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Abundance- and functional-based mechanisms of plant diversity loss with fertilization in the presence and absence of herbivores

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Abstract Nutrient supply and herbivores can regulate plant species composition, biodiversity and functioning of terrestrial ecosystems. Nutrient enrichment frequently increases plant productivity and decreases diversity while herbivores tend to maintain plant diversity in productive systems. However, the mechanisms by which nutrient enrichment and herbivores regulate plant diversity remain unclear. Abundance-based mechanisms propose that fertilization leads to the extinction of rare species due to random loss of individuals of all species. In contrast, functional-based mechanisms propose that species exclusion is based on functional traits which are disadvantageous under fertilized conditions. We tested mechanistic links between fertilization and diversity loss in the presence or absence of consumers using data from a 4-year fertilization and fencing experiment in an alpine meadow. We found that both abundance- and functional-based mechanisms simultaneously affected species loss in the absence of herbivores while only abundance-based mechanisms affected species loss in the presence of herbivores. Our results indicate that an abundance-based mechanism may consistently play a role in the loss of plant diversity with fertilization, and that diversity decline is driven primarily by the loss of rare species regardless of a plant's functional traits and whether or not herbivores are present. Increasing efforts to conserve rare species in the context of ecosystem eutrophication is a central challenge for grazed grassland ecosystems.

Keywords Alpine meadow · Community composition · Competition · Functional traits · Rare species

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Introduction

Nutrient availability and herbivore abundance can regulate plant species composition, biodiversity and productivity of terrestrial ecosystems (Proulx and Mazumder 1998; Worm et al. 2002; Hillebrand et al. 2007; Borer et al. 2014). However, current human activities are altering ambient nutrient supplies and the number and identity of herbivores worldwide (Young et al. 2013). In particular, terrestrial N inputs are currently more than double the pre-industrial rate and are predicted to reach triple or quadruple the pre-industrial rate by 2050 (Tilman 1999; Vitousek et al. 2002; Galloway et al. 2004). Large herbivores are disappearing from many ecosystems worldwide through land use changes

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and increasing habitat fragmentation (WallisdeVries et al. 1998; Young et al. 2013). These anthropogenic pressures can cause dramatic changes in plant community composition and species diversity. Understanding the mechanisms by which nutrients and herbivores regulate plant diversity is especially critical to developing policies that minimize the loss of plant diversity under future global climate change scenarios.

Numerous observational studies and fertilization experiments, particularly in grasslands, have demonstrated that fertilization simultaneously increases productivity, favors competitively dominant species, and decreases plant species diversity (Tilman and Pacala 1993; Stevens and Carson 1999; Rajaniemi 2002; Suding et al. 2005; Niu et al. 2008; Hautier et al. 2009; Yang et al. 2011; Lan and Bai 2012; Isbell et al. 2013; Borer et al. 2014). Several hypotheses have been proposed to explain this decline in diversity with fertilization (Oksanen 1996; Stevens and Carson 1999; Rajaniemi 2002; Hautier et al. 2009) and can be categorized into two major types. Random loss of species, an abundance-based mechanism, assumes that all species suffer equal probability of loss of individuals, leading to community thinning and extinction of rare species due to their small population size (Oksanen 1996; Stevens and Carson 1999; Luo et al. 2006). Alternatively, species loss may be a function of specific traits that competitively favor some species under nutrient enrichment, a functional-based mechanism also referred to as "environmental filtering" in community assembly studies (Westoby 1998; Diaz and Cabido 2001; Pakeman 2004; Wright et al. 2004; Püttker et al. 2014). In particular, fertilization may favor faster growing or taller species that reduce light availability to plants in the understory leading to their exclusion through increased competition for light (Grime 1973; Tilman 1982; Rajaniemi 2002; Suding et al. 2005; Dybzinski and Tilman 2007; Hautier et al. 2009; Dickson and Foster 2011). Fertilization can further affect plant diversity by modifying species turnover resulting from local extinction (loss) of resident species and colonization (gain) of new species from the regional species pool (Tilman 1993; Olff and Ritchie 1998). A decrease in diversity with fertilization can thus result from decreased probability of successful colonization, increased probability of local extinction, or both.

