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REGULAR ARTICLE

Impacts of seasonal grazing on net ecosystem carbon exchange in alpine meadow on the Tibetan Plateau

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Abstract
Background and aims Understanding the effect of grazing season on net ecosystem CO_2 exchange (NEE) and its components is crucial to predict the feedback of grazing management to climate change.
Methods We estimated NEE, gross primary productivity (GPP) and ecosystem respiration (Re) under different seasonal grazing practices (i.e. no-grazing (NG), warm season grazing (WG) and cold season grazing (CG)) by sheep during the growing seasons from 2008 to 2012 on the Tibetan Plateau.
Results Our results show that the impacts of seasonal grazing on daily GPP, Re and NEE in the alpine meadow ecosystem varied with sampling date and year. Compared with NG and CG, WG significantly reduced average seasonal NEE by 22.7 %, because grazing impact was exacerbated by drought in July in 2010. Soil temperature only explained 19–31 % of the variation in daily GPP, Re and NEE for all grazing treatments. The interannual variabilities of GPP, Re and NEE were mainly determined by root biomass and/or average soil temperature during the growing season.
Conclusions Our results suggest that although WG may decrease sequestration of CO_2 under continuous drought conditions after grazing, it would have little impact on CO_2 sequestration during the growing season under conditions of future warming with greater rainfall in alpine meadows on the Tibetan Plateau.

Keywords Warm season grazing · Cold season grazing · GPP · Re and NEE · Alpine meadow · Tibetan Plateau

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Introduction

Grazing is a main land use of natural grasslands in the world and has a great effect on carbon (C) cycling of grassland ecosystems (Conant et al. 2001; Lecain et al. 2002; Wilsey et al. 2002; Wang et al. 2011, 2012; Thomas 2012; Lin et al. 2011; Dong et al. 2013; Mai et al. 2014; Cui et al. 2014a). Grazing has a negative (Rogiers et al. 2005) or positive (Ward et al. 2007) impact, or no impact (Lecain et al. 2002; Wilsey et al. 2002; Risch and Frank 2006) on net ecosystem CO_2 exchange (NEE) of grasslands, depending on grazing intensity and history, climate and grassland types (Lecain et al. 2000; Wilsey et al. 2002; Thomas 2012; Dong et al. 2013). Alpine meadows are a weak C sink under natural conditions (Gu et al. 2003; Kato et al. 2006; Zhao et al. 2006).. Grazing, especially overgrazing, may reduce the strength of the C sink, and even turn it into a C source because of greater losses in ecosystem respiration (Re) relative to photosynthetic CO_2 uptake in the region (Cao et al. 2004; Li et al. 2005). However, Lin et al. (2011) found that moderate grazing did not significant affect seasonal average Re in the alpine meadow. Moreover, regardless of warm season grazing (WG) and cold season grazing (CG), moderate grazing significantly increased plant production but had little influence on soil respiration relative to no-grazing (NG) in this alpine region (Cui et al. 2014a). Although many studies suggest that moderate grazing (i.e. consuming about half and leaving about half of total biomass) benefits biodiversity, annual net primary productivity and community composition in semiarid grasslands with a long history of grazing (Milchunas and Laurenroth 1993; LeCain et al. 2000; Wang et al. 2003; Cui et al. 2014a, b), few studies are available about the effect of grazing season on sequestration of CO_2 in grassland ecosystems under moderate grazing, especially for alpine meadows. Therefore, to reduce uncertainty in our understanding of the global C cycle, it is important to identify the factors that determine the strength of the carbon source/sink of grassland ecosystems.

Because overgrazing predominantly results in degradation of grasslands in China (Wang et al. 2003; Zhao et al. 2011), the countries central government has promoted a series of ecological construction programs since 2000. In order to balance feed demand from grazing animals and ecosystem service provision in grassland regions, a program of moderate grazing has been implemented since 2011. As the largest grassland unit on the Eurasian continent, the Tibetan Plateau covers an area of approximately 2.5 million km^2 at 3500 m or more above sea level (a.s.l.) (Zheng et al. 2000). Generally, alpine meadows in the region are divided into two grazing seasons, i.e. warm season grazing (WG) from June to September and cold season grazing (CG) from October to May. Our previous results in the same experimental platform showed that annual cumulative forage utilization rates (i.e., total sheep intake as a percentage of aboveground net primary production) were 57, 53, 62, 45 and 48 % for the WG treatment (averaging 53 %), and 47, 65, 66, 52, 52 % for the CG treatment (averaging 56 %) in 2008, 2009, 2010, 2011 and 2012, respectively (Cui et al. 2014a). These average forage utilization rates can be considered as representing moderate grazing intensity in the region (Zhao et al. 2011). Most studies indicated that moderate grazing may result in maximum plant production (Milchunas and Laurenroth 1993; LeCain et al. 2000; Wang et al. 2003; Cui et al. 2014a). Thus, we hypothesized that both WG and CG under moderate grazing intensity is conducive to CO_2 sequestration, and that biotic (i.e. biomass) rather than abiotic factors (i.e. soil temperature and soil moisture) are the main factors affecting CO_2 sequestration. Because both WG and CG have no significant impacts on bare soil temperature (i.e. after removing vegetation) or soil moisture (Cui et al. 2014a) in the alpine meadow. The objectives of the study were to determine the impacts of different seasonal grazing patterns on NEE and its main components (i.e. grass primary production (GPP) and ecosystem respiration (Re)), and to identify the main factors affecting NEE in the alpine meadow ecosystem under moderate grazing over a 5-year period.

Materials and methods

Experimental site and experimental design

The experimental site is located at the Haibei Alpine Meadow Ecosystem Research Station (HBAMERS) (latitude 37° 37′N, longitude 101° 12′E). The mean elevation of the valley bottom is 3200 m a.s.l. A detailed site description can be found in Zhao and Zhou (1999). Average maximum and minimum temperature were 15.5 and −7.0 °C over the 5 years, and mean temperature and total rainfall were 6.7, 6.8, 7.3, 6.8 and 6.8 °C, and 348.0, 350.2, 442.6, 339.2 and 325.8 mm during the growing seasons from 1 May to 31 October in 2008, 2009, 2010, 2011 and 2012, respectively (Table 1). Each winter, there is snow cover for short periods and soils freeze. Compared to average rainfall during the growing seasons in the region (i.e. 450 mm) from 1981 to 2012, these years can be typified as normal in 2010, and under drought conditions in 2008, 2009, 2011 and 2012 (i.e. 22–28 % less than average rainfall) (Cui et al. 2014a). The plant community at the experimental site is dominated by graminoids and forbs, the average coverage of graminoids, forbs, and legumes was about 86 %, 86 %, and 28 %, respectively (Wang et al. 2012). The soil is a

