REPORT

Experimental warming causes large and rapid species loss, dampened by simulated grazing, on the Tibetan Plateau

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Abstract

We investigated the independent and combined effects of experimental warming and grazing on plant species diversity on the north-eastern Tibetan Plateau, a region highly vulnerable to ongoing climate and land use changes. Experimental warming caused a 26–36% decrease in species richness, a response that was generally dampened by experimental grazing. Higher species losses occurred at the drier sites where N was less available. Moreover, we observed an indirect effect of climate change on species richness as mediated by plant–plant interactions. Heat stress and warming-induced litter accumulation are potential explanations for the species' responses to experimental warming. This is the first reported experimental evidence that climate warming could cause dramatic declines in plant species diversity in high elevation ecosystems over short time frames and supports model predictions of species losses with anthropogenic climate change.

Keywords

Alpine, biodiversity, climate warming, global change, grazing, rangelands, species richness, Tibetan Plateau.

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INTRODUCTION

Species diversity plays an essential role in sustaining Earth system processes and providing basic goods and services to human society. Stresses from human activities, however, are having a profound effect on the Earth system, including loss of species diversity, which is proceeding at unprecedented rates (Sala *et al.* 2000). One such stress is climate alteration (Parmesan & Yohe 2003; Root *et al.* 2003; Thomas *et al.* 2004), but climate warming is superimposed upon other global changes, such as land surface modification, species invasions and nitrogen deposition. Most projections of species loss under climate change are based either on modelling or correlation studies. Moreover, the feedbacks and interactions among global changes are poorly understood; recent evidence suggests that co-occurring global changes can confound results from single factor studies (Shaw *et al.* 2002). Subsistence based societies may be unduly vulnerable to global environmental changes, but we currently have little knowledge regarding how the ecosystems of the world on which subsistence based societies still depend will be affected by global environmental changes.

Thus, study of the ecological effects of global change in these potentially vulnerable regions of the world can add greatly to our ability to model and predict human resource use patterns in response to global changes.

In this paper, we investigate the independent and combined effects of experimental warming and grazing on several measures of plant diversity on the alpine rangelands of the north-eastern Tibetan Plateau. More than 25% of the world's population lives in mountainous regions and their foothills and more than 40% depend on mountain resources. The alpine zone – the highest limit of plant life – covers *c.* 4 million km^2 or 3% of the terrestrial surface. Alpine ecosystems may be particularly vulnerable to climate changes because of (1) the strong role of climate in structuring and regulating alpine ecosystems; (2) inhibited migration as a result of topography and lack of soil formation; (3) short growing seasons; and (4) snow-albedo feedbacks. The Tibetan Plateau is an extensive alpine zone where humans rely on the natural vegetation for their main form of subsistence, animal husbandry. Several lines of evidence indicate the Tibetan Plateau is experiencing climatic warming (Thompson *et al.* 1993; French & Wang

1994). Moreover, this region is predicted to experience 'much greater than average' increases in surface temperatures in the future (Giorgi *et al.* 2001). The IMAGE model predicts that the Tibetan Plateau and the Himalayan alpine tundra will be reduced to 20% of its current area with climate warming (Walker *et al.* 2001). Concurrent with the climate changes, there are profound changes to the pastoral land use dynamic which are increasing grazing pressures on the Tibetan rangelands (Williams 1996; Miller 1999).

Studies from arctic, alpine and sub-alpine meadow ecosystems have illustrated significant changes in vegetative cover, biomass, growth and phenology in response to experimental warming (Chapin *et al.* 1995; Harte & Shaw 1995; Arft *et al.* 1999). Changes in belowground resource availability, duration of snow cover and plant development and allocation pattern have been cited as causes for these observed changes (Arft *et al.* 1999; Bret-Harte *et al.* 2001; Dunne *et al.* 2003). While species diversity has been correlated with temperature, elevation and latitude at various scales (Huston 1994; Francis & Currie 2003), to our knowledge, there are no reported changes in species diversity with short-term (< 5 years) experimental warming. Experimental studies have, however, documented changes in species diversity as a result of altered N, biomass and litter regimes (Tilman 1987, 1993; Zavaleta *et al.* 2003) – all of which are potential indirect effects of climate warming. Grazing also alters species diversity, although the magnitude and direction of the response is contingent on site and historical factors (Milchunas & Lauenroth 1993; Hofgaard 1997; Collins *et al.* 1998; Austrheim 2001; Vujnovic *et al.* 2002; Gelbard & Harrison 2003; Harrison *et al.* 2003).