Ecological theory suggests that herbivores can help maintain plant diversity (Aarssen et al. 2006; Hillebrand et al. 2007; Yang et al. 2013). However, experiments performed in many ecosystems find highly variable effects of herbivores on plant species diversity, with the greatest effects frequently observed in productive ecosystems (Proulx and Mazumder 1998; Olff and Ritchie 1998; Worm et al. 2002; Bakker et al. 2006; Hillebrand et al. 2007; Borer et al. 2014). Two categories of mechanism may underlie these changes. Functional-based mechanisms suggest that herbivores maintain plant diversity as productivity increases by consuming the dominant light competitors and increasing light availability to less-effective competitors (Olff and Ritchie 1998; Borer et al. 2014). In contrast, abundance-based mechanisms suggest that the chance that a species is lost is proportional to its initial abundance, and diversity decline is driven primarily by the loss of rare species as productivity increases and density declines (Suding et al. 2005). Herbivores can also affect plant species diversity by modifying the probability of extinction or colonization, or both (Glenn and Collins 1992; Olff and Ritchie 1998; Bakker et al. 2006). For example, herbivores could increase the probability of extinction by selecting palatable species or they could increase the probability of colonization by creating bare soil patches (Bakker and Olff 2003; Bakker et al. 2006).

Here, we analyzed the evidence for both abundance- and functional-based mechanisms of species loss after fertilization in a 4-year field experiment performed in the presence or absence of herbivores. We quantified the effects of fertilization on functional traits optimal for resource use by calculating community-weighted means of functional trait values (CWM) as mean trait values weighted by species relative abundances within a community (Garnier et al. 2004; Shipley et al. 2006; Cohen et al. 2014). CWM therefore simplifies the community into an average trait value that is strongly determined by the functional trait values of the more abundant species; changes in CWM in response to fertilization summarize shifts in functional traits of dominant species. We further assessed species turnover based on extinction (losses) and colonization (gains) of species with fertilization. We tested the following predictions:

1. Functional-based mechanisms underlie species loss in the absence of herbivores, i.e., diversity decline is driven primarily by shifts in community composition to species with functional traits competitively advantageous under nutrient enrichment. In particular, species that most effectively capture light [species that are tall or have higher specific leaf area (SLA) (Lambers et al. 2006; Craine 2009)] should most frequently outcompete species that are less effective at light capture (forbs and species in the lower canopy or with lower SLA) or species suffering reduced recruitment under low light, (e.g., annuals).

2. Abundance-based mechanisms underlie species loss in the presence of herbivores, i.e., diversity decline is driven primarily by the loss of rare species and the probability that a species will be lost is proportional to its initial abundance.

3. The decrease in diversity caused by fertilization is due not only to an increase in the number of species lost, but also to a decline in the number of species

gained. In our study system, we show that the decrease in diversity caused by fertilization was not due to a decline in the numbers of species gained, but instead to an increase in the numbers of species lost.

We further demonstrate that both abundance- and functional-based mechanisms act simultaneously to reduce plant diversity in the absence of herbivores, while abundance-based mechanisms dominate species loss in the presence of herbivores.

Materials and methods

Study site

The study was carried out on a broad and flat site at the Alpine Meadow and Wetland Ecosystems research station of Lanzhou University. The station is located at Maqu (35°58′N, 101°53′E, 3500 m a.s.l.), Gansu, China, on the eastern Tibetan Plateau. The mean annual temperature is 1.2 °C, with the monthly average ranging from of −10 °C in January to 11.7 °C in July. The mean annual precipitation over the previous 35 years is 620 mm, mainly distributed during the short, cool summer (June–September). The area has 2580 h year^{-1} of sunshine and more than 270 days year^{-1} of frost (Yang et al. 2013). The mean aboveground biomass is approximately 70 g 0.25 m^{-2} (280 g m^{-2} dry weight). There are, on average, 30 vascular plant species 0.25 m^{-2}. The grassland vegetation is dominated by *Kobresia graminifolia*, *Poa botryoides*, *Elymus nutans* and *Anemone rivularis* (Yang et al. 2013).

Experimental design

In October 1999, a field (450 by 220 m) located in a flat area (the slope is less than 1°) was fenced. The height of the woven wire fence was 1.3 m. Yaks and sheep were excluded by the fence, while smaller mammals, such as marmots and field mice, could access the fenced plot. Outside of the exclosure, vegetation was moderately grazed by ungulates, with 110 yaks (0.18 ha^{-1}) and 2200 sheep (3.68 ha^{-1}) during all months except for 40 days between the end of July and mid-September when the animals were moved to higher elevation pastures. The fence excluded herbivores from mid-April to the end of November of each year. The productivity estimate here is approximately the median of global grassland productivity (Borer et al. 2014), and species diversity is at the very top end of that reported in Borer et al. (2014). The densities of sheep and yak correspond to moderate grazing in the area (Gao et al. 2007; Niu et al. 2008) and can result in 30–60 % utilization of annual forage production.

