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Experimental Warming Increases Seasonal Methane Uptake in an Alpine Meadow on the Tibetan Plateau

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Abstract

Increased understanding of the response of soil methane (CH_4) uptake in alpine meadow ecosystems to warming and grazing could reduce uncertainty in estimates of the soil-atmospheric CH_4 budget. To determine the effects of warming and grazing on soil CH_4 uptake at different timescales (that is, daily, monthly, seasonal, and annual), we conducted a controlled warming and grazing experiment [that is, no warming with no grazing (NWNG), no warming with grazing (NWG), warming with no grazing (WNG), and warming with grazing (WG)] in an alpine meadow on the Tibetan plateau from 2006 to 2009. Soil CH_4 uptake was mainly affected by warming and sample date and their interaction. Warming treatment regardless of grazing significantly increased seasonal average CH_4 uptake by 31-39% during the growing season (from May to September) and by 162% during the non-growing season (from October to April next year) in 2007–2008, whereas only WNG increased seasonal average CH_4 uptake by 87–138% compared with NWNG during the non-growing seasons in 2006–2007 and 2008–2009. Warming in WNG and WG increased annual CH_4 uptake by 50–87% compared with NWNG or NWG. Moreover, warming regardless of grazing and warming with grazing (compared with NWNG) significantly increased the contribution to annual uptake of CH_4 uptake during the non-growing season in 2007–2008 and 2008–2009. However, moderate grazing did not significantly influence soil CH_4 uptake, although grazing with warming decreased CH_4 uptake by 43% during the growing season in 2006. Soil moisture explained 16–25% of the CH_4 variation during the growing season, but there was no significant relationship between soil CH_4 uptake and soil moisture during the non-growing season. Our results suggest that more attention should be paid to the stimulating effect of warming on soil CH_4 uptake during the non-growing season due to its greater response to warming and different stimulating mechanisms compared to responses during the growing season in the alpine meadow.

Key words: warming; grazing; CH_4 uptake; alpine meadow; Tibetan plateau; climate change.

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Author contributions X.L. conducted field experiments, analyzed data, and wrote the manuscript. S.W., Z.X., and H.N. conceived of or designed the study and contributed to writing. Y.H., C.L., and Z.Z. conducted field experiments.

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Introduction

Methane (CH_4) is the second most important anthropogenic greenhouse gas, contributing roughly 20% to observed global warming, and CH_4 has a global warming potential about 25 times greater than that of CO_2 over 100 years (IPCC 2007). Its concentration is increasing at a rate of 1% per year and the global budget of methane sources and sinks is currently out of balance (Dlugokencky and others 2011). Atmospheric methane consumption by upland soils is regarded as an important global sink for atmospheric CH_4 through the activity of aerobic CH_4-oxidizing bacteria, which consume about 30 Tg of methane from the atmosphere annually, consuming about 10% of the atmospheric CH_4 (Mosier and others 1991; IPCC 2007). Although methane uptake by soils is small compared with consumption in the atmosphere (470 Tg y^{-1}) and total CH_4 emission (525 Tg y^{-1}) (Le Mer and Roger 2001), it is an important flux in the global budget of atmospheric methane and any change in CH_4 uptake by upland soils may modify the rate of increase of atmospheric CH_4 (King 1997; Dutaur and Verchot 2007; Liu and others 2007). The magnitude of atmospheric CH_4 uptake depends on soil physical properties (texture and structure), soil environmental conditions (soil moisture and temperature), soil gas diffusion rate, soil chemical features (pH and mineral-N), and soil biological characteristics (soil microbial activity and population size) (King 1997; Verchot and others 2000; Price and others 2004; Castaldi and Fierro 2005; Livesley and others 2011). These attributes would be expected to respond to environmental change and land-use change (King 1997; van den Pol-van and others 1998; Dutaur and Verchot 2007).

Grassland soils are thought to be the second largest sink for atmospheric CH_4 after forest soils (Potter and others 1996). As the largest grassland unit on the Eurasian continent, the Tibetan plateau is mostly situated at more than 3,500 m meters above sea level (m.a.s.l.), covering an area of approximately 2.5 million km^2 (Zheng and others 2000) with about half of its area covered by alpine meadow (Xie and others 2003). Therefore, methane oxidation in alpine meadow soils would be one important flux affecting the methane budget of the Tibetan plateau. However, the Tibetan plateau is experiencing climatic warming and the region is predicted to experience ''much greater than average'' increases in surface temperatures in the future (IPCC 2007). Moreover, the Tibetan plateau is one of the most sensitive areas to global climate change (Liu and Chen 2000). Grazing has been practiced on the Tibetan plateau for about 4,000 years and continues to be one of the most prevalent land uses in alpine meadow areas, where open grazing by more than 12 million domestic yaks and 30 million sheep and goats is practiced, and pastoralists have probably been raising stock on the Tibetan steppe for about 4,000 years (Sheehy and others 2006). However, the responses of atmospheric methane consumption to elevated mean temperature and grazing is uncertain in this region.

Warming stimulates soil CH_4 uptake in some grasslands and forests (Peterjohn and others 1994; Sjögersten and Wookey 2002; Hart 2006), shows no effect on CH_4 uptake in other forests (Christensen and others 1997; Rustad and Fernandez 1998) and even decreases CH_4 uptake in semiarid grasslands (Blankinship and others 2010a, b; Dijkstra and others 2011). This inconsistency may be attributed to different responses of soil CH_4 uptake in different regions to changes in soil water status, soil gas diffusion, soil/rhizosphere environment, and methanotroph community induced by warming (King 1997; Sjögersten and Wookey 2002; Zheng and others 2012). Grazing can lead very quickly to changes in nutrient pools and fluxes, vegetation cover, plant community composition, and changes in soil temperature and soil gas diffusion (Saggar and others 2007; Lin and others 2011; Wang and others 2012), which would probably affect soil CH_4 uptake. In some studies, grazing reduced atmospheric CH_4 uptake (Liu and others 2007; Saggar and others 2007), whereas grazing showed no significant impact on CH_4 uptake in other studies in temperate semi-arid steppes (Zhou and others 2008; Chen and others 2011). Therefore, the responses of CH_4 uptake to warming and grazing are significantly different among different ecosystems characterized by different vegetation types and climate conditions (Rustad and Fernandez 1998; Hart 2006; Saggar and others 2007; Chen and others 2011; Dijkstra and others 2011). This underscores the need to understand the relative importance of specific factors to soilCH_4 uptake at different spatial and temporal scales.

