

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 dif-

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,

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ni-29.5-12.69Tw -22.742 -1.90 eN chmo812 (w)-2a(w)-256 (-10.4 (u)-2:)(o)-256 (-10. w)-2m.9 (v)11x.9 (a52.69T4m)0..1 (m)4.r (N)11o(e)-f. (ch)-.9

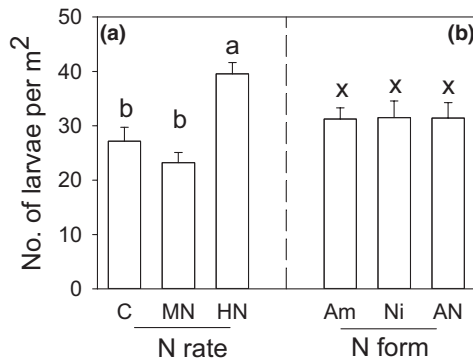


FIGURE 1 Effects of N rate (a) and N form (b) on number of *Gynaephora menyuanensis* larvae in the alpine meadow. MN and HN are N addition at rates of 1.5 and 7.5 g m⁻² year⁻¹, respectively, and C for no N addition. Am, Ni and AN are addition of ammonium-N, nitrate-N and both ammonium-N and nitrate-N, 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 arcsine 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).

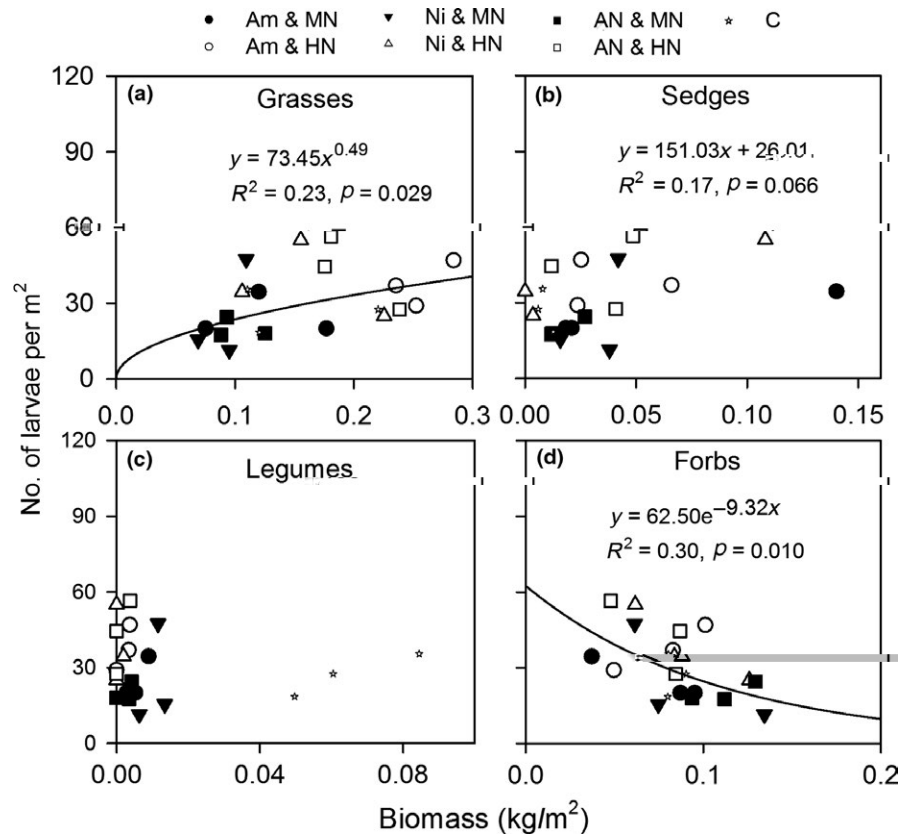
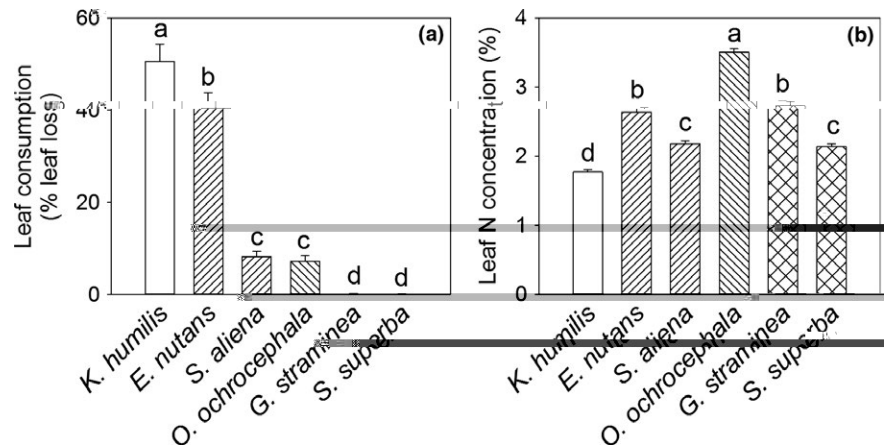


FIGURE 2 Relationships of number of *Gynaephora menyuanensis* larvae to the abundance (above-ground biomass) of each of the four plant functional groups (a–d). Treatment codes are described as in Figure 1

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

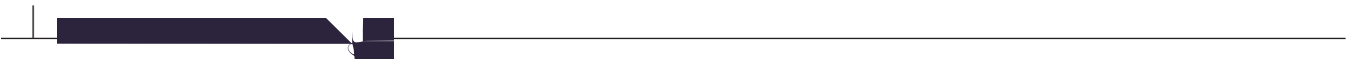


3.3 | Effects of plant species, N rate and form on feeding preference and leaf quality

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



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