We hypothesized that experimental warming would have no large-scale direct effect on total species richness (SR) of a long-lived primarily perennial vegetative community over a short-term experimental manipulation. We hypothesized that plots in which grazing is simulated by clipping would have higher SR than non-clipped plots because more species will move into the clipped plots from the surrounding species pool and because species numbers in the non-clipped plots will decline because of the increasing dominance of fewer species. As a result of the long evolutionary history of grazing on the Tibetan Plateau, we predicted the magnitude of these species changes would be small. We assumed that the treatment effects would be additive and hypothesized that combined experimental warming and grazing would result in an increase in SR similar to that experienced under the clipping alone scenario. Based on the idea that higher species richness confers more compositional stability (Ives *et al.* 2000), that higher species richness is associated with lower resource availability (Rajaniemi 2002; Zavaleta *et al.* 2003) and that more fertile sites are more responsive to climate change (Grime *et al.* 2000), we hypothesized that if we did observe

species richness changes with warming, these would occur in sites with higher N and overall resource availability. In this study, we focus on the number of species, regardless of the identity and functional characteristics of those species. In a subsequent paper, we will focus on the importance of species' traits.

MATERIAL AND METHODS

Study region

We conducted our research at the Haibei Research Station (HARS), which is situated at latitude 37°37′ N, longitude 101°12′ E (Fig. 1). Mean annual temperature is −1.6 °C, mean annual precipitation is 562 mm, over 80% of which falls during the summer monsoon season. Mean elevation of the valley bottom is 3200 m. There are two main habitats in the region: the warmer winter-grazed meadow situated along the valley floor and the cooler summer-grazed shrubland situated on the higher slopes encircling the valleys. The meadow is dominated by an assemblage of forbs and graminoids; the shrubland is dominated by a deciduous shrub, *Potentilla fruticosa*. Forbs, grasses and sedges occur in all sites; however, the specific vegetative assemblages depend on habitat and grazing history. Approximately 87% of all plant species at the sites are perennial and most species use the C3 photosynthetic pathway. The alpine meadow and shrub vegetation, which occur in this region, comprise *c.* 35% of the area of the Tibetan Plateau (Zhao & Zhou 1999).

Within both habitats, we identified sites with 'low' and 'high' grazing intensity histories, for a total of four sites: low grazing intensity history meadow site (LG meadow), high grazing intensity history meadow site (HG meadow), low grazing intensity history shrubland site (LG shrubland) and high grazing intensity history shrubland site (HG shrubland). Both the grazing intensity and grazing duration differed among grazing history sites. Within each habitat, the low and high grazing history sites were similar in other features – such as slope, aspect, soil type and distance to the river.

We fenced each of the four 30 × 30 m sites within which we laid out 16 plots in a 4 × 4 matrix (for a total of 64 plots). Within each site, we established a complete factorial experimental design where we simulated warming using fibreglass open top chambers (OTCs) and simulated the defoliation effects of grazing through clipping (Fig. 1). The OTCs, which were 1.5 m diameter and 40 cm height, were constructed of Sun-Lite HP (Solar Components Corporation, Manchester, NH, USA) 1.0 mm thick fibreglass and remained on the plots year-round. All other plots were of similar dimensions. There were *c.* 2 m between each plot. OTCs are commonly employed to study the effects of climate warming on ecosystems (Marion *et al.* 1997). The
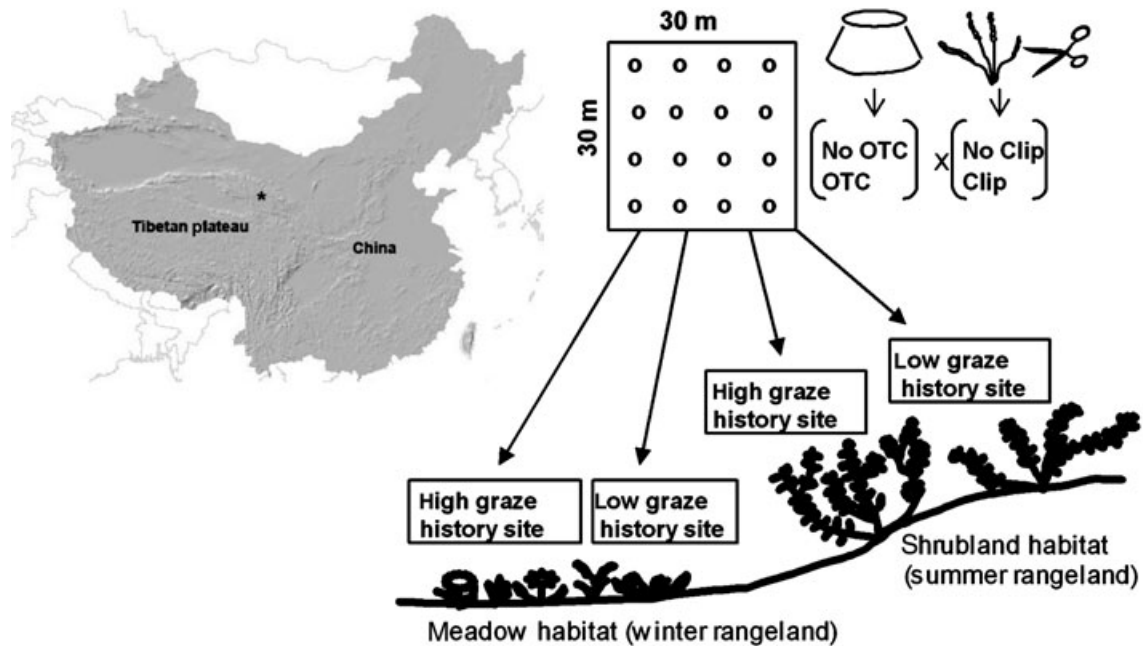
Figure 1 Left side: Study site region. The asterisk indicates the approximate location of the experiment. Right side: Experimental design. We conducted this study in two habitats: a summer-grazed shrubland and a winter-grazed meadow. Within each habitat, we identified sites with 'low' grazing (LG) and 'high' grazing (HG) intensity history sites, for a total of four study sites (LG meadow, HG meadow, LG shrubland and HG shrubland). In each site, we fenced a 30 × 30 m area, within which we established a fully factorial experimental design, with 'open top chambers' (OTCs) and 'clipping' as our main treatments. We had four replicates per treatment for a total of 16 plots per site × 4 sites = 64 plots.