Accurate prediction of the effect of future climate change on the CH_4 budget of the Tibetan plateau strongly depends on understanding of the response of terrestrial ecosystems to grazing under warming conditions. In some humid and semi-humid soils, CH_4 uptake has been found to be much more sensitive to changes in soil water status than to changes in temperature (King 1997; Dijkstra and others 2011). Therefore, we hypothesized that soil water status was the main driving factor for methane uptake and that a warming treatment would promote CH_4 uptake in this semi-humid meadow because of lower soil moisture caused by warming which would enhance the diffusivity of CH_4 into the soil during the growing season and increase methanotrophic activity in winter (that is, the dry season). We also hypothesized that grazing would decrease CH_4 uptake because of limited gas diffusivity caused by trampling, ammonium toxicity, and lower methanotrophic activity due to reduced carbon input from plant to soil microbes. However, there is much uncertainty regarding how soil CH_4 uptake in the alpine meadow ecosystem will respond to warming and grazing and their interactive effects at different timescales. To better understand the response of alpine meadow CH_4 uptake to warming, grazing and their interaction, we conducted a study in an alpine meadow on the Tibetan plateau with controlled asymmetrical warming and moderate simulated grazing on the field scale (Kimball and others 2008; Lin and others 2011). The warming manipulations were intended to mimic as closely as possible anticipated major environmental changes at the site in the year 2075. Most field studies have been conducted to examine the effects of warming on CH_4 fluxes during the growing season in forests and grasslands (Christensen and others 1997; Rustad and Fernandez 1998; Hart 2006; Blankinship and others 2010a, b; Dijkstra and others 2011). There is still much uncertainty about how CH_4 uptake in meadows is affected by warming in winter. Therefore, we examined the effects of warming and grazing and their interaction on CH_4 fluxes at different timescales (that is, daily, monthly, seasonally (growing season and non-growing season), and inter-annually). By examining the effects of warming and grazing on soil water and the relationship between soil CH_4 uptake and environmental variables (that is, soil temperature and soil moisture), we aimed to gain insight into how changes in environmental variables caused by warming influence the response of CH_4 uptake to climate warming.

Materials and Methods

Study Site

The experiment was conducted in an alpine meadow located in the Haibei Alpine Meadow Ecosystem Research Station, Northwest Plateau Institute of Biology, Chinese Academy of Sciences (37°37′N, 101°120′E; 3,250 m.a.s.l.). The local climate is characterized by strong solar radiation, long cold winters, and short cool summers, with annual mean air temperature of −1.7°C and annual mean precipitation of 580 mm. Mean temperature and total rainfall during the growing season (from May to September) in 2006, 2007, and 2008 were 8.4, 8.5, and 8.1°C, and 449, 398, and 339 mm, respectively (Figure 1). The plant community at the experimental site is dominated by *Kobresia humilis*, *Festuca ovina*, *Elymus nutans*, *Poa* spp., *Carex* spp., *Scripus distigmaticus*, *Gentiana straminea*, *Gentiana farreri*, *Leontop odiumnanum*, and *Potentilla nivea*. The soil is a clay loam and is classified as Mat Crygelic Cambisols (Institute of Soil Science of the Chinese Academy of Sciences 2001) corresponding to Gelic Cambisol (WRB 1998). The study site is flat with initial soil properties measured before the

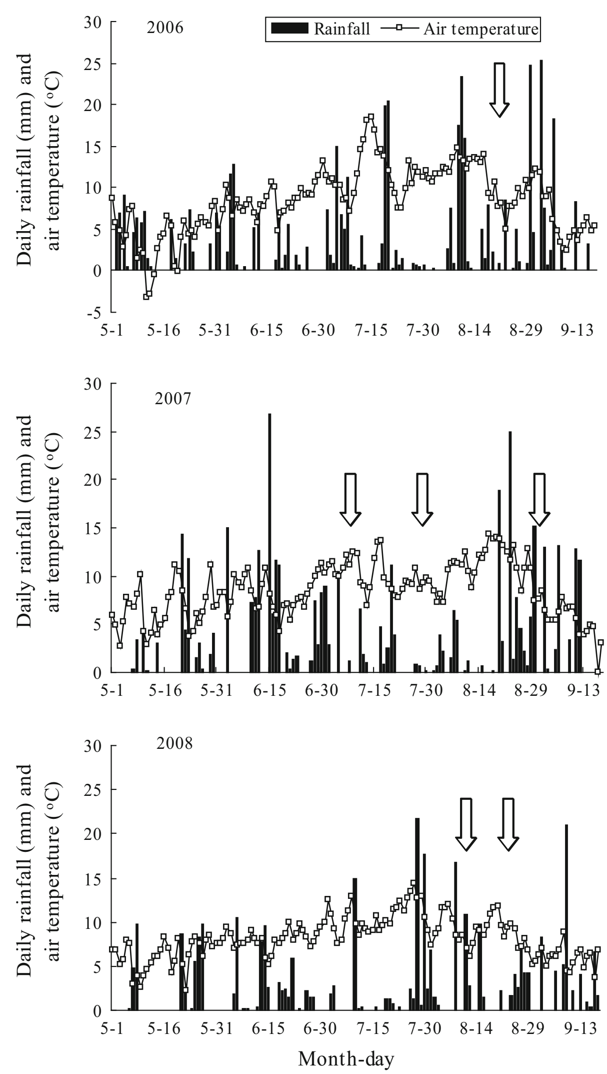

Figure 1. Distributions of rainfall, air temperature, and grazing events during the growing seasons from 2006 to 2008. *Arrow* indicates grazing events in the figures.