To quantify abundance- and functional-based mechanisms of extinction on species loss after fertilization in the presence or absence of herbivores, we applied two levels of nutrients (no nutrient addition and nutrient addition {30 g [(NH$_4$)$_2$HPO$_4$] m^{-2}}) in two different spatial locations—inside [ungrazed (E; with enclosure)] and outside [grazed (G; no enclosure)] a single enclosure—resulting in two treatments in each location (E0 and E30; G0 and G30). Thirty-two permanent 8 × 4-m plots were established on 30 May 2007 with 16 plots in the fenced exclosure and the other 16 plots outside the fence. Plots were arranged in a regular 4 × 4 matrix with a 2-m buffer zone between plots. Nutrients were randomly applied to eight plots inside the fence and eight plots outside the fence in May each year during a rain event using slow-release (NH$_4$)$_2$HPO$_4$ pellets (Tianjin International Trading, Tianjin, China) at the rate of 6.3 g N m^{-2} year and 7.0 g P m^{-2} year. These rates are comparable to, although slightly lower than, those for other grassland experiments that sought to overcome nutrient limitation (Harpole et al. 2007; Borer et al. 2014). We acknowledge the non-independence of plots clustered together within and outside of the enclosure. However, pre-fencing data collected in 1999 show no difference in species richness (*t*-test, $t_{1,7} = -0.314$, $p = 0.76$) and aboveground biomass ($t_{1,7} = 0.309$, $p = 0.77$) for plots that were subsequently assigned to fenced and unfenced treatments, suggesting that spatial heterogeneity at the site is unlikely to act as a confounding factor, particularly for the plot sizes used here.

Vegetation monitoring

We measured aboveground plant biomass and species composition at peak biomass in early September 2007 and 2010 by clipping the inner 50 × 50 cm of each plot at ground level (Yang et al. 2013). The clipping quadrats were at least 50 cm from the edge of the plot to avoid edge effects. We only clipped and collected species rooted within each quadrat; species rooted outside, but overhanging the plot, were not included. Biomass was then sorted to species, dried at 80 °C, weighed and species richness and identity were recorded. The density of each species was determined as the number of individuals of each species that appeared in each quadrat prior to clipping for biomass collection. For clonal plants, we considered clusters of stems as an individual [a ramet, in most cases (Luo et al. 2006)].

Trait measurements

We chose 48 common species in G0, forty-six in G30, forty-seven in E0 and 42 in E30 (Table S1, online appendix) for trait measurements and sampled them at fruiting time from 20 July to 5 September in 2008 and 2009. These

species accounted for more than 95 % of the aboveground biomass in these communities. Only aboveground components were sampled due to the difficulties in collecting entire roots in an alpine meadow. In each treatment, we randomly sampled 20 individuals of each species. A total of 3660 individuals were investigated each year.

Height was measured for the 20 randomly selected individuals per species in each treatment. We measured SLA (the ratio of leaf area to leaf dry mass) by randomly sampling one whole leaf from each of the 20 individuals of each species in each treatment in 2008 and 2009. The fresh leaves were scanned to measure leaf area using ImageJ (Schneider et al. 2012), then dried at 80 °C and weighed. In each plot, we measured light with a Decagon Sunfleck ceptometer (Decagon, Pullman, DC) at the time of vegetation monitoring on 25 August 2009. The ceptometer integrates 80 sensors along a 84-cm probe wand. Light readings were taken on a cloudless day (1100–1300 hours). The probe wand was oriented north–south across each plot and photosynthetically active radiation (PAR) was recorded at ground level, and above the top of the canopy. The ratio between these two measurements was taken as a proxy for understory PAR transmission.

Trait characterization

Plant functional composition within each treatment was quantified using CWM trait values calculated using species' relative abundance. CWM values of functional traits were calculated using the FD packages (Laliberté and Legendre 2010) in R 2.14.0. Based on our predictions, CWM was calculated for functional groups (forb, grass, legume or sedge), growth forms (basal, rosette or erect), life history (annual, biennial or perennial), height, and SLA in control conditions. Results for CWM trait values (i.e., height, and SLA) are reported as mean shifts for dominant species with their 95 % confidence intervals (CIs), while results for categorical CWM trait values (i.e., functional groups, growth forms and life history) are described as shifts in these traits for dominant species.

Response metrics

We used the number of plant species per 0.25 m^2 as our measure of plant species richness, and aboveground biomass per 0.25 m^2 as our measure of plant biomass. We calculated fertilization-induced changes in species richness, aboveground biomass, light transmission, and CWM of functional traits at the end of the experiment in 2010 as the difference between fertilized and control plots (unfertilized) within each of the herbivory treatment. Values of zero indicate no change in response due to fertilization. Species turnover was assessed based on extinction (losses) and colonization (gains) of species with fertilization. A species was considered lost from a fertilized plot if it was present in the control plot (unfertilized) but absent in the associated fertilized plot and gained if it was absent in the control plot but present in the associated fertilized plot.

