Ecology, 93(11), 2012, pp. 2365–2376

Effects of warming and grazing on soil N availability, species composition, and ANPP in an alpine meadow

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Abstract. Uncertainty about the effects of warming and grazing on soil nitrogen (N) availability, species composition, and aboveground net primary production (ANPP) limits our ability to predict how global carbon sequestration will vary under future warming with grazing in alpine regions. Through a controlled asymmetrical warming (1.2/1.7°C during daytime/nighttime) with a grazing experiment from 2006 to 2010 in an alpine meadow, we found that warming alone and moderate grazing did not significantly affect soil net N mineralization. Although plant species richness significantly decreased by 10% due to warming after 2008, we caution that this may be due to the transient occurrence or disappearance of some rare plant species in all treatments. Warming significantly increased graminoid cover, except in 2009, and legume cover after 2008, but reduced non-legume forb cover in the community. Grazing significantly decreased cover of graminoids and legumes before 2009 but increased forb cover in 2010. Warming significantly increased ANPP regardless of grazing, whereas grazing reduced the response of ANPP to warming. N addition did not affect ANPP in both warming and grazing treatments. Our findings suggest that soil N availability does not determine ANPP under simulated warming and that heavy grazing rather than warming causes degradation of the alpine meadows.

Key words: alpine meadow; global warming; herbivore; N addition; plant functional group; primary production; species diversity; Tibetan Plateau.

Introduction

Climate change, especially warming, directly affects plant growth and shifts in plant species across a range of tundra ecosystems (Arft et al. 1999, Dormann and Woodin 2002, van Wijk et al. 2003, Walker et al. 2006, Elmedorf et al. 2012). Several studies indicate that plant aboveground net primary production (ANPP) has variable responses to warming in arctic and alpine regions, with reported increases, decreases, or no change (Arft et al. 1999, Rustad et al. 2001, Wan et al. 2005, Klein et al. 2007, Post and Pedersen 2008). Livestock grazing is one of the main land uses of natural grasslands and its interaction with climate change affects plant biomass, soil nutrient dynamics, and species composition of plant communities (Milchunas and Lauenroth 1993, Wang et al. 2003, Klein et al. 2007, Post and Pedersen 2008). In particular, contrasting responses of plant community composition to warming with and without herbivore grazing have been observed (Klein et al. 2007, Post and Pedersen 2008).

The effect of soil N availability on ANPP in grasslands depends on plant species composition (Bear and Blair 2008, Ren et al. 2010, Chen et al. 2011). Climate change (Dormann and Woodin 2002, van Wijk et al. 2003, LeBauer and Treseder 2008) and grazing (Seagle et al. 1992) significantly affect nutrient dynamics, especially nitrogen, in soil organic matter. Many studies have investigated the role of organic nitrogen (N) for plant production in arctic, boreal, temperate, Mediterranean, and alpine ecosystems (see review from Gärdenäs et al. 2011). However, uncertainty about how warming and grazing affect soil N availability, species composition, and ANPP limits our ability to predict how global carbon sequestration will vary under future warming in alpine regions. Thus, understanding the response of these processes to the abiotic and biotic effects of warming with grazing is important for

Manuscript received 3 August 2011; revised 2 May 2012; accepted 14 May 2012. Corresponding Editor: X. Xiao.
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predicting future responses of grasslands to warming in alpine areas, such as the Tibetan Plateau.

Degradation of alpine meadows is one of the main environmental problems in the Tibetan Plateau (Zhao 2009), although the amount and causes of degradation are highly disputed (Harris 2010). The causes of degradation are still uncertain, with different studies demonstrating the role of climate change, particularly warming (Klein et al. 2004, 2007) and heavy grazing (Zhou et al. 2005, Zhao 2009). In spite of the relatively robust literature on the response of individual ecological processes to changes in temperature and grazing (Klein et al. 2004, 2007, Luo et al. 2009, 2010, Zhao 2009, Hu et al. 2010, Lin et al. 2011), this study is to examine the effects of warming and grazing on soil N availability, species composition, and ANPP through a controlled warming-grazing experiment in the region. The experiment was conducted over five years from 2006 to 2010, including an N addition treatment under the warming-grazing experiment for one year in 2010. Our research objectives were to address the following three questions: (1) How does a free-air temperature enhancement (FATE) system with asymmetrical warming (1.2/1.7°C during daytime/nighttime) and grazing affect N availability in soil? (2) How does plant species composition respond to asymmetrical warming and grazing? (3) How does ANPP respond to biotic (i.e., shifts in plant species composition) and abiotic (i.e., soil temperature, soil moisture, and soil N availability) conditions in the region? Our results suggest that soil N availability does not influence ANPP in the alpine region under simulated warming conditions, and suggest that heavy grazing rather than warming results in degradation of alpine meadows in the region.

Materials and Methods

Experimental site

The experimental site is located at the Haibei Alpine Meadow Ecosystem Research Station (HBAMERS; 37°37′ N, 101°12′ E). The mean elevation of the valley bottom is 3200 m. A detailed site description can be found in Zhao and Zhou (1999). Mean temperature and total rainfall were 8.4, 8.5, 8.1, 8.5, and 9.1°C, and 449.2, 397.6, 339.4, 282.6, and 376.2 mm during the growing seasons (1 May to 20 September) in 2006, 2007, 2008, 2009, and 2010, respectively (Appendix B: Fig. B1). Compared to average rainfall during the growing season in the region (i.e., 450 mm), our site had normal rainfall in 2006 and tended a little toward drought from 2007 to 2010 (i.e., 11–37% below average rainfall).

The plant community at the experimental site is dominated by *Kobresia humilis*, *Festuca ovina*, *Elymus nutans*, *Poa pratensis*, *Carex scabrirostris*, *Scripus distigmaticus*, *Gentiana straminea*, *Gentiana farreri*, *Blysmus sinocompressus*, and *Potentilla nivea*.

Controlled warming-grazing experiment

The design of the controlled warming (i.e., free-air temperature enhancement [FATE] system with infrared heaters) with grazing experiment was described previously by Luo et al. (2010). In brief, in May 2006, eight hexagonal arrays of Mor FTE (1000W, 240V; Mor Electric Heating Association, Comstock Park, Michigan, USA) infrared heaters were deployed over vegetation canopy that had previously been heavily grazed by sheep during cool seasons from October to May of prior years at the Haibei Alpine Meadow Ecosystem Research Station, with eight dummy arrays over reference plots. The heaters were controlled using the proportional-integral-derivative-outputs (PID) control system so as to ensure constant warming between heated and reference plots. The set point differences of the vegetation canopy between heated and corresponding reference plots were 1.2°C during daytime and 1.7°C at night in summer (Kimball et al. 2008, Luo et al. 2010). A two-way factorial design (warming and grazing) was used with four replicates of each of four treatments: no warming with no razing (i.e., control, C), no warming with grazing (G), warming with no grazing (W), and warming with grazing (WG). In total, 16 plots of 3 m diameter were fully randomized throughout the study site.

