al. 2004; Harpole and Tilman 2007; Clark and Tilman 2008; Manning et al. 2008; Duprè et al. 2010; Bobbink et al. 2010). Additions of extraneous N are a good approach to unravel the effects of N deposition on plant diversity. A number of mechanisms have been identified, i.e. long-term negative effect of ammonia and ammonium, soil-mediated effects of acidification and increased susceptibility to secondary stress, disturbance factors and direct toxicity of N gases (Bobbink et al. 2010). Apparently, however, this is not appropriate for investigating the effects of soil N availability on plant diversity through adding extraneous N. Besides, microbial community and activities are also affected by N deposition (Johnson et al. 1998; Waldrop et al. 2004; Bradley et al. 2006; Allison et al. 2007). Consequently, the conclusions obtained from N fertilization observation can not represent the effects of N enrichment derived from enhanced SOM decomposition by soil warming. Second, studies in this regard mainly focused on loss of plant diversity caused by N enrichment. Harpole and Tilman (2007) ascribed grassland plant species loss to reduced niche dimension caused by nutrient enrichment. The expanding of high stature (or N-demanding) species at the expense of low stature (or less competitive) species in grassland ecosystems was regarded as a second mechanism (Berendse and Elberse 1990; Klanderud 2008). In contrast, in this study we focused on species coexistence among dominant plant species when natural nutrient enrichment occurred. Actually, this indicates that nutrient enrichment may have greater impacts on non-dominant plant species than dominant plant species. Recent warming experiments also showed rapid loss of non-dominant species in alpine meadows (Klein et al. 2004; Li et al. 2004). Therefore, further studies should focus on responses of non-dominant species to nutrient enrichment for a better understanding of effects of nutrient enrichment

on plant species diversity, as species-specific responses to warming and nutrient addition were observed in alpine ecosystems (Klanderud 2008). Third, so far the observation regarding niche shift of dominant plant species is obtained from empirical studies in invasive species ecology (Dietz and Edwards 2006; Broennimann et al. 2007; Harrington et al. 2009). Invasive plants often produce certain biochemicals that depress the growth and development of other neighboring plants (Callaway and Aschehoug 2000; Bais et al. 2003; Thorpe et al. 2009). In this case, it is difficult to obtain real processes of niche shift in native ecosystems. Nonetheless, our results also suffered from some deficiencies. First, in this study we only studied a single fairy ring produced by *Agaricus* because it was difficult to find several fairy rings at the same site. Second, biotic and abiotic changes occurred with decomposer fungal growth and activity which influence interactions between plant species. A previous study in the Tibetan alpine meadow showed that *Agaricus* significantly increased inorganic N content and available phosphorus in soil, but didn't alter pH in this type of meadow. The aboveground biomass of graminoids, sedges, legumes and forbs was significantly higher in the annulus zone than outside the fairy ring (Liu 1997). In this study we also observed an increase of total biomass between in the annulus zone compared with the outer zone of the fairy ring. This implies that the basidiomycete fungus *Agaricus campestris* doesn't depress the growth of the five dominant plant species (Fig. 2). Therefore, our results at least reflect how dominant plant species acclimate to high available N level. As the development of fairy rings of this size normally takes decades, we can assume that the observed chemical niche shifts occur within annual to decadal time scales. This indicates that chemical niche shifts can occur rapidly in alpine meadows, and challenges the core of niche-based geographical models which have employed niche conservatism as an important assumption (Peterson et al. 1999; Pearman et al. 2008).

In summary, this study demonstrates that dominant species can acclimate to N enrichment via chemical niche shifts which may indirectly contribute to the maintenance of species coexistence. This finding has important implications with regard to understanding the mechanisms responsible for species coexistence when nutrient enrichment is induced by climate warming.