OTCs elevated growing season averaged daily air temperature by 0.6–2.0 °C, growing season averaged maximum daily air temperature by 1.9–7.3 °C, and diurnal air range by 0.9–7.0 °C. In some sites and years, OTCs increased growing season averaged soil temperature, measured at 12 cm depth, by 0.3–1.9 °C. The OTC effects on soil moisture, measured at 12 cm depth, were inconsistent and depended on habitat, grazing history and the presence or absence of clipping. However, even when significant effects occurred, the change in growing season averaged soil moisture with OTCs was < 3%.

In the winter-grazed meadows, traditionally grazed during the winter months, we clipped the plots prior to initiation of growth in the early spring. In the summer-grazed shrubland sites, traditionally grazed during the summer months, we clipped the plots in mid-July. We clipped plots to *c.* 3 cm in height, which is the height of the vegetation outside of our fenced plots in the sites with a high grazing history. We removed *c.* 30% of total live peak aboveground biomass in the shrubland sites and 15% of total peak aboveground biomass in the meadows. We did not clip plants that yak and sheep do not graze (such as *Oxytropis* spp. and *Stellera chamaejasme*). We plucked the shrub leaves from the stems to simulate sheep browsing.

To examine the efficacy of the simulated grazing treatments, and to examine the extent to which the control plots within the fenced areas represented recovery from grazing, in 2000 we established four replicated 'grazing control' (GC) plots situated outside of the fenced areas in all four sites. We established these plots > 5 m but < 15 m away from the fence to eliminate any 'fence' effect but to be representative of the plots within the fenced area. Pairs of GC plots were *c.* 2 m apart from each other.

We sampled species richness (SR) in 1998, 1999, 2000 and 2001 in 75 × 75 cm quadrats centered in the plots by identifying all species present in the plot. We collected plant community data in 2000 and 2001 using the point method where we divided the 75 × 75 cm quadrat into a 10 × 10 grid with 100 intersecting points. We recorded all plant species that touched a point placed vertically from the grid to the ground at each of 100 intersecting points. We used the data to compute the Shannon–Wiener (H') diversity index, which combines both species richness and evenness in a single index. We chose to use the Shannon–Wiener rather than the Simpson's index since the former places more weight on rare species than the latter. As there was a high average number of species per plot (36 species) at our sites, the Shannon–Wiener was more appropriate for this analysis. To facilitate the interpretation of H', we took the

antilog of the Shannon index ($e^{H'}$), which is the number of species that would, if each were equally common, produce the same H' as the sample (Ludwig & Reynolds 1988).

To control for potential covariates in the study, we monitored small mammal activity in our experimental plots. We estimated the activity of these small mammals within the 75×75 cm grid. The two indices we employed were: (1) areal coverage of soil disturbance because of small mammal activity and (2) areal coverage of small mammal tunnels in the vegetation. We calculated small mammal activity as the sum of these two indices. We also recorded microclimate data and estimated nutrient availability, soil carbon storage and vegetative productivity (Klein 2003). We measured microclimate variables using HOBO PRO dataloggers, which were situated 10 cm aboveground and 12 cm belowground from April to October. We measured nitrate and ammonium availability using buried mixed ion exchange resin bags (Giblin *et al.* 1994).