Table 1 Monthly air temperature (°C) and rainfall (mm) from April to October over the experimental period from 2008 to 2012 and their average values at the experimental site from 1981 to 2012

Year Item	Month	Apr	May	Jun	Jul	Aug	Sep	Oct
Air temperature	2008	0.8	6.1	8.0	10.6	8.8	6.6	0.3
	2009	2.7	5.1	8.4	11.0	8.9	7.8	−0.1
	2010	−0.3	5.0	9.0	12.6	10.7	7.2	−0.6
	2011	1.7	4.8	9.3	10.0	10.0	6.2	0.4
	2012	0.3	5.4	8.5	11.3	10.6	5.4	−0.6
Rainfall	2008	40.4	49.2	53.2	70.2	76.4	90.4	8.6
	2009	0.4	1.6	35.6	83.6	111.2	84.6	34.2
	2010	15.2	62.4	53.0	53.6	170.2	73.4	30.0
	2011	15.4	75.1	83.8	96.2	80.9	65.9	31.4
	2012	14.6	44.8	40.2	95.6	85.0	64.8	11.6
Average monthly air temperature from 1981 to 2012		0.4	4.7	8.2	10.5	9.4	5.4	−0.6
Average monthly rainfall from 1981 to 2012		30.5	60.9	83.0	100.7	106.8	76.1	27.9

clay loam with an average thickness of 65 cm, and is perennially wet. The soils are classified as Mat Cry-gelic Cambisols according to the Chinese national soil survey classification system (Chinese Soil Taxonomy Research Group 1995). Organic carbon content is 7.3 % in surface soil, soil pH is 6.9 and average surface soil bulk density is 0.88 g cm^{-3} (Wu et al. 2005).

Grazing experiment

The experimental design has previously been reported by Cui et al. (2014a). In brief, the experimental site was an overgrazed winter meadow without litter biomass before the start of the experiment. Nine plots of 5 m× 5 m were fenced in August 2006 and fully randomized throughout the study site. The grazing experiment started in 2007 with three replicates of each of three treatments: no-grazing (i.e. control-NG), grazing during the warm season (WG) and grazing during the cold season (CG). Four adult Tibetan sheep were used for the WG and CG treatments in each grazing plot. The CG treatments took place on two grazing dates just before and after the start of the growing season, and the WG treatments took place on two dates which depended on vegetation conditions during the growing season. For WG, the canopy heights were about 6–7 before and 3–4 cm after each grazing event. The canopy height of the vegetation was measured at 50 points within the plots before and after grazing, and in both WG and CG treatments, 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 per plot was set up inside each plot for each grazing event. The forage utilization rate was calculated as the difference between biomass present inside and outside the cage after each grazing event.

The average annual cumulative forage utilization rates (i.e., total sheep intake as a percentage of aboveground net primary production (ANPP)) were 52 % for the WG treatment and 59 % for the CG treatment over the experimental period (Cui et al. 2014a). There were no significant differences between treatments in the annual cumulative forage utilization rates for WG and CG over the experimental period (Cui et al. 2014a).

Measurement of soil temperature and soil moisture

Volumetric soil moisture (%) was measured within all treated plots using Time Domain Reflectometry (TDR) (CS615) with four probes (15 cm in length). Soil temperatures at the 10 cm depth at the experimental site were measured using digital thermometers at the same time as the collection of gas samples. Soil moisture was measured from July to October in 2010 and from June to October in 2011.

Measurement of aboveground and root biomass

Plant production was determined using the cage comparison method (Cook and Stubbendieck 1986). A 50×

50 cm cage was set up in each plot and clipped inside and outside cages after each grazing event. All samples were oven-dried and weighed to determine plant production. Sheep intake was calculated using the difference between standing biomass inside and outside the cage after each grazing event. The sum of the standing biomass at the end of the grazing period and sheep intake during each grazing period was used as aboveground net primary production (ANPP) (Wang et al. 2012). Root biomass was measured using a 4-cm diameter soil drill sampler to take 0–10, 10–20, and 20–40 cm soil samples at the end of August each year. These root samples were immediately washed, dried at 80 °C, and weighed. Total root biomass was the sum of the root biomass in each soil layer each year.

Net ecosystem CO_2 exchange measurements

Net ecosystem CO_2 exchange (NEE) was measured using a manual transparent chamber (0.4 m×0.4 m×0.6 m) with a wall material of acrylic sheet, allowing over 90 % light transmittance, attached to an infrared gas analyzer (IRGA) (LI-6400; LiCor, Lincoln, NE, USA). The chamber had two small electric fans that ran continuously to promote air mixing during measurement, and a vent for pressure equilibration (75 cm long plastic tube, 0.7 cm inner diameter). A temperature and photosynthetically active radiation (PAR) sensor were put into the chamber and used for real-time measurement of gas temperature and PAR within the chamber, and atmospheric temperature and PAR outside the chamber were measured at the same time with a probe. The difference between the temperatures inside and outside of the chamber was less than 0.2 °C during the measurement process. A LI-6400 analyzer (IRGA) was connected to the chamber via inlet and outlet tubings (4 mm inner diameter), and gas circulation was ensured by an electric pump attached to the LI-6400, running at a flow rate of 500 ml min^{-1}. This chamber method has been successfully used to evaluate plot level fluxes of CO_2 in grassland ecosystems (Niu et al. 2008; Xia et al. 2009). The square steel frame structure was inserted directly into the meadow soil about 5 cm below the soil surface at a location about 0.5 m away from the edge of the plots. The frames provided a flat base between the soil surface and the CO_2 sampling chamber (Lin et al. 2009, 2011). When measuring NEE, the transparent chamber was put on the metal base rim and sealed with water. NEE was measured every 2 h on each sampling day at 1-week intervals from April to October each year. Six consecutive recordings of CO_2 and water vapor concentrations were taken on each frame at 10-s intervals during a 60-s period. Meanwhile, Re was also measured using a lightproof cloth mantle to cover the transparent chamber immediately after measuring NEE. CO_2 concentrations were allowed to build up or draw down over time, from which the changes of CO_2 concentration rate were used to calculate NEE and Re, respectively. Daily NEE was the average NEE throughout the day. Monthly NEE was the average daily NEE in each month and seasonal NEE was the average daily NEE measured within the growing season of each year.