Prior to implementing the fencing treatment, the vegetation composition in the study area inside the fence and outside the fence did not differ (Fig. S1). Dominant species in both fields were *Potentilla anserina, K. graminifolia, Tibetia himalaica, Poa crymophila* and *Anemone obtusiloba*. Exclusion of herbivores resulted in a decrease in species richness and by 2007, at the time the fertilization treatment was implemented, species richness was reduced by 4.8 species 0.25 m^{-2} (95 % CIs = 3.4 to −6.1) in the fenced field relative to the unfenced field. The decrease in species richness did not differ among plots that were subsequently randomly assigned to fertilized and unfertilized treatments. This suggests that assessing fertilization-induced changes within each of the herbivory treatments as differences between the fertilized and unfertilized treatments in 2010 is a reasonable and valid approach as it reflects compositional divergence that occurred during the course of the experiment.

Data analysis

The response to fertilization of species richness, aboveground biomass, light transmission, CWM of functional traits and species turnover in the absence (ungrazed) or presence (grazed) of herbivores was tested by ANOVAs. We estimated the importance of initial abundance, measured as mean plant species density for each treatment in 2007 under controlled conditions, for the probability of species loss with fertilization in the absence or presence of herbivores by logistic regressions. Compositional responses of the 32 communities in 2010 to nutrient addition (fertilized and control plots) and herbivory (both in grazed and ungrazed plots) were evaluated using permutational multivariate ANOVA (Anderson 2001) employing a modified Gower distance with a logarithmic transformation (Anderson et al. 2006). Species' responses to treatments were further assessed as changes in species' relative abundance. To evaluate different cluster analyses based on the experimental treatments, we calculated the Akaike information criterion corrected for small sample size (McQuarrie and Tsai 1998) using a multivariate analogue of residual sums of squares and calculated as the sum of all eigenvalues minus the sum of the canonical eigenvalues of **Y** (the species table) on **X** (the matrix of fertilization and exclosure treatment levels) described by Legendre and Anderson (1999). All analyses were performed in R 2.14.0 (Oksanen et al. 2011). A table of species present in each of the four treatments is provided in Table S1.

Fig. 1 Effects of 4 years of fertilization on grassland diversity, species turnover, productivity and light transmission (photosynthetically active radiation; PAR) at ground level in the absence (*ungrazed*) or presence (*grazed*) of herbivores. Fertilization-induced changes in **a** species richness, **b** species turnover, **c** aboveground plant biomass, and **d** light transmission were calculated as the difference between fertilized and control plots within each of the herbivory treatments in 2010. *Points* denote treatment means and *error bars* denote 95 % confidence intervals (CIs) (treatments with intervals not including 0 indicate a significant effect of nutrient addition)
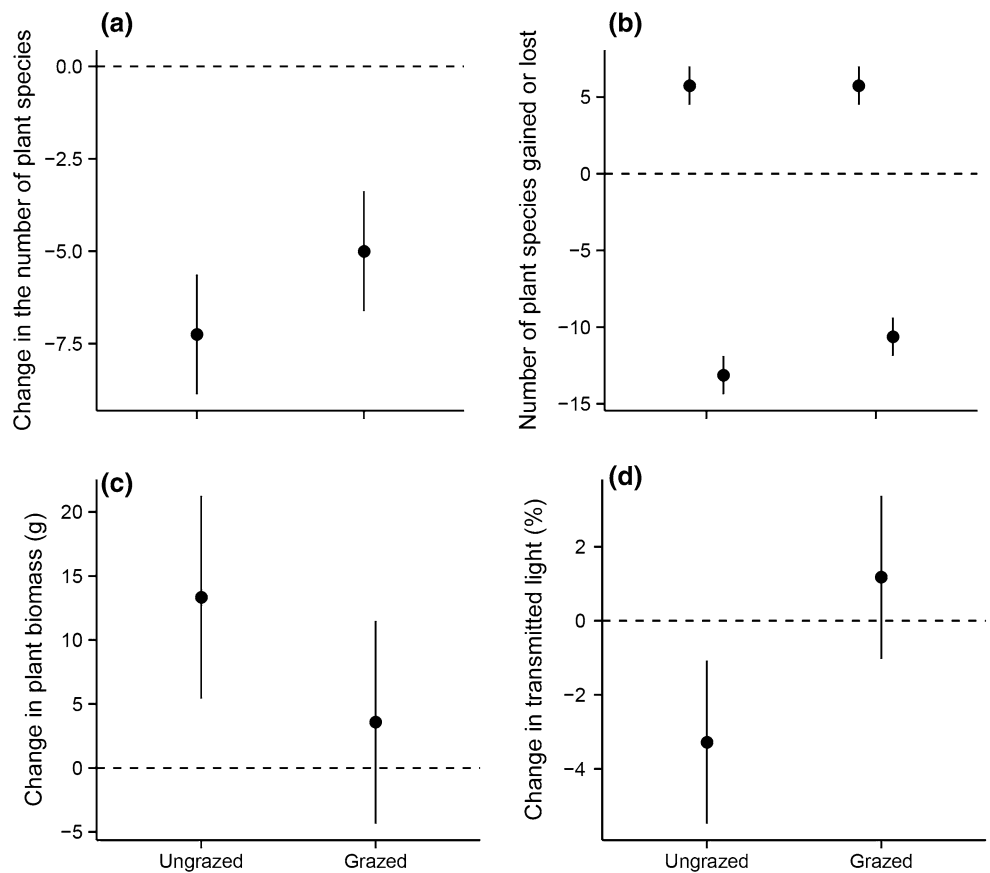
Results

