

RESEARCH ARTICLE

Changes in quantity rather than palatability of alpine meadow species induce cascading effects of long-term nitrogen fertilization on phytophagous insect abundance

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Abstract

Questions: Do changes in plant tissue quality mediated by long-term N enrichment cascade to affect the palatability and thus abundance of phytophagous insects? Do shifts in the abundance of plant functional groups mediated by long-term N enrichment cascade to affect abundance of phytophagous insects?

Location: An alpine meadow on the Tibetan Plateau, China.

Methods: We measured the abundance of larvae of a phytophagous moth (*Gynaephora menyanensis*) in plots subjected to 8 years of fertilization with different N rates and chemical forms in an alpine meadow. In a feeding experiment with the larvae, we tested whether N rate and N form affected leaf consumption of six abundant plant species in the alpine meadow through leaf quality.

Results: High N rate increased larval density by 45.6%. It increased leaf N concentration and decreased leaf C:N of four plant species, but did not affect leaf consumption on any of the six species. High N increased the abundance of more favoured graminoids, but decreased that of less favoured legumes and some forbs. Larval density was positively related to graminoid abundance and negatively related to forb abundance.

Conclusions: Long-term N fertilization induced changes in both leaf quality and abundance of plant species in the community. However, the abundance of phytophagous insects was associated with shifts in plant functional group abundance but not with changes in plant palatability. These findings suggest that N-mediated changes in plant community composition may have cascading effects on insect quantity.

KEYWORDS

cascading effect, caterpillar, feeding preference, feeding test, grassland, nitrogen fertilization, palatability, Tibetan Plateau

1 | INTRODUCTION

Nitrogen (N) is a limiting nutritional element in most terrestrial ecosystems (Elser et al., 2007; Vitousek & Howarth, 1991). However, ecosystems are facing increasing N enrichment, either by atmospheric N deposition or agricultural N fertilization (Fowler, Kilsby, O'Connell, & Burton, 2005; Galloway et al., 2004). N enrichment

causes large changes in structure and function of plant communities, resulting in, for instance, loss of plant species (Borer et al., 2014; Suding et al., 2005; Tilman, Reich, & Knops, 2006), shifts in the relative dominance of species (Bobbink et al., 2010; Phoenix et al., 2012; Suding et al., 2005) and increases in productivity (Song et al., 2012; Xia & Wan, 2008; Zhou, Zhang, & Niklas, 2014). Furthermore, N enrichment modifies N to carbon (C) stoichiometry of plant species (Lu



et al., 2011; Reich et al., 2001). All these changes may have cascading effects on the next trophic level by, for instance, affecting the abundance and feeding preference of phytophagous insects (Barros, Thuiller, Georges, Boulangeat, & Münkemüller, 2016; Mundim, Costa, & Vasconcelos, 2009; Throop & Lerdau, 2004). Knowledge about cascading effects of N enrichment on higher trophic levels may deepen our understanding of how components of ecosystems interact and what proportion of primary productivity may be lost due to, e.g., herbivory, and may thereby better inform sustainable ecosystem management in the face of increasing N deposition.

Leaf N concentration and C:N are closely associated with plant palatability, which has been linked to the performance and population dynamics of phytophagous insects (Clissold, Sanson, & Read, 2006; Clissold, Sanson, Read, & Simpson, 2009; Mattson, 1980). If plant tissue N has not reached the optimal N concentration for herbivores, an increase in plant tissue N concentration or a decrease in C:N may enhance the performance of the herbivores (Mattson, 1980; Throop & Lerdau, 2004; White, 1993). However, if plant tissue N has exceeded the optimal N concentration, then a further increase in plant tissue N may decrease the performance of the herbivores (Cease et al., 2012; Leroy et al., 2013; Raubenheimer & Simpson, 1993; Raubenheimer & Simpson, 2003). We therefore hypothesize that if long-term N fertilization causes changes in leaf N concentration or leaf C:N of dominant plant species (Reich et al., 2001; Stevens, Dise, Mountford, & Gowing, 2004; Xia & Wan, 2008), then it will affect the abundance and feeding preference of phytophagous insects.

Nitrogen-mediated changes in plant community structure may also have a profound effect on the performance and population dynamics of phytophagous insects because many phytophagous insects selectively feed on particular plant species or functional groups (Lewinsohn, Novotny, & Basset, 2005; Novotny & Basset, 2005; Schädler, Jung, Auge, & Brandl, 2003). This could be an important source of either positive or negative feedback on plant community composition (La Pierre, Joern, & Smith, 2015; Throop & Lerdau, 2004). N fertilization generally increases the abundance of graminoids (grasses and/or sedges) and decreases that of legumes (Song et al., 2012; Suding et al., 2005; Xia & Wan, 2008). Furthermore, rare forb species are often at great risk of being lost upon N fertilization (Suding et al., 2005). Such changes in quantity of plant species in different functional groups may further alter the abundance and feeding behaviour of phytophagous insects. We therefore hypothesize that long-term N fertilization will alter the abundance and feeding preference of phytophagous insects also through N-mediated shifts in the abundance of plant species in different functional groups.

Soil N is available in different chemical forms (e.g., ammonium, nitrate and free amino acids; McKane et al., 2002; Martens-Habbena, Berube, Urakawa, De La Torre, & Stahl, 2009; Song, Zheng, Suding, Yin, & Yu, 2015). Atmospheric deposition enriched with certain N forms and long-term addition of agricultural fertilizers with different N forms can increase the availability of ammonium or nitrate in soil disproportionately (Fowler, Flechard, Skiba, Coyle, & Cape, 1998; Galloway et al., 2004; Stevens et al., 2004)

and alter the original proportion of soil available N forms (Wathes, Demmers, & Xin, 2003). Different N forms could induce distinct responses of N uptake in different plant species (McKane et al., 2002; Song et al., 2015) and thus may drive differences in their abundance (Song et al., 2012) and leaf quality. In response to changes in the availability of 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 (Martens-Habbena et al., 2009; McKane et al., 2002; Song et al., 2015). In contrast, species characterized by niche conservatism (Diekmann & Lawesson, 1999) may be less flexible and may continue to rely on a specific N form regardless of changes in availability of alternative forms. Such differences may result in changes in species or functional composition of communities, which may further affect the abundance of phytophagous insects. However, no previous study appears to have examined potential cascading effects of N forms on the abundance and feeding preference of phytophagous insects.

We measured the abundance and feeding preference of larvae of a phytophagous moth (*Gynaephora menyuanensis* Yan & Chou) in an alpine meadow on the Tibetan Plateau, after 8 years of fertilization with different N supply rates and different chemical N forms (ammonium, nitrate or ammonium and nitrate). In this alpine meadow, N fertilization and release from grazing caused some loss of species richness and an increase in above-ground productivity (Song et al., 2012). Here, we examine whether N-mediated changes in leaf nutrient concentration and shifts in abundance of plant functional groups can cascade to affect the abundance of herbivorous insects in this system. To evaluate this, we needed to understand whether and how long-term N fertilization drives cascading effects on the abundance and feeding preference of phytophagous insects, via variation both in leaf quality within given species and in the abundance of plant species in different functional groups that are selectively eaten. We thus specifically examined (a) leaf quality responses of different plant species and their relationships to feeding preference of *G. menyuanensis*, and (b) the population quantity response of *G. menyuanensis* and its relationship to abundance of functional groups relative to their palatability after 8 years of different experimental fertilization treatments in this alpine meadow.