Table 1. Selected Soil Characteristics in Each Plot Before the Warming Experiment Manipulation in Early 2006

Plot	Treatment	Total C ($g\ kg^{-1}$)	Total N ($g\ kg^{-1}$)	Bulk density ($g\ cm^{-3}$)	Soil porosity (%)
1	WNG	74.5	5.9	0.95	64.1
2	NWG	71.0	5.1	1.15	56.7
3	NWNG	73.2	5.7	1.14	57.0
4	WG	74.9	5.3	1.03	61.3
5	NWG	72.5	5.1	1.14	57.0
6	WNG	73.6	5.8	1.10	58.4
7	WG	73.2	5.3	1.04	60.7
8	NWNG	74.4	5.6	0.97	63.6
9	WG	74.4	5.3	1.04	60.7
10	NWNG	74.3	5.9	1.13	57.4
11	WNG	74.5	6.0	1.14	57.0
12	NWG	71.6	5.2	1.17	55.7
13	NWNG	72.1	5.7	1.12	57.7
14	WG	74.8	5.2	1.15	56.6
15	NWG	70.1	5.1	1.14	57.0
16	WNG	73.5	5.8	1.10	58.6

experimental manipulation in 2006 as shown in Table 1. Basic soil properties at 10 soil depths were: soil total carbon 73.3 $g\ kg^{-1}$, total nitrogen 5.5 $g\ kg^{-1}$, bulk density 1.09 $g\ cm^{-3}$, and soil porosity 58.7%.

Controlled Warming-Grazing Experiment

We developed sixteen plots of 3 m diameter assigned to 4 treatments (no warming with no grazing (NWNG), no warming with grazing (NWG), warming with no grazing (WNG), and warming with grazing (WG)) with four replicates in a complete randomized block distribution. The design of the controlled warming with grazing experiment heated by a free-air temperature enhancement system (FATE) has been described by Kimball and others (2008) in detail. In brief, in May 2006 eight hexagonal arrays of Mor FTE (1,000 W, 240 V) infrared heaters were deployed over vegetation canopy with eight dummy arrays over reference plots. The heaters were controlled by a proportional-integral derivative output (PID) control system. The canopy temperature was sensed using an infrared thermometer (Model IRT-P5, Apogee Instruments, Logan, UT, USA). The set-point difference of vegetation canopy temperatures between heated and corresponding reference plots was 1.2°C during daytime and 1.7°C at night during the growing season (from May to September). Because some infrared thermometers could not work during the non-growing season (from October to April next year), the power outputs of the heaters were manually set at 1,500 W per plot. In the grazing plots, one adult Tibetan sheep was fenced on 17 August 2006 for approximately 2 h. Similarly, two adult Tibetan sheep were fenced for approximately 1 h in the grazing plots on 12 July, 3 August, and 12 September in 2007, and 8 July and 20 August in 2008. The sheep were removed from the grazing plots when the canopy height was reduced to approximately half of the initial height. The annual forage utilization rates during the growing seasons were 32–60.8% for the grazing treatments in 2006, 2007, and 2008, roughly corresponding to a moderate-stocking rate (Wang and others 2012).

At 50 cm inside the edge of each plot (nearby the chambers for measuring CH_4 flux), soil temperatures at depths of 5, 10, and 20 cm and soil moisture at depths of 10, 20, 30, and 40 cm were automatically measured by type-K thermocouples (Campbell Scientific, Logan, Utah, USA) and manually measured through a tube in the ground down to 40 cm depth using a frequency domain reflectometer (FDR; Model Diviner-2000, Sentek Pty Ltd., Australia). The average soil temperatures were stored in a CR1000 datalogger every 1 min, and 15 min. The soil moisture was expressed as a volume percentage (%) and measured at 8:00, 14:00, and 20:00 every day. During the freezing period, the data measured by FDR were the unfrozen soil water contents.

Measurement of CH_4 Fluxes

During the growing season, CH_4 fluxes were measured every 3-5 days in 2006 and every 7–10 days (CH_4 fluxes were not measured on heavy rain days and were measured when heavy rain had stopped, which showed little impact on the estimation of the cumulative fluxes) in 2007 and 2008 using opaque, static, manual stainless steel chambers. During the non-growing season, gas samples were taken at approximately one-month intervals, with 6 sampling occasions from October 2006 to April 2007, 7 sampling occasions from October 2007 to April 2008, and 7 occasions from October 2008 to April 2009. The dimensions (40 cm × 40 cm × 40 cm) and architecture of the chambers were the same as those reported by Lin and others (2009). There was one chamber at 50 cm inside the edge of each plot, that is, four chambers for every treatment. Moreover, these plots were randomly distributed in the field for each treatment, so replicates could reduce the systematic errors as far as possible. Based on investigation of diurnal gas flux variation (data not shown), the fluxes of CH_4 between 9:00 and 11:00 a.m. could represent one-day average flux. Gas samples (100 ml) were taken using plastic syringes at 0, 10, 20, and 30 min after chamber closure. Methane concentrations of gas samples in plastic syringes were analyzed with gas chromatography equipped with a flame ionization detector (HP Series 4890D, Hewlett Packard, USA) within 24 h following gas sampling. The gas chromatography configurations for analyzing concentrations of CH_4 were the same as those described by Song and others (2003). Flux rates were calculated from the linear ($r^2 > 0.9$) decrease in CH_4 concentrations over 30 min in the chamber headspace. The growing seasonal, non-growing seasonal, and annual (from May to April next year) cumulative fluxes were estimated by linear interpolation and average fluxes were calculated from the cumulative fluxes divided by the number of days in each period.

Data Analysis

General linear model-repeated measures define factors (SPSS 13.0, SPSS Inc., Chicago, IL, USA) was applied with warming and grazing as the main (between-subject) factors and with sampling date as the within-subject factor, including their interactions, to test the effects of the main factors on CH_4 flux (repeated measures) by sampling date. The statistical power of the test was calculated by choosing ''Option-Observed power'' of the general linear model-repeated measures in SPSS. When testing the significance of the main factors or their interactions, the result had a statistical power larger than 0.80 (Table 2) meaning that the test is statistically powerful and is highly likely to detect an effect if it actually exists (Krebs 1999). Multi-comparison of least standard difference (LSD) was conducted for all measured variables within each sampling date using a two-way ANOVA (general linear model univariate in SPSS) with CH_4 as the dependent variable and warming and grazing as the fixed factors. Because the non-grazing treatment was applied on all plots before 16 August 2006, the data during the growing season before and after 16 August 2006 were analyzed separately. The influences of warming and grazing on monthly average, seasonal average, seasonal cumulative, and yearly cumulative CH_4 fluxes were investigated using a two-way ANOVA. Simple correlation and stepwise regression analysis were performed to test the possible dependency of CH_4 fluxes on soil water-filled pore space (WFPS) and soil temperature at a depth of 10 cm. All significances mentioned in the text were at the 0.05 level, unless otherwise noted.