Mechanisms of diversity loss with fertilization in the absence of herbivores

After 4 years of chronic nutrient addition, fertilization significantly decreased plant diversity by an average of 7.3 species 0.25 m^{-2} (95 % CIs = −8.5 to −6.0) relative to the control, corresponding to approximately one quarter of the original species richness (Fig. 1a, Fig. S2a). The decrease in diversity caused by fertilization was not due to a decline in the numbers of species gained (Fig. 1b; number of species gained = 5.8, 95 % CIs = 4.6–6.9), but instead to an increase in the numbers of species lost (Fig. 1b; number of species lost = −13.1, 95 % CIs = −14.5 to −11.8).

Abundance-based mechanisms predict that random extinction of rare species should account for most of the decline in species diversity with fertilization. The likelihood of species loss with fertilization in 2010 was negatively associated with the mean abundance at the start of the experiment in 2007 (Fig. 2), thus providing some support for this mechanism in the absence of herbivores. However, extinction also occurred for some abundant species (e.g., *K. graminifolia*) suggesting that species loss was not only due to rarity.
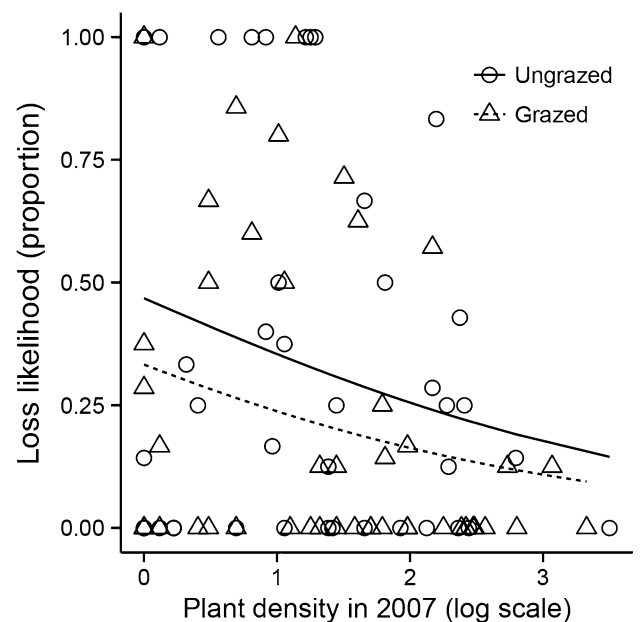

Fig. 2 Likelihood of species extinction with fertilization in the absence (*ungrazed*) or presence (*grazed*) of herbivores as a function of initial density measured as mean plant species density in control plots for each treatment in 2007. Logistic regressions on species loss significantly decreased with initial abundance both in the absence and presence of herbivores (slopes = −0.47, 95 % CIs = −0.90 to −0.06)