Our treatments were: $T_{CON} = $ CONTROL (no OTC, no clip); $T_{WM} = $ WARM (+OTC, no clip); $T_{CL} = $ CLIP (no OTC, +clip); $T_{WM \times CL} = $ (+OTC, +clip). If there was no warm × clip interaction, we refer to an overall warming effect, where the 'warming effect' = $[(T_{WM} + T_{WM \times CL})/2 - (T_{CON} + T_{CL})/2]$. If a warm × clip interaction was present, we present the effects of 'warm (no clip)' = $(T_{WM} - T_{CON})$ and 'warm (+clip)' = $(T_{WM \times CL} - T_{CL})$ separately. We follow the same convention for the clipping effects. The 'combined effects of warming and clipping' = $(T_{WM \times CL} - T_{CON}) = [(T_{WM} - T_{CON}) + (T_{CL} - T_{CON})] + $ interaction effect. This is the effect of combined warming and clipping on a non-warmed, non-clipped landscape. If an interaction was present (interaction effect $\neq 0$), we compare the direction and magnitude of the combined effect (additive effect + interaction) to the strictly additive treatment effects.

We used SPSS version 11.5 (SPSS Inc., Chicago, Illinois, USA) to conduct all statistical analyses. We began our

analysis by conducting multi-factorial, repeated measures ANOVAs for each site, assuming a heterogeneous first order auto-regressive covariance matrix structure, where 'year' was the repeated measure and 'warming' and 'clipping' were the main factors. We tested for interactions between main factors and the within-subjects repeated measure. We followed up on significant findings by examining multiple comparisons using the Tukey test. We also conducted two-way ANOVAs (where the main effects were warm and clip) for each year and site separately and followed significant findings with Tukey's *post hoc* test. We conducted simple linear regressions between the change in species richness with warming and the initial site variables using the mean values for each site. We conducted the analysis separately for clipped and non-clipped plots in order to remove the influence of clipping from the relationship between site variables and change in SR with warming.

RESULTS

Habitat and grazing history effects: control plots only

With all sites combined, there was an average of 36 species per plot (average of years 1999–2001). Averaged over 1999–2001, there was no difference in SR numbers between habitats. The high grazing intensity history sites had eight more species than the low grazing history sites.

Treatment effects

There was a strong interannual effect on SR treatment responses. There was a year × warm interaction at most sites, a year × clip interaction at the LG meadow site and a year × warm × clip interaction at the HG shrubland site.

Warming decreased total species richness (SR) by five to 14 species (depending on habitat and grazing history) in
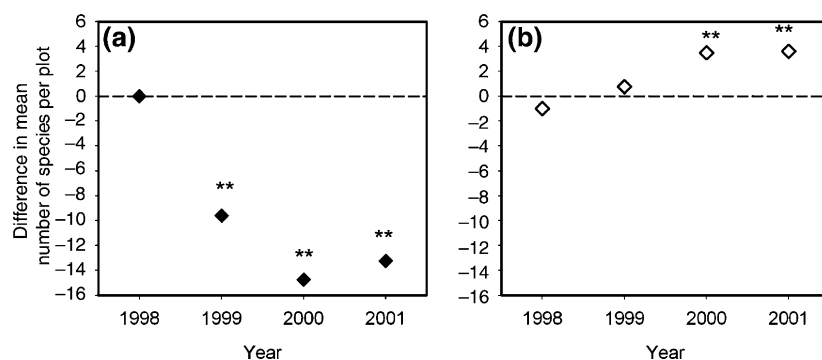

Figure 2 Effect of (a) warming on total species richness (SR) at the low grazing intensity history shrubland site and (b) clipping on total SR at the low grazing intensity history meadow site, from 1998 to 2001. '**' indicates significance at $P < 0.05$ based on within year ANOVAs. Solid diamond represents the overall warming effect, both in the presence and absence of clipping; open diamond represents the overall clipping effect, both in the presence and absence of warming, $n = 8$.

1999 and by nine to 15 species in 2001 (Fig. 2a). This represents a 16–30% decline in SR in 1999 and a 26–39% decline in SR by 2001. The larger absolute and relative declines in total SR occurred at the HG meadow and LG shrubland sites. Warming similarly decreased the Shannon diversity index by four species (34%) in 2001 averaged across all sites.

There was no SR difference between grazing control (GC) plots and clipped plots at all sites. This suggests that – with respect to SR – simulated and actual grazing had similar effects on the vegetation. However, simulated grazing may differ from actual grazing in how it affects other aspects of the soil and vegetation. At two of the four sites, there were no differences among fenced control plots and GC plots (the plots situated outside of the fenced area). At the LG meadow site, the GC plots had seven more species than the fenced control plots. By contrast, at the HG shrubland site, the GC plots had *c.* four species less than the fenced control plots ($P = 0.09$).