Results

Soil Moisture

The effects of warming and grazing treatments on soil WFPS at 10 cm depth varied with months and years and no interaction between warming and grazing was found. Generally, warming tended to decrease WFPS during the soil-thawing period (from May to November) (Figure 2). For example, warming significantly decreased the monthly average WFPS by 19% after grazing in August 2006. Especially in the relatively dry year of 2008, warming significantly decreased the monthly average WFPS by 16–21% from July to November. Contrary to the results during the thawing period, warming tended to increase WFPS during the soil-freezing period (from December to April next year). For example, warming tended to increase the monthly average WFPS by 14% in December 2007 ($P = 0.055$). Warming significantly increased the monthly average WFPS by 21% in January 2008, by 63% in March 2008, and by 21% in April 2008. Grazing showed no significant influence on soil WFPS in most months, but grazing significantly reduced WFPS by 16% in June 2008. The soil moistures at 20, 30, and 40 depth showed the same trends as that at 10 cm depth under different treatments (data not shown).

Effects of Warming and Grazing on CH_4 Uptake

Before grazing in the first year (2006), warming treatment increased CH_4 uptake only in 3 out of 20 sampling dates, marginally increasing CH_4 uptake

Table 2. Summary of the Analysis of Variance on CH_4 Fluxes From Repeated-Measure ANOVAs Using Year and Sampling Day as Repeated-Measures Conducted Separately for the Growing and the Non-growing Season from 2006 to 2009

Year	Period	Model	*F*	*P*	Power
2006	Before grazing	Warming (W)	4.51	0.052	0.507
		Date (D)	22.64	<0.001	>0.999
		W × D	1.34	0.156	0.595
	After grazing	W	10.44	0.007	0.842
		Grazing (G)	14.29	0.003	0.934
		W × G	11.10	0.006	0.864
		D	15.43	0.000	>0.999
		W × D	2.59	0.018	0.866
		G × D	2.03	0.061	0.755
		W × G × D	1.15	0.341	0.466
2007–2008	During growing season	W	17.71	0.002	0.966
		G	0.07	0.798	0.057
		W × G	0.003	0.955	0.050
		Year (Y)	66.89	<0.001	>0.999
		W × Y	2.35	0.156	0.284
		G × Y	1.28	0.284	0.177
		W × G × Y	2.06	0.181	0.256
		D	13.98	0.000	>0.999
		W × D	0.94	0.535	0.658
		G × D	1.27	0.211	0.826
		W × G × D	0.88	0.606	0.621
		Y × D	5.19	0.000	>0.999
		W × Y × D	1.02	0.444	0.705
		G × Y × D	0.47	0.968	0.324
		W × G × Y × D	0.63	0.876	0.442
2006–2009	During non-growing season	W	21.05	0.001	0.987
		G	0.05	0.835	0.054
		W × G	2.03	0.180	0.259
		Year (Y)	4.66	0.034	0.815
		W × Y	1.39	0.290	0.261
		G × Y	0.03	0.974	0.054
		W × G × Y	1.17	0.348	0.274
		D	3.38	0.062	0.889
		W × D	3.13	0.074	0.736
		G × D	0.83	0.560	0.349
		W × G × D	0.85	0.550	0.306
		Y × D	2.85	0.211	0.991
		W × Y × D	0.32	0.928	0.644
		G × Y × D	0.29	0.941	0.310
		W × G × Y × D	0.47	0.838	0.237

Power is the statistical power.

($P = 0.052$) by 17.0% compared with the no warming treatment (Table 2; Figure 3A). After grazing in 2006, the warming and grazing treatment significantly impacted on soil CH_4 uptake during the growing season. Their effects varied with sampling date, and there were interactions between warming, grazing, and sampling date during this period. For example, warming significantly increased CH_4 uptake in 5 out of 8 sampling dates (Figure 3B), whereas grazing significantly decreased CH_4 uptake in 4 out of 8 sampling dates. However, during this period the stimulative effect of warming on CH_4 uptake was only apparent when WNG was compared with NWNG, which increased average CH_4 uptake by 67%, and the inhibitive effect of grazing was only apparent in WG compared with WNG, which decreased average CH_4 uptake by 43%.

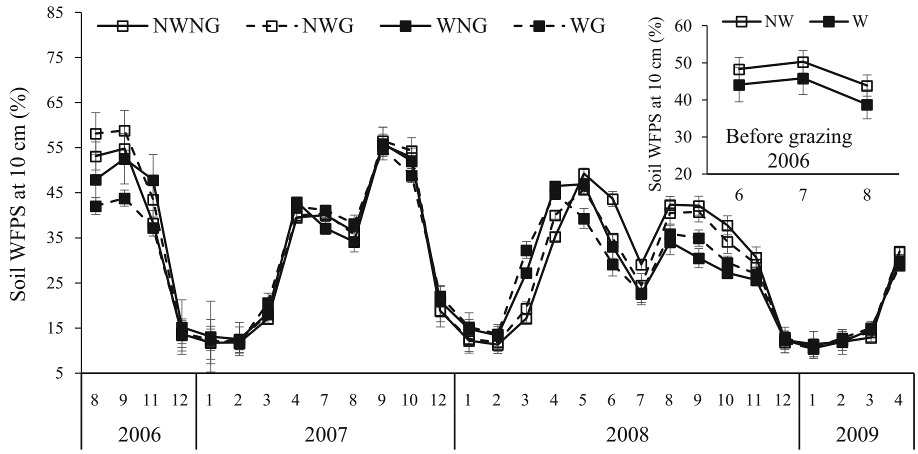

Figure 2. Dynamics of soil WFPS at 10 cm depth under different treatments from June 2006 to April 2009. *NWNG* no warming with no grazing, *NWG* no warming with grazing, *WNG* warming with no grazing, *WG* warming with grazing. The *panel* shows the dynamics of soil WFPS at 10 cm depth before grazing in 2006. *Bars* indicate standard errors.