Initially, one adult Tibetan sheep was fenced in each of the grazing plots on the morning of 15 August 2006 for approximately 2 h. The canopy height was about 8–9 cm and 4–5 cm before and after grazing, respectively. Two adult Tibetan sheep were fenced for approximately 1 h in each of the grazing plots on the mornings of 12 July, 3 August, and 12 September in 2007, 8 July and 20 August in 2008, 9 July and 24 August in 2009, and 7 July and 23 August in 2010 (Appendix B: Fig. B1). The canopy heights were about 6–7 and 3–4 cm before and after grazing, respectively. The canopy height of the vegetation was measured at 50 points within the plots before and after grazing, and the sheep were removed from the grazing plots when the canopy height was reduced to approximately half of the initial height. A 50×50 cm cage was set up inside each plot for each grazing event. The forage utilization rate was calculated using the difference between biomass present inside and outside cage after each grazing event. The annual cumulative forage utilization rates during the growing seasons were 32.4%, 44.3%, 60.8%, 56.7%, and 55.5% for the G treatment, and 31.7%, 49.9%, 56.4%, 53.7%, and 57.7% for the WG treatment in 2006, 2007, 2008, 2009, and 2010, respectively. All experimental sheep were fenced into three additional 5×5 m fenced plots for one day before the beginning of the grazing experiment to help them adapt to small plots.

Nitrogen (N) fertilization

In order to eliminate the mixed effect between species composition and N fertilization on ANPP, N fertilization and no-fertilization treatments using PVC tubes were conducted in each of the 16 plots for C, G, W, and WG treatments because a change in species composition under different treatments had been found before beginning the fertilization experiment in 2010. Two PVC tubes (i.e., one for N fertilization and another one for no-fertilization treatment in each plot) of 30 cm inner diameter and 45 cm length were set up in each plot in early May 2010, i.e., in total 32 PVC tubes were used. The height of the PVC tubes was kept at about 5 cm above the surface soil. In total, three fertilization events were performed, with 1, 1, and 2 g N/m^2 (as NH_4NO_3-N) applied uniformly in the middle of June, July, and August in 2010 using a small spray with solution to the vegetation canopy. For the non-fertilized treatment, equal amounts of deionized water were applied. The community selected inside the PVC tubes was the same as that of the treated plot.

Soil temperature and soil moisture measurements

The effects of warming and grazing on soil temperature and soil moisture from 2006 to 2008 were reported by Luo et al. (2010) and Hu et al. (2010). In brief, both warming and grazing alone significantly increased mean seasonal soil temperature at 10 cm from May to September by 1.9° and 1.0°C from 2006 to 2010 (Appendix B: Fig. B2A), whereas only warming significantly decreased soil moisture at 10 cm by 15.6%, 19.1%, and 17.8% in 2008, 2009, and 2010 during the growing season (Fig. B2B).

Estimation of aboveground net primary production (ANPP) and plant species diversity

A nondestructive sampling method was described by Klein et al. (2007) to estimate ANPP. The mean height and mean cover of the vegetation canopy were measured using a 100 × 100 cm quadrat divided into 400 5 × 5 cm squares in each plot. This process was also conducted off-plot from 2006 to 2009 on ungrazed and grazed fields during the growing seasons, after which we harvested, dried, separated, and weighed the vegetation. The equation used to simulate aboveground living present biomass (APB) was $APB = -5.7575 + 0.0839C + 5.6656H$ ($r^2 = 0.84$, $n = 210$, $P < 0.001$), where C is the total canopy cover and H is mean canopy height based on the weight of different species cover in the community. Mean frequency of different species was the percentage of plots with species presence during the five years divided by the total number of plots (i.e., 80). Mean cover of different species was calculated as the total coverage in squares with species presence divided by the total number of 5 × 5 cm squares in each plot (i.e., 400). The peak of APB in the year for no-grazing treatments (i.e., C and W) was taken to represent annual ANPP at the end of August each year. The intake by sheep during each grazing period was estimated through the difference in APB before and after grazing. The sum of peak APB and total intake by sheep during the growing season was taken to represent the annual ANPP for grazing treatments (i.e., G and WG treatments). Species diversity at the end of August each year was calculated using Shannon's index $H' = -\sum_i p_i \log p_i$, where p_i is the proportional abundance of the ith species out of S species.

N mineralization measurement

Net nitrogen mineralization rates were measured using the in situ soil core incubation method. A PVC cylinder 50 cm length and 6 cm in diameter was driven into the soil to a depth of 40 cm in each plot. The measurement was conducted from 14 August to 28 August in 2006, from 4 to 31 July in 2008, and from 10 July to 8 August in 2010. All soil cores (initial and after incubation) were cut into four layers at intervals of 10 cm and were sieved through a 2-mm screen immediately and then temporarily stored in a refrigerator at 4°C. Fresh soil (10 g) was extracted with 50 mL of 2 mol/L KCl on a rotary shaker for 1 h within 24 h. The filtrate made using Whatman no.1 filter paper was analyzed for NH_4-N and NO_3-N using a Skalar flow analyzer (Skalar Analytical, Breda, The Netherlands). Net N mineralization rates during the incubation periods were calculated from the difference in inorganic N concentrations between the initial and incubated samples divided by the number of days of incubation. We only show data at the 10 cm soil depth because there were no significant differences among treatments at 20, 30, and 40 cm soil depths.

Data analysis

Repeated-measures analyses of variance (ANOVA), with warming and grazing as the main factors (between-subject factors) and with sample date as within-subject factors including interactions, was applied to test the effects of the main factors on soil temperature and moisture at 10 cm, daily net N mineralization rate, relative change of plant species and plant functional group (PFG) cover, and ANPP using the GLM procedure and type III sum of squares in SPSS version 12.0 (IBM, Armonk, New York, USA). A two-way ANOVA followed by multiple comparisons using Turkey's HSD test was conducted for all measured variables, except for fertilization. A paired test was used for the effect of N addition in 2010 on ANPP regardless of warming and grazing in order to eliminate the effect of change of plant species composition on ANPP. Stepwise regression analysis was used to measure the relationships between ANPP and average soil temperature, soil moisture, cover of different plant species and PFGs. Simple correlation was performed between ANPP and daily net N mineralization rates using three years of data. All significant differences were at $P \leq 0.05$, unless otherwise stated.