Additionally, because of rapid chemical niche shifts in terrestrial ecosystems as shown here greater caution should be taken when drawing conclusions using niche-based geographical models to predict how species respond to global warming. Considering there are still some deficiencies, further research needs to investigate more fairy rings and effects of interactions between AMF and basidiomycete fungi on plant N acquisition.

**Acknowledgements** We thank Dr. Douglas Schaefer for his English improvement. We also thank the two anonymous reviewers for their helpful comments that helped us to greatly improve the manuscript. This study was supported by the National Natural Science Foundation of China (Grant No. 30870424) and National Basic Research Program of China (Grant No. 2010CB833501).

## References

- Allison SD, Hanson CA, Treseder KK (2007) Nitrogen fertilization reduces diversity and alters community structure of active fungi in boreal ecosystems. *Soil Biol Biochem* 39:1878–1887
- Bais HP, Vepachedu R, Gilroy S, Callaway RM, Vivanco JM (2003) Allelopathy and exotic plant invasion: from molecules and genes to species interactions. *Science* 301:1377–1380
- Berendse F, Elberse WT (1990) Competition and nutrient availability in heathland and grassland ecosystems. In: Grace JB, Tilman D (Eds) *Perspectives on Plant Competition*, Academic Press, pp 93–116
- Bobbink R, Hicks K, Galloway J, Spranger T, Alkemade R, Ashmore M, Bustamante M, Cinderby S, Davidson E, Dentener F, Emmett B, Erisman J-W, Fenn M, Gilliam F, Nordin A, Pardo L, De Vries W (2010) Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. *Ecol Appl* 20:30–59
- Bradley K, Drijber RA, Knops J (2006) Increased N availability in grassland soils modifies their microbial communities and decreases the abundance of arbuscular mycorrhizal fungi. *Soil Biol Biochem* 38:1583–1595
- Broennimann O, Treier UA, Müller-Schärer H, Thuiller W, Peterson AT, Guisan A (2007) Evidence of climatic niche shift during biological invasion. *Ecol Lett* 10:701–709
- Callaway RM, Aschehoug ET (2000) Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. *Science* 290:521–523
- Chesson P (2000) Mechanisms of maintenance of species diversity. *Ann Rev Ecol Syst* 31:343–366
- Chinese Soil Taxonomy Research Group (1995) *Chinese soil taxonomy*. Science Press, Beijing, pp 58–147
- Clark CM, Tilman D (2008) Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. *Nature* 451:712–715
- Cooke RC, Rayner ADM (1984) *The ecology of saprotrophic fungi*. Longman, London