2 | METHODS

2.1 | Study area and experiment description

Our long-term experimental site was an alpine meadow located in the northeastern Tibetan Plateau, Qinghai Province, China. This alpine meadow belongs to Haibei Alpine Meadow Ecosystem Research Station (37°37' N, 101°19' E, 3,200 m a.s.l.) of the Chinese Academy of Sciences. It has a plateau continental monsoon climate, with mean annual temperature of -1.7°C and mean annual precipitation of 580 mm. The meadow is dominated by the sedge *Kobresia humilis* Serg. The phytophagous moth *Gynaephora*

menyuanensis (Lepidoptera, Lymantriidae) is common in the meadow, and feeding by its generalist larvae constitutes one of the main factors that can significantly hamper plant growth (Yan, Liu, & Mei, 1995).

Because low temperature restricts decomposition, most N is bound in organic forms (Cao & Zhang, 2001). Jiang (2010) measured the atmospheric wet N deposition through precipitation in the same alpine meadow from May 2008 to May 2009 at $0.46 \pm 0.03 \text{ g m}^{-2} \text{ year}^{-1}$. However, the current atmospheric wet N deposition may be higher in this region because precipitation from May 2008 to May 2009 was lower than usual (Jiang, 2010).

The long-term N fertilization experiment, which was started in 2005 and coincided with cessation of long-term grazing by placing a fence around the experiment, had three N supply rates (0.375, 1.5 and $7.5 \text{ g N m}^{-2} \text{ year}^{-1}$, referred to as LN, MN and HN, respectively) crossed with three N chemical forms (ammonium-N, nitrate-N and a 50:50 mixture of ammonium-N and nitrate-N, referred to as Am, Ni and AN, respectively), with no N addition as the control (C). LN was close to the current atmospheric wet N deposition ($0.46 \pm 0.03 \text{ g m}^{-2} \text{ year}^{-1}$) through precipitation in the alpine meadow (Jiang, 2010). Because atmospheric N deposition is expected to increase due to increased impacts of anthropogenic activities in China (Zhang et al., 2008; Jiang, 2010) and soil organic matter mineralization is expected to accelerate due to climate warming, soil N availability in this region is expected to increase greatly in the future. Only MN and HN were thus included here to simulate potential N enrichment in the future. In total, therefore, there were seven treatments and each treatment was applied in three replicate $2 \text{ m} \times 2 \text{ m}$ plots. Previous results showed that LN did not cause significant changes in plant species composition or leaf C and N concentration in the alpine meadow. Therefore, and to reduce the workload and expenditure, we excluded LN from this study. Further details of the N fertilization experiment are described in Song et al. (2012).

2.2 | Plant species richness and abundance measurements

A $1 \text{ m} \times 1 \text{ m}$ quadrat was established in the centre of each plot, and occurrence of each vascular species in each quadrat was recorded in the middle of August each year. For biomass measurement, we clipped above-ground shoots within a $0.25 \text{ m} \times 0.25 \text{ m}$ quadrat outside the central $1 \text{ m} \times 1 \text{ m}$ quadrat but within the plot in the middle of August every year when biomass peaked. The quadrat for clipping shifted each year within the plot to avoid harvesting the same area in successive years. Shoots were clipped at ground level and sorted into four plant functional groups, i.e., grasses, sedges, legumes and forbs. All shoots were oven-dried at 60°C for 48 hr and then weighed (see Song et al., 2012 for more information). We recorded a total of 32 species in our plots. In the $1 \text{ m} \times 1 \text{ m}$ quadrats, species richness was 25 on average, ranging from 23 to 27, and may fluctuate from year to year (Song et al., 2012).

2.3 | Moth larva survey

For each of the seven treatments used in this study, we counted the number of *G. menyuanensis* larvae in two $50 \text{ cm} \times 50 \text{ cm}$ quadrats in each plot on 29 and 30 June 2012. Observations were performed twice per day, at around 10:00 and 15:00 hours, when the larvae were very active. The data for the two observations per day were averaged. The larvae were in the third instar during this period. Density of the larvae during 2012 was typical of most years (Ming-Hua Song *pers obs*).

2.4 | Feeding test

A feeding test was carried out at the end of June 2012, the 8th year after the start of the N fertilization. For the test, we selected six abundant plant species that occurred in all the plots, i.e., one sedge (*K. humilis*), two grasses (*Elymus nutans* and *Stipa aliena*), one legume (*Oxytropis ochrocephala*) and two forbs (*Gentiana straminea* and *Saussurea superba*). For each of these six species, its above-ground biomass was no less than 5% of above-ground biomass of the community (i.e., summed biomass of all species in the community) since the experiment started in 2005. Also, summed above-ground biomass of the six species amounted to more than 63% of above-ground biomass of the community. Thus, they well represented the abundance of the community. In each of the seven treatments, we set up two quadrats of $50 \text{ cm} \times 50 \text{ cm}$ at least 1 m apart in each plot. These quadrats were different from those for the larva survey to avoid disturbance effects of leaf collecting on larval densities. In each quadrat, we collected fresh leaves of the six target species in the morning and stored them in sealed polythene bags at 4°C before the feeding test. One part of each leaf sample was oven-dried at 65°C for 48 hr and used for measuring leaf C and N concentrations with an elemental analyser (vario MACRO cube, Elementar, Frankfurt, Germany).

We collected fresh leaves from one quadrat in each of the seven treatments on 24–29 June 2012 and collected third-instar, similar-sized larvae of *G. menyuanensis* from the unfertilized areas close to our experimental plots. The feeding test employed a “cafeteria” approach (Pérez-Harguindeguy et al., 2013). The larvae were starved for 24 hr before the test. The leaves of the target species were cut into pieces of 50 mm^2 . Leaves of species with relatively wide, short ones (*O. ochrocephala*, *G. straminea*, and *S. superba*) were cut into pieces of $5 \text{ mm} \times 10 \text{ mm}$; leaves of species with narrower and longer ones (*K. humilis*, *E. nutans* and *S. aliena*) were cut into pieces of $2.5 \text{ mm} \times 20 \text{ mm}$ or $2 \text{ mm} \times 25 \text{ mm}$ depending on leaf width. The leaf pieces were fixed with steel pins on arenas made of foam boards installed at the inner bottom of six glass boxes ($1\text{-m long} \times 1\text{-m wide} \times 0.25\text{-m high}$) designed by us. Each box, which was treated as a block, contained eight pieces of each of the six plant species from each treatment that had been collected on the same day. The 336 leaf pieces (7 treatments \times 6 species \times 8 pieces) in each box were randomly arranged in 19 rows and 19 columns.