Positive and negative NEE values represent net C release by, and uptake from, the ecosystem, respectively. The relationship between NEE (μmol CO_2 m^{-2} s^{-1}) and GPP (μmol CO_2 m^{-2} s^{-1}) was assessed as follows (e.g. Kowalski et al. 2003):

$$NEE = Re - GPP \text{ or } GPP = Re - NEE$$

Statistical analysis

General Linear Model-Repeated Measures Define Factors (SPSS 13.0, SPSS Inc., Chicago, IL, USA) was used to assess the significance of the impacts of treatment, year, month, and their interactions on GPP, Re, NEE, soil temperature, soil moisture and ANPP, with year and/or sampling date as within subject variables and treatment as a between-subject variable. For each sampling date and each year, significant differences in GPP, Re and NEE, soil temperature, soil moisture and ANPP between treatments were assessed using a one-way ANOVA and Least Significance Difference (LSD) tests. To test the correlations between soil temperature and soil moisture and NEE, Pearson's correlation and partial correlation analysis were performed. A multiple hypothesis approach (Burnham and Anderson 2002) using AIC (Akaike Information Criterion) was used in order to test the possible dependency of NEE, Re and GPP on soil moisture, soil temperature, and aboveground biomass and root biomass (0–40 cm). AIC considers the fitting quality and the number of variables included in the model. Hence, smaller AIC values indicate better models (Mazerolle 2006). AIC along with *p*-value were used as criteria for selecting model that best fitted. The model with the lowest AIC or *p*-value is chosen, since the two criteria tended to reach the lowest synchronically in the research. All significances mentioned in the text are at the 0.05 level, unless otherwise noted.

Results

Soil temperature and soil moisture and biomass

Compared with NG, both WG and CG significantly increased seasonal average soil temperature by 0.45–0.91 °C, except in 2011 and 2012, and compared with CG, WG increased seasonal average soil temperature by 0.39 °C only in 2009 (Fig. 1a). Compared with NG, WG significantly reduced seasonal average soil moisture in 2009, whereas the opposite results were found in 2011 and 2012, and there were no significant differences in soil moisture between NG and CG (Fig. 1b). WG significantly increased ANPP compared with NG, except in 2012, and compared with CG, except in 2010, and there were no significant differences between NG and CG, except in 2008 and 2009, when CG significantly decreased ANPP compared with NG (Fig. 2a). Compared with NG, WG significantly increased root biomass at 0–40 cm depth, except in 2011, and similar results were observed for CG, except in 2008 and 2011 (Fig. 2b).

Net ecosystem CO_2 exchange (NEE)

Grazing season alone did not significantly affect GPP and Re (Table 2), whereas interactive effects were observed between grazing season and year on GPP (Table 2 and Fig. 3) and on Re (Table 2 and Fig. 4), and between grazing season and sampling date on GPP (Table 2 and Fig. 3). Significant impacts on NEE were found for grazing season alone and for interactions between grazing season and sampling date, and between year and sampling date (Table 2 and Fig. 5). The daily

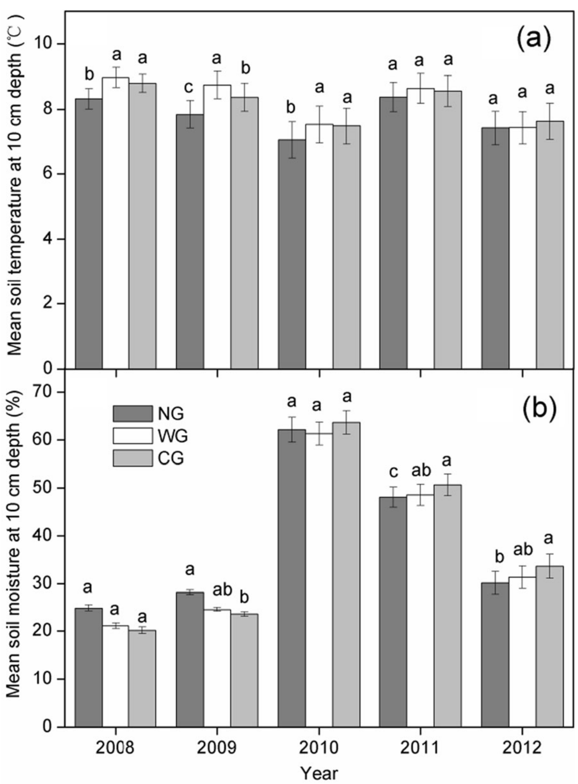

Fig. 1 Seasonal average soil temperature and soil moisture at a depth of 10 cm under seasonal grazing from 2008 to 2012. *NG* no-grazing, *WG* warming season grazing, *CG* cold season grazing. *Bars* indicate mean±1SE. *Different letters* indicate significant difference at p=0.05 level

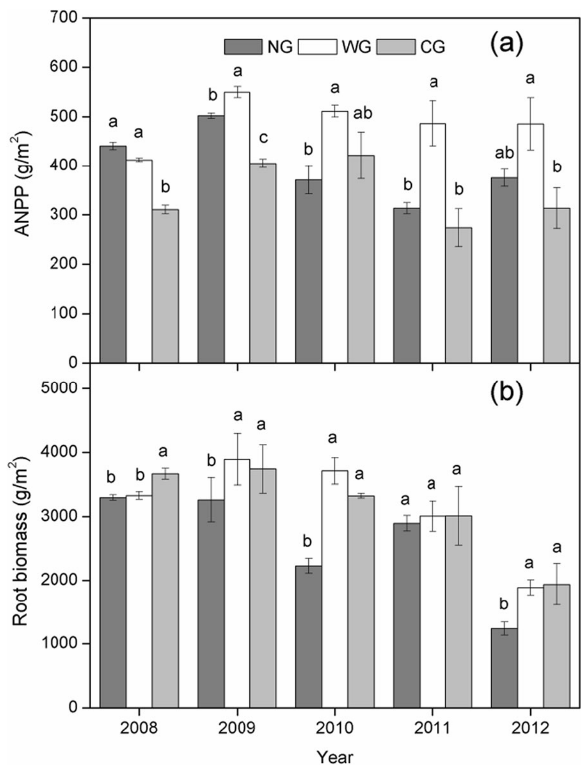

Fig. 2 ANPP and root biomass for NG, WG and CG from 2008 to 2012. *NG* no-grazing, *WG* warming season grazing, *CG* cold season grazing. *Bars* indicate mean±1SE. *Different letters* indicate significant difference at p=0.05 level

Table 2 Gross primary production (GPP), ecosystem respiration (Re) and net ecosystem exchange of CO_2 (NEE) from repeated-measure ANOVAs using NG, WG, and CG as the main factors from 2008 to 2012

Model	GPP		Re		NEE	
	F	*P*	*F*	*p*	*F*	*P*
Treatment (T)	1.623	0.273	0.374	0.703	13.413	**0.006**
Year (Y)	82.027	**<0.001**	805.935	**<0.001**	55.714	**<0.001**
Y × T	5.992	**0.037**	7.077	**0.026**	0.576	0.590
Day (D)	280.544	**<0.001**	143.380	**<0.001**	0.094	0.769
D × T	42.769	**<0.001**	3.515	0.098	23.310	**0.001**
Y × D	73.292	**<0.001**	66.088	**0.001**	109.853	**<0.001**
Y × D × T	5.104	0.051	3.864	0.083	1.215	0.361

Significant *p* values are in bold

NG no-grazing, *WG* warm season grazing, *CG* cold season grazing

dynamics of GPP, Re and NEE followed a one-peak pattern with higher values in the middle of the growing seasons. The maximum GPP, Re and NEE values in the alpine meadow occurred in July, except for NEE in 2010 due to drought (Figs. 3, 4, and 5).