- Dietz H, Edwards PJ (2006) Recognition that causal processes change during plant invasion helps explain conflicts in evidence. *Ecology* 87:1359–1367
- Duprè C, Stevens CJ, Ranke T, Bleeker A, Pepler-Lisbach C, Gowing DJG, Dise NB, Dorland E, Bobbink R, Diekmann M (2010) Changes in species richness and composition in European acidic grasslands over the past 70 years: the contribution of cumulative atmospheric nitrogen deposition. *Glob Chang Biol* 16:344–357
- Edwards PJ (1988) Effects of the fairy ring fungus *Agaricus arvensis* on nutrient availability in grassland. *New Phytol* 110:377–381
- French HM, Wang B (1994) Climate controls on high altitude permafrost, Qinghai-Xizang (Tibet) Plateau, China. *Permafrost Periglac Process* 5:87–100
- Giorgi F, Hewitson B, Christensen J, Hulme M, Von Storch H, Whetton P, Jones R, Fu C et al (2001) Climate change 2001: regional climate information—evaluation and projections. In: Houghton JT, Griggs DJ, Noguer M, van der Linden PJ, Dai X, Maskell K, Johnson CA (eds) *Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, pp 585–636
- Gough L, Osenberg CW, Gross KL, Collins SL (2000) Fertilization effects on species density and primary productivity in herbaceous plant communities. *Oikos* 89:428–439
- Govindarajulu M, Pfeffer PE, Jin H, Abubaker J, Douds DD, Allen JW, Bücking H, Lammers PJ, Shchar-Hill Y (2005) Nitrogen transfer in the arbuscular mycorrhizal symbiosis. *Nature* 435:819–823
- Gramss G, Voigt K-D, Bergmann H (2005) Factors influencing water solubility and plant availability of mineral compounds in the tripartite fairy rings of *Marasmius oreades* (Bolt.:Fr.) Fr. *J Basic Microbiol* 45:41–54
- Griffith GW, Roderick K (2008) Saprotrophic basidiomycetes in grasslands: distribution and function. In: Boddy L, Frankland JC, van West P (eds), *Ecology of saprotrophic basidiomycetes*. British Mycological Society Symposia Series. Elsevier Ltd., pp 275–297
- Harpole WS, Tilman D (2007) Grassland species loss due to reduced niche dimension. *Nature* 446:791–793
- Harrington LA, Harrington AL, Yamaguchi N, Thom MD, Ferreras P, Windham TR, Macdonald DW (2009) The impact of native competitor on an alien invasive: temporal niche shifts to avoid interspecific aggression. *Ecology* 90:1207–1216
- He X, Critchley C, Bledsoe C (2003) Nitrogen transfer within and between plants through common mycorrhizal networks (CMNs). *Crit Rev Plant Sci* 22(6):531–567
- Hodge A, Fitter A (2010) Substantial nitrogen acquisition by arbuscular mycorrhizal fungi from organic material has implications for N cycling. *PNAS* 107:13754–13759
- Hodge A, Campbell CD, Fitter AH (2001) An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic matter. *Nature* 413:297–299
- Johnson D, Leake JR, Lee JA, Campbell CD (1998) Changes in soil microbial biomass and microbial activities in response to 7 years simulated pollutant nitrogen deposition on heathland and two grasslands. *Environ Pollut* 103:239–250
- Jones DL, Kielland K (2002) Soil amino acid turnover dominates the nitrogen flux in permafrost-dominated taiga forest soils. *Soil Biol Biochem* 34:209–219
- Kahmen A, Renker C, Unsicker SB, Buchmann N (2006) Niche complementarity for nitrogen: an explanation for the biodiversity and ecosystem functioning relationship? *Ecology* 87(5):1244–1255
- Kaiser P (1998) Relations of *Leucopaxillus giganteus*, basidiomycete of fairy rings, with soil microflora and grassland plants. *Cryptogam Mycol* 19:45–61
- Kaiser K, Miede G, Barthelmes A, Ehrmann O, Scharf A, Schult M, Schlütz F, Adamczyk S, Frenzel B (2008) Turf-bearing topsoils on the central Tibetan Plateau, China: pedology, botany, geochronology. *Catena* 73:300–311
- Klanderud K (2008) Species-specific responses of an alpine plant community under simulated environmental change. *J Veg Sci* 19(3):363–372
- Klein JA, Harte J, Zhao X (2004) Experimental warming causes large and rapid species loss, dampened by simulated grazing, on the Tibetan Plateau. *Ecol Lett* 7:1170–1179
- Lambers H, Raven JA, Shaver GR, Smith SE (2008) Plant nutrient-acquisition strategies change with soil age. *Trends Ecol Evol* 23(2):95–103
- Li Y, Zhao L, Zhao X, Zhou H (2004) Effects of a 5-year mimic temperature increase to the structure and productivity of *Kobresia humilis* meadow. *Acta Agrestia Sin* 12(3):236–239
- Liu ZK (1997) A comparison between mushroom sphere and plants outside the sphere and soil in alpine meadow. *Pratacult Sci* 14(3):68–70
- Lükewille A, Wright RF (1997) Experimentally increased soil temperature causes release of nitrogen at a boreal forest catchment in southern Norway. *Glob Chang Biol* 3:13–21
- Manning P, Morrison SA, Bonkowski M, Bardgett RD (2008) Nitrogen enrichment modifies plant community structure via changes to plant–soil feedback. *Oecologia* 157:661–673
- McKane RB, Johnson LC, Shaver GR, Nadelhoffer KJ, Rastetter EB, Fry B, Giblin AE, Kielland K, Kwiatkowski BL, Laundre JA, Murray G (2002) Resource-based niches provide a basis for plant species diversity and dominance in arctic tundra. *Nature* 415:68–71
- Miller AE, Bowman WD (2002) Variation in nitrogen-15 natural abundance and nitrogen uptake traits among co-occurring alpine species: do species partition by nitrogen form? *Oecologia* 130:609–616
- Miller AE, Bowman WD, Suding KN (2007) Plant uptake of inorganic and organic nitrogen: neighbor identity matters. *Ecology* 88(7):1832–1840
- Nasholm T, Sandberg G, Ericsson A (1987) Quantitative-analysis of amino-acids in conifer tissues by high-performance liquid-chromatography and fluorescence detection of their 9-fluorenylmethyl chloroformate derivatives. *J Chromatogr* 396:225–236
- Pearman PB, Guisan A, Broennimann O, Randin CF (2008) Niche dynamics in space and time. *Trends Ecol Evol* 23(3):149–158