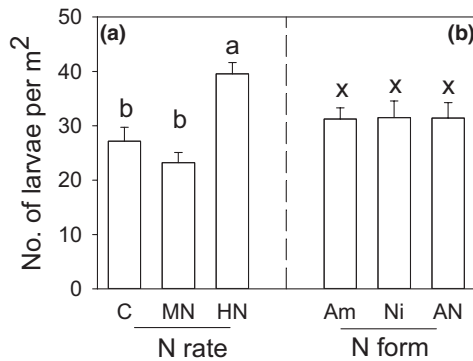


FIGURE 1 Effects of N rate (a) and N form (b) on number of *Gynaephora menyuanensis* larvae in the alpine meadow. MN and HN are N addition at rates of 1.5 and 7.5 g m⁻² year⁻¹, respectively, and C for no N addition. Am, Ni and AN are addition of ammonium-N, nitrate-N and both ammonium-N and nitrate-N, respectively. Bars and vertical lines are mean \pm SE. For N form and N rate, respectively, bars sharing the same letter are not different at $p = 0.05$

The feeding tests were conducted on 6 days from 25 to 30 June 2012. On each test day at 09:00 a.m., 20 third-instar larvae of *G. menyuanensis* were randomly placed on each arena. To keep plant material fresh and moist, we installed wet filter paper on the foam board under the leaf pieces (Cornelissen, Pérez-Harguindeguy, Cabido, Vendramini, & Cerabolini, 1999) and carefully sprayed a small amount of water into the boxes at noon each day during the experiment. The boxes were put close to the experimental site during the day and covered with plastic film at night to avoid damage from freezing or rain. The feeding test ran for 72 consecutive hours, and leaf consumption by the larvae was calculated as percentage loss of leaf area and mass at the end of the feeding test.

2.5 | Data analyses

Data on number of *G. menyuanensis* larvae, leaf consumption, leaf N concentration, C concentration and C:N were analysed using general linear models. In particular, we used four-way nested ANOVA with repeated measures to examine the effects of N supply rate (MN or HN), N chemical form (Am, Ni or AN), survey date (29 or 30 June 2012) and plot on number of *G. menyuanensis* larvae (Sokal & Rohlf, 1995). N rate and N form were treated as fixed factors. Survey date was treated as a repeated factor as the data in the same plots were repeatedly collected on 2 days, and these data collected on the 2 days were thus not independent. Plot was included as a random factor nested within N form because, in each plot, the data were collected from two quadrats. In this analysis, the control (C) was not included as the experiment was not a factorial design and the control belonged to none of three treatments of N form. We then used linear contrasts to examine the overall differences (averaged across the two dates) among the three N form treatments (Am, Ni and AN; Sokal & Rohlf, 1995), and among the three N rate treatments (C, MN, and HN) in which the control was included.

We employed four-way nested ANOVA to test the effects of species, N rate, N form and plot (as a random factor) on leaf consumption, leaf N concentration, C concentration and C:N (Sokal & Rohlf, 1995). Species was treated as a fixed factor because the six species occurred in all the plots. Again, the control was not included as it did not belong to any of three treatments of N form. We then used Tukey post-hoc tests to examine the differences among the six species. For each species, we also used Tukey tests to examine the differences among the three N form treatments, and among the three N rate treatments in which the control was included (C, MN and HN). Data on leaf consumption were transformed to the arc-sine of the square root to improve normality and homogeneity of variance.

Regression analyses were performed to examine the relationships of number of *G. menyuanensis* larvae with the abundance (above-ground biomass) of each of the six target species and of each of the four functional groups (grasses, sedges, legumes and forbs) across all treatments. For the regression analyses, we used linear, power, exponential and logarithmic functions and selected the one that gave the largest R^2 and the smallest p -value. We chose these four models as they are among the simplest regression models and their biological meanings are easy to interpret. Also, these four regression models are most widely used in ecological research. Analyses were performed using SAS v 9.2 (SAS Institute Inc., 2009).

3 | RESULTS

3.1 | Effects of N rate and form on larval abundance

Nitrogen supply rate affected number of *G. menyuanensis* larvae ($F_{1,6} = 18.8$, $p = 0.005$). High N rate increased the number of larvae by 45.6% compared to the control (Figure 1a; Supporting Information Appendix S1). However, neither N form ($F_{2,6} < 0.01$, $p = 0.999$) nor its interaction with N rate ($F_{2,6} = 0.8$, $p = 0.514$) affected number of larvae (Figure 1b; Supporting Information Appendix S1).

3.2 | Relationships of larval abundance with species and functional group abundance

At the functional group level, number of *G. menyuanensis* larvae was positively related to the abundance of grasses (Figure 2a), and negatively related to the abundance of forbs (Figure 2d). It was not related to the abundance of sedges (Figure 2b) or legumes (Figure 2c).

At the species level, number of *G. menyuanensis* larvae was significantly or marginally positively related to the abundance of the grasses *E. nutans* (Supporting Information Appendix S5b) and *S. aliena* (Supporting Information Appendix S5c). It was negatively related to the abundance of the forb *S. superba* (Supporting Information Appendix S5f), but not related to the abundance of the sedge *K. humilis*, the legume *O. ochrocephala* or the forb *G. straminea* (Supporting Information Appendix S5a, d, and e).

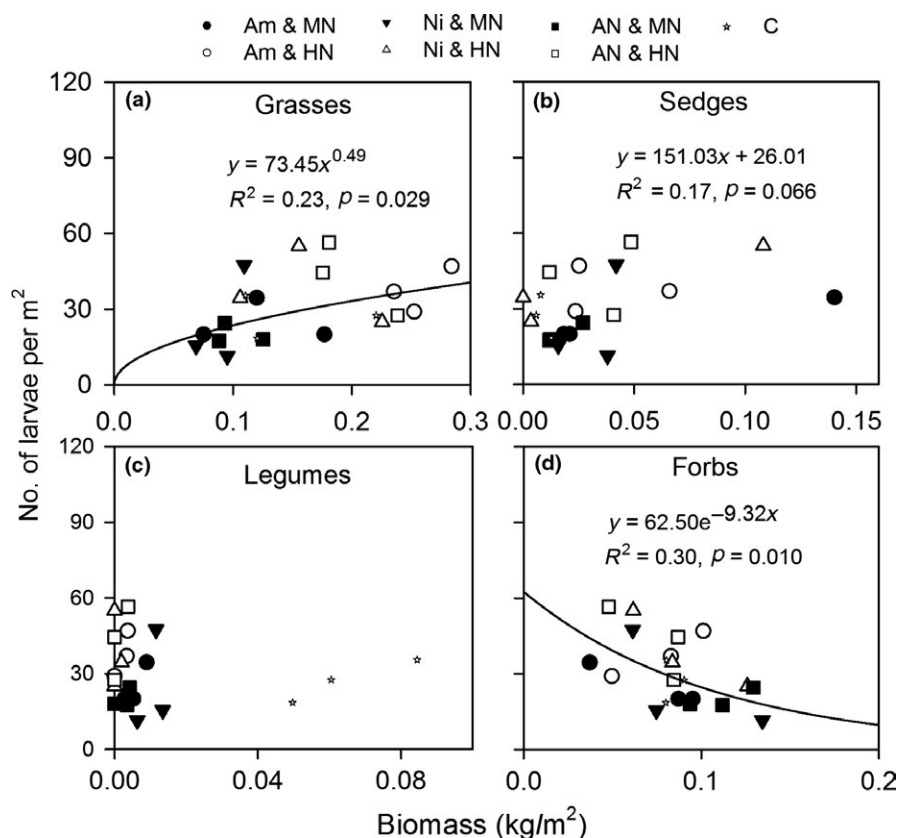
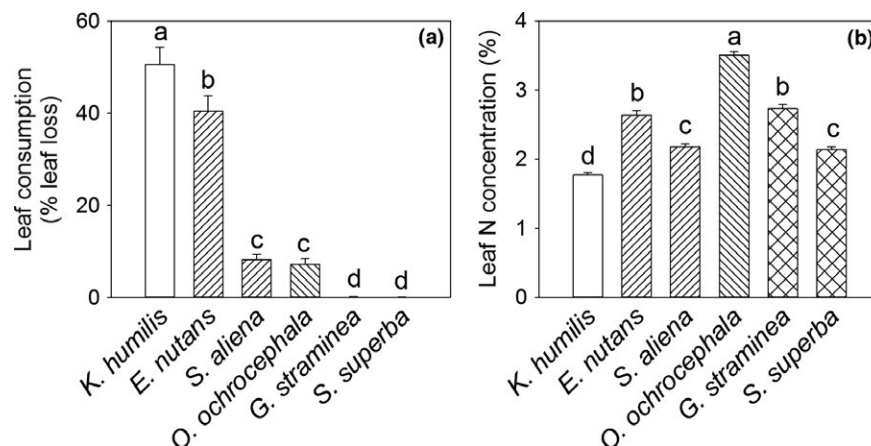


