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REGULAR ARTICLE

Asymmetric responses of methane uptake to climate warming and cooling of a Tibetan alpine meadow assessed through a reciprocal translocation along an elevation gradient

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Abstract
Aims A lacking of understanding about cooling effects on methane (CH_4) fluxes and potential asymmetrical responses to warming and cooling causes uncertainty about climate change effects on the atmospheric CH_4 concentration. We investigated CH_4 fluxes in an alpine meadow on the Tibetan Plateau in response to climate warming and cooling.

Methods A 2-year reciprocal translocation experiment was implemented to simulate climate warming (i.e. downward translocation) and cooling (i.e. upward translocation) along an elevation gradient with four different

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vegetation types (at 3200, 3400, 3600 and 3800 m elevation) during the growing season (May to October) in 2008 and 2009.

Results Although the effects of warming and cooling varied depending on vegetation type, elevation and timescale (i.e., daily and seasonally), warming increased average seasonal CH_4 uptake by 60 %, while cooling reduced it by 19 % across all vegetation types, based on a 1.3–5.1 °C difference in soil temperature at 20 cm depth. Soil temperature over the range of 4–10 °C explained 11–25 % of the variation in average seasonal CH_4 fluxes, while there was no relationship with soil moisture over the range of 13–39 % and soil NH_4^+-N and NO_3^-N content. Methane uptake was more sensitive to warming than to cooling.

Conclusions Because of warming and cooling spells in the alpine region, warming effects on CH_4 uptake would be over-estimated by 64 % if cooling effects on it are not considered. Our findings suggest that asymmetrical responses of CH_4 fluxes to warming and cooling should be taken into account when evaluating the effects of climate change on CH_4 uptake in the alpine meadow on the Tibetan plateau.

Keywords Climate change · Reciprocal translocation · Methane uptake · Asymmetry · Alpine meadow · Tibetan plateau

Introduction

Methane (CH_4) is the second most powerful greenhouse gas with a global warming potential that is about 25 times larger compared to CO_2 over one century (IPCC 2007). Its concentration in the atmosphere has exceeded pre-industrial levels by about 150 % to 1803 ppb in 2011 causing approximately 20 % of global warming (IPCC 2007; Kirschke et al. 2013). Soil is the second largest sink of CH_4 after oxidation by hydroxyls in the troposphere. It is estimated that about 24–40 Tg atmospheric CH_4 is consumed annually by biological oxidation in soil (IPCC 2007; King 1997), although large uncertainties still remain. The soil sink of CH_4 can be altered by climate change and anthropogenic activity (Dijkstra et al. 2013; Zürcher et al. 2013) and thus produce an important potential feedback to climate change (Torn and Harte 1996). Accordingly, investigation of changes in CH_4 fluxes in response to variation in soil temperature and moisture is of vital importance to evaluate the potential of terrestrial ecosystems to absorb atmospheric CH_4.

CH_4 is primarily produced by methanogens under anaerobic conditions (Dalal and Allen 2008; Le Mer and Roger 2001) while atmospheric CH_4 is oxidized by methanotrophs under aerobic conditions (Hanson and Hanson 1996). Abiotic and biotic factors including temperature and moisture (Dijkstra et al. 2013; Wang et al. 2009), soil inorganic N content (Fang et al. 2010; Zhuang et al. 2013), microbial community attributes (McCalley et al. 2014; Shrestha et al. 2012) and vegetation composition (Zhang et al. 2012) affect the exchange of CH_4 between atmosphere and soil. Previous studies have found that warming increased CH_4 uptake in a temperate forest (Peterjohn et al. 1994), in subarctic systems (Sjögersten and Wookey 2002) but weakened the sink of CH_4 in a semiarid grassland (Dijkstra et al. 2013) and in a peatland (Yang et al. 2014). These contradictory findings reveal that the response of CH_4 exchange between atmosphere and soil may be ecosystem dependent. Our previous study has found that experimental warming increased CH_4 uptake in a Tibetan alpine meadow (Lin et al. 2015) because warming increased the abundance of soil methanotrophs (Zheng et al. 2012), and also facilitated diffusion of more atmospheric oxygen and CH_4 into soil due to reduced soil moisture (Lin et al. 2015; Wang et al. 2009). However, most of these studies only focused on warming effects without exclusion of drying effects caused by experimental warming and ignored cooling effects on CH_4 fluxes. In fact, compared to historical temperature records, higher and lower temperature spells accompanied by variable rainfall frequently occur on the Tibetan Plateau (Li et al. 2004). The annual average surface temperature in 22 out of the last 44 years was lower than the average, while it was higher in 19 out of the 44 years, with no long-term trend (Li et al. 2004). This indicates that both warming and cooling occurs on the Tibetan Plateau. The Tibetan Plateau is an important CH_4 sink in China (Wei et al. 2014) consuming about 44 % of the total CH_4 uptake of all grasslands in China (Wang et al. 2014c). However, a lack of studies on CH_4 uptake in response to climate change, especially climate cooling on the Tibetan Plateau, has limited our ability to estimate the temporal variation of CH_4 fluxes in this region and its contribution to absorbing atmospheric CH_4.