While the overall effects of experimental warming on species diversity were large and robust among sites, the effects of simulated grazing, and of combined simulated warming and grazing, exhibited more site specific heterogeneity. Simulated grazing had no effect on SR at all sites in 1999. By 2000 and 2001, most sites exhibited an increase of *c.* two to four species with clipping; this represents an increase in SR of 5–14%. For example, clipping increased

SR by four species at the LG meadow site in 2001 (Fig. 2b). By contrast, clipping had no effect on SR at the LG shrubland site in all years. In 2000, clipping increased the Shannon index by three species at most sites, with no effects in 2001.

Interactions between warming and clipping were present at some sites. The presence of clipping tended to reduce the species decrease with warming; the presence of warming tended to enhance the species increase with clipping. Warming on a clipped landscape generally dampened the species losses from warming alone. For example, at the high grazing history shrubland site in 2001, warming on a non-clipped landscape decreased SR by nine species, whereas warming on a clipped landscape had no effect on SR (Fig. 3). At the low grazing history meadow site in 2001, the magnitude of the warming induced decline in the Shannon–Wiener index was three times greater in the absence of clipping than the decline in the presence of clipping. Furthermore, clipping on a warmed landscape increased diversity more than clipping on a non-warmed landscape. For example, at the HG shrubland site in 2001, clipping on a non-warmed landscape had no effect on SR, while clipping on a warmed landscape increased total SR by five, $P = 0.1$ (Fig. 3). Moreover, at the LG meadow site, clipping on a non-warmed landscape decreased by three, while clipping on a warmed landscape increased Shannon diversity by two species.

Combined treatments decreased total SR by four to 16 species from 1999 to 2001, depending on site and year (Fig. 4). This represents a decline of *c.* 14–38% in 1999 and
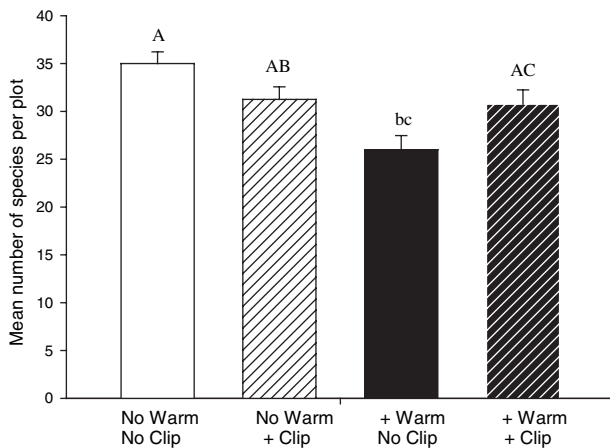
Figure 3 Mean number of species per plot at the high grazing (HG) shrubland site in 2001. Bars represent mean total species richness (SR) values plus standard error. White bars indicate non-open top chamber (non-OTC) plots and black bars indicate OTC plots. Non-hatched bars represent non-clipped plots and hatched bars represent clipped plots. Bars that have different capitalized letters are significantly different from each other at $P < 0.05$; bars that have the same letter (but one is capitalized and one is lower-cased) are different at $0.05 < P < 0.1$ based on ANOVA followed by Tukey test for multiple comparisons.
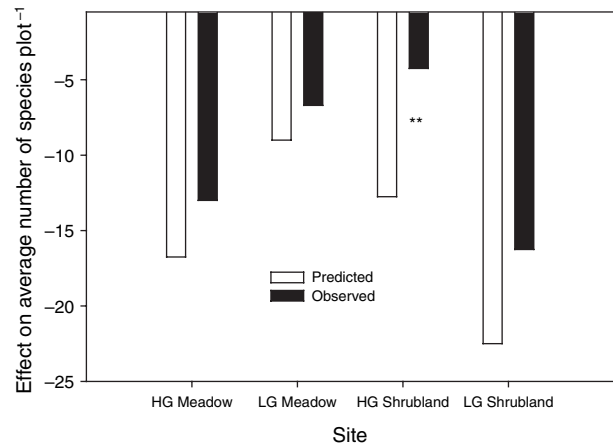
Figure 4 Combined (observed) vs. additive (predicted) effects of experimental warming and clipping at all four sites in 2001. The observed effect $= (T_{WM \times CL} - T_{CON})$, the difference between the warm × clip vs. the no warm × no clip plots. The predicted effect $= (T_{WM} - T_{CON}) + (T_{CL} - T_{CON})$, the sum of the warm (no clip) and clip (no warm) effects. Asterisks indicate that the interaction term for the ANOVA was significant at $P < 0.05$.