Results

Net nitrogen (N) mineralization rate

Net N immobilizations were observed during August 2006 and July 2008, while net N mineralization occurred in July 2010 for all treatments (Fig. 1). There were no significant effects of warming, grazing, and their interaction on net N mineralization rates except for the G and WG treatments in 2006. In this case, WG was associated

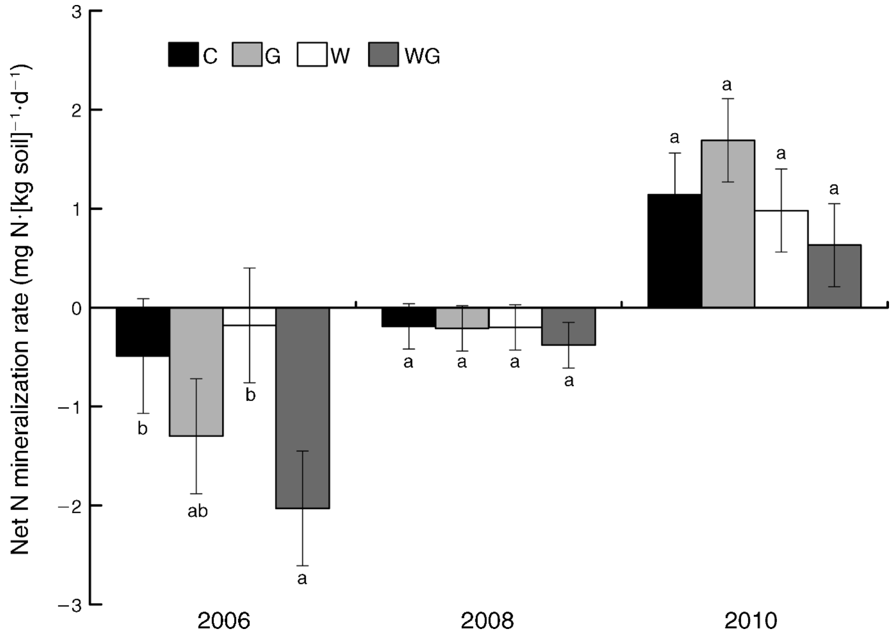

FIG. 1. Net nitrogen mineralization rates at 10 cm soil depth under different treatments. Bars show ±SE. Abbreviations are: C, no warming with no grazing; G, no warming with grazing; W, warming with no grazing; and WG, warming with grazing. Bars with different letters are significantly different ($P \leq 0.05$).

with greater net N immobilization. The effect of year on net N mineralization rates was significant (Fig. 1).

Plant species and plant functional group (PFG) composition

Generally, plant species richness was only significantly affected by the interaction between warming and year, whereas the species diversity index was significantly affected by interactions between year and warming and between warming and grazing (Table 1). For example, warming significantly decreased plant species richness by about 10% only since 2008 and decreased the plant diversity index only in 2010, whereas moderate grazing did not significantly affect them (Table 1 and Fig. 2). Only 13 out of about 30 plant species in each plot were significantly influenced by warming and/or grazing and their interaction with year and the effects were in both directions (Fig. 3). For example, warming alone significantly increased cover of the two non-legume forbs (i.e., *Lancea tibetica* except in 2010 and *S. distigmaticus* in 2007 and 2008) and two legumes (i.e., *Trigonella ruthenica* after 2008 and *Gueldenstaedtia diversifolia* in 2010), whereas warming alone decreased cover of the two forbs (*Potentilia anserina* after 2008 and *Thalictrum alpinum* in 2007) (Fig. 3). Grazing alone decreased the cover of the three grasses and grass-like plants (i.e., *P. pratensis*, *Festuca ruba*, and *C. scabrirostris*), the forb *T. alpinum*, and the two legumes *S. distigmaticus* and *G. diversifolia*, whereas it increased the coverage of the two non-legume forbs (*S. distigmaticus* and *P. anserina* after 2008) (Fig. 3). Interactive effects between warming and grazing significantly affected coverage of four plant species (i.e., two grasses of *E. nutans* and *Stipa aliena* and two non-legume forbs of *G. straminea* and *Taraxacum mongolicum*) (Fig. 3). For example, warm-

TABLE 1. Summary of repeated-measure ANOVAs for species richness and species diversity index using warming and grazing as main factors from 2006 to 2010.

Source	df	Species richness		Species diversity index	
		F	*P*	*F*	*P*
Warming (W)	1, 12	3.206	0.098	2.315	0.154
Grazing (G)	1, 12	2.251	0.159	0.112	0.743
W × G	1, 12	1.707	0.216	1.868	0.197
Year	4, 48	1.478	0.224	62.871	<0.001
Year × W	4, 48	6.554	<0.001	4.144	0.006
Year × G	4, 48	1.478	0.224	3.366	0.017
Year × W × G	4, 48	0.319	0.864	0.926	0.457

ing with grazing increased the coverage during the experimental period, but warming alone decreased the coverage of *G. straminea* and *T. mongolicum* even for *E. nutans* in 2010. Grazing alone decreased the coverage of *E. nutans* and *G. straminea* except in 2010, *S. aliena* and *G. straminea*, but grazing with warming increased the coverage *G. straminea* in 2010 and *T. mongolicum* after 2009 (Fig. 3).

The average coverage of graminoids, forbs, and legumes was about 86%, 86%, and 28%, respectively, for the control treatment over the experimental period. Warming, grazing, year, and the interactions between year and grazing and/or between year and warming significantly affected coverage of PFGs in the community ($P < 0.005$) (Table 2). Warming significantly increased graminoid coverage except in 2009 and legume coverage after 2008, but reduced forb cover in the community. Grazing significantly decreased coverage of all PFGs before 2009 but increased forb cover in 2010 (Fig. 4). There was only interactive effect between warming and grazing on forb cover ($P < 0.05$). For example, warming without grazing decreased forb cover during the experimental period, but warming with grazing increased forb cover after 2009. Similarly, grazing without warming decreased forb cover except in 2010, whereas grazing with warming increased forb cover after 2009.