FIGURE 2 Relationships of number of *Gynaephora menyuanensis* larvae to the abundance (above-ground biomass) of each of the four plant functional groups (a–d). Treatment codes are described as in Figure 1

FIGURE 3 Differences in leaf consumption by the *Gynaephora menyuanensis* larvae measured as percentage of leaf area loss (a) and leaf N concentration (b) in the six species representing the most abundant species in four plant functional groups in the alpine meadow. Bars and vertical lines are mean \pm SE. Bars sharing the same letter are not different at $p = 0.05$. Functional groups are indicated by differences in symbol fills: open—sedges; left hatched—grasses; right hatched—legumes; crossed—forbs



3.3 | Effects of plant species, N rate and form on feeding preference and leaf quality

Leaf consumption by the larvae differed greatly among the six species (Figure 3a; Supporting Information Appendix S2). The sedge *K. humilis* (51%) sustained the highest consumption, followed by the grass *E. nutans* (40%) and then the grass *S. aliena* (8%) and the legume *O. ochrocephala* (7%; Figure 3a). The forbs *G. straminea* and *S. superba* were barely consumed (Figure 3a). Neither N rate nor N form affected leaf consumption by the larvae in any of the six species (Supporting Information Appendices S2, S6).

Leaf N concentration and leaf C:N differed among the six species (Figure 3b; Supporting Information Appendices S3, S7). *O. ochrocephala* (3.53%) had the highest leaf N concentration, followed by *G. straminea* (2.40%) and *E. nutans* (2.42%), and then by *S. aliena* (2.07%) and *S. superba* (2.10%); *K. humilis* (1.68%) had the lowest content (Figure 3b). Leaf C:N showed exactly the opposite pattern because absolute values of leaf C (ranging from 42% to 46%) were very similar among the six species (Supporting Information Appendix S7).

Effects of N supply rate on leaf N concentration and leaf C:N varied among species (Figure 4; Supporting Information Appendices S3, S8). Compared to the control, high N rate increased leaf N

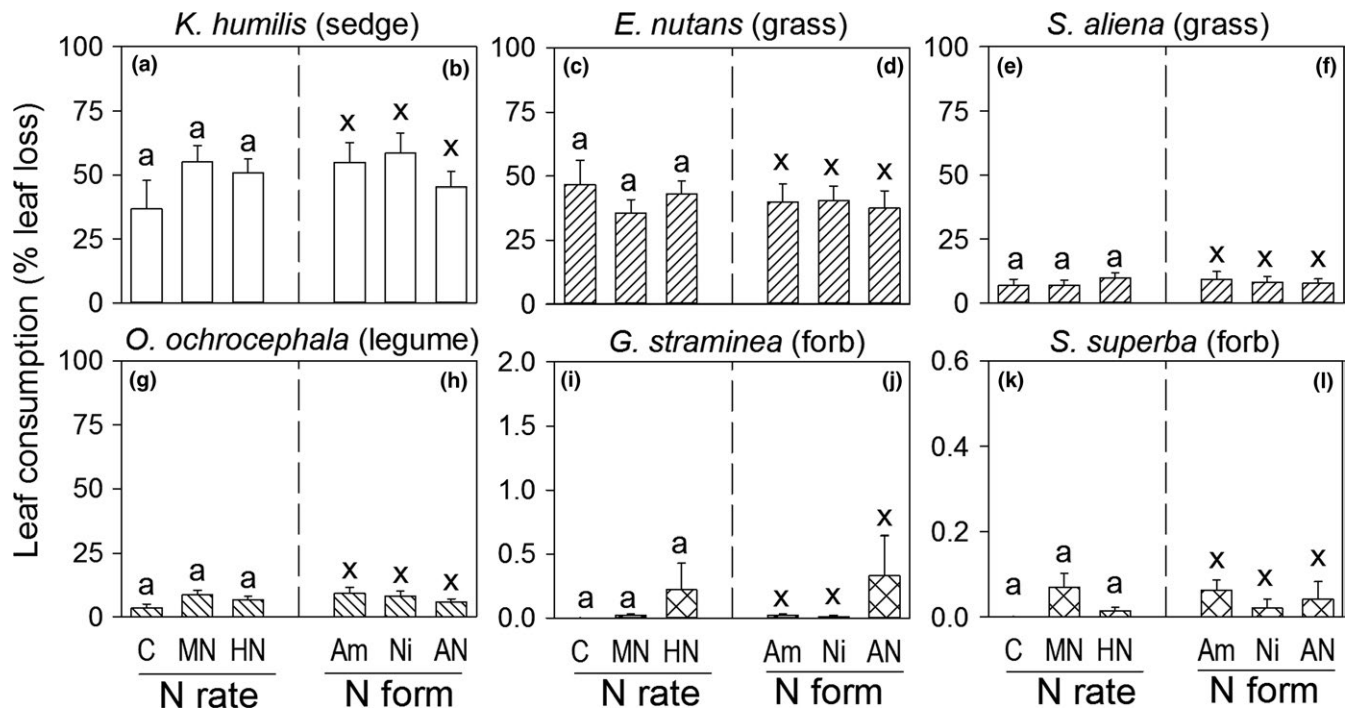


FIGURE 4 Effects of N form and N rate on leaf N concentration for each of six abundant plant species in the alpine meadow. Treatment codes are described as in Figure 1. Bars and vertical lines are mean \pm SE. For N form and N rate, respectively, bars sharing the same letter are not different at $p = 0.05$. Functional groups are also indicated by differences in fills (see Figure 3)

concentration and decreased leaf C:N in four species (*K. humilis*, *E. nutans*, *S. aliena* and *G. straminea*; Figure 4a,c,e,i; Supporting Information Appendix S8m,o,q,u), but had no effect in the other two species (Figure 4g, k; Supporting Information Appendix S8s, w). Moderate N rate did not affect leaf N concentration or leaf C:N in any of the six species (Figure 4; Supporting Information Appendix S8).

Effects of N form on leaf N concentration and leaf C:N also varied among species (Figure 4; Supporting Information Appendices S3, S8). Leaf N concentration was lower in Ni than in Am in *K. humilis* (Figure 4b), but higher in Ni and AN than in Am in *S. aliena* (Figure 4f) and *S. superba* (Figure 4l), and also higher in Ni than in Am in *G. straminea* (Figure 4j). Leaf C:N again showed the opposite pattern (Supporting Information Appendix S8).