- Peterson AT, Soberón J, Sánchez-Cordero V (1999) Conservatism of ecological niches in evolutionary time. *Science* 285:1265–1267
- Rustad LE, Campbell JL, Marion GM, Norby RJ, Mitchell MJ, Hartley AE, Cornelissen JH, Gurevitch J, GCTE-NEWS (2001) A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia* 126:543–562
- Sardans J, Peñuelas J, Estiarte M (2006) Warming and drought alter soil phosphatase activity and soil availability in a Mediterranean shrubland. *Plant Soil* 289:227–238
- Schoener TW (1974) Resource partitioning in ecological communities. *Science* 185:27–39
- Silvertown J (2004) Plant coexistence and the niche. *Trends Ecol Evol* 19:605–611
- Song MH, Xu XL, Hu QW, Tian YQ, Ouyang H, Zhou CP (2007) Interactions of plant species mediated plant competition for inorganic nitrogen with soil microorganisms in an alpine meadow. *Plant Soil* 297:127–137
- Stevens CJ, Dise NB, Mountford JO, Gowing DJ (2004) Impact of nitrogen deposition on the species richness of grasslands. *Science* 303:1876–1879
- Thompson LG, Mosley-Thompson E, Davis M, Lin PN, Yao T, Dyurgerov M (1993) Recent warming: ice core evidence from tropical ice cores with emphasis on Central Asia. *Glob Planet Change* 7:145–156
- Thorpe AS, Thelen GC, Diaconu A, Callaway RM (2009) Root exudate is allelopathic in invaded community but not in native community: field evidence for the novel weapons hypothesis. *J Ecol* 97:641–645
- Tilman D (1982) Resource competition and community structure. Princeton Univ. Press, Princeton
- Vierheilig H, Schweiger P, Brundrett M (2005) An overview of methods for the detection and observation of arbuscular mycorrhizal fungi in roots. *Physiol Plant* 125:393–404
- von Felten S, Hector A, Buchmann N, Niklaus PA, Schmid B, Scherer-Lorenzen M (2009) Belowground nitrogen partitioning in experimental grassland plant communities of varying species richness. *Ecology* 90(5):1389–1399
- Waldrop MP, Zak DR, Sinsabaugh RL (2004) Microbial community response to nitrogen deposition in northern forest ecosystems. *Soil Biol Biochem* 36:1443–1451
- WRB (1998) World reference base for soil resources. FAO/ISRIC/ISSS, Rome
- Zheng D (2000) Mountain geoecology and sustainable development of the Tibetan Plateau. Kluwer, Dordrecht
- Zhou XM (2001) Alpine *Kobresia* meadows in China. Science Press, Beijing, pp 51–62