Generally there were no significant differences in the seasonal average GPP values between NG (i.e. 6.8 μmol CO_2 m^{-2} s^{-1}), WG (i.e. 6.6 μmol CO_2 m^{-2} s^{-1}) and CG (i.e. 7.1 μmol CO_2 m^{-2} s^{-1}) during the growing seasons over the 5-year period (Fig. 6a). However, WG significantly decreased GPP compared with NG and CG in 2008 and compared with NG in 2010 (Fig. 6a). Grazing treatment significantly affected GPP on 10, 8, 7, 4 and 9 out of 30 sampling days in 2008, 2009, 2010, 2011 and 2012, respectively (Fig. 3a–e).

Similarly, there were also no significant differences in seasonal average Re values between NG (i.e. 4.6 μmol CO_2 m^{-2} s^{-1}), WG (i.e. 4.9 μmol CO_2 m^{-2} s^{-1}) and CG (i.e. 4.9 μmol CO_2 m^{-2} s^{-1}) during the growing seasons over the 5-year period, but NG significantly reduced seasonal average Re in 2010 compared with CG (Fig. 6b). Grazing season only significantly affected Re on 2, 3, 7, 3 and 4 out of 30 sampling days in these years (Fig. 4a–e).

Generally, WG significantly increased seasonal average NEE by about 22.7 % ($p<0.05$) compared with NG (i.e. −2.1 μmol CO_2 m^{-2} s^{-1}) and CG (i.e. −2.2 μmol CO_2 m^{-2} s^{-1}), indicating that WG significantly decreased sequestration of CO_2 during the growing seasons over the 5-year period, because negative NEE values represent net CO_2 uptake by the ecosystem. However, actually, compared with NG and CG, WG significantly reduced CO_2 sequestration only in 2010, and there were no significant differences between NG and CG over the 5-year experimental period (Fig. 6c). Grazing season significantly affected NEE on 8, 8, 14, 5 and 10 out of 30 sampling days in these years (Fig. 5a–e). Moreover, WG significantly reduced daily NEE immediately after grazing, except on July 22 in 2011 and on July 26 in 2012 (Fig. 7).

Relationships between GPP, Re and NEE and soil temperature, soil moisture and plant biomass

Generally, positive correlations were found between daily GPP and soil temperature, and between daily Re and soil temperature and soil moisture, but when all data are pooled negative correlations were found between daily NEE and soil temperature. Soil temperature alone explained about 31, 19 and 29 % of the variation in daily GPP, Re and NEE, respectively (Table 3). However, the regression coefficients indicate that the dependency of daily GPP, Re and NEE on soil temperature (i.e. the slopes of the regression equations) varied with grazing treatment (Table 3). For NG, WG and CG treatments, soil temperature explained about 36, 21 and 39 % of the variation in daily GPP; 22, 15 and 20 % of the variation of daily Re; and 32, 18 and 39 % of the variation in daily NEE (Table 3).

Seasonal average GPP, Re and NEE were significantly correlated with soil temperature, root biomass and soil moisture (Table 4). When all data were pooled, soil temperature explained about 62 and 11 % of the variation in GPP and Re, respectively, and root biomass

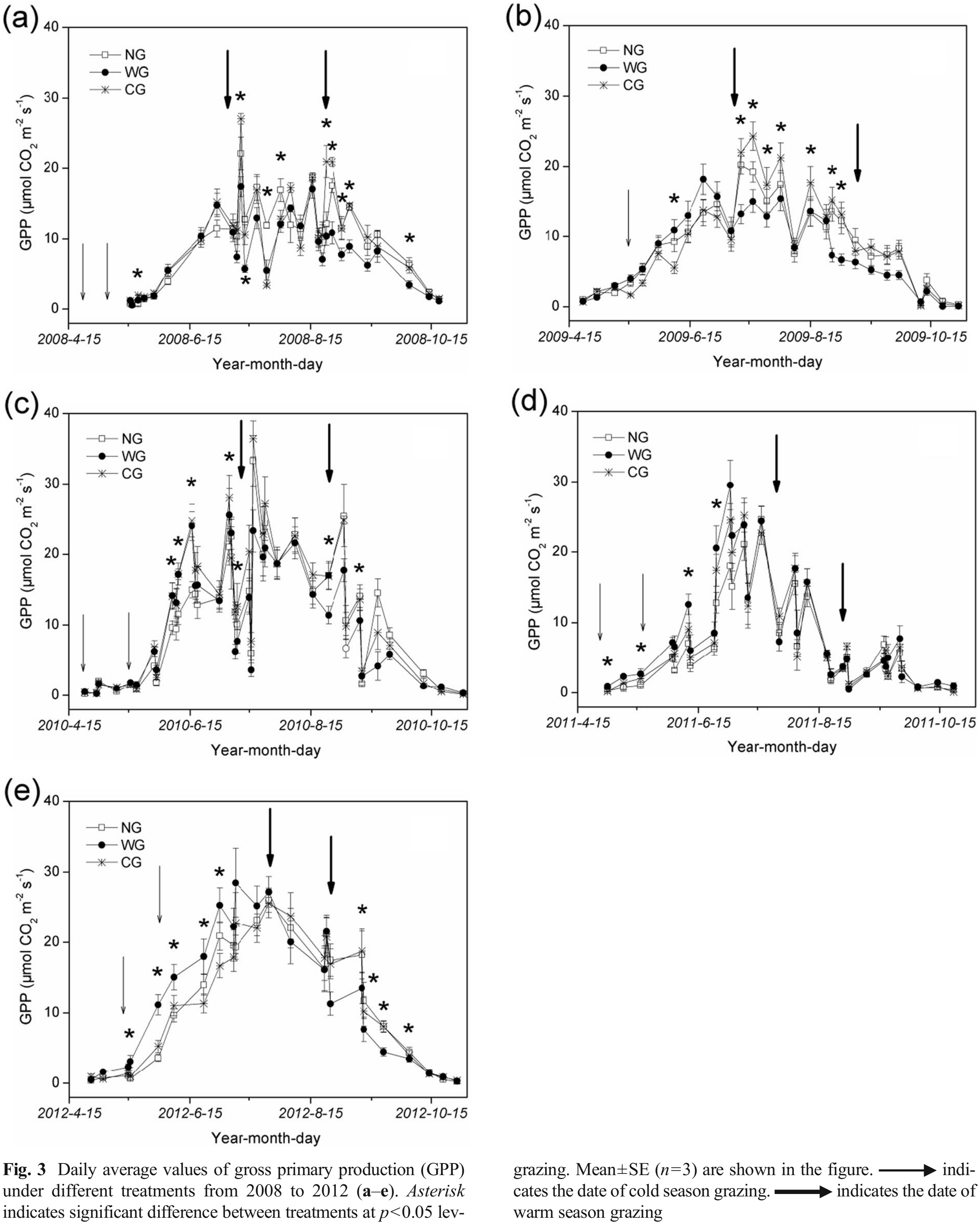

Fig. 3 Daily average values of gross primary production (GPP) under different treatments from 2008 to 2012 (**a**–**e**). *Asterisk* indicates significant difference between treatments at $p<0.05$ level; *NG* no-grazing, *WG* warm season grazing, *CG* cold season grazing. Mean±SE ($n=3$) are shown in the figure. ⟶ indicates the date of cold season grazing. ➞ indicates the date of warm season grazing