4 | DISCUSSION

Eight years of N fertilization with high supply rates increased the abundance but did not affect the feeding preference of the phytophagous moth *G. menyuanensis* in the alpine meadow. Interestingly, the increased abundance of the larvae was associated with the N-mediated shifts in the abundance of functional groups, but not with the N-mediated changes in leaf nutrient quality. The results suggest that effects of long-term N input on plant communities can partly explain the population dynamics of phytophagous insects in this alpine meadow (La Pierre & Smith, 2016; La Pierre et al., 2015; Throop & Lerdau, 2004; Wookey et al., 2009).

4.1 | Effects of N-mediated shifts in functional group abundance on larval abundance

High N rate caused changes in the abundance of plant functional groups and in the abundance of the *G. menyuanensis* larvae in the alpine meadow. Resource availability is the main determinant of the dynamics and distribution of phytophagous insects (Didham & Springate, 2003; Grimbacher & Stork, 2007). Generally, the distribution pattern of phytophagous insects follows the pattern of their favoured host plant species (Didham & Springate, 2003; Grimbacher & Stork, 2007), which may be favoured at least partly for their lack of secondary compounds. Compared to monocots (e.g., grasses and sedges), eudicots (e.g., legumes and forbs) generally contain a broad spectrum of both specific and generic secondary compounds, especially phenolics, which have likely evolved as deterrents against herbivores (Harborne, 1997; Lambers, Chapin, & Pons, 2008). The leaves of the forbs in our study likely contained secondary compounds that made them unpalatable to the larvae irrespective of their N content. This is especially true for the legume *O. ochrocephala*, a species in the genus *Oxytropis*. Species in this genus, known as locoweeds, are notorious for producing swainsonine, a phytotoxin that negatively affects grazing animals such as insect herbivores (Descombes et al., 2017; Ralphs & James, 1999). Therefore, the abundance of insects is expected to be proportionally higher in areas with a high abundance of their favoured host plants (Futuyma & Wasserman, 1980; Peter & Johnson, 2014), i.e., grasses and sedges in our study.

High N rate increased the abundance of grasses that were generally preferred by the *G. menyuanensis* larvae and also most

abundant in this alpine meadow (Supporting Information Appendix S9). Consumption of the dominant grass species by the larvae also overrode the consumption on the most favoured sedge species, suggesting a trade-off between food quantity and palatability as drivers of larval abundance. On the other hand, high N rate decreased the abundance of legumes and did not change that of forbs (Supporting Information Appendices S4, S9), which were little favoured by the larvae. Furthermore, the abundance of the larvae in the field was positively related to the abundance of grasses, but negatively related to that of forbs (Figure 2). Our results suggest that a N-mediated shift in functional group abundance, especially the increase in abundance of grasses, can partly explain the increase in abundance of the larvae (Figure 4). Thus, long-term fertilization with high N rate increased the resources favoured by the phytophagous larvae and subsequently promoted consumer abundance. However, regression analysis can only provide a correlation between grass and larvae abundance, suggesting the cascade to herbivorous insects in the long-term N addition through alterations in plant community composition. The 23% explanatory power of the grass above-ground biomass to the variation in the larvae abundance indicates that N-induced alterations in other biotic or abiotic factors may also drive changes in larvae abundance. Moreover, the low replications for each treatment could lead to high within-group variances in abundance of both larvae and plant functional groups, which would subsequently weaken their relationship. Further manipulative experiments are needed to test the factors driving the cascade to the phytophagous moth in this N-limited alpine meadow.

Nitrogen chemical form did not affect the abundance of the *G. menyuanensis* larvae, likely because N form did not affect the abundance of *S. aliena* or *E. nutans* (Supporting Information Appendix S4d, f), the species favoured by the *G. menyuanensis* larvae. Although the abundance of grasses was lower in the nitrate than in the ammonium treatment (Supporting Information Appendix S9), nitrate-N increased the abundance of the most favoured species (*K. humilis*; Supporting Information Appendix S4b); the different responses of the three favoured species (*K. humilis*, *E. nutans*, and *S. aliena*) led to no significant difference in their total abundance among the three N form treatments. Consequently, we failed to detect an effect of N form on larval abundance.

4.2 | Lack of effects of N-mediated changes in leaf quality on larva feeding preference

Many phytophagous insects show strong host preference (Fontana, Partridge, & Longo, 2010; Wan & Zhang, 2006). Our feeding test also showed that larvae of *G. menyuanensis*, despite being moderately generalist herbivores, strongly preferred the dominant graminoids (Figure 3). Within 72 hr, the larvae consumed 51% and 40% of the leaves of the sedge *K. humilis* and the abundant grass *E. nutans*, respectively, very little of the abundant legume *O. ochrocephala*, and virtually none of the abundant forb *G. straminea*. Below the optimal N concentration, plant palatability to herbivores often increases with increasing N concentration and decreasing C:N in plant

tissues (Grime Cornelissen, Thompson, & Hodgson, 1996) because herbivore performance is often constrained due to the limitation of available nutrient elements or proteins (Andersen, Kristensen, Loeschcke, Toft, & Mayntz, 2010; Cornelissen et al., 1999; Throop & Lerdau, 2004). However, although high N rate increased leaf N concentration and decreased leaf C:N of most of the study species, including *K. humilis* and *E. nutans* (Figure 4; Supporting Information Appendix S8), this change in leaf nutritional quality had no effect on the feeding preference of the *G. menyuanensis* larvae (Supporting Information Appendix S6). Similarly, although N form changed leaf N concentration or leaf C:N, it did not affect the feeding preference of the larvae. Our principal explanation for this is that any subtle changes in leaf quality within species in terms of N concentration and C:N stoichiometry were overruled by the strong larval preference ranking of the different plant species, with a large division between palatable monocots (graminoids) and unpalatable eudicots (legumes and forbs).

An additional explanation for lack of feeding response to intra-specific leaf nutritional quality may lie in chemical stoichiometry. Proteins and carbohydrates are sources of amino acids and energy that are important for survival, growth and reproduction of herbivores (Karasov & Martinez del Rio, 2007; Simpson & Raubenheimer, 2012). Most herbivores have their own species-specific forms of proteins and carbohydrates to ensure their optimal performance (Behmer, 2009; Raubenheimer & Simpson, 2003; Simpson & Raubenheimer, 2012; Throop & Lerdau, 2004). Finding foods that contain proteins and carbohydrates in optimal ratios is a challenge to many herbivores because plants can be highly variable with respect to their protein and digestible carbohydrate concentrations (Behmer & Joern, 2012; Clissold et al., 2009). Herbivores can, however, overcome some of this variation (Clements, Raubenheimer, & Choat, 2009; Felton et al., 2009; Wright, Simpson, Raubenheimer, & Stevenson, 2003). In our study, leaf C:N of the most favoured plant (*K. humilis*) was 26 and that of the least favoured plant (*O. ochrocephala*) was 12. This broad C:N range suggests the *G. menyuanensis* larvae are able to feed on plants with a wide protein/carbohydrate range (Yan et al., 1995). Although high N rate increased leaf N concentration, it reduced leaf C:N only from 26 to 23 in *K. humilis* and from 18 to 15 in *E. nutans*. Therefore, for the same plant species, the increased leaf N concentration and decreased leaf C:N under high N supply rate were not too extreme, so that leaf N concentration and C:N were still within the broad protein/carbohydrate range that is functionally optimal for the larvae. Consequently, such changes could not induce a significant change in feeding preference of the larvae. An alternative explanation might be that the C quality was also affected by the fertilization treatments, i.e., high N concentration might in theory have been accompanied by less digestible or less "tasty" chemical forms of C.