12–43% by 2001. While warming consistently decreased SR, the large range in SR responses to the combined treatments is due to whether a strong clipping effect and/or a strong warm × clip interaction was present. The presence of a strong clipping effect dampened the species losses compared with warming alone. The presence of a strong warm × clip interaction dampened the species losses compared with warming alone and enhanced the species increases compared with clipping alone. The sites where the largest clipping effects occurred (such as the LG meadow site) and where the strongest clip × warm interactions occurred (such as the HG shrubland site) had the smallest species losses (four to seven species in 2001) with combined treatments. The sites where the warming effect was strong, where clipping effects were absent and/or where warm × clip interactions were weak or absent (such as the LG shrubland site) had the largest species declines (16 species in 2001) with combined treatments. While the combined treatments had fewer species losses than the strictly additive treatment effects at all sites, this was only statistically significant at the HG shrubland site in 2001 (Fig. 4).

Site variables and warming-induced SR changes

Within the non-clipped plots, sites with less available N had greater species losses with warming when compared with sites with higher levels of available N. Within the clipped plots, sites with less soil moisture were more prone to species losses with warming as compared with the moister sites. At the LG shrubland site, decreases in SR were positively associated with aboveground shrub biomass. As the shrub *Potentilla fruticosa* was so dominant (in terms of percent cover and biomass) at the LG shrubland site, but

Table 1 Simple linear regression results where change in total species richness (SR) was the dependent variable and initial site factors were the independent variables

Plot type	Independent variable	R^2	Standard coefficient	P-value
No clip	N availability	0.95	−0.98	0.025
	Forb SR*	0.99	0.99	0.037
Clip	Soil moisture	0.88	−0.94	0.06
	Air temperature*	1.00	−1.00	0.012
No clip and clip	Shrubland biomass**	0.99	0.99	0.004

Each regression value represents the mean site value, $n = 4$.
R^2 values should be interpreted with caution because of the low sample size.
'*' indicates that this analysis omitted the low grazing shrubland site, $n = 3$.
'**' is from the shrubland sites only, and includes both clipped and non-clipped plots, $n = 4$.

was absent from the meadow sites and comprised a small percent of the vegetative cover at the HG shrubland site, we also examined the relationship between change in SR with warming and initial site variables without data from the LG shrubland site in the analysis. When excluding the LG shrubland site from the analysis, sites with cooler air temperatures (clip plots) and also sites with a higher number of forbs (non-clip plots) experienced larger species losses with warming (Table 1).

DISCUSSION

In this study, we found that total SR declined dramatically and rapidly with experimental warming. This effect was sometimes dampened by simulated grazing, which tended to increase SR at some sites. Interactions between warming and clipping were also present, such that the species losses under the combined treatments were less than that predicted from the sum of the independent warm and clip effects. Sites with less available N, less soil moisture, cooler air temperatures and a higher initial number of forbs exhibited the largest species losses with experimental warming. High species losses were also associated with increasing shrub biomass.

There are several potential explanations for the observed plant species diversity responses to experimental warming. These include changes to belowground resources, increased litter accumulation, enhanced small mammal activity, reduced reproductive output and success, vegetative heat stress and improper tissue cold hardening.

Ecosystem responses to experimental warming have previously been attributed to changes in belowground resource availability (Oechel *et al.* 1998; Luo *et al.* 2001; Melillo *et al.* 2002). Our soil temperature and moisture results did not show a strong and consistent directional change in soil temperature and moisture at 12 cm depth that was sustained over the entire growing season with experimental warming. Therefore, it is unlikely that warming fostered a less favourable belowground environment for plant species with respect to soil temperature and moisture. Rather, we observed increases in N availability with warming. Averaged over all sites and years, warmed plots had more available nitrate and ammonium than non-warmed plots (+2.3 μg per bag per day, $P = 0.004$). In this region, where over 80% of the precipitation occurs during the growing season, warming could positively affect the resource balance of the ecosystem by making N more available (with no ecologically significant decrease in soil moisture). These ameliorated belowground resource conditions could indirectly decrease diversity (Tilman 1987; Zavaleta *et al.* 2003). However, N additions likely decrease diversity through an increase in vegetative productivity (Tilman 1993). We did not observe increases in above-

ground net productivity with experimental warming, despite increases in N availability. Therefore, changes in below-ground resources are likely not directly responsible for the large SR declines with experimental warming.