The reciprocal translocation method can synchronously estimate warming and cooling effects by making use of differences in natural environmental gradients. This method has been used to test key carbon and nitrogen processes (Hu et al. 2016; Link et al. 2003; van de Weg et al. 2013) and microbial community dynamics (Liang et al. 2015; Zhao et al. 2014; Zumsteg et al., 2013) in response to climate change. Although CH_4 fluxes have been reported to be exponential, linear or not correlated to soil temperature in different ecosystems (Dijkstra et al. 2013; Fang et al. 2010; Fang et al. 2014; Jiang et al. 2010; Lin et al. 2009; Lin et al. 2015; Wang et al. 2009; Wei et al. 2014) where CH_4 fluxes are plotted against temperature variability, the production and oxidation of CH_4 have been considered as inherently nonlinear processes and to obey exponential dependency on temperature (Dunfield et al. 1993; Segers 1998). Thus, linear models are usually failed to fit data well as the range of data increases. However, our recent study from a reciprocal translocation experiment has found that a linear model can well fit the relationship between difference in ecosystem respiration and temperature difference (ranged from 0.2 to 4.0 °C) in the Tibetan alpine meadow that shows an asymmetric response to warming and cooling (Hu et al., 2016), and the CH_4 fluxes are highly negative related to ecosystem respiration (r = 0.734, P = 0.007). Moreover, higher shift rate of microbial community to warming than to cooling were also observed in an Arctic ecosystem (Zumsteg et al., 2013) and an agricultural ecosystem (Liang et al. 2015), respectively. Thus, it has reasons to extrapolate that CH_4 flux of Tibetan alpine meadow may show similar responses to warming and cooling. Here, we tested the following hypotheses: (1) soil CH_4 uptake decreases with an increase in elevation due to a decrease in soil temperature along the natural elevation gradient; (2) warming (through translocation of soil and vegetation to lower elevation) increases CH_4 uptake but cooling (translocation to higher elevation) has the opposite effect; and (3) the response of CH_4 fluxes of this alpine meadow to climate warming and cooling is asymmetrical with greater sensitivity to warming than to cooling.

Materials and methods

Study site and experimental design

The experiment was conducted using a reciprocal translocation climate change experimental platform, situated along an elevation gradient from 3200 m to 3800 m on the southern slope of Qilian Mountains in the northeastern Tibetan Plateau near the Haibei Alpine Meadow Ecosystem Research Station (HBAMERS) (37°37′N, 101°12′E) of the Chinese Academy of Sciences. The mean annual air temperature and precipitation from 1981 to 2000 were −1.7 °C and 561 mm, respectively. The soil is a clay loam and classified as Mat Cry-gelic Cambisols according to the Chinese national soil survey classification system (The Institute of Soil Science and the Chinese Academy of Sciences, 2001). For details about the site, we refer to Zhao and Zhou (1999).

In 2006, four 20-m long × 8-m wide areas were fenced at 3200 m (37°36′42.3″N, 101°18′47.9″E), 3400 m (37°39′55.1″N, 101°19′52.7″E), 3600 m (37°41′46.0″N, 101°21′33.4″E) and 3800 m (37°42′17.7″N, 101°22′09.2″E) to avoid grazing from animals. We named these four sites vegetation type A, B, C and D at 3200, 3400, 3600 and 3800 m, respectively, due to their obvious differences in vegetation community composition. At 3200 m, the vegetation is dominated by *Kobresia humilis*, *Festuca ovina*, *Elymus nutans*, *Poa spp.*, *Carex spp.*, *Scripus distigmaticus*, *Gentiana straminea*, *Gentiana farreri*, *Leontopo diumnanum*, and *Potentilla nivea.* At 3400 m, the vegetation is dominated by alpine shrub *Potentilla fruticosa*, *K. capillifolia*, *K. humilis*, *Saussurea superba*. At 3600 m, the vegetation is dominated by *K. humilis*, *Saussurea katochaete Maxim*, *P. nivea*, *Thalictrum alpinum*, *Carex spp.*, *Poa spp.*, and *P. fruticosa*. At 3800 m, the vegetation is dominated by *K. humilis*, *L. odiumnanum* and *Poa spp*. Details of the experimental design were reported by Wang et al. (2014a,b).

In May 2007, 12 intact soil blocks (length × width × depth = 1.0 × 1.0 × 0.3–0.4 m, with 30 cm depth at 3800 m due to a shallower soil layer) with vegetation from each elevation were cut off for reciprocal translocation. The translocation process caused only minimal damage to plant roots that are mostly distributed in the top 10 cm (Wang and Shi 1999). Three of these 12 soil blocks were reinstated at the same site (i.e. home plot) and handled similarly as blocks moved to other elevations. The other 9 intact soil blocks were equally distributed among the other 3 elevation sites (i.e. away plots). All intact soil blocks were installed in a fully randomized design, surrounded by plastic to prevent any exchange with the ambient soil environment. Thus, there were a total of 48 plots in our study (36 away plots and 12 home plots at 4 elevations and 3 replicates).

Soil temperature and moisture

At the center of the fenced experimental area at each elevation site, HOBO weather stations (Onset Computer Corporation, Cape Cod, Massachusetts, USA) were installed to monitor soil temperature and soil moisture at 20 cm soil depth. The sensors were connected to a CR1000 datalogger. Soil temperature and soil moisture were measured every 1 min, and then 30-min averages were stored.

Soil mineral nitrogen (N)

Soil samples were collected from the topsoil (0–30 cm) of all 48 plots on 13 August 2009. Three soil cores (2 cm in diameter) at 0–10, 10–20 and 20–30 cm depth were randomly taken from each plot. Soil samples from the same plot and depth were mixed as a single sample and packed in polyethylene bags and immediately stored in an ice chest until they were transported to the laboratory and stored in a refrigerator at 4 °C prior to analyses. The composite samples were then sieved through a 2 mm screen, and any visible plant materials were manually removed from the sieved soil. Soil NH_4^+-N and NO_3^--N were extracted by shaking soil (5 g) in 25 mL 2 M KCl within a day or two and filtered using Whatman #40 filter paper. Their concentrations in the KCl extracts were determined on an automated segmented flow analyzer (San^{++}, Skalar Analytical, B.V., Netherlands).