Aboveground net primary production (ANPP)

Generally, ANPP varied with warming ($F_{1,12}$ = 332.8, $P < 0.001$), grazing ($F_{1,12}$ = 4.7, P = 0.051), year ($F_{4,48}$ = 191.1, $P < 0.001$), and the interactions between warming and grazing and/or year (Appendix A: Table A1 and Fig. 5A). Warming significantly increased average ANPP by 27.8% regardless of grazing during the five-year experiment, but grazing reduced the response of ANPP to warming (Fig. 5B). An interaction between grazing and warming was observed only in 2008 ($F_{1,12}$ = 34.0, $P < 0.001$) and 2009 ($F_{1,12} = 9.2$, $P = 0.010$) and it was weak in 2007 ($F_{1,12} = 4.1$, $P = 0.066$). For example, in 2008 and 2009, warming alone increased ANPP by 65.9% and 41.4% compared with the control treatment, but only by 18.2% and 20.2% for WG compared with the grazing-alone treatment (Fig. 5A).

The effect of grazing on ANPP varied with warming and year ($F_{4,48}$ = 12.5, $P < 0.001$; Table A1). For example, grazing did not affect ANPP in 2006, 2009, or 2010 but significantly reduced ANPP in 2007 (i.e., 6.8% and 5.8% with and without warming) regardless of warming (Fig. 5A). However, in 2008, grazing alone increased ANPP compared with the control treatment (i.e., 14.8%), but WG decreased ANPP compared with the warming alone treatment (i.e., 18.2%). Moreover, N addition did not significantly influence ANPP under all treatments in 2010 (Fig. 5C).

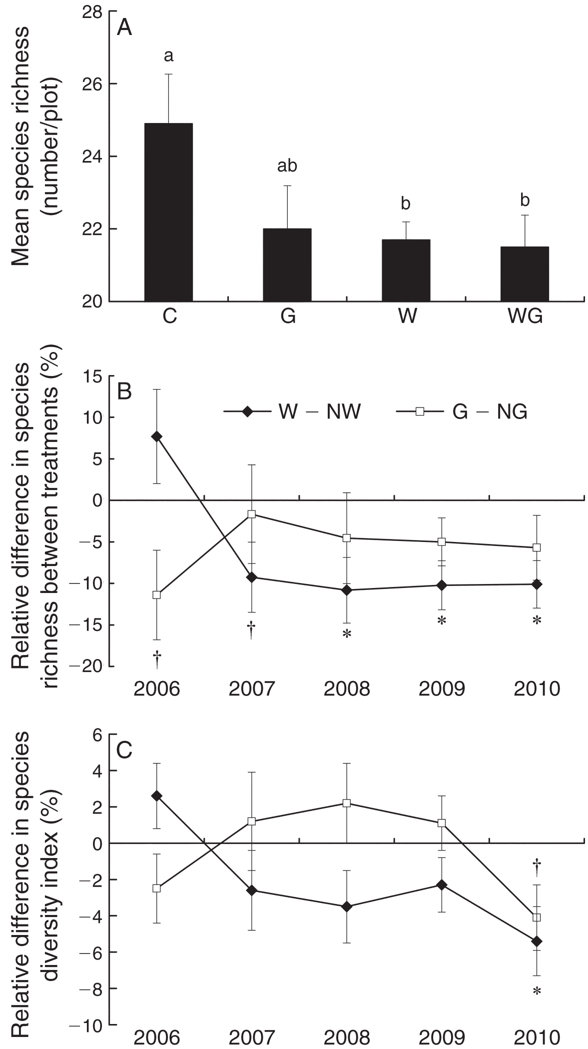

FIG. 2. (A) Total plant species richness across all years in each plot, (B) their relative difference, and (C) plant diversity index under different treatments. Bars show ±SE. In panel A, abbreviations are: C, no warming with no grazing; G, no warming with grazing; W, warming with no grazing; and WG, warming with grazing. In panel A, bars with different letters are significantly different ($P \leq 0.05$). In panels B and C, solid diamonds show the relative difference between warming (W) and no warming (NW), across grazing treatments, and open squares show the difference between grazing (G) and no grazing (NG) across warming treatments. Asterisks indicate significant difference at $P \leq 0.05$, and daggers indicate significant difference at $P \leq 0.10$.

Factors affecting ANPP

Although significant positive correlation between ANPP and soil temperature ($r^2 = 0.04$, $P = 0.081$) and negative correlation between ANPP and soil moisture ($r^2 = 0.07$, $P = 0.015$) at 10 cm were found, the r^2 values were small. However, strong positive correlation between ANPP and average air temperature ($r^2 = 0.35$, $P = 0.006$; Fig. 6A) and negative correlation between ANPP and total rainfall ($r^2 = 0.30$, $P = 0.011$; Fig. 6B) were