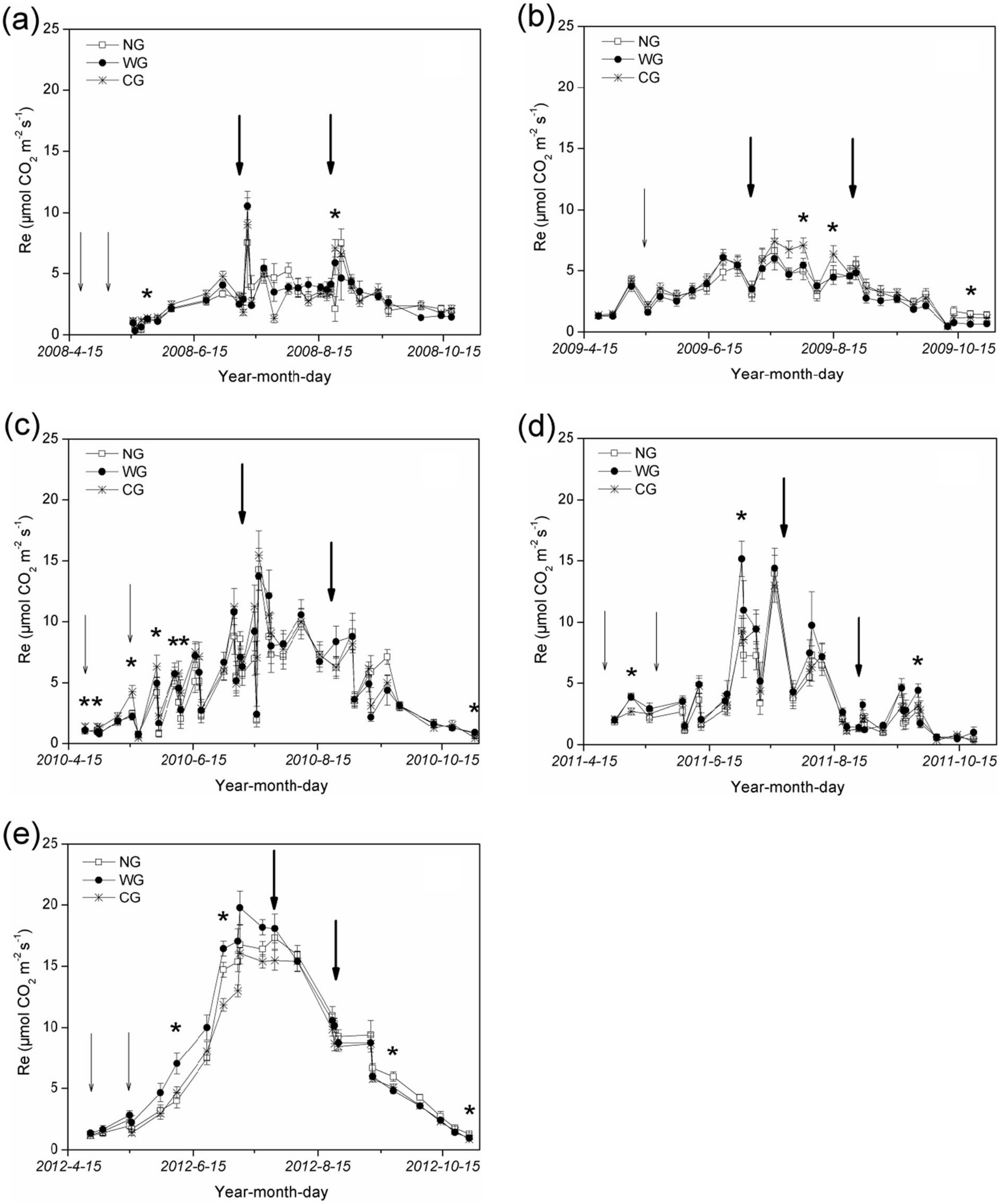

Fig. 4 Daily average values of ecosystem respiration (Re) under different treatments from 2008 to 2012 (**a**–**e**). *Asterisk* indicates significant difference between treatments at $p<0.05$ level; *NG* no-grazing, *WG* warm season grazing, *CG* cold season grazing. Mean ±SE ($n=3$) are shown in the figure. ⟶ indicates the date of cold season grazing. ⟶ indicates the date of warm season grazing