During the growing season in 2007 and 2008, warming treatment showed significant impacts on daily CH_4 uptake, whereas grazing treatment did not significantly influence CH_4 fluxes. There was no interaction between warming and grazing during this period (Table 2). There were 4 occasions in 2007 and 8 occasions in 2008 when warming was observed to significantly increase CH_4 uptake (Figure 4A, B). Regardless of grazing, warming significantly increased seasonal average CH_4 uptake by 31 and 39.0% during the growing season in 2007 and 2008, respectively. However, grazing did not significantly affect the seasonal average CH_4 flux during the growing season in 2007 and 2008. During the non-growing season in 2006–2007, 2007–2008, and 2008–2009, only warming significantly impacted CH_4 uptake and no interaction between warming and grazing was found. Warming significantly increased CH_4 uptake on 1 occasion in 2006–2007, on 2 occasions in 2007–2008 and on 2 occasions in 2008–2009 during the non-growing season (Figure 5A, B, C). Warming (WNG + WG vs. NWNG +NWG) in the winter of 2007–2008 significantly increased the seasonal average CH_4 flux by 162%, whereas only warming in WNG compared with NWNG significantly increased the seasonal average CH_4 by 87 and 138% during the non-growing season in 2006–2007 and 2008–2009, respectively.

On a monthly scale, the peak of monthly average CH_4 uptake occurred in July for all treatments during the growing season in 2007 and 2008, whereas there were ''bursts'' of CH_4 uptake for WNG in December 2007, March 2008, and October 2008 during the non-growing season (Figure 6). The effects of warming and grazing treatments on soil CH_4 uptake varied with month. For example, warming in WNG significantly increased monthly average CH_4 uptake by 39% in June 2007 and 135% in September 2008 compared with NWNG. Compared with NWG, warming in WG significantly increased monthly average CH_4 uptake by 39% in July 2007, by 37% in June 2008, and by 66% in September 2008. During the non-growing season, WNG sharply increased the monthly average CH_4 flux by 22.3, 10.5, 1.6, and 28.0 times in December 2007, March 2008, October 2008, and March 2009 compared with NWNG, respectively. Warming (WNG + WG vs. NWNG +NWG) also tended to increase the monthly average CH_4 uptake in the other months from 2007 and 2008. In contrast, only grazing in NWG significantly decreased monthly average CH_4 uptake by 27.0% in July 2007 compared with NWNG and no significant impacts of grazing on CH_4 uptake were found in other months.

Annual cumulative CH_4 fluxes were -165 mg m^{-2} for NWNG, -169 mg m^{-2} for NWG, -309 mg m^{-2} for WNG, and -279 mg m^{-2} for WG in 2007–2008, respectively (Figure 6A, B). In 2008–2009, annual cumulative CH_4 fluxes were -221 mg m^{-2} for NWNG, -269 mg m^{-2} for NWG, -405 mg m^{-2} for WNG, and -354 mg m^{-2} for WG, respectively. Regardless of grazing, warming (WNG + WG vs. NWNG + NWG) significantly enhanced annual cumulative CH_4 uptake by 65–87% in 2007–2008, whereas compared with NWNG and NWG, WNG significantly increased by 50–83%, and WG significantly increased by 60% compared with NWNG in 2008–2009. Grazing with and without warming

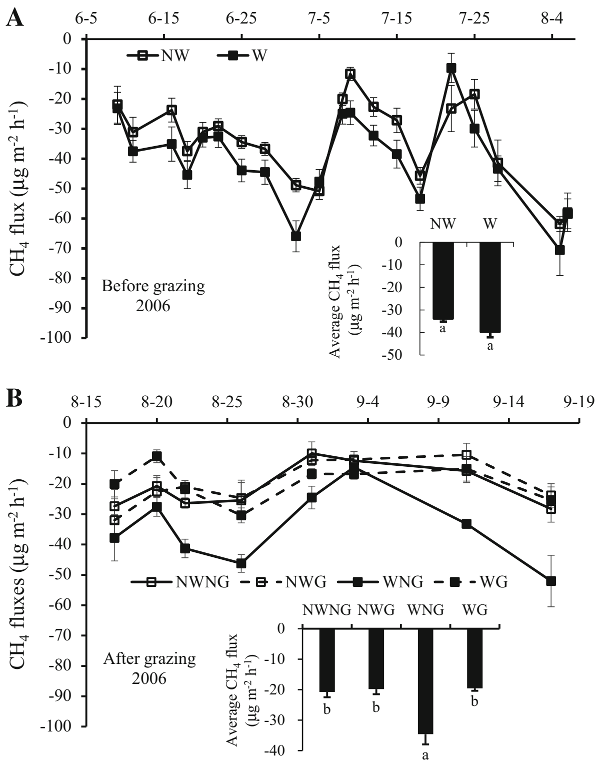

Figure 3. Effects of warming and grazing on CH_4 fluxes before and after grazing during the growing season in 2006. *W* warming, *NW* no warming. *Panels* inside the figures show average values of CH_4 fluxes under different treatments. *Bars* indicate standard errors. *Different letters* indicate significant difference at 0.05 level.

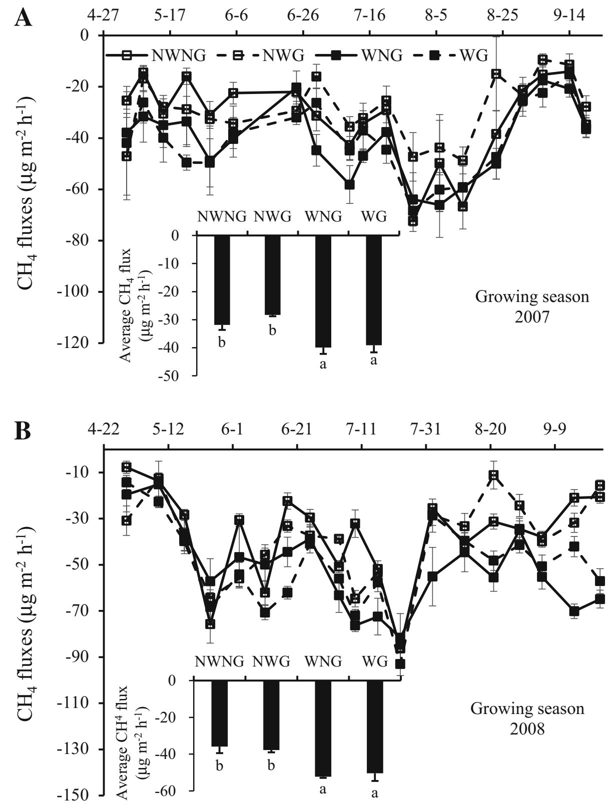

Figure 4. Effects of warming and grazing on CH_4 fluxes during the growing season in 2007 and 2008. *Panels* inside the figures show average values of CH_4 fluxes under different treatments. *Bars* indicate standard errors. *Different letters* indicate significant difference at 0.05 level.