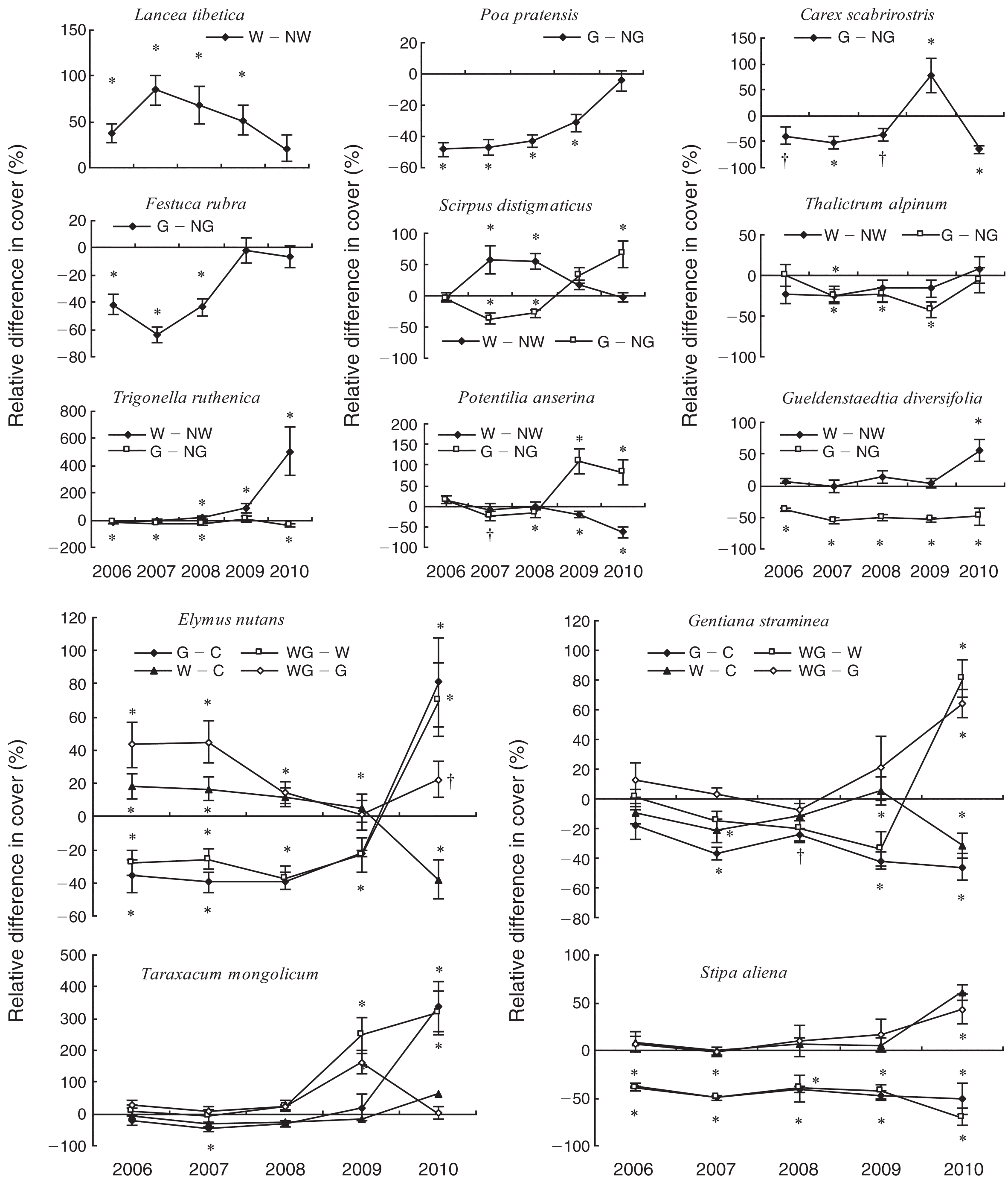

FIG. 3. Relative difference in plant species coverage in the community under different treatments. Bars show ±SE. Asterisks indicate significant difference at $P \leq 0.05$, and daggers indicate significant difference at $P \leq 0.10$.

found during the growing season from May to September. There were significant negative correlations between ANPP and species richness (Fig. 7A) and species diversity index (Fig. 7B). However, the coverage of three plant species (i.e., *E. nutans*, *T. ruthenica*, and *C. scabrirostris*) (Fig. 7C) and the coverage of graminoid PFG (Fig. 7D) were positively correlated with ANPP. In particular, grass *E. nutans* cover explained 32% of the variation in ANPP (Fig. 7C). Based on stepwise regression analysis when considering species composition, ANPP = −530.310 + 99.786AT − 8.204SM + 2.882EN + 6.937CS + 15.7974ST ($r^2 = 0.86$, $P < 0.001$), where AT is average air temperature from May to September; SM is average soil moisture at 10 cm; EN is

TABLE 2. Summary of repeated-measure ANOVAs for coverage of graminoids, forbs, and legumes using warming and grazing as main factors from 2006 to 2010.

		Graminoid cover		Forb cover		Legume cover	
Source	df	*F*	*P*	*F*	*P*	*F*	*P*
Warming (W)	1, 12	13.702	0.003	16.480	0.002	17.264	0.001
Grazing (G)	1, 12	94.787	<0.001	10.046	0.008	50.547	<0.001
W × G	1, 12	0.245	0.629	22.217	<0.001	1.752	0.210
Year	4, 48	8.985	<0.001	9.479	<0.001	2.125	0.092
Year × W	4, 48	1.360	0.262	5.219	0.001	7.560	<0.001
Year × G	4, 48	21.275	<0.001	9.130	<0.001	1.441	0.234
Year × W × G	4, 48	0.975	0.430	0.459	0.765	0.086	0.986

cover of *E. nutans*; CS is cover of *C. scabrirostris*; and ST is soil temperature at 10 cm. When considering PFG composition, ANPP = −672.108 + 129.930AT − 3.974SM + 1.518GC + 17.954ST − 0.522*R* −0.982FC ($r^2 = 0.87$, $P < 0.001$), where AT is average air temperature from May to September; SM is average soil moisture at 10 cm; GC is grasses coverage; ST is soil temperature at 10 cm; *R* is total rainfall from May to September; and FC is forb coverage. There was no significant correlation between ANPP and net N mineralization rate ($P > 0.05$).

DISCUSSION

Air temperature and rainfall and ANPP

Many studies show that the main control on primary production in grasslands on a regional scale is precipitation (Fang et al. 2001, Knapp and Smith 2001). However, in our study, we generally found positive correlations between ANPP and average air temperature and negative correlations between ANPP and total rainfall from May to September, suggesting that generally low average air temperature rather than total rainfall during the growing season may limit plant growth in this cold and wet region. This finding is consistent with previous reports (Dormann and Woodin 2002, Shen et al. 2002). For example, although growing season rainfall was less in 2008 and 2009 than in 2006, 2007, and 2010 (Appendix B: Fig. B1), ANPP in 2008 was almost the same as in 2006 and 2007 and ANPP in 2009 was even greater than in 2006, 2007, and 2008 for the control treatment (Fig. 5A). We also found that the response of ANPP under different treatments to average air temperature and total rainfall during the growing season was different. For example, significant negative correlation between ANPP and total rainfall was only observed for the warming-alone treatment ($r^2 = 0.91$, $P = 0.011$) and positive correlation was only found between ANPP and average air temperature for the control treatment ($r^2 = 0.90$, $P = 0.013$) during the growing season. Probably the change of species composition alter the response pattern of ANPP to climate conditions (Yahdjian and Sala 2006) because warming increased the proportion of grasses in the community and grazing reduced the response of grasses to