Measurement of CH_4 fluxes

During the growing seasons from May to September in 2008 and 2009, CH_4 was measured in each plot on the same day using static chambers and gas chromatography techniques every 7–10 days depending on weather conditions. For the dimension (40 cm length × 40 cm width × 40 cm height) and structure of chambers, and method of gas sampling and analysis we followed the description by Lin et al. (2009) and Hu et al. (2010). Based on our previous investigation of diurnal gas flux variation (Lin et al. 2015), the fluxes of CH_4 between 9:00 and 11:00 a.m. could represent one-day average flux. Chambers were closed for 30 min and 4 gas samples (about 100 ml) were manually collected from the closed chamber every 10 min using 100 ml plastic syringes between 9:00 and 11:00 a.m. The CH_4 concentration of all gas samples was analyzed using gas chromatography (HP Series 4890D, Hewlett Packard, USA) within 24 h after sampling.

Here, we made a CH_4 convention that the positive/negative values of CH_4 fluxes present the emission/uptake of CH_4. To calculate the temperature sensitivity of CH_4 fluxes (% °C^{-1}), we took the slope of a linear regression equation between the difference (%) in CH_4 fluxes and the difference (°C) in soil temperature between the away plots and the home plot from the elevation where the away plots came from.

Statistical analysis

A univariate General Linear Model was used to analyze effects of elevation (between-subject factor) and depth (within-subject factor) on soil ammonium (NH_4^+-N) and nitrate (NO_3^-N) in the home plot with SPSS version 16.0 (SPSS Inc. Chicago, USA). Vegetation type was included as a within-subject factor for analysis of NH_4^+-N and NO_3^-N in the translocation plots. Linear mixed models with repeated measurements were used for analysis of daily CH_4 fluxes. Type III SS was adopted since there were missing data at 3600 m in 2009 for CH_4 fluxes. To test for elevation, date and year effects on CH_4 fluxes in the home plot, elevation was taken as the between-subject factor and date and year as the within-subject factors. For warming and cooling effects caused by translocation, elevation and vegetation type were taken as the between-subject factor, date and year were within-subject factors. One way ANOVA and Least Significance Difference (LSD) were used to test the significance of differences in soil mineral N content and the average seasonal CH_4 fluxes during the growing seasons between the away plots and the home plot at each elevation. Linear regressions were performed between daily/average seasonal CH_4 fluxes and environmental factors (i.e., soil temperature and soil moisture) and soil mineral N, and also between the differences in average seasonal CH_4 fluxes and the differences in soil temperature between away plots and home plots. All significances mentioned in the text were at the 0.05 level. Data of CH_4 fluxes at 3600 m in 2009 were not measured because experimental plots were destroyed by alpine pikas (*Ochotona curzoniae*).

Two types of regression model (i.e., linear regression and segmented linear regression) were used to fit the responses of CH_4 fluxes to the change in soil temperature. Segmented linear regression was fitted in R-3.1.0 (R Development Core Team 2013) with R package “Segmented (ver 0.5–1)”. The fitness of the models was evaluated based on Akaike’s information criterion

(AIC) in R-3.1.0. Asymmetry was supported when the difference in AIC between linear regression and segmented regression was larger than 2.

Results

Soil temperature and moisture

Soil temperature at 20 cm depth decreased with an increase in elevation (Fig. 1a, b), similar to previous reports (Wang et al. 2014a,b). The average soil temperatures at 20 cm depth between 9:00 and 11:00 a.m. during the sampling period were 9.5, 7.3, 5.9 and 4.5 °C in 2008, and 10.1, 7.8, 6.5 and 5.3 °C in 2009 at 3200 m, 3400 m, 3600 m and 3800 m, respectively. The average soil moistures at 20 cm depth were 34.5, 29.6, 35.0 and 12.9 % in 2008 at 3200 m, 3400, 3600 and 3800 m, respectively, and they were 35.6, 29.3, 43.6 and 16.1 % in 2009. The highest soil moisture observed at 3600 m was due to the topography of this site, being situated at the foot of the mountain.

Soil mineral nitrogen

For the home plots (i.e., not transplanted), soil NH_4^+-N content was only affected by soil depth and soil NO_3^--N content was affected by elevation and depth (Table S1). Both soil NH_4^+-N and NO_3^--N content decreased with an increase in soil depth (Fig. 2). There was no significant difference in soil NH_4^+-N content among different elevation sites. Significant differences in soil NO_3^--N content were only found between 3200 and 3600 m elevation at 10–20 cm depth, with higher contents at 3600 m. No relationship was found between average soil NH_4^+-N content within 30 cm depth and soil temperature ($P = 0.114$) and soil moisture ($P = 0.495$) at 20 cm depth, and between average soil NO_3^--N content within 30 cm depth and soil temperature ($P = 0.082$) and soil moisture at 20 cm depth ($P = 0.667$) averaged during the growing season in 2009.

In the translocation plots, soil NH_4^+-N content was affected by soil depth, elevation and the interaction of elevation and vegetation type, and soil NO_3^--N content