We recorded larval consumption on an area basis, but long-term N addition could cause alteration in leaf mass per area (LMA). Therefore, the same variation in leaf area consumption by the *G. menyuanensis* larvae may not match that of the leaf mass consumed. Also, the larvae might have a preference for low LMA,



i.e., less tough leaves, and there is the theoretical possibility of herbivore feedback to LMA. On the one hand, there might be an "induced resistance response" (Morris, Traw, & Bergelson, 2006) by which concentrations of secondary compounds in leaves, and thereby LMA, are raised by the plant as a defence against further herbivory. On the other hand, herbivory on relatively old leaves might lead to compensatory expansion of new, more productive leaves with relatively low LMA (Belsky, 1986; Cornelissen, 1993). However, based on our results, none of the possible mechanisms is likely to have been a significant factor in our experiment: LMA of the six species measured on 1 July 2015 showed that N addition effects on LMA were small, which had little effect on the consumption patterns (Supporting Information Appendix S10). The only significant effects of N form and N rate on LMA were observed in the legume *O. ochrocephala* and the forb *S. superba* (Supporting Information Appendix S10h, k), which were barely consumed by the larvae. As a consequence, N addition did not cause significant changes in the consumption of leaf mass within any of the plant species (Supporting Information Appendices S2, S11).

5 | CONCLUSIONS

In alpine meadows, long-term N fertilization can have a cascading effect on higher trophic levels such as phytophagous insects, and such an effect could be partly realized through N-mediated changes in quantity of different functional groups in terms of differences in palatability between species rather than in terms of changes in leaf quality within species. However, while the regression results demonstrate a positive relationship between grass and larvae abundance, it also indicates that additional factors may drive the increase in larvae abundance in the high N plots. Identifying and quantifying these other factors require follow-up experiments. Nevertheless, the current study is a first step to test the cascading effect of long-term N addition on larvae feeding preference. Further investigations should aim to examine whether the negative moth feedback shown here could arguably add a further mechanism for plant species co-existence and maintenance of diversity in these alpine meadow ecosystems.

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CONFLICT OF INTEREST

The authors have no competing interests.

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REFERENCES

- Andersen, L. H., Kristensen, T. N., Loeschcke, V., Toft, S., & Mayntz, D. (2010). Protein and carbohydrate composition of larval food affects tolerance to thermal stress and desiccation in adult *Drosophila melanogaster*. *Journal of Insect Physiology*, 56, 336–340. <https://doi.org/10.1016/j.jinsphys.2009.11.006>
- Barros, C., Thuiller, W., Georges, D., Boulangeat, I., & Münkemüller, T. (2016). N-dimensional hypervolumes to study stability of complex ecosystems. *Ecology Letters*, 19, 729–742. <https://doi.org/10.1111/ele.12617>
- Behmer, S. T. (2009). Insect herbivore nutrient regulation. *Annual Review of Entomology*, 54, 165–187. <https://doi.org/10.1146/annurev.ento.54.110807.090537>
- Behmer, S. T., & Joern, A. (2012). Insect herbivore outbreaks viewed through a physiological framework: Insights from *Orthoptera*. In P. Barbosa, D. K. Letourneau, & A. A. Agrawal (Eds.), *Insect outbreaks revisited* (pp. 1–29). Oxford, UK: Blackwell.
- Belsky, A. J. (1986). Does herbivory benefit plants? A review of the evidence. *The American Naturalist*, 127, 870–892. <https://doi.org/10.1086/284531>
- Bobbink, R., Hicks, K., Galloway, J., Spranger, T., Alkemade, R., Ashmore, M., ... Dentener, F. (2010). Global assessment of nitrogen deposition effects on terrestrial plant diversity: A synthesis. *Ecological Applications*, 20, 30–59. <https://doi.org/10.1890/08-1140.1>
- Borer, E. T., Seabloom, E. W., Gruner, D. S., Harpole, W. S., Hillebrand, H., Lind, E. M., & Yang, L. H. (2014). Herbivores and nutrients control grassland plant diversity via light limitation. *Nature*, 508, 517–520. <https://doi.org/10.1038/nature13144>
- Cao, G. M., & Zhang, J. X. (2001). Soil nutrition and substance cycle of *Kobresia* meadow. In X. M. Zhou (Ed.), *Chinese Kobresia meadows* (pp. 58–147). Beijing, China: Science Press.
- Cease, A. J., Elser, J. J., Ford, C. F., Hao, S., Kang, L., & Harrison, J. F. (2012). Heavy livestock grazing promotes locust outbreaks by lowering plant nitrogen content. *Science*, 335, 467–469. <https://doi.org/10.1126/science.1214433>
- Clements, K. D., Raubenheimer, D., & Choat, J. H. (2009). Nutritional ecology of marine herbivorous fishes: Ten years on. *Functional Ecology*, 23, 79–92. <https://doi.org/10.1111/j.1365-2435.2008.01524.x>
- Clissold, F. J., Sanson, G. D., & Read, J. (2006). The paradoxical effects of nutrient ratios and supply rates on an outbreaking insect herbivore, the Australian plague locust. *Journal of Animal Ecology*, 75, 1000–1013.
- Clissold, F. J., Sanson, G. D., Read, J., & Simpson, S. J. (2009). Gross vs. net income: How plant toughness affects performance of an insect herbivore. *Ecology*, 90, 3393–3405. <https://doi.org/10.1890/09-0130.1>
- Cornelissen, J. H. C. (1993). Growth, morphology and leaf characteristics after simulated herbivory in Chinese subtropical evergreen saplings. *Ecological Research*, 8, 143–150. <https://doi.org/10.1007/BF02348526>
- Cornelissen, J. H. C., Pérez-Harguindeguy, N., Cabido, M., Vendramini, F., & Cerabolini, B. (1999). Leaf structure and defence control litter decomposition rate across species and life forms in regional floras on two continents. *New Phytologist*, 143, 191–200. <https://doi.org/10.1046/j.1469-8137.1999.00430.x>
- Descombes, P., Marchon, J., Pradervand, J. N., Bilat, J., Guisan, A., Rasmann, S., & Pellissier, L. (2017). Community-level plant palatability increases with elevation as insect herbivore abundance declines. *Journal of Ecology*, 105, 142–151. <https://doi.org/10.1111/1365-2745.12664>
- Didham, R. K., & Springate, N. D. (2003). Determinants of temporal variation and insect herbivory: Patterns in a successional old-field plant community. *Oikos*, 103, 121–132.