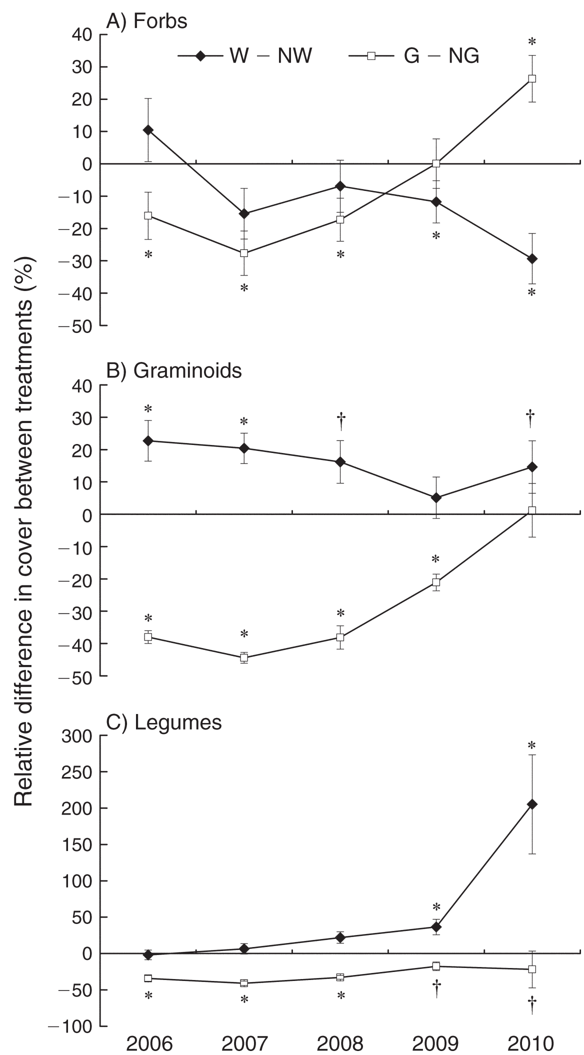

FIG. 4. Relative difference in coverage of different plant functional groups (PFGs) in the community under different treatments: (A) forbs PFG; (B) grasses PFG; and (C) legumes PFG. Bars show ±SE. Asterisks (*) indicate significant difference at $P \leq 0.05$, and daggers (†) indicate significant difference at $P \leq 0.10$.

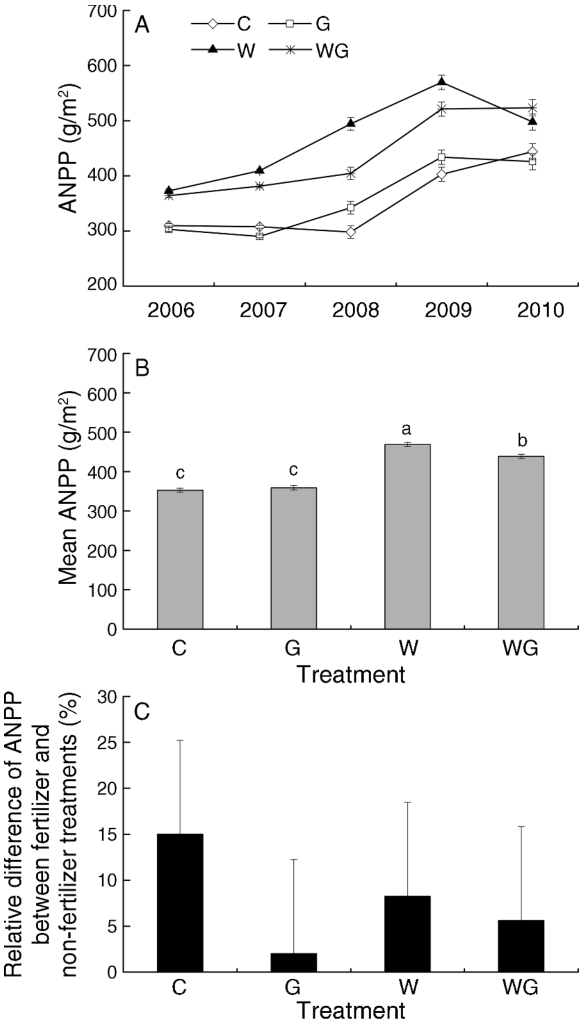

FIG. 5. (A) Aboveground net primary production (ANPP) of different treatments and different years, (B) average ANPP over five years, and (C) the effect of nitrogen fertilizer on ANPP under different treatments in 2010. Bars show standard error. Different lowercase letters in panel (B) indicate significant difference at 0.05 level. Abbreviations are: C, no warming with no grazing; G, no warming with grazing; W, warming with no grazing; WG, warming with grazing; NG, no grazing; and NW, no warming.

warming. In our study, we found the grasses usually were taller, which strongly affected ANPP compared with forbs. In particular, different plant species have different responses through time to temperature and different water use efficiency at a single location (Shen et al. 2002).

Plant species diversity and ANPP

Most studies across tundra ecosystems reported that warming caused about ~30% species loss (Arft et al. 1999, Klein et al. 2004, 2007, Walker et al. 2006, Post and Pedersen 2008). However, in our study, warming alone did not significantly affect plant species richness, and the effect of warming on plant species richness varied with year. Although we also observed that warming resulted in a significant reduction of plant species richness by about 10% in our study since 2008, this could be due to transient occurrences or disappearance. We noticed that there were a total of 42 plant species in the 16 plots during the five-year experiment (Appendix B: Fig. B3), whereas the frequency and coverage of 16 plant species was less than 20% and 1%, respectively (Fig. B3). The occasional appearance or disappearance of these 16 plant species (Appendix A: Table A2) resulted in the differences we observed in plant species richness between warming and no-warming treatments. For example, *Plantago depressa* was only observed in one plot for G in 2010, and its frequency and coverage were only 1.3% and 0.01% (Table A2). Similarly, *Morina chinensis* was only found for WG before 2009 and disappeared in 2010, and its frequency and coverage were 5.0% and 0.1% (Table A2). Overall, we found that there were no significant differences in species diversity index between treatments, except in 2010. Therefore, these findings caution against the conclusion of species loss induced by warming from short-term warming experiments.

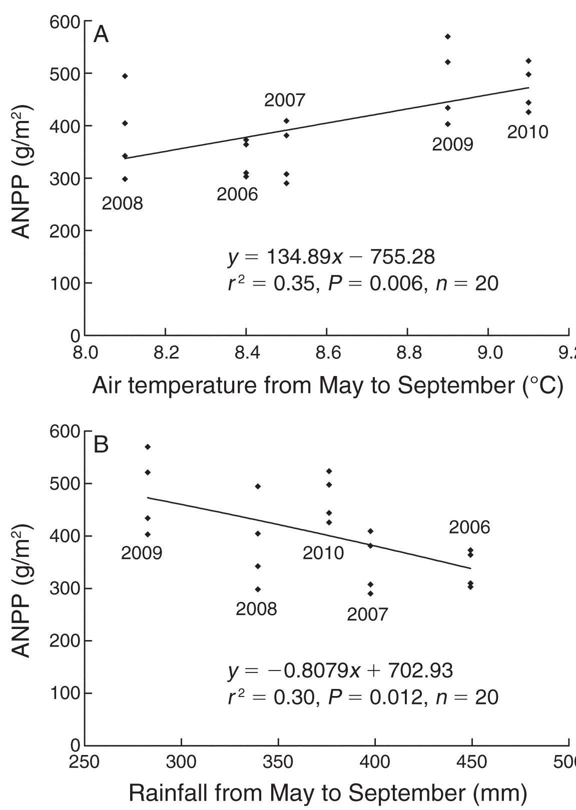

FIG. 6. Relationships between aboveground net primary production (ANPP) across all treatments and (A) average air temperature and (B) total rainfall during the growing season from May to September each year.