showed no significant influence on annual cumulative CH_4 uptake in 2007–2008 and 2008–2009. The contributions of CH_4 uptake during the non-growing seasons to total annual CH_4 uptake were 29% for NWNG, 32% for NWG, 52% for WNG, and 48% for WG in 2007–2008, respectively. In 2008–2009, the contributions of CH_4 uptake during the non-growing seasons to total annual CH_4 uptake were 27% for NWNG, 46% for NWG, 52% for WNG, and 47% for WG, respectively. Warming treatment (WNG + WG vs. NWNG + NWG) significantly increased the contribution of CH_4 uptake during the non-growing season to annual CH_4 uptake in 2007–2008, and warming treatment also tended to increase it in 2008–2009 ($P = 0.150$), whereas grazing did not affect it.

Factors Affecting Temporal Variation of CH_4 Uptake

Seasonal variation in CH_4 uptake showed no significant relationship with soil temperature in the alpine meadow. Methane fluxes were positively correlated with soil WFPS at 10 cm depth ($P < 0.001$) during the growing season, which could explain 16–25% of the seasonal CH_4 variation for all treatments over the 3-year period (Figure 7). These results show that seasonal variation in CH_4 uptake during the growing season was mainly controlled by soil moisture rather than soil temperature in the alpine meadow, and that the amount of soil CH_4 uptake increased linearly with a decrease in soil moisture. There was no significant correlation between seasonal variation in CH_4 uptake and soil WFPS for all treatments during the non-growing season. When pooled data of all treatments were analyzed, soil WFPS and precipitation explained 67% ($P = 0.001$) and 46% ($P = 0.016$) of variation in CH_4 uptake during the growing season, respectively. For example, soil CH_4 uptake was the greatest during the growing season in 2008, probably because the experimental site experienced a relative drought in 2008 and had lower soil moisture, whereas the rainfall was normal in 2006 and 2007.

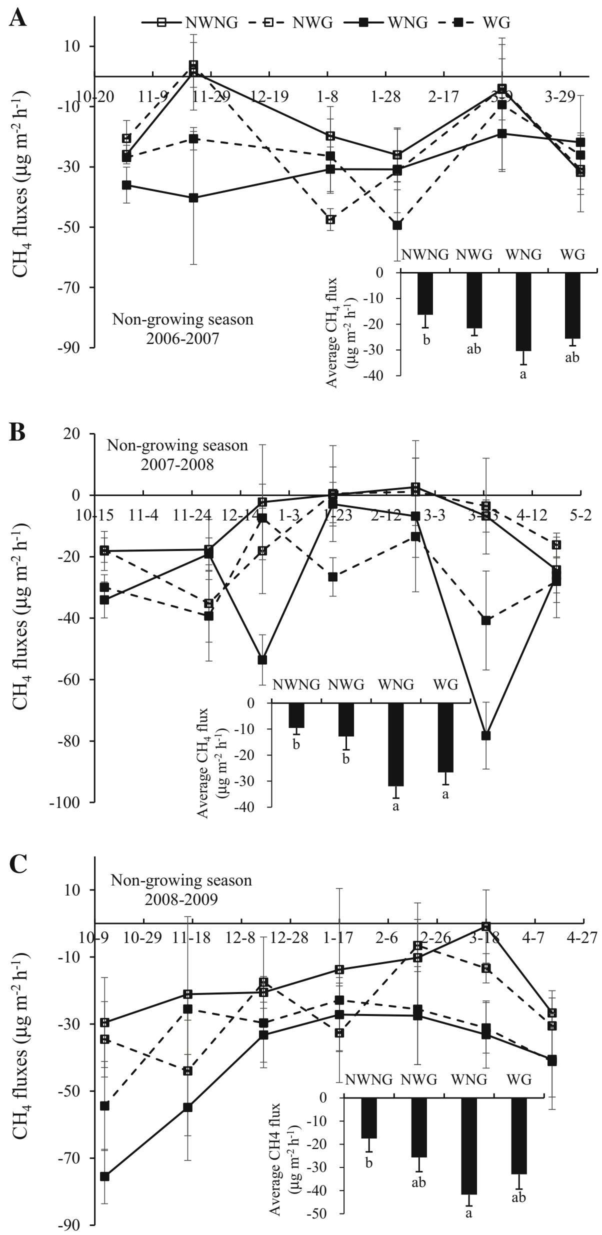

Figure 5. Effects of warming and grazing on CH_4 fluxes during the non-growing season in 2006–2007, 2007–2008, and 2008–2009. *Panels* inside the figures show average values of CH_4 fluxes under different treatments. *Bars* indicate standard errors. *Different letters* indicate significant difference at 0.05 level.

Discussion

Warming Effects

Similar to the results in other semi-humid ecosystems (Sjögersten and Wookey 2002; Hart 2006), warming treatment significantly increased soil CH_4 uptake by 50–87% in the semi-humid alpine meadow in our study. This result falls within the range of the positive effects reported by Sjögersten and Wookey (2002) and Hart (2006). However, the effects of soil warming on CH_4 uptake vary with climate types. For example, soil warming (increased by 2.4–2.5°C) stimulated soil CH_4 uptake by 0.6–2.1 times in some semiarid or semi-humid forests (Sjögersten and Wookey 2002; Hart 2006), whereas soil warming (increased by 1.5–5°C) had no effects on CH_4 uptake in some humid forests (Christensen and others 1997; Rustad and Fernandez 1998). On the other hand, soil warming (increased by 1.0–3.0°C) significantly decreased soil CH_4 uptake by 13–32% in some semiarid grasslands and forests (Blankinship and others 2010a; Dijkstra and others 2011) and in a grassland during the dry growing season (Blankinship and others 2010b).