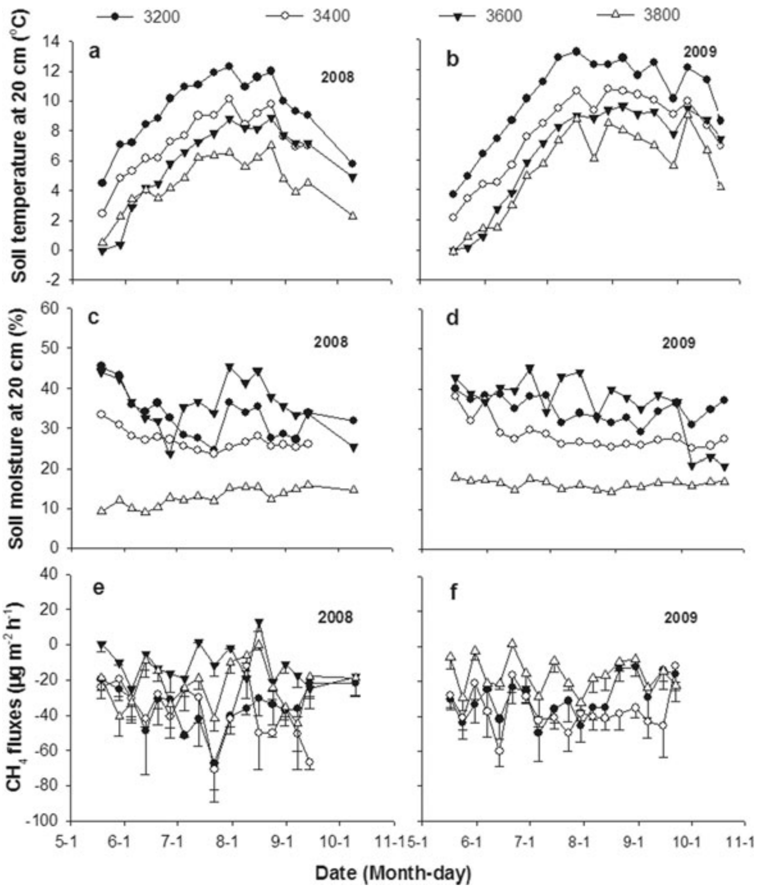


Fig. 1 Soil temperature (**a,b**), soil moisture (**c,d**) at 20 cm depth for different elevations, and daily mean CH_4 fluxes (**e,f**) of the home plots during the growing seasons from May to October in 2008 and 2009. Soil temperature and soil moisture are the mean values from 9:00 to 11:00 a.m. on the same CH_4 sampling date

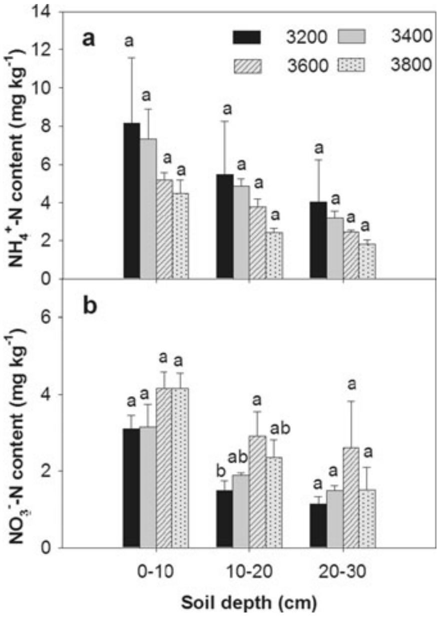


Fig. 2 Soil NH_4^+-N and NO_3^-N content (mean ± se) of the home plots in 2009 at the four elevation sites (3200, 3400, 3600 and 3800 m). Different lower-case letters indicate significant differences at the 0.05 significant level

was affected by soil depth and vegetation type (Table S2). The effects of warming and cooling on soil NH_4^+-N and NO_3^-N content varied with elevation (Fig. 3). Cooling effects were determined by comparing away plots from higher elevation to home plots at a specific elevation, while warming effects were determined by comparing away plots from lower elevation to home plots at a specific elevation. Soil NH_4^+-N content increased by 0.8 mg kg^{-1} to warming (averaged across all away plots brought to higher elevation, ranging between −0.3 and 1.7 mg kg^{-1}), and by 2.2 mg kg^{-1} to cooling (averaged across all away plots brought to lower elevation, ranging between −1.6 and 5.2 mg kg^{-1}). Soil NO_3^-N content decreased on average by 0.2 mg kg^{-1} to warming (ranging between −1.5 and 1.4 mg kg^{-1}), but increased by 0.8 mg kg^{-1} to cooling (ranging between −1.1 and 3.2 mg kg^{-1}). However, significant cooling effects on NH_4^+-N and NO_3^--N were only found at 3600 m.

Temporal variation of CH_4 fluxes in the natural elevation gradient

In home plots, daily mean CH_4 fluxes varied from −107 to 31 μg CH_4-C m^{-2} h^{-1} across all four elevations and was significantly affected by sampling date and elevation (Table 1). In general, CH_4 uptake (negative fluxes) occurred most of the time and its uptake tended to peak in July - August (Fig. 1e, f). Mean seasonal CH_4 fluxes significantly decreased linearly with an increase in soil temperature at 20 cm depth across all elevation sites (i.e., increased CH_4 uptake with increased temperature) and it explained 30.9 % of the variation in seasonal CH_4 fluxes (Fig. 4b). No relationship was found between mean seasonal CH_4 fluxes and soil moisture at 20 cm depth over the 2-year period across all elevation sites (Fig. 4c), but significant positive relationships between daily CH_4 fluxes and soil moisture at 20 cm were observed for each elevation separately (Table S3).

Mean CH_4 fluxes during the growing seasons over the 2-year period at 3200 and 3400 m were lower (i.e, higher CH_4 uptake) than at 3600 and 3800 m. Mean CH_4 uptake was significantly lower at 3600 m than at 3200 and 3400 m in 2008, and significantly lower at 3800 m than at 3400 m in 2009 (Fig. 4a). There was no significant difference in mean CH_4 uptake between 2008 and 2009.