Warming increased litter cover by *c.* 25%. An increase in litter could result from increased annual inputs to the litter pool or from decreased decomposition of the litter pool. We have evidence for the latter effect (Klein 2003) as decomposition rates, represented by the decay constant k (month^{-1}), were slower (k was smaller) in warmed plots compared with non-warmed plots during a 1 year litter decomposition experiment in 2000–2001 (averaged over vegetation types and sites, $k_{non-warm} = 0.051$, $k_{warm} = 0.039$, $P = 0.001$). Litter accumulation substantially reduces the photosynthetically active radiation (PAR) that reaches plants and also creates a mechanical barrier. Both of these effects inhibit the growth and establishment of vegetation and hence reduce diversity (Tilman 1993; Foster & Gross 1998). There was a strong negative relationship between litter cover and SR ($R^2 > 0.80$, $P < 0.002$) at the high grazing history meadow site (Fig. 5a) and at most other sites. Litter cover could, therefore, be an important indirect link between warming and decreases in SR. The removal of this litter mass with grazing could also explain the opposite direction of biodiversity response to warming and clipping. While this is a plausible explanation, the increase in litter cover with warming was primarily in the non-clipped plots; however, warming decreased SR in both clipped and non-clipped plots. Therefore, increased litter coverage with warming is only a partial explanation of the SR losses with warming.

Some researchers assert increasing small mammal activity could result in biodiversity losses on the Tibetan Plateau (Wageningen & Wenjun 2001). At both meadow sites, we observed an increase in small mammal disturbance with experimental warming. However, at the low grazing history shrubland site, there was a positive relationship between small mammal disturbance and SR ($R^2 = 0.45$, $P = 0.07$, $n = 8$). Small mammal activity may create small-scale

disturbances that enhance, rather than decrease, diversity at this site. Therefore, enhanced small mammal activity with warming does not explain the diversity losses at our system; rather, our results suggest that in some situations, warming-enhanced small mammal activity could actually enhance plant SR.

Open top chambers could potentially reduce plant species diversity by decreasing plant reproductive output and success. However, the reported effects of OTCs on plant reproductive output and success are mixed. Across a range of arctic and alpine sites, reproductive effort (e.g. number of flowers) and success (e.g. number of seeds and seed weight) increased with OTC warming after three to four years of the manipulation (Arft *et al.* 1999). To our knowledge, there are no reports of OTC effects on seed dispersal. Molau (2000) found that OTCs limited, had no effect and increased reproductive success (in terms of fertilization level), depending on species. Totland & Eide (1999) observed decreased pollinator visitation rates inside OTCs for *Ranunculus acris*. Alpine plants, including those at our sites, possess a range of reproductive strategies that fall into the broader categories of sexual reproduction and clonal propogation. Some species can reproduce by several strategies; other species rely on a specific mode of reproduction. It is possible that pollination or seed dispersal was limited for plants that solely rely on those processes for reproduction and, therefore, the OTCs could have reduced SR through this artifactual mechanism. However, given the small number of species likely affected by this artifact, given the evidence that OTCs can also increase reproductive output and success, and given the consistent and large declines in SR we observed, this mechanism may contribute to, but is not solely responsible for, diversity declines with experimental warming.

Diversity declines with experimental warming may be attributed to OTC induced heat stress in aboveground plant tissue. Alpine plants have evolved heat-trapping morphology, which can result in plant tissue temperatures that exceed the heat tolerance threshold of the plant. Prostrate
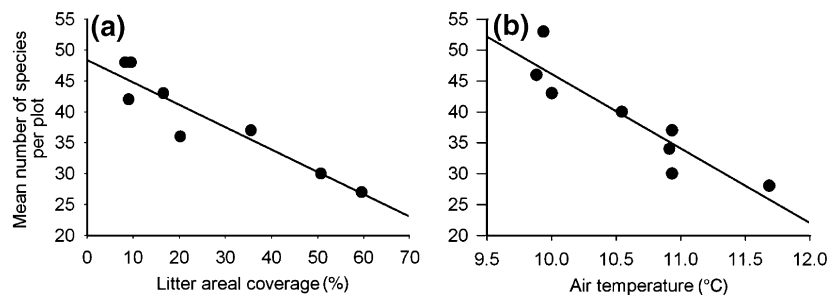

Figure 5 (a) Relationship between total species richness (SR) and growing season averaged % litter cover in 1999, $R^2 = 0.87$. (b) Relationship between total SR and growing season averaged air temperature in 2000, $R^2 = 0.83$. Both relationships were observed at the non-clipped plots of the high grazing intensity history meadow site, $n = 8$.

growth and other specialized mechanisms enable alpine plants to decouple from their environment, an adaptation that can make alpine plants susceptible to occasional overheating (Korner 1999). The difference between ambient air temperature and leaf temperature is larger for alpine plants as compared with plants from all other biomes. While we did not directly measure leaf temperature in our plots, the maximum 24 h July air temperature measured 10 cm above the soil surface was 25 °C for the control plots and 33 °C for the warm plots at the high grazing history meadow site in 1999; similar results were found in other sites and years. The maximum 24 h air temperature in 1999 and 2000 for warmed plots ranged from 45–47 °C. These values, which are lower than actual leaf temperature, are at the leaf temperature threshold defined as the heat tolerance temperature (50% survival) for plants growing in several alpine and sub-alpine environments (Larcher & Wagner 1976; Gauslaa 1984; Loik & Harte 1996). Moreover, there is evidence that experimental warming can decrease the heat tolerance temperature of leaves (Loik & Harte 1996). OTC warming also generally delayed the onset of senescence in the non-clipped plots (Klein 2003). This shift in phenology could prevent proper cold hardening and retranslocation of carbohydrates, nutrients and water from the plants, making them susceptible to freezing damage (Korner 1999). A positive correlation between susceptibility to heat and frost damage has been previously documented (Gauslaa 1984). The strong negative relationship we observed between air temperature and SR at most sites supports these hypotheses (Fig. 5b).