Functional-based mechanisms predict that species extinction with fertilization is due to an increase in competition for light. Nutrient addition significantly increased productivity in the absence of herbivores by approximately 20 % (Fig. 1c, Fig. S2b; change in plant biomass = 13.3 g 0.25 m^{-2}, 95 % CIs = 6.8–19.8) and decreased light availability by approximately 50 % from 6.7 to 3.4 % (Fig. 1d, S2c; change in percentage of transmitted light = −3.3 %, 95 % CIs = −5.2 to −1.4) relative to the control. This result is consistent with increased competition for light as a mechanism of diversity loss with eutrophication (Fig. S2d). We also found large shifts in functional traits of dominant species (Fig. 3) and in the composition of plant communities (Fig. 4), supporting the expectation that strong competitors for light (grasses and species with higher SLA or in the higher canopy) increased with fertilization. Redundancy analysis biplots of the 2009 data (Fig. 4) support the strong differences in composition between ungrazed communities without nutrient addition and those receiving nutrient addition. Ungrazed communities shifted from a mixture of forbs (*Bupleurum malconense, Tibetia himalaica, Oxytropis kansuensis, Scirpus pumilus, Anemone obtusiloba, Potentilla fragarioides*), grass (*Stipa aliena*) and sedge (*Kobresia graminifolia)* when no nutrients were added to a community dominated by two tall grass species (*Poa crymophila, Elymus nutans*; Fig. 3a; change in CWM height = 24.4 cm, 95 % CIs = 22.1–26.6) with high SLA (Fig. 3b; change in CWM SLA = 30.4 cm, 95 % CIs = 22.4–38.4) when nutrients were added. No differences in CWM of growth forms and life history were observed.

Mechanisms of diversity loss with fertilization in the presence of herbivores

Fertilization decreased grassland plant diversity in the presence of herbivores by an average of 5.0 species 0.25 m^{-2} (Fig. 1a; S2a; 95 % CIs = −6.3 to −3.7) relative to the control. The decrease in diversity caused by fertilization in these plots was not due to a decline in the number of species gained (Fig. 1b; number of species gained = 5.8, 95 % CIs = 4.6–6.9), but instead to an increase in the number of species lost (Fig. 1b; number of species lost = −10.6, 95 % CIs = −12.0 to −9.3).

We found some support for abundance-based mechanisms in the presence of herbivores as the likelihood of species loss with fertilization in 2010 was negatively associated with the mean abundance at the start of the experiment in 2007 (Fig. 2). However, species loss was less likely in the presence of herbivores than when herbivores were excluded, independent of initial abundance (Figs. 1a, 2).

With grazing, we found little evidence that species extinction with fertilization was due to an increase in competition for light, thus providing very little support for
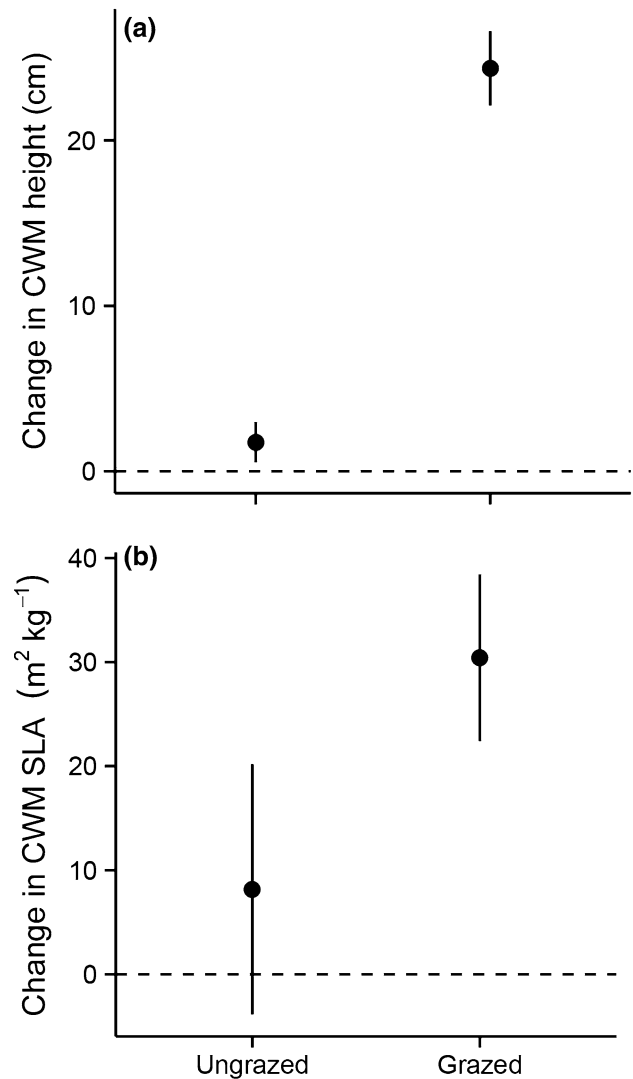