- Diekmann, M., & Lawesson, J. (1999). Shifts in ecological behaviour of herbaceous forest species along a transect from northern central to North Europe. *Folia Geobotanica*, 34, 127–141. <https://doi.org/10.1007/BF02803080>
- Elser, J. J., Bracken, M. E., Cleland, E. E., Gruner, D. S., Harpole, W. S., Hillebrand, H., ... Smith, J. E. (2007). Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters*, 10, 1135–1142. <https://doi.org/10.1111/j.1461-0248.2007.01113.x>
- Felton, A. M., Felton, A., Raubenheimer, D., Simpson, S. J., Foley, W. J., & Wood, J. T. (2009). Protein content of diets dictates the daily energy intake of a free-ranging primate. *Behavioral Ecology*, 20, 685–690. <https://doi.org/10.1093/beheco/arp021>
- Fontana, L., Partridge, L., & Longo, V. D. (2010). Dietary restriction, growth factors and aging: From yeast to humans. *Science*, 328, 321–326.
- Fowler, D., Flechard, C., Skiba, U., Coyle, M., & Cape, J. N. (1998). The atmospheric budget of oxidized nitrogen and its role in ozone formation and deposition. *New Phytologist*, 139, 11–23. <https://doi.org/10.1046/j.1469-8137.1998.00167.x>
- Fowler, H. J., Kilsby, C. G., O'Connell, P. E., & Burton, A. (2005). A weather type conditioned multi-site stochastic rainfall model for generation of scenarios of climatic variability and change. *Journal of Hydrology*, 308, 50–66. <https://doi.org/10.1016/j.jhydrol.2004.10.021>
- Futuyma, D. J., & Wasserman, S. S. (1980). Resource concentration and herbivory in oak forests. *Science*, 210, 920–922. <https://doi.org/10.1126/science.210.4472.920>
- Galloway, J. N., Dentener, F. J., Capone, D. G., Boyer, E. W., Howarth, R. W., Seitzinger, S. P., ... Vörösmarty, C. J. (2004). Nitrogen cycles: Past, present, and future. *Biogeochemistry*, 70, 153–226. <https://doi.org/10.1007/s10533-004-0370-0>
- Grimbacher, P. S., & Stork, N. E. (2007). Vertical stratification of feeding guilds and body size in beetle assemblages from an Australian tropical rainforest. *Austral Ecology*, 32, 77–85. <https://doi.org/10.1111/j.1442-9993.2007.01735.x>
- Grime, J. P., Cornelissen, J. H. C., Thompson, K., & Hodgson, J. G. (1996). Evidence for a causal connection between anti-herbivore defence and the decomposition rate of leaves. *Oikos*, 77, 489–494.
- Harborne, J. B. (1997). Plant secondary metabolism. In M. J. Crawley (Ed.), *Plant ecology*, 2nd ed (pp. 132–155). Oxford, UK: Blackwell.
- Jiang, C. M. (2010). *Effect of increasing nitrogen deposition on the key ecological processes in the Haibei alpine meadow on Qinghai-Tibetan Plateau*. Ph.D. dissertation. Beijing, China: Institute of Geographical Sciences and Natural Resources Research, Chinese Academy of Sciences.
- Karasov, W. H., & Martinez del Rio, R. C. (2007). *Physiological ecology: How animals process energy, nutrients, and toxins*. Princeton, NJ: Princeton University Press.
- La Pierre, K. J., Joern, A., & Smith, M. D. (2015). Invertebrate, not small vertebrate, herbivory interacts with nutrient availability to impact tallgrass prairie community composition and for biomass. *Oikos*, 124, 842–850. <https://doi.org/10.1111/oik.01869>
- La Pierre, K. J., & Smith, M. D. (2016). Soil nutrient additions increase invertebrate herbivore abundances, but not herbivory, across three grassland systems. *Oecologia*, 180, 485–497. <https://doi.org/10.1007/s00442-015-3471-7>
- Lambers, H., Chapin III, F. S., & Pons, T. L. (2008). *Plant Physiological Ecology (2nd ed.)* (pp. 445–477). New York, NY: Springer. <https://doi.org/10.1007/978-0-387-78341-3>
- Leroy, C., Carrias, J. F., Corbara, B., Pélozuelo Dézerald, O., Brouard, O., Dejean, A., & Céréghino, R. (2013). Mutualistic ants contribute to tank-bromeliad nutrition. *Annals of Botany*, 112, 919–926. <https://doi.org/10.1093/aob/mct147>
- Lewinsohn, T. M., Novotny, V., & Basset, Y. (2005). Insects on plants: Diversity of herbivore assemblages revisited. *Annual Review of Ecology, Evolution, and Systematics*, 36, 597–620. <https://doi.org/10.1146/annurev.ecolsys.36.091704.175520>
- Lu, M., Yang, Y., Luo, Y., Fang, C., Zhou, X., Chen, J., ... Li, B. (2011). Responses of ecosystem nitrogen cycle to nitrogen addition: A meta-analysis. *New Phytologist*, 189, 1040–1050. <https://doi.org/10.1111/j.1469-8137.2010.03563.x>
- Martens-Habbena, W., Berube, P. M., Urakawa, H., De La Torre, J. R., & Stahl, D. A. (2009). Ammonia oxidation kinetics determine niche separation of nitrifying archaea and bacteria. *Nature*, 461, 976–979. <https://doi.org/10.1038/nature08465>
- Mattson, W. J. (1980). Herbivory in relation to plant nitrogen content. *Annual Review of Ecology, Evolution and Systematics*, 11, 119–161. <https://doi.org/10.1146/annurev.es.11.110180.001003>
- McKane, R. B., Johnson, L. C., Shaver, G. R., Nadelhoffer, K. J., Rastetter, E. B., Fry, B., ... Murray, G. (2002). Resource-based niches provide a basis for plant species diversity and dominance in arctic tundra. *Nature*, 415, 68–71. <https://doi.org/10.1038/415068a>
- Morris, W. F., Traw, M. B., & Bergelson, J. (2006). On testing for a tradeoff between constitutive and induced resistance. *Oikos*, 112, 102–110. <https://doi.org/10.1111/j.0030-1299.2006.14253.x>
- Mundim, F. M., Costa, A. N., & Vasconcelos, H. L. (2009). Leaf nutrient content and host plant selection by leaf-cutter ants, *Atta laevigata*, in a Neotropical savanna. *Entomologia Experimentalis et Applicata*, 130, 47–54. <https://doi.org/10.1111/j.1570-7458.2008.00789.x>
- Novotny, V., & Basset, Y. (2005). Host specificity of insect herbivores in tropical forests. *Proceedings of the Royal Society of London series B, Biological Sciences*, 272, 1083–1090. <https://doi.org/10.1098/rspb.2004.3023>
- Pérez-Harguindeguy, N., Díaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., ... Cornelissen, J. H. C. (2013). New handbook for standardized measurement of plant functional traits worldwide. *Australian Journal of Botany*, 61, 167–234. <https://doi.org/10.1071/BT12225>
- Peter, C. I., & Johnson, S. D. (2014). A pollinator shift explains floral divergence in an orchid species complex in South Africa. *Annals of Botany*, 113, 267–275.
- Phoenix, G. K., Emmett, B. A., Britton, A. J., Caporn, S. J. M., Dise, N. B., Helliwell, R., ... Power, S. A. (2012). Impacts of atmospheric nitrogen deposition: Responses of multiple plant and soil parameters across contrasting ecosystems in long-term field experiments. *Global Change Biology*, 18, 1197–1215. <https://doi.org/10.1111/j.1365-2486.2011.02590.x>
- Ralphs, M. H., & James, L. F. (1999). Locoweed grazing. *Journal of Natural Toxins*, 8, 47–51.
- Raubenheimer, D., & Simpson, S. J. (1993). The geometry of compensatory feeding in the locust. *Animal Behavior*, 45, 953–964. <https://doi.org/10.1006/anbe.1993.1114>
- Raubenheimer, D., & Simpson, S. J. (2003). Nutrient balancing in grasshoppers: Behavioural and physiological correlates of dietary breadth. *Journal of Experimental Biology*, 206, 1669–1681. <https://doi.org/10.1242/jeb.00336>
- Reich, P. B., Tilman, D., Craine, J., Ellsworth, D., Tjoelker, M. G., Knops, J., ... Lee, T. D. (2001). Do species and functional groups differ in acquisition and use of C, N and water under varying atmospheric CO₂ and N availability regimes? A field test with 16 grassland species. *New Phytologist*, 150, 435–448. <https://doi.org/10.1046/j.1469-8137.2001.00114.x>
- SAS Institute Inc. (2009). *SAS/STAT 9.2 User's Guide*, 2nd edn. Cary, NC, USA.
- Schädler, M., Jung, G., Auge, H., & Brandl, R. (2003). Palatability, decomposition and insect herbivory: Patterns in a successional old-field plant community. *Oikos*, 103, 121–132. <https://doi.org/10.1034/j.1600-0706.2003.12659.x>
- Simpson, S. J., & Raubenheimer, D. (2012). *The nature of nutrition: A unifying framework from animal adaptation to human obesity*. Princeton, NJ: Princeton University Press. <https://doi.org/10.1515/9781400842803>
- Sokal, R. R., & Rohlf, F. J. (1995). *Biometry*, 3rd ed. New York, NY: W. H. Freeman and Co..