Effects of warming and cooling on temporal variation in CH_4 fluxes

Sampling date, year, elevation, vegetation type, and interactions of vegetation type with elevation, date, and year significantly affected daily mean CH_4 fluxes in the translocation plots (Table 2). Similar temporal patterns of daily mean CH_4 fluxes were observed as in the home plots (Fig. S1). The effects of warming and cooling varied depending on vegetation type and sampling date. For example, warming increased daily mean CH_4 uptake in 14, 9 and 3 out of 17 sampling dates in vegetation type D brought down to 3200, 3400 and 3600 m in 2008, respectively, but decreased it in 8 out of 16 sampling dates in 2008 for vegetation type B brought down to 3200 m. Cooling decreased daily mean CH_4 uptake in 7 out of 16, 15 and 3 out of 17 sampling dates for vegetation type A brought up to 3400, 3600 and 3800 m in 2008, respectively, but increased it in 3 and 6 out of 16 sampling dates for vegetation type B brought up to 3600 and 3800 m in 2008, respectively.

The effects of warming and cooling on average seasonal CH_4 uptake during the growing seasons over the 2-year period varied with vegetation type and elevation (Fig. S2). Warming increased CH_4 uptake in most combinations of vegetation type and elevation, with an increase ranging between 20 (vegetation type C brought down to 3400 m) and 275 % (vegetation type C brought

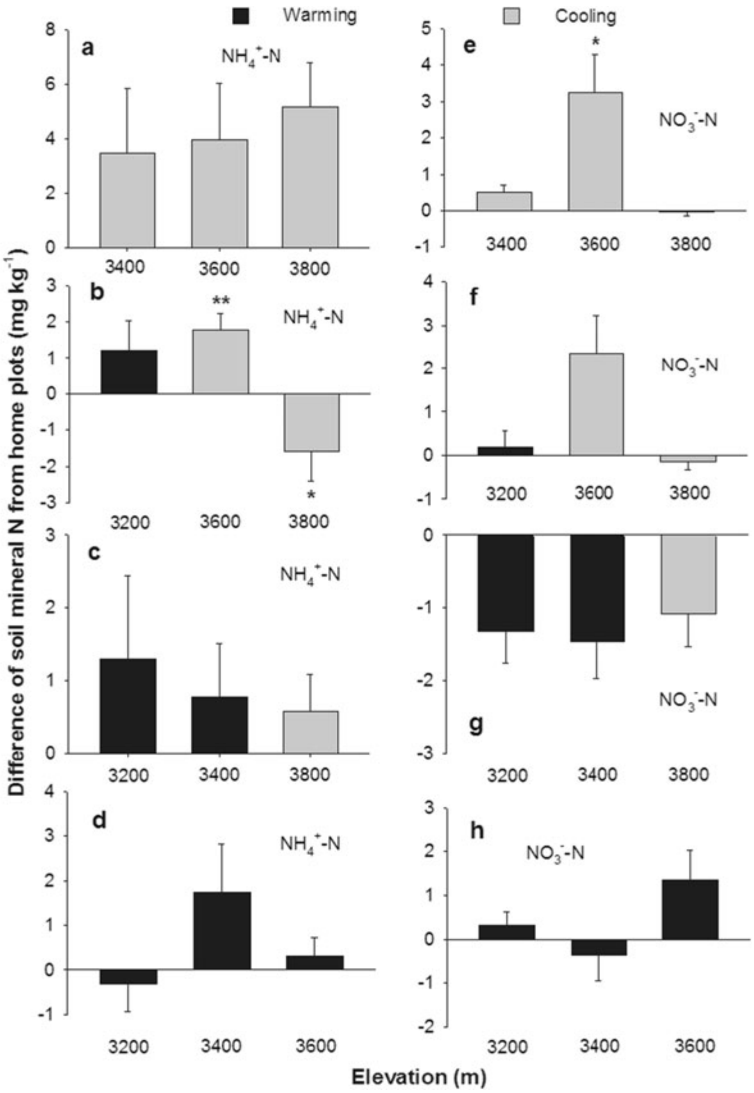


Fig. 3 The difference in soil ammonium (NH_4^+-N) and nitrate (NO_3^-N) content between away and home plots (mean ± se) for vegetation type A (**a, e**), B (**b, f**), C (c, g), and D (**d, h**) at each elevation.* and ** indicate significant differences at 0.05 and 0.01 significant level

down to 3200 m), but slightly decreased CH_4 uptake at two other occasions by 24 and 17 % (vegetation type B brought down to 3200 m and vegetation type D brought down to 3600 m). Cooling decreased average seasonal CH_4 uptake in most cases, with decreases ranging between 8 (vegetation type B brought up to 3800 m) and 54 % (vegetation type A brought up to 3600 m). Cooling only increased average seasonal CH_4 uptake in vegetation type A brought up to 3400 m (8 % increase). Significant difference between away plots and home plots was found for vegetation type D brought down to 3200 m over 2-year, and also for other vegetation types when separated by elevation of away plots and year (Fig. S1).

Factors affecting average seasonal CH_4 uptake

No significant relationship was found between average seasonal CH_4 fluxes and soil mineral N (i.e. NH_4^+-N and NO_3^-N) content across all three depths. The seasonal average CH_4 uptake showed a significant positive relationship with soil temperature at 20 cm depth in plots with warming and cooling across all vegetation types, explaining 11–25 % of the variation in average seasonal CH_4 fluxes over the range of 4–10 °C (Fig. 5a). However, no significant relationship between seasonal average CH_4 uptake and soil moisture was found over the range of 13–39 % (Fig. 5b).