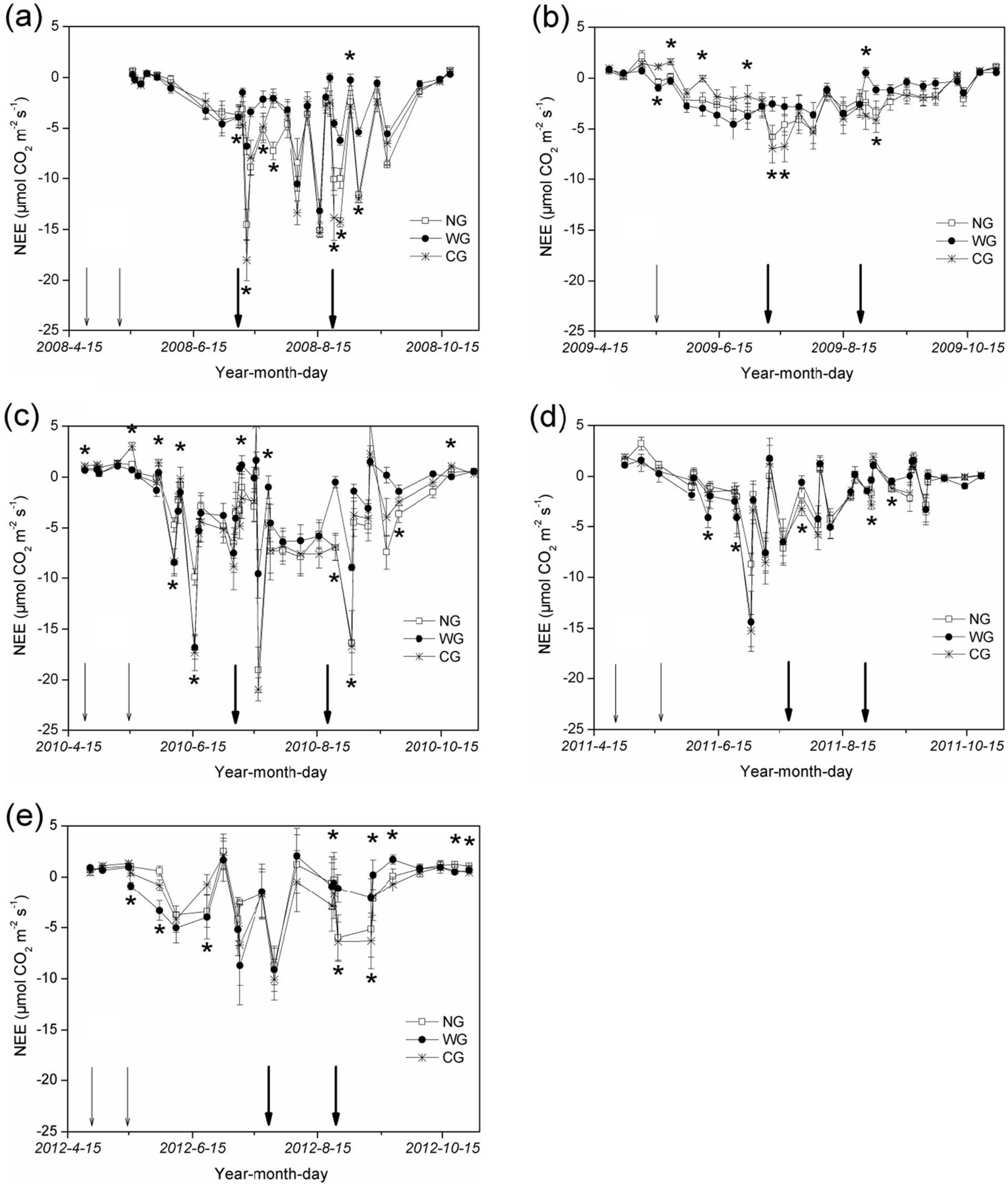

Fig. 5 Daily average values of net ecosystem exchange of CO_2 (NEE) under different treatments from 2008 to 2012 (**a**–**e**). *Asterisk* indicates significant difference between treatments at $p<0.05$ level; *NG* no-grazing, *WG* warm season grazing, *CG* cold season grazing. Mean±SE ($n=3$) are shown in the figure. ⟶ indicates the date of cold season grazing. ➡ indicates the date of warm season grazing

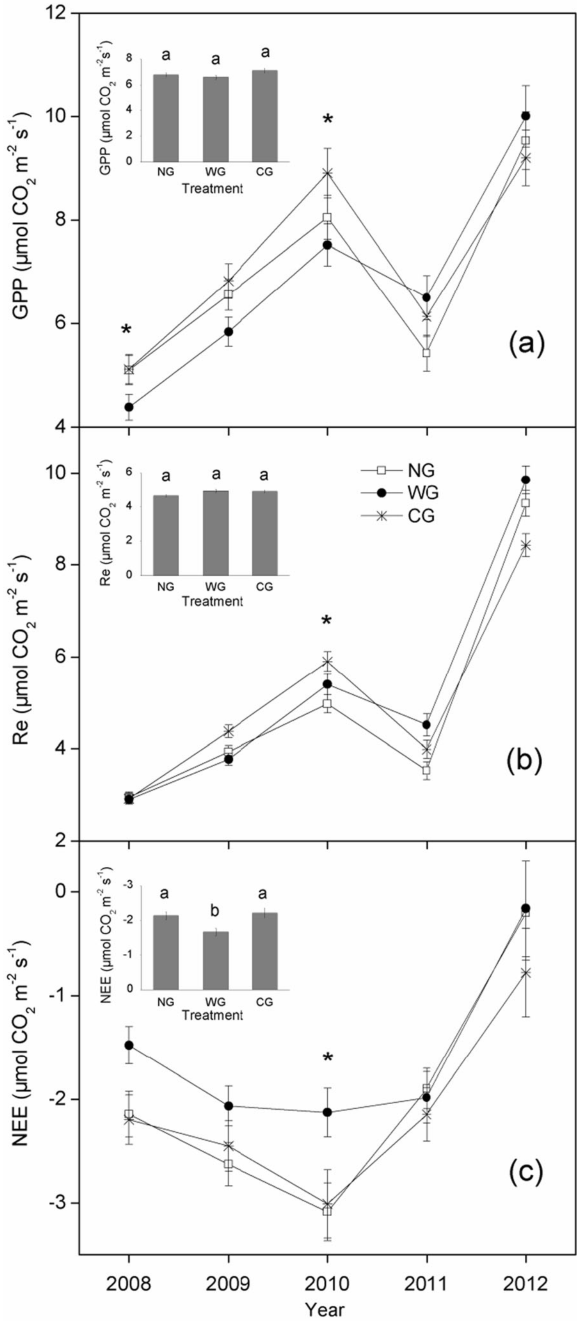

Fig. 6 Seasonal average values of gross primary production (GPP), ecosystem respiration (Re) and net ecosystem exchange of CO_2 (NEE) under different grazing treatments from 2008 to 2012. The panels are seasonal average GPP (**a**), Re (**b**) and NEE (**c**). *NG* no-grazing, *WG* warming season grazing, *CG* cold season grazing. *Asterisk* indicates significant difference between treatments at $p<0.05$ level; Mean±SE (n=3) are shown in the figure. *Different letters* indicate significant differences at $p<0.05$ level

explained about 4, 50 and 28 % of the variation in GPP, Re and NEE, respectively. However, their influences on seasonal average GPP, Re and NEE varied with grazing treatment (Table 4). Seasonal average GPP was primarily affected by soil temperature for all grazing treatments, which explained about 66 % of the variation in seasonal GPP for NG, WG and CG. Seasonal average NEE was mainly affected by root biomass for NG and WG, which explained about 46 and 40 % of the variation in seasonal NEE, respectively. However, for CG, seasonal NEE was mainly affected by soil temperature, soil moisture and ANPP, which explained 66 % of the variation in seasonal NEE. For WG and CG, seasonal average Re was mainly affected by soil temperature, which explained 60 and 67 % of the variation in seasonal Re, respectively.