It is possible that the OTCs caused an unrealistic buildup of heat on the leaf surface and that under 'actual' climate warming conditions the heat build-up at the leaf surface would be dissipated by wind. While our experiment may represent an 'extreme' case, the mechanism remains: alpine plant species richness is vulnerable to climate warming. Under a less extreme scenario, the decreases in diversity may be more gradual and occur over a longer time-scale.

As the control plots were fenced just prior to this study, the SR in these plots could represent a recovery from grazing. However, at two of the four sites, there was no SR difference between the control plots within the fenced areas and the GC plots outside of the fenced area. At these sites, the fence did not have a significant effect on SR. At the LG meadow site, the fenced control plots had fewer species than the GC plots. The only 'recovery from grazing' effect was therefore to dampen the magnitude of the species loss with experimental warming. At the HG shrubland site, where the GC plots had four fewer species than the fenced control plots (averaged over 2000 and 2001), there were still five fewer species in the OTC than in the GC plots (averaged over 2000 and 2001), representing a 17% decline in SR.

Sites with lower soil moisture and available N and cooler air temperatures were associated with greater species losses with warming. It is possible that the vegetation in these relatively resource poor sites have less buffering capacity to cope with climate warming or increased climate variability. Sites with lower N availability were also associated with a larger initial species pool (linear regression between available NH_4 and total SR in non-warmed plots, $R^2 = 0.89$, $P < 0.0005$, $n = 8$; and between available NO_3 and total SR in non-warmed plots, $R^2 = 0.87$, $P = 0.001$, $n = 8$). Moreover, sites with higher initial forb SR were prone to higher species losses with warming. These results are contrary to the prediction that higher SR confers more compositional stability in response to environmental perturbations, such as climate warming. This suggests that species' identities, rather than numbers, may be more important with respect to resilience to perturbations (Sankaran & McNaughton 1999). The plots with initially high total and forb SR (and lower N availability) may have experienced more species losses because of these initial conditions and the probability of having more rare species present. However, when we examine the Simpson diversity index (which is more sensitive to abundant species than the Shannon–Wiener index), the Simpson diversity index also decreased significantly at all sites with experimental warming. This suggests warming may have important consequences for species diversity, regardless of initial species' abundance.

We not only observed a direct effect, but also an indirect effect of climate on species richness as mediated through plant–plant interactions at the LG shrubland site. The LG shrubland site, which had a large initial shrub biomass pool, experienced larger species losses than that predicted by initial site conditions. Across the shrubland sites, higher species losses were associated with larger initial shrub biomass pools. As shrub biomass increased with experimental warming, we observed a climate induced, plant competition mediated feedback to species diversity.

Our study provides the first experimental evidence that climate warming can cause dramatic declines in plant species diversity at a high elevation ecosystem over a relatively short time frame. The species decline with experimental warming was robust among the alpine meadow and deciduous shrubland habitats, which comprise c. 35% of the Tibetan Plateau vegetation (Zhao & Zhou 1999) and among sites that differed by grazing intensity history and grazing season of use. These results support the IMAGE model predictions of climate warming effects on the Tibetan Plateau flora and general predictions of large species losses with climate warming; however, further study is necessary to test the generality of these responses across other regions of the Tibetan Plateau and other high altitude ecosystems. The potential mechanisms we identify are occasional heat stress

and improper cold hardening of plant tissue, phenomena to which alpine flora are particularly vulnerable. Warming-induced litter accumulation may also contribute to these species losses. Cooler, more resource poor sites may be more prone to these rapid species losses; moreover, biotically mediated interactions can feedback to increase these losses. Declining biodiversity may make high elevation ecosystems, and their inhabitants, more vulnerable to future changes.

Our findings suggest that grazing management can be used to mitigate some of these warming-induced species losses. This study, which is the first to explicitly examine the independent and combined effects of experimental warming and grazing on the rangelands of the Tibetan Plateau, suggests that the future species diversity in this region will depend on both climate change and grazing. These findings underscore the need to investigate the multiplicity of factors causing vegetation change in this and other pastoral regions of the world.

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