Fig. 3 Effects of 4 years of fertilization on community weighted mean (*CWM*) of functional traits. Fertilization-induced changes in **a** CWM specific leaf area (*SLA*) and **b** CWM height were calculated as the difference between fertilized and control plots within each herbivory treatment. *Points* denote treatment means and *error bars* denote 95 % CIs (treatments with intervals not including 0 indicate a significant effect of nutrient addition)

functional-based mechanisms of species loss in the presence of herbivores. In particular, productivity (Fig. 1c, S2b; change in productivity = 3.6, 95 % CIs = −4.4 to 11.5) and light availability at ground level (Fig. 1d; Fig. S2c; change in percentage of transmitted light = 1.2, 95 % CIs = −0.7 to 3.1) remained unchanged with fertilization in the presence of herbivores. Moreover, where herbivores were present, there were minimal shifts in functional traits of dominant species (Fig. 3; change in CWM height = 24.4 cm, 95 % CIs = 22.1–26.6; change in CWM SLA = 1.8 cm, 95 % CIs = 0.5–3.0), in CWM values of functional traits, growth forms and life history, and in the
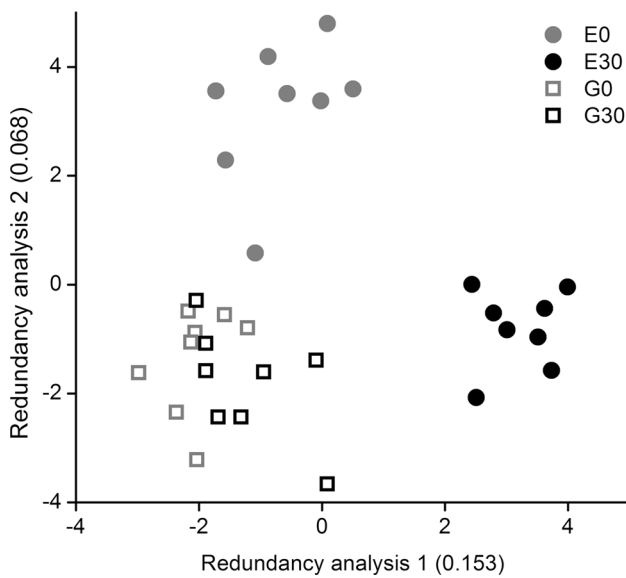
Fig. 4 Redundancy analysis of the effects of fertilization on alpine plant community composition in the absence (*ungrazed*) or presence (*grazed*) of herbivores in 2009. *Numbers in parentheses* indicate the proportion of inertia accounted for by the first two constrained axes. *G0* No enclosure, no nutrient; *G30* no enclosure, nutrient (30 gm^{-2}); *E0* enclosure, no nutrient; *E30* enclosure, nutrient (30 gm^{-2})

composition of plant communities (Fig. 4). Composition did not differ between grazed communities without nutrient addition and those receiving nutrient addition (Fig. 4). Grazed communities were dominated by nine forbs (*Pedicularis kansuensis, Heracleum millefolium, Leontopodium souliei, Plantago depressa, Lomatogonium carinthiacum, Potentilla anserina, Taraxacum mongolicum, Halenia elliptica, A. obtusiloba*).

Discussion

The results of our grassland manipulation extend past work on diversity maintenance and loss due to fertilization by demonstrating the differing importance of functional- and abundance-based mechanisms of diversity loss in the presence and absence of herbivores. Here, plant diversity declined with fertilization, as in many past studies (Tilman and Pacala 1993; Stevens and Carson 1999; Rajaniemi 2002; Suding et al. 2005; Niu et al. 2008; Hautier et al. 2009; Yang et al. 2011; Lan and Bai 2012; Isbell et al. 2013; Borer et al. 2014), but our results demonstrate that this fertilization-induced decrease in diversity was not due to a decline in the numbers of species gained (Tilman 1993; Foster and Gross 1998; Stevens et al. 2004; Bakker et al. 2006) but instead to an increase in the numbers of species lost. As in past studies, both abundance- and functional-based mechanisms influenced the decline in plant diversity

with fertilization (Suding et al. 2005); however, whereas fertilization reduced plant diversity both in the presence and absence of herbivores in our study, it impacted plant biomass, light availability, functional traits of dominant species, and community composition only in the absence of herbivores. Thus, our work extends the literature examining trait-based and trait-neutral mechanisms of species loss (Suding et al. 2005) by suggesting that the extent and identity of species lost in response to fertilization are contingent on the presence of grazers. In particular, herbivores appear to mediate the effects of nutrients on plant biomass, ground-level light resources, and the resulting competitive environment. In our plots, herbivores achieved this by reducing the biomass of grasses that dominated fertilized plots (in particular *Poa crymophila* and *Elymus nutans*), thereby partially counteracting the increased primary productivity induced by fertilization, increasing the relative availability of light at ground level, and promoting the persistence of a grassland species pool with diverse functional traits, growth forms, and life histories. Although fertilization reduced diversity in the presence and absence of herbivory, herbivores partially offset this effect, ameliorating diversity losses primarily by reducing the importance of functional-based mechanisms.