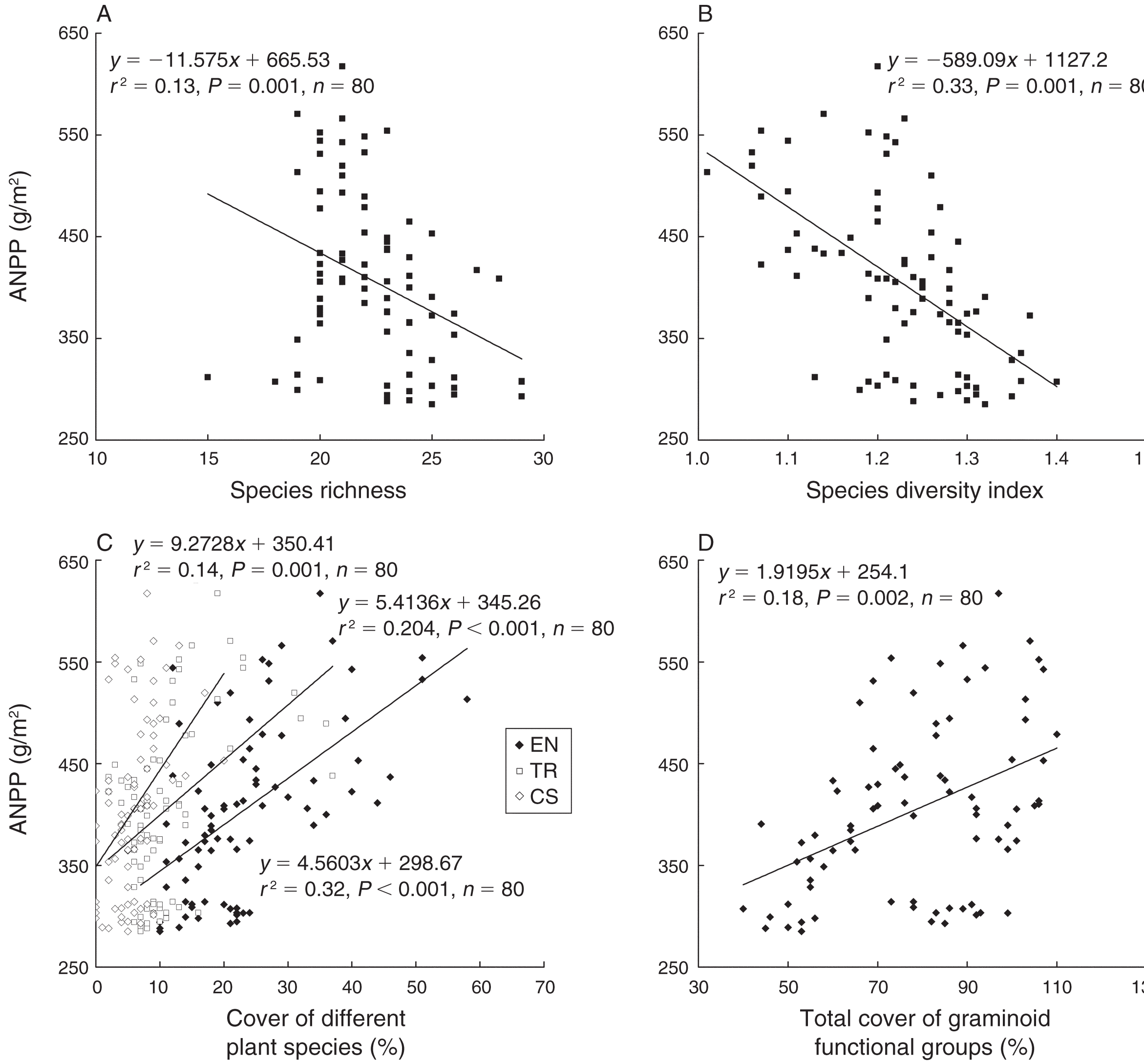

FIG. 7. Relationships between aboveground net primary production (ANPP) across all treatments and (A) species richness, (B) species diversity index, (C) cover of different plant species, and (D) cover of graminoid functional group. Abbreviations are: EN, *Elymus nutans*; TR, *Trigonella ruthenica*; and CS, *Carex scabrirostris*.

Similar to meta-analysis of 13 sites (Arft et al. 1999), we observed that warming increased coverage of graminoids and legumes but decreased forb coverage, and grazing decreased coverage of graminoids and legumes. However, Klein et al. (2004, 2007) found opposite results in the same alpine meadow using an open-top-chamber (OTC) warming and clipping experiment. These differences probably arise from the different warming patterns between the OTC and our FATE system. OTCs increase air temperature to a maximum air temperature during daytime (Klein et al. 2005), while the FATE system only increases soil temperature during daytime and nighttime (Kimball et al. 2008). Especially at an air temperature difference of up to 7.3°C (Klein et al. 2005), the extreme high temperature may cause heat stress in alpine plants, and graminiods may be more sensitive to heat stress compared with forbs because they are taller and provide shelter to the forbs in the plant community. Decrease of graminiods resulted in a decrease in ANPP with OTC warming (Klein et al. 2007) due to the greater contribution to total ANPP of graminiods compared to forbs in the community. On the other hand, Klein et al. (2007) found that clipping increased the proportion of forbs but did not affect other PFGs in the community. However, we found that grazing reduced graminoid and legume coverage. These differences could result from the difference between the effects of clipping and grazing on species composition because sheep have differential preferences between different plant species (Wang et al. 2003), but clipping is just done based on the average canopy height although Klein et al. (2008)

reported that some species were selected to be clipped. Moreover, vegetation was clipped prior to initiation of growth in the early spring and 15% of total peak aboveground biomass in the meadows was removed (Klein et al. 2007), whereas in our study grazing occurred two or three times during the growing season (except in 2006) and average forage utilization rates by grazing sheep were about 50% (ranging from about 30% to 60%), and was not affected by warming in our study. Forage utilization was up to 70% or more in this region previously, causing the heavy degradation of natural alpine meadow in the region (Zhou et al. 2005, Zhao 2009). Meanwhile, forbs are too short to clip in spring and forbs may benefit from clipping when higher grasses are clipped, which could result in an increase in forbs in the community (Klein et al. 2007). Our findings suggest that grazing, and especially heavy grazing, rather than warming may cause degradation of alpine meadow because the basic characteristic of degraded alpine meadow is a reduction of graminoids and palatable legumes, which decreases ANPP in the community in the region (Zhou et al. 2005, Zhao 2009).