- Song, M. H., Yu, F. H., Ouyang, H., Cao, G. M., Xu, X. L., & Cornelissen, J. H. C. (2012). Different responses to availability and form of nitrogen in space and time explain species coexistence in an alpine meadow community after release from grazing. *Global Change Biology*, 18, 3100–3311. <https://doi.org/10.1111/j.1365-2486.2012.02738.x>
- Song, M. H., Zheng, L. L., Suding, K. N., Yin, T. F., & Yu, F. H. (2015). Plasticity in nitrogen form uptake and preference in response to long-term nitrogen fertilization. *Plant and Soil*, 394, 215–224.
- Stevens, C. J., Dise, N. B., Mountford, J. O., & Gowing, D. J. (2004). Impact of nitrogen deposition on the species richness of grasslands. *Science*, 303, 1876–1879. <https://doi.org/10.1126/science.1094678>
- Suding, K. N., Collins, S. L., Gough, L., Clark, C., Cleland, E. E., & Gross, K. L. (2005). Functional- and abundance-based mechanisms explain diversity loss due to N fertilization. *Proceedings of the National Academy of Sciences of the United States of America*, 102, 4387–4392. <https://doi.org/10.1073/pnas.0408648102>
- Throop, H. L., & Lerdau, M. T. (2004). Effects of nitrogen deposition on insect herbivory: Implications for community and ecosystem processes. *Ecosystems*, 7, 109–133.
- Tilman, D., Reich, P. B., & Knops, J. M. H. (2006). Biodiversity and ecosystem stability in a decade-long grassland experiment. *Nature*, 441, 629–632. <https://doi.org/10.1038/nature04742>
- Vitousek, P. M., & Howarth, R. W. (1991). Nitrogen limitation on land and in the sea: How can it occur? *Biogeochemistry*, 13, 87–115.
- Wan, X. L., & Zhang, W. G. (2006). Feeding habit and spatial pattern of *Gynaephora alpherakii* larvae. *Acta Agrestia Sinica*, 14, 84–88.
- Wathes, C. M., Demmers, T. G. M., & Xin, H. (2003). *Ammonia concentrations and emissions in livestock production facilities: Guidelines and limits in the USA and UK*. In: ASAE Annual International Meeting (July 27–30, 2003, Las Vegas, Nevada). St. Joseph, MI: The ASAE.
- White, T. R. (1993). *The inadequate environment: Nitrogen and the abundance of animals*. New York, NY: Springer. <https://doi.org/10.1007/978-3-642-78299-2>
- Wookey, P. A., Aerts, R., Bardgett, R. D., Baptist, F., Bråthen, K., Cornelissen, J. H. C., ... Shaver, G. R. (2009). Ecosystem feedbacks and cascade processes: Understanding their role in the responses of Arctic and alpine ecosystems to environmental change. *Global Change Biology*, 15, 1153–1172. <https://doi.org/10.1111/j.1365-2486.2008.01801.x>
- Wright, G. A., Simpson, S. J., Raubenheimer, D., & Stevenson, P. C. (2003). The feeding behavior of the weevil, *Exophthalmus jekelianus*, with respect to the nutrients and allelochemicals in host plant leaves. *Oikos*, 100, 172–184. <https://doi.org/10.1034/j.1600-0706.2003.11270.x>
- Xia, J. Y., & Wan, S. Q. (2008). Global response patterns of terrestrial plant species to nitrogen addition. *New Phytologist*, 179, 428–439. <https://doi.org/10.1111/j.1469-8137.2008.02488.x>
- Yan, L., Liu, Z. K., & Mei, G. R. (1995). Feed selection and utilization of grassland caterpillar in the field cage condition. *Acta Agrestia Sinica*, 3, 257–268.
- Zhang, Y., Zheng, L., Liu, X. J., Jickells, T., Cape, J. N., Goulding, K., ... Zhang, F. (2008). Evidence for organic N deposition and its anthropogenic sources in China. *Atmospheric Environment*, 42, 1035–1041.
- Zhou, X. B., Zhang, Y. M., & Niklas, K. J. (2014). Sensitivity of growth and biomass allocation patterns to increasing nitrogen: A comparison between ephemerals and annuals in the Gurbantunggut Desert, north-western China. *Annals of Botany*, 113, 501–511. <https://doi.org/10.1093/aob/mct275>

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1 Effects of N form, N rate, survey date and sampling plot on number of *Gynaephora menyuanensis* larvae in the alpine meadow

Appendix S2 Effects of species, N form, N rate and sampling plot on leaf consumption

Appendix S3 Effects of species, N form, N rate and sampling plot on leaf consumption, leaf N concentration, C concentration and C:N

Appendix S4 Effects of N form and N rate on above-ground biomass of each of the six abundant plant species

Appendix S5 Relationships of number of *Gynaephora menyuanensis* larvae to above-ground biomass of each of the six species representing most of the abundance of the four functional groups of plants

Appendix S6 Effects of N rate and N form on leaf consumption (percentage leaf area loss) in each of the six species representing most of the abundance of the four functional groups of plants

Appendix S7 Differences in leaf C concentration (a) and leaf C:N (b) between the six abundant species

Appendix S8 Effects of N form and N rate on leaf C concentration (a–l) and C:N (m–x) for each of the six abundant plant species

Appendix S9 Effects of N rate and N form on above-ground biomass of each of the four functional groups

Appendix S10 Effects of N rate and N form on leaf mass per area in each of the six abundant species

Appendix S11 Effects of N rate and N form on leaf consumption (measured as leaf dry mass loss) in each of the six abundant species

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