Climate change influences CH_4 uptake fluxes directly through changes in the rates of methanogenesis and methanotrophy (King 1997). In a previous incubation experiment (soil sampled from our experiment site) (Zheng and others 2012), the stimulative effect of warming on CH_4 uptake was mostly due to its effects on the abundance of methanotrophs, whereas there was no change in methanotroph community composition and diversity. In general, methane consumption in humid and semi-humid soils appears to be diffusion-limited and much more sensitive to changes in soil water status than to changes in temperature (King 1997; Dijkstra and others 2011). A decrease in surface soil water content would likely decrease resistance to atmospheric CH_4 transport (that is, increase rates of diffusion), increase substrate (that is, CH_4) for methanotrophic organisms, and promote methanotrophic abundance (Torn and Harte 1996; Hart 2006; Dijkstra and others 2011; Zheng and others 2012). In our study, we also found negative correlations between soil WFPS and soil CH_4 uptake during the growing season. During the growing season, soils were relatively humid (monthly average WFPS varied from 29 to 70% for all treatments) and the warming treatment consistently reduced soil moisture in the upper 10 and 20 cm soil in the alpine meadow during the growing season (Luo and others 2009; Hu and others 2010). Therefore, the increase in CH_4 diffusion as a result of warming-induced soil drying would be one potential mechanism explaining the positive effects of warming on CH_4 uptake in the alpine meadow. However, CH_4 uptake would be limited by methanotroph activity when soils are dry, and a higher soil moisture would likely increase CH_4 uptake (Dijkstra and others 2011). Although we did not find a significant correlation between soil moisture and CH_4 uptake in the alpine

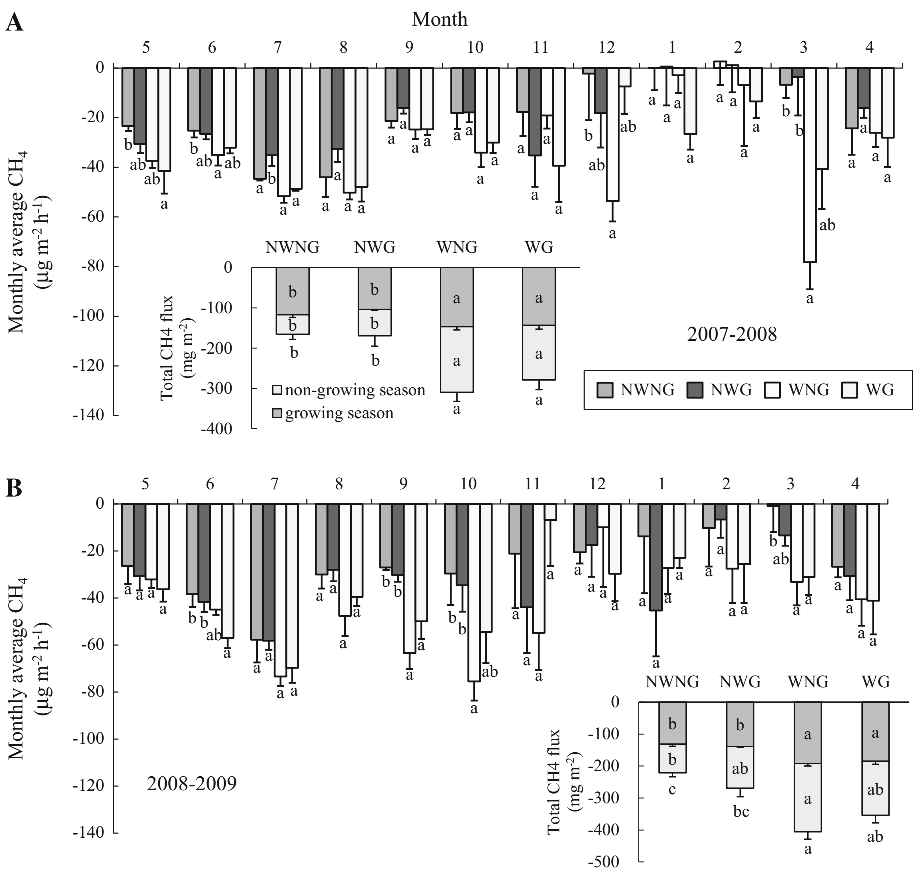

Figure 6. Effects of warming and grazing on monthly average, seasonal cumulative and annual cumulative CH_4 fluxes. *Bars* indicate standard errors. *Different letters* indicate significant difference at 0.05 level.

meadow during the drier non-growing season (WFPS was below 25%), probably due to the limited number of sampling occasions, warming plots showed greater soil moisture and more CH_4 uptake compared with non-warming plots in some months during the freezing period. We speculate that, compared with the non-warming plots, soil warming probably strengthens CH_4 uptake in alpine meadows with greater soil moisture in the warming plots in the relatively drier winter season. When soils are below soil optimum moisture for methane uptake, CH_4 uptake responses to soil moisture are more sensitive (Dijkstra and others 2011), which may explain why the increased percent of CH_4 uptake induced by warming during the non-growing season is greater than that during the growing season.

Changes in soil chemical properties (such as soil NH_4^+ and labile C) and vegetation process as a result of soil warming may also be responsible for the effects of soil warming on CH_4 uptake (Sjögersten and Wookey 2002; Dijkstra and others 2011). Some researchers have reported a positive relationship between net N mineralization and net nitrification and CH_4 consumption in forests (Peterjohn and others 1994; Hart 2006). They hypothesize that increased availability of NH_4^+ resulting from soil warming-induced increases in net N mineralization leads to larger autotrophic nitrifier populations that enhance the capacity of these soils to oxidize CH_4. After 3 years of treatment, warming significantly increased soil total nitrogen, microbial biomass carbon and nitrogen, and soil organic nitrogen at the 10–20 and 20–30 cm soil depths, but showed no significant impacts on net N mineralization in our study site (Rui and others 2011; Wang and others 2012). Regardless of those changes in the soil environment caused by warming, our results suggest that the soil moisture environment changed by warming could be the first factor that affect methanotroph abundance and the amount of soil CH_4 uptake in the Tibetan alpine meadow.