A meta-analysis of 20 warming experiments demonstrated an average of 19% increase in aboveground plant productivity under warming in comparison to that under control (Rustad et al. 2001). Our results indicated that regardless of grazing the response of the average ANPP to warming was higher (i.e., about 28%) than the average value in the world (Rustad et al. 2001), and was even up to 41–66% for the warming alone treatments in 2008 and 2009. Warming stimulated ANPP but this did not result from higher species diversity because there were significant negative correlations between ANPP and species richness and species diversity index in our study, which is inconsistent with previous reports (Tilman et al. 1996). This could result from shifts in species or PFG composition induced by warming (Luo 2007). For example, in our study, warming significantly increased the coverage and height of a dominant species (*E. nutans*) and graminoid coverage in the community increased, and these changes explained 32% and 18% of the variation in annual ANPP during the five-year experimental periods. Based on the stepwise regression and N addition experiments, our results suggest that plant species composition, rather than soil N availability, may determine ANPP of the alpine meadow under future warming conditions. This finding may be attributed to the following causes. (1) Plants with shallow roots are more sensitive to drought induced by warming compared to plants with deep roots (Klein et al. 2008). Roots of most forbs are shallower than roots of graminoids, with the exception of a few tap-rooted forbs, and graminoids have higher utilization efficiencies of water and N than forbs in the region (Shen et al. 2002). (2) There is a trade-off or compensation in ANPP between graminoids and forbs (Shen et al. 2002). In our study, the decreased coverage of forbs was compensated for by increased coverage of graminoids, which is consistent with other reports (Shen et al. 2002, Cross and Harte 2007). Taller and higher density graminoids shade the lower forbs, which limits light resource competition for forbs under warming treatments. The total ANPP of the community increased with increased coverage of graminoids when the contribution to total ANPP in the community from increased graminoids overrode the contribution from decreased forbs.

Soil N availability and ANPP

Generally, we found that warming did not affect soil N availability in our study. Existing studies indicate inconsistent results, i.e., warming increases N mineralization (Melillo et al. 2002) or leads to an increase or decrease (Shaw and Harte 2001, Wan et al. 2005), or has no effects (Jonasson et al. 1993, Hovenden et al. 2008) on N mineralization across different biomes (Rustad et al. 2001). Year-to-year variation in N mineralization was observed in our study and could be attributed to two reasons. One reason is different rainfall amounts during the growing seasons in 2006, 2008, and 2010. Rainfall was high in 2006, and high precipitation could lead to more N fixation by microorganisms under warming with grazing, causing net N immobilization. The increase in soil temperature, which may lead to higher microorganism biomass compared with other treatments, was highest for warming with grazing and grazing promoted root exudation of carbon, which was quickly assimilated into a burgeoning microbial population in the rhizosphere of grazed plants (Hamilton and Frank 2001). However, 2008 and 2010 were drought years, and the especially higher biomass in 2010 may have needed more nutrients, which could drive net N mineralization. Another possible cause of the variation is the different incubation periods between 2008, 2010, and 2006. In 2006, only two weeks of incubation was conducted but, in 2008 and 2010, four weeks of incubation was conducted. An alternative explanation would be that the sampling was conducted later in the season when plants had already taken up relatively more of the mineralized N pool, particularly under higher rain conditions.

Many studies reported that N limits ANPP in the majority of terrestrial ecosystems (LeBauer and Treseder 2008). In our study, warming consistently increased ANPP regardless of grazing, whereas N availability in soils did not significant change between treatments, except for between the WG and W treatments in 2006. Therefore, a decoupling of soil N availability and ANPP occurred in our study, which is consistent with other previous reports under experimental warming conditions (Rustad et al. 2001, Shaw and Harte 2001, Wan et al. 2005). Moreover, N addition did not affect ANPP regardless of warming and grazing in 2010 in our study. Another N fertilization experiment in the region also revealed that N fertilization did not significantly affect the ANPP of annual oat pasture (Appendix B: Fig. B4A) or alpine meadow (Fig. B4B) from 2008 to 2010. Therefore, consistent with previous reports at the same site (Shen et al. 2002), these results suggest that the alpine meadow ecosystem may be N replete with a high organic N content in the soil (Xu et al. 2006). Some plants develop strategies to use organic N, and the average contribution of organic N to plant total N uptake is 22.5% (range 13–35%; Xu et al. 2004, 2006). Organic N is more important in cool and wet environments than in hot and dry environments (Warren 2006, Gärdenäs et al. 2011). Meanwhile, increased legume coverage due to warming may provide more biotic-fixed N for the plant community in our study. This suggests that warming effects on ANPP are not due to warming effects on soil N availability and soil N availability does not limit plant production in the alpine meadow (Warren 2006, Gärdenäs et al. 2011).

In summary, although we found that the interaction between warming and year significantly affected plant species richness, our results caution against conclusions regarding the effect of warming and grazing on plant species richness based on the occasional appearance or disappearance of some plant species during short-term experimental periods. Warming significantly increased coverage of graminoids and legumes, but grazing reduced them. Although warming and grazing did not influence net N mineralization, warming increased ANPP and grazing modified the response of ANPP to warming. Our results indicate that soil N availability was not a dominant factor controlling the response of ANPP to future warming and grazing in the alpine community in the region.

Acknowledgments

This research was funded by the Key Program of National Natural Science Foundation of China (41030105), National Basic Research Program (2010CB833502), the Special Program of Carbon Sequestration of Chinese Academy of Sciences (XDA05070205), and Kunlun Scholarship of Qinghai Province. We thank the anonymous reviewers for their valuable contributions. Shiping Wang, Jichuang Duan, Guangping Xu, and Yanfen Wang made equal contributions to this paper.

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Supplemental Material

Appendix A

Tables showing statistical results for aboveground net primary production (ANPP) and dynamics of species composition changed under warming and grazing treatments from 2006 and 2010 (*Ecological Archives* E093-223-A1).

Appendix B

Figures showing dynamics of rainfall and air temperature, mean soil temperature and soil moisture at 10 cm, mean frequency and coverage of different plant species during the 5-year experiment under warming and grazing treatments, and aboveground net primary production of natural alpine *Kobresia* meadow and an annual oat crop under nitrogen fertilization (*Ecological Archives* E093-223-A2).