Table 1 Summary of linear mixed model analysis on daily CH_4 fluxes in the home plots during the growing seasons in 2008 and 2009

Source	df	*F*	*P*
Year (Y)	1	3.382	0.067
Date (D)	17	2.040	0.010
Elevation (E)	3	29.277	<0.001
Y * D	17	1.517	0.089
Y * E	2	1.283	0.279
D * E	51	1.210	0.173
Y * D * E	33	0.601	0.960

Temperature sensitivity of CH_4

Based on AIC, the segmented linear regression was a better fitted model for all pooled plots with warming and cooling (Table 3). There were positive relationships between the differences in average seasonal CH_4 fluxes between away plots and home plot (%) and soil temperature differences measured in these plots during the growing seasons in plots with warming and cooling (averaged across all vegetation types). The CH_4 temperature sensitivity values (i.e. the slope of the regression equation) were 16, 35 and 24 % $^{o}C^{-1}$ for vegetation type A, C and D (Fig. 6), and they were 26 % $^{o}C^{-1}$ for all pooled plots with warming, 10 % $^{o}C^{-1}$ for all pooled plots with cooling and 16 % $^{o}C^{-1}$ for all pooled plots with warming and cooling, respectively (Fig. 7). The difference in AIC between linear regression and segmented regression was larger than 2 for all pooled plots with warming and cooling (Table 3), suggesting that the responses of CH_4 fluxes to warming and cooling were not symmetrical.

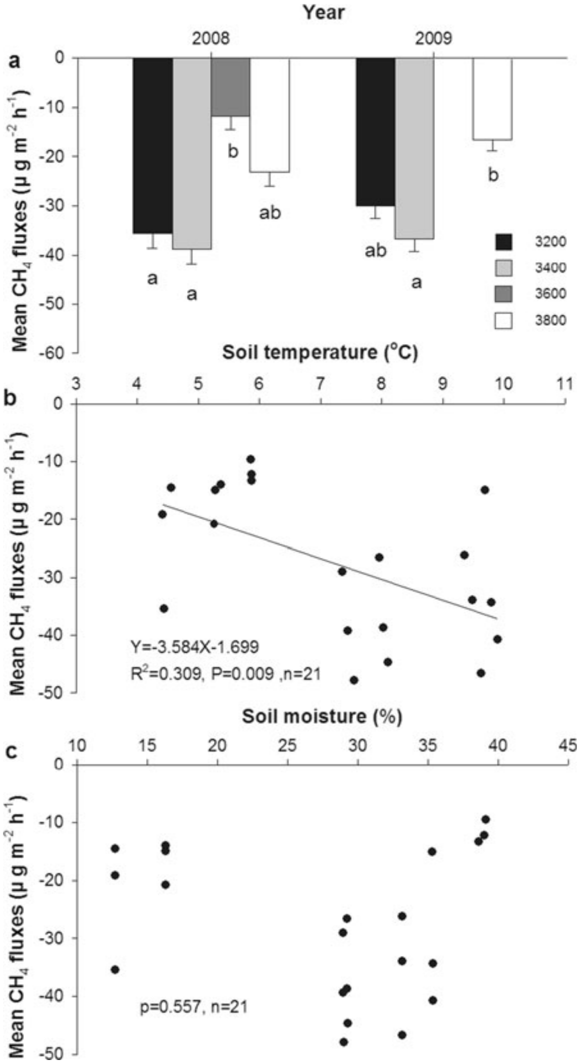


Fig. 4 Average seasonal CH_4 fluxes (mean ± se) (**a**), the relationship between average seasonal CH_4 fluxes and soil temperature at 20 cm (**b**) and soil moisture (**c**) in home plots at four elevation sites (3200, 3400, 3600 and 3800 m) in 2008 and 2009. Different lower-case letters indicate significant differences at the 0.05 significant level

Discussion

Temporal variation of CH_4 fluxes and its affecting factors

Similar to previous studies (Lin et al. 2015; Wang et al. 2009), our results showed that alpine meadow was a sink of CH_4 with the average uptake rate of 25.5 μg m^{-2} h^{-1} across the natural gradient (i.e. non-translocation). However, the magnitude of CH_4 uptake across the natural gradient varied largely (ranging from −31 to 107 μg m^{-2} h^{-1}) depending on vegetation type, elevation, and sampling date, reflecting the high variability of CH_4 uptake in time and space. Average seasonal CH_4 uptake rates were significantly positively related to soil temperature across all vegetation types and elevations (Fig 4b and 6a), which supported our first hypothesis. Similar temperature effects were also observed in other studies (Fang et al. 2014; Jiang et al. 2010; Lin et al. 2015; Shrestha et al. 2012; Wang et al. 2009). No relationship between daily and average seasonal CH_4 uptake and soil moisture was observed when all treatments were pooled together, but daily mean CH_4

Table 2 Summary of linear mixed model analysis on daily CH_4 fluxes in the translocation plots during the growing seasons in 2008 and 2009

Source	df	*F*	*P*
Date (D)	17	8.181	<0.001
Year (Y)	1	28.608	<0.001
Elevation (E)	3	28.518	<0.001
Vegetation type (V)	3	56.692	<0.001
D * Y	16	3.352	<0.001
D * E	51	0.464	1.000
D * V	50	1.532	0.011
Y * E	3	0.632	0.594
Y * V	2	4.069	0.017
E * V	9	11.975	<0.001
D * Y * E	48	0.652	0.968
D * Y * V	31	1.723	0.009
D * E * V	150	0.826	0.929
Y * E * V	6	0.971	0.444
D * Y * E * V	93	0.762	0.951

uptake significantly decreased with an increase in soil moisture when separated by elevation (Table S3). Probably, higher moisture caused more blocked soil-pores and depressed the diffusion of atmospheric oxygen and CH_4 into soil and consequently limited the oxidation ability of methanotrophs (Wang et al. 2009; Zhuang et al. 2013). Methanotroph abundance and activity can also directly increase in response to a rise in temperature (Topp and Pattey 1997; Zheng et al. 2012). Previous studies in the same region have found that soil temperature and/or soil moisture explained 17–27 % and 16–47 % of the variation of daily CH_4 fluxes, respectively (Fang et al. 2014; Lin et al. 2015; Lin et al. 2009; Wang et al. 2009). We found that soil temperature was the main factor controlling seasonal CH_4 fluxes and explained 11–25 % of the variation (Fig 6a) along the elevation gradients. Because soil moisture in each home and away plot at each site was probably different due to the differences in soil physical properties of their original site that was not measured, no relationship between CH_4 fluxes and soil moisture was found for the elevation gradients (Fig 6b). However, a weak negative relationship between CH_4 uptake and soil moisture at each single elevation (Table S3) indicated that higher soil moisture could enhance CH_4 emission from the alpine meadow.