Discussion

Our results indicated that the impacts of seasonal grazing on daily GPP, Re and NEE in the alpine meadow ecosystem varied with sampling date and year during the growing seasons over the 5-year period. Grazing season alone had no significant impacts on seasonal average GPP or Re, but significantly reduced seasonal average NEE over the 5-year experimental period (Fig. 6) because grazing impact was exacerbated by drought in July in 2010.

Studies of temperate and arctic ecosystems have found that ecosystem CO_2 uptake and respiration are functions of temperature (Oechel and Vourlitis 1994; Wohlfahrt et al. 2008) and plant standing biomass (Bellisario et al. 1998; Morris and Jensen 1998). By removing plant biomass, grazers may modify canopy structure and the energy balance of grasslands, with resulting feedbacks on soil temperature (ST) increase and soil water content decrease (Zhou et al. 2007), and ultimately on net CO_2 uptake (Owensby et al. 2006; Soussana et al. 2007; Wohlfahrt et al. 2008). Although WG significantly increased ANPP (Cui et al. 2014a; Fig. 2), we found that WG alone did not affect average seasonal Re, a finding that is similar to other reports (Rogiers et al. 2005; Lin et al. 2011; Peichl et al. 2012). We suspect that reduced autotrophic respiration following biomass removal by grazing may have been counterbalanced by elevated heterotrophic respiration due to an increase in soil temperature (Conant et al. 2011; Peichl et al. 2012). Although grazing exerted a short-term negative impact on daily NEE for WG (Fig. 7), regrown leaves after defoliation are often more

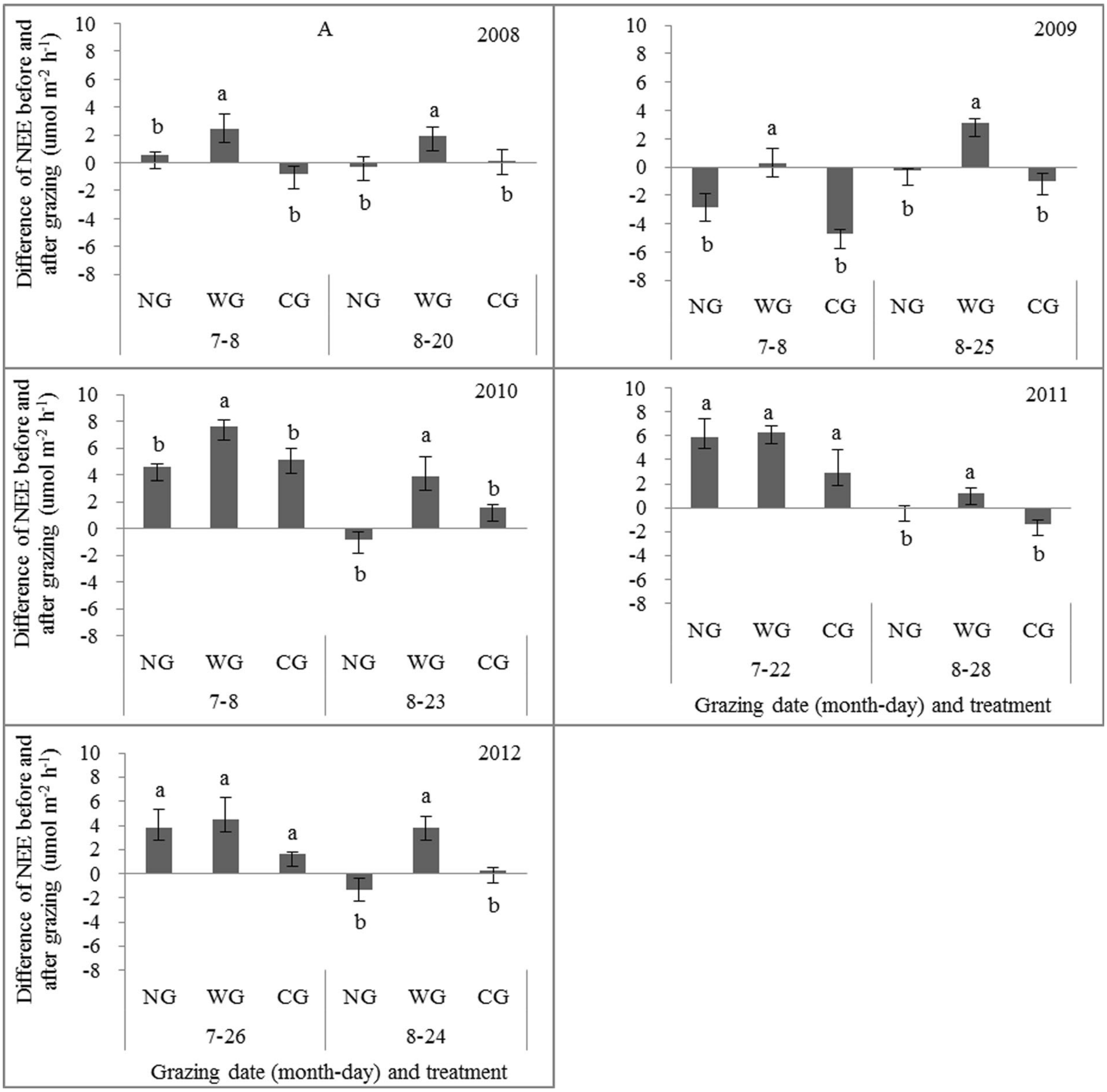

Fig. 7 Differences in daily average net ecosystem exchange of CO_2 (NEE) before and after grazing under different grazing treatments during the 5-year experimental period. *NG* no-grazing, *WG* warming season grazing, *CG* cold season grazing. Mean±SE (n= 3) are shown in the figure. *Different letters* indicate significant difference at $p<0.05$ level

physiologically active than the older leaves in ungrazed grasslands (Owensby et al. 2006), so photosynthetic capacity could recover within a short period of time, and the alpine grassland could recover its capacity for carbon sequestration after grazing. However, if drought occurs after defoliation, the regrowth of new leaves could be hampered, which could prolong the duration of low levels of carbon sequestration and even turn the grassland into a carbon source for a short time during the summer (Fig. 7). WG significantly decreased NEE which suggests that the positive effect of WG on Re is greater than WG's negative effect on GPP. However, CG significantly increased seasonal average NEE compared with WG. This could be attributed to two reasons. First, CG had no significant influence on ANPP (Cui et al. 2014a; Fig. 2), possibly because grazing occurred in winter, when grazing has a small effect on plant growth. On the other hand, animal trampling in winter

Table 3 Models among daily gross primary production (GPP), ecosystem respiration (Re) and net ecosystem exchange (NEE), soil temperature and soil moisture with smallest AIC and high goodness-of fit R^2 under three treatments