In the presence of herbivores, diversity declined less in response to fertilization than when herbivores were absent, and shifts in functional traits of dominant species and structure of the plant communities were limited, thus suggesting that consumers partially counteracted the light competition induced by fertilization. This result is concordant with recent work that demonstrates that where grassland herbivores increase light at ground level, they can reduce diversity losses with or without fertilization (Borer et al. 2014), but our current study extends this to quantify the association with species traits. Here, in plots outside the fence, trait turnover was minimal, whereas within the fence, plant traits changed dramatically in response to nutrients. Taken together, these results suggest a key role of herbivores in mediating functional-based diversity losses, pointing to the importance of plant traits (Quétier et al. 2007; Eskelinen et al. 2012) and tradeoffs between competition and defense in maintaining species composition and diversity in these grasslands (Viola et al. 2010; Adler et al. 2013). Our results demonstrate that in a field setting, consumers can reduce diversity losses due to competitive exclusion, slowing extinction rates caused by functional-based mechanisms.

When herbivores were excluded, fertilization led to substantially altered composition and function of the plant community. In particular, fertilization increased plant productivity, favored two grasses, *P. crymophila* and *E. nutans*, and decreased light availability. As a result, species with functional traits which are disadvantageous under increased shading were excluded. For example, species with lower

SLA such as *Kobresia setchwanensis* and *Scirpus pumilus* or with shorter stature such as *Kobresia humilis* and *Lancea tibetica* declined with fertilization. This result supports one of the functional-based mechanisms predicting that diversity loss with fertilization is due to an increase in competition for light (Grime 1977; Tilman 1982; Hautier et al. 2009; Borer et al. 2014). Recent empirical estimates from sites spanning six continents found an average grassland species loss due to fertilization that is somewhat lower than what we found, although we used slightly lower rates of N and P addition (Borer et al. 2014), suggesting that grasslands in the eastern Tibetan Plateau will be more sensitive to future nutrient enrichment than many grasslands of the world.

In addition to functional-based mechanisms of diversity loss with fertilization, we found that rare species were more susceptible to exclusion regardless of their functional traits and of the presence of herbivores. This result suggests that abundance-based species loss was consistently an important predictor of the decline in plant diversity with fertilization. The importance of abundance-based mechanisms for diversity maintenance is becoming well established (Suding et al. 2005; Adler et al. 2013). However, functional traits and relative abundance often are correlated with each other and underlying environmental gradients (e.g, Goldberg and Miller 1990; Diaz et al. 1998; Garnier et al. 2004; Shipley et al. 2011), suggesting that functional- and abundance-based mechanisms likely act in concert in maintaining diversity in this and other grasslands.

Our results therefore indicate that abundance-based mechanisms leading to the loss of rare species play a key role in community responses to eutrophication, whereas functional-based mechanisms are most important in determining the consequences of the interaction between fertilization and herbivory for plant community diversity, productivity, and trait turnover. These findings have important implications for the management of species and trait diversity in this grassland and others. For example, herbivores could help maintain species and trait diversity as productivity increases by consuming the dominant resource competitors and increasing ground-level light availability (Holt et al. 1994; Olff and Ritchie 1998; Dybzinski and Tilman 2007; Borer et al. 2014). However, herbivores did not fully rescue plant diversity in our study; even in the presence of herbivores, fertilization induced local extinctions primarily via the loss of rare species. Our results illustrate that eutrophication could lead to plant diversity loss even when herbivores reduce net productivity and ground-level light limitation because of the random loss of small individuals of all species. Given the association of rarity with functional traits associated with the light and nutrient environment, management focused on counteracting productivity increases in response to nutrient deposition in parallel with management aimed at increasing the relative abundance of rare species and rare functional traits could jointly serve to maintain grassland diversity (Diaz and Cabido 2001; Suding et al. 2005) and ecosystem function (Hooper and Vitousek 1997; Loreau et al. 2001; Cadotte et al. 2011).

A growing body of experimental studies of fertilization and observational studies of N deposition in terrestrial ecosystems show that the increased availability of nutrients reduces plant diversity. Predicted increases in nutrient inputs globally in the coming decades (Vitousek et al. 1997; Galloway et al. 2004, 2008) will likely reduce biodiversity with potentially dramatic consequences for ecosystem functioning (Isbell et al. 2013; Hautier et al. 2014). We have demonstrated that abundance-based and functional-based mechanisms can be important in species losses with eutrophication, but the relative importance of these mechanisms can be modified via grazing, with promising effects on the maintenance of grassland species, their traits and compositional diversity. This insight could be used to develop mechanistically informed management of grassland diversity.

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