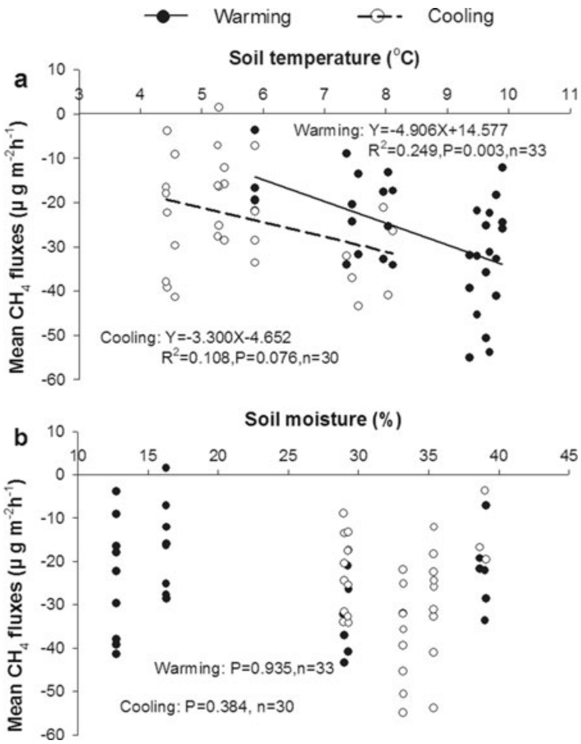


Fig. 5 Relationships between average seasonal CH_4 fluxes during the growing seasons in 2008 and 2009 and soil temperature (**a**) and soil moisture (**b**) at 20 cm depth for plots with warming and cooling effects

Many field N addition experiments found that CH_4 uptake may be limited by accumulation of mineral N (i.e. NH_4^+-N and NO_3^-N) (Fang et al. 2014; Jiang et al. 2010) due to a competitive inhibition effect of NH_4^+ on CH_4 oxidation, and by toxicity effects of NO_3^- on methanotrophs. Therefore, a reduced CH_4 sink of terrestrial ecosystems in response to N deposition at the global scale is expected (Zhuang et al. 2013). However, inconsistent with a previous report by Fang et al. (2010), no relationship between CH_4 uptake and soil mineral N was found along the elevation gradient in our study. But only one sampling of soil mineral N in this work made it difficult to determine their relationship. Thus, it needs a further study about the relationship between soil mineral N content caused by climate change and CH_4 uptake in the Tibetan alpine meadow.

Warming and cooling effects

Although the effects of warming and cooling on CH_4 uptake varied with vegetation type and elevation, our results generally supported the second hypothesis that

Table 3 Comparison of two fitted models based on Akaike's information criterion (AIC)

Vegetation type	Linear	Segmented
A	145.986	-
B	148.766	151.296
C	96.207	87.468
D	170.038	-
all	578.213	575.266

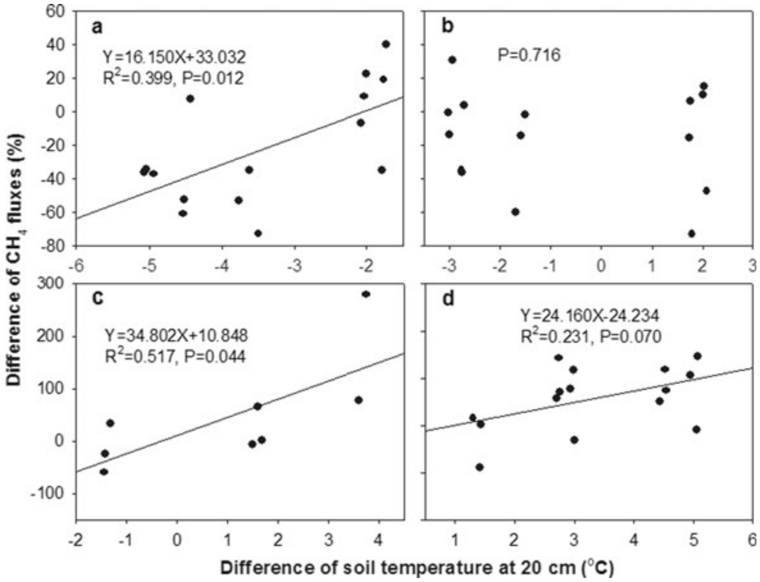
Notes: Values are considered to be the same if the difference in AIC values (⊿AIC) < 2

warming increased but cooling reduced CH_4 uptake. As explained above, increased CH_4 uptake in response to warming may have been caused by direct temperature effects on methanotroph activity, and by indirect effects through reduced soil moisture enhancing the diffusive transport of atmospheric oxygen and CH_4 into soil (Lin et al. 2015; Wang et al. 2009). Similarly, reduced soil temperature may have reduced CH_4 oxidation in the soil. The increase in CH_4 uptake with warming is consistent with observations in a temperate forest (Peterjohn et al. 1994), subarctic systems (Sjögersten and Wookey 2002) and an alpine meadow (Lin et al. 2015) but in contrast to observations in a semiarid grassland (Dijkstra et al. 2013) and a peatland (Yang et al. 2014). However, we also observed that warming did not always increase CH_4 uptake, while cooling did not always reduce CH_4 uptake (Fig. S2). This reflects that the effects of soil temperature on CH_4 fluxes of alpine meadow depended on vegetation type that might be influenced by changes in soil moisture, which is difficult to separate from each other in this study. A possible explanation for the variable effects is that soil moisture did not show a clear increase with elevation, but was highest at 3600 m (Fig. 1), which may have caused variable warming and cooling effects among vegetation types. For instance, the decrease in CH_4 uptake with warming by bringing vegetation type D down to 3600 m may have resulted from an increase in soil moisture. It is also possible that the variable effects of warming and cooling on soil inorganic N (Fig. 3) may have contributed to the variation in CH_4 uptake among vegetation types and elevation, although no significant relationship was found between average seasonal CH_4 fluxes and soil mineral N. Increased soil mineral N (Fig. 3) might inhibit CH_4 uptake for vegetation type B brought to 3200 m. Our results suggest that CH_4 uptake in the alpine meadow on the Tibetan Plateau could be affected by co-variation in soil moisture and temperature. Soil moisture could strengthen the effects of cooling on CH_4 uptake with the expectation that more rainfall in lower temperature years would cause a larger reduction of the CH_4 sink in this alpine meadow.

Asymmetry of temperature sensitivity of CH_4 fluxes

The slope of the regression equation between the difference in CH_4 fluxes (%) and difference in soil temperature between home and away plots can be used as an

Fig. 6 Relationships of difference in average seasonal CH_4 (%) and temperature difference at 20 cm depth (°C) between away and home plots for vegetation type A (**a**), B (**b**), C (**c**) and D (**d**). Positive/negative values of CH_4 mean that the CH_4 uptake at away plot is greater/smaller than at home plot. The slopes of the regression equations represent the CH_4 fluxes sensitivity to temperature variation



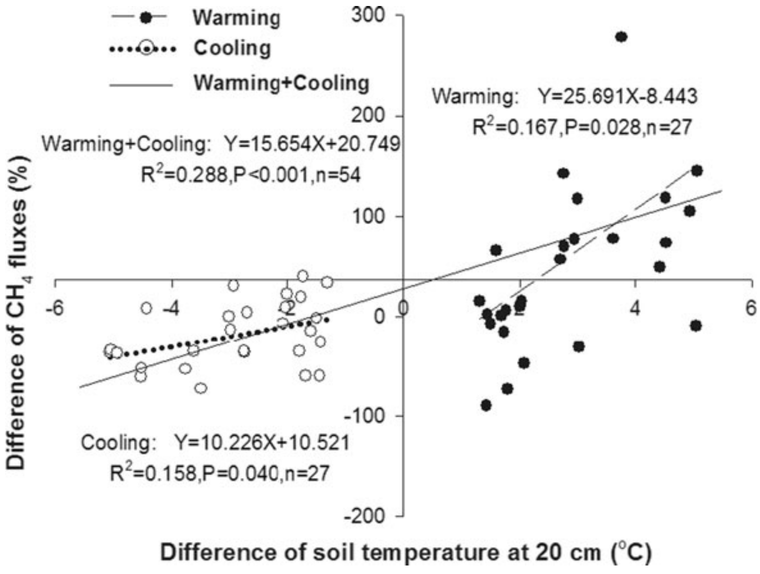


Fig. 7 Relationships of difference in average seasonal CH_4 fluxes (%) and temperature difference at 20 cm depth (°C) between away and home plots with warming, cooling and pooled warming + cooling effects across all vegetation types. Positive/negative values mean that the CH_4 uptake at away plot is greater/smaller than at home plot. The slopes of the regression equations represent the CH_4 fluxes sensitivity to temperature variation

indication of CH_4 temperature sensitivity (Hu et al. 2016; Luo et al. 2010; Wang et al. 2014a). We found that a temperature increase of about 4 °C would correspond to a 100 % increase while a decrease of 4 °C in temperature correspond to a 30 % decrease in methane uptake (Fig. 7). These results supported the third hypothesis (i.e., asymmetrical response of CH_4 uptake to warming and cooling with greater sensitivity to warming). This phenomenon was also found with regards to ecosystem respiration (Hu et al. 2016) for this alpine meadow, and microbial succession rate for an agricultural ecosystem (Liang et al. 2015) and an Arctic ecosystem (Zumsteg et al., 2013). There are several explanations for the asymmetrical response in CH_4 uptake. Firstly, CH_4 uptake in this meadow was caused by the net effect of CH_4-consuming methanotrophs and CH_4-producing methanogens, which have asymmetrical responses to temperature change. Methanogens tend to be more responsive to temperature changes than methanotrophs, and methanogenesis is much more responsive to temperature than is methane oxidation (Topp and Pattey 1997). The microbial succession rate showed asymmetrical responses to warming and cooling, and differences in sensitivity to warming and cooling depended on taxonomic microbial groups in a soil transplant experiment in an agricultural system (Liang et al. 2015). Accordingly, the responses of methanotrophs and methanogens in the soil may be different to climate warming and cooling. Secondly, the response of aboveground biomass was more sensitive to warming than to cooling (unpublished results). This differential response may have resulted in a larger supply of substrates to methanotrophs in response to warming than the corresponding reduction in response to cooling. Probably, warming caused more accumulation of aboveground biomass (Lin et al. 2011) and stimulated the growth and reproduction of methanotrophs (Zheng et al. 2012). Thirdly, changes in soil chemical properties including soil inorganic N content caused by warming and cooling may also have been responsible for the asymmetry of CH_4 uptake. A positive relationship between N mineralization and