Treatment	Linear model	r^2	*F*-value	*p*-value
Pooled data	GPP=−0.636+1.567ST	0.31	346.55	<0.001
	Re=−0.050+0.532ST+0.022SM	0.20	94.08	<0.001
	NEE=1.223-1.023ST	0.29	308.56	<0.001
NG	GPP=−0.208+1.567ST	0.36	144.21	<0.001
	Re=0.575+0.582ST	0.22	73.203	<0.001
	NEE=−0.449-1.009ST+0.041SM	0.33	62.76	<0.001
WG	GPP=0.484+1.328ST	0.21	67.62	<0.001
	Re=−0.202+0.503ST+0.037SM	0.17	26.68	<0.001
	NEE=0.381-0.808ST	0.18	57.725	<0.001
CG	GPP=−2.381+1.835ST	0.39	165.51	<0.001
	Re=0.259+0.538ST	0.20	65.229	<0.001
	NEE=2.640-1.296ST	0.39	165.25	<0.001

SM soil moisture at 10 cm, *ST* soil temperature at 10 cm, *NG* no-grazing, *WG* warm season grazing, *CG* cold season grazing

causes less damage to the structure of frozen soils compared with grazing during the growing season. Grazing caused soil temperature to increase which further promotes soil respiration (Cui et al. 2014a). The reduction in aboveground biomass negatively affected carbon absorption after each grazing event (Fig. 7), especially when drought occurred after grazing in 2010 (Fig. 5), which is consistent with other research showing that abiotic and biotic stress combinations reduce plant growth to a much greater extent than either abiotic or biotic stresses applied individually (Suzuki et al. 2014). For the WG treatment, grazing occurred during the plants' vigorous growing stage, and grazing immediately reduced aboveground biomass and directly affected CO_2 assimilation (Haferkamp and Macneil 2004; Novick et al. 2004; Rogiers et al. 2005). The pattern of net carbon gain was interrupted by biomass removal, even turning the grassland from a sink into a short-term carbon source (Figs. 5 and 7), but with re-growth of biomass the grassland gradually became a net sink of

Table 4 Relationships between seasonal average gross primary production (GPP), ecosystem respiration (Re) and net ecosystem exchange (NEE) and seasonal average soil temperature and moisture at 10 cm soil depth, above ground net primary productivity (ANPP) and root biomass at 0–40 cm (RB) with smallest AIC and high goodness-of-fit R^2

Treatment	Linear model	r^2	*F*-value	*p*-value
Pooled data	GPP=29.380-2.127ST+0.0001RB	0.66	41.39	<0.001
	Re=22.496-0.001RB-1.683ST-0.030SM	0.64	24.50	<0.001
	NEE=0.637+0.0001RB-0.019SM	0.41	14.29	<0.001
NG	GPP=27.883-1.906ST-0.001RB	0.74	17.30	<0.001
	Re=12.011-0.002RB-0.042SM	0.81	25.07	<0.001
	NEE=4.031+0.0001RB-0.046SM-0.006ANPP	0.83	17.93	<0.001
WG	GPP=32.369-2.301ST+0.0001RB	0.81	25.677	<0.001
	Re=24.309-1.818ST-0.001RB	0.83	29.08	<0.001
	NEE=0.605+0.0001RB	0.40	8.761	0.011
CG	GPP=35.241-2.978ST	0.85	76.01	<0.001
	Re=29.046-2.547ST+0.0001RB-0.029SM	0.87	23.67	<0.001
	NEE=11.247-1.248ST-0.030SM-0.005ANPP	0.66	7.06	0.006

SM seasonal average soil moisture at 10 cm, *ST* seasonal average soil temperature at 10 cm, *NG* no-grazing, *WG* warm season grazing, *CG* cold season grazing

CO_2 after grazing. Thus, because grazing reduces aboveground biomass, grazing should be considered to be a main factor affecting average seasonal NEE. Interseasonal variation suggests that the timing of rain events and temperature change could also have strong impacts on NEE.

Consistent with other studies (Knapp and Seastedt 1986; Polley et al. 2008; Ford et al. 2012), we found that grazing increased soil temperature (Fig. 1), which could explain about 39, 20 and 39 % of the variation in daily GPP, Re, and NEE for CG in this study. Soil temperature rather than soil moisture was the main factor affecting Re in our study, probably because daily Re resulted from soil respiration, which was mainly affected by soil temperature (Cui et al. 2014a). Similarly, we found that there were positive correlations between daily NEE and soil temperature, which is consistent with previous reports in the region (Saito et al. 2009), indicating that soil temperature could also be a main factor controlling daily average NEE in alpine meadows.

In the alpine meadows on the Tibetan Plateau, greater ecosystem CO_2 uptake (GPP) relative to CO_2 release (Re) led to a net CO_2 sink (negative NEE values) for all grazing treatments during the growing seasons in all 5 years of the study (Fig. 6), and grazing season alone did not have a significant effect on average GPP and Re during the 5 years (Fig. 6). Generally, net carbon gain was highest during peak biomass, but large interannual differences related to interannual variability in precipitation have been found in other long-term studies (e.g., Flanagan et al. 2002; Hunt et al. 2004). Temperature and plant biomass have significant influences on interannual variation in CO_2 exchange in alpine meadows (Kato et al. 2006; Wohlfahrt et al. 2008). Comparing grazed with non-grazed plots, although grazing exert a short-term negative impact on daily NEE, the impact of grazing on NEE was different in different years. For example, after grazing in 2010, excessive drought (Table 1) hampered plant regeneration after grazing, which further decreased the ability of plants to absorb CO_2. Thus, the negative effects of WG on NEE were exacerbated due to drought that occurred after grazing. The large seasonal variations in CO_2 fluxes (Figs. 3, 4, and 5) indicate that the alpine meadow is sensitive to climate change. Our results suggest that, regardless of grazing season, under moderate intensities grazing would have no significant impact on NEE under normal rainfall conditions, but moderate grazing followed by drought could reduce NEE during the growing season. An increase in future drought events could turn temperate ecosystems into carbon sources, contributing to positive carbon-climate feedbacks already anticipated in the tropics and at high latitudes (Ciais et al. 2005). However, the impact of WG on carbon sequestration could decrease because warming with greater rainfall is predicted in the future in the region, which will improve plant production (Wang et al. 2012). Thus, both moderate WG and CG are potentially suited to balancing food security and animal product production with the maintenance of other ecosystem services (i.e. plant production and carbon sequestration) in the future.

Conclusion

Compared with NG and CG, WG only significantly decreased average seasonal NEE due to drought after grazing in 2010. There were no significant differences in GPP, Re and NEE between NG and CG in the alpine meadow during the 5 growing seasons studied. Root biomass was the main factor affecting seasonal NEE in the alpine region. However, caution should be taken in extrapolating our results from this short-term (i.e. 5-year) experiment to the long-term or to larger scales, because we did not investigate the effects of seasonal grazing during winter in the region.

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Conflict of interest The authors declare that they have no conflict of interest.

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