Grazing Effects

Atmospheric methane consumption is especially sensitive to anthropogenic disturbances that partially or fully modify soil features. Anthropogenic disturbances typically decrease CH_4 uptake (King 1997; Merino and others 2004). In some studies, grazing as the main anthropogenic disturbance to grasslands significantly reduced atmospheric CH_4 uptake (Liu and others 2007; Saggar and others

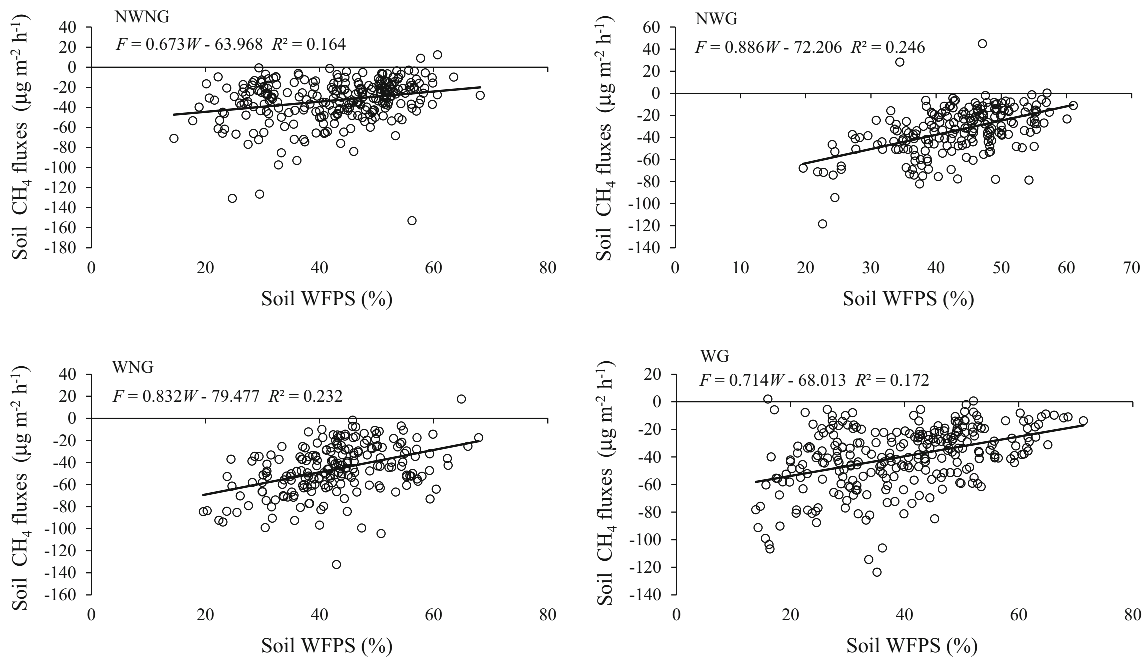

Figure 7. Linear regression models for the relationship between soil CH_4 fluxes (*F*) and soil WFPS (*W*) at 10 cm depth in different treatments during the growing season across 3 years. R^2 is the coefficient of determination.

2007). However, in accordance with other studies in temperate semi-arid steppes (Zhou and others 2008; Chen and others 2011), we found that the moderate grazing treatment in our study showed no significant impact on soil CH_4 oxidation, except during the growing season in 2006, which was the first year of our experiment. These conflicting responses of CH_4 uptake to grazing may be due to differences in grazing intensity.

Ammonium toxicity is generally thought to restrict soil CH_4 consumption (Nesbit and Breitenbeck 1992; King 1997; Saggar and others 2007). However, in some soils fertilizer addition shows no inhibition of CH_4 oxidation and positive correlations between soil NH_4^+ concentrations and CH_4 oxidation have been observed, which demonstrates that the direction of response is soil-dependent (Mosier and others 1998; Schellenberg and others 2012). Although grazing increased soil NO_3^--N and total inorganic N at the 0–10 cm depth in our study site after 3 years (Rui and others 2011), our previous experiment nearby this warming experiment site showed that urine application with large N inputs did not significantly affect soil CH_4 uptake (Lin and others 2009). Therefore, we could not deduce that there would be adverse effects of increasing N fertilization following grazing on soil CH_4 consumption in the meadow. Livestock trampling probably causes soil compaction, which may be another factor that reduces CH_4 uptake by decreasing gas diffusion into the soil and limiting CH_4 and O_2 availability for the oxidation process (Saggar and others 2007; Liu and others 2007). However, our study site had been under grazing before the experiment was conducted and we could not quantify the effects of the compaction and nutrients applied by the sheep and whether they were homogeneously spatially distributed. Therefore, the impacts of compaction and N input caused by sheep trampling and excreta on CH_4 uptake could not be confirmed in our study in the first few years.

In our study, grazing increased average soil temperature (grazing reduced vegetation cover and could increase both inwards and outwards heat flux) and tended to decrease soil moisture during the growing seasons in 2007 and 2008 (Luo and others 2009; Hu and others 2010). The reduction in soil moisture due to grazing was likely to cause more CH_4 uptake. So, the response of soil CH_4 uptake to grazing may depend on grazing intensity and the balance between inhibitive effects due to soil compaction caused by trampling and stimulative effects through decreasing soil moisture due to soil warming. For example, if the inhibitive effect was offset by the stimulative effect, or if none of the suggested effects were significant under light grazing intensity, then sheep grazing would have little effect on methanotrophic abundance (for example, Zheng and others 2012) and soil CH_4 uptake, as was seen in 2007 and 2008 in our study.

Conclusions

Generally, warming treatment significantly increased soil CH_4 uptake and the magnitude of the effect of warming on CH_4 uptake was greater during the non-growing season compared with the growing season over the experimental period in the semi-humid alpine meadow on the Tibetan plateau. However, moderate-simulated grazing treatment showed no significant impacts on CH_4 uptake except for grazing under warming conditions, which reduced CH_4 uptake for a short time during the growing season in 2006. The response of CH_4 uptake in the meadow to warming treatment could depend on soil moisture, which is changed by soil warming. Warming treatment stimulated CH_4 uptake by combining with greater soil moisture in the relatively drier winter season and lower soil moisture during the growing season. The stimulating effect of the warming treatment was greater in the relatively drier year, which implied that the impact of warming treatment on CH_4 uptake could be influenced by variation in annual precipitation. Moreover, the response of soil CH_4 uptake to warming during the non-growing seasons should be considered due to the higher contribution of soil CH_4 uptake to total annual soil CH_4 uptake in the warming plots. These results imply that the response of CH_4 uptake to the warming treatment is more sensitive in winter and that the stimulating mechanism of warming on soil CH_4 uptake could be different between the growing season and the non-growing season due to different relationships between soil CH_4 uptake and soil moisture in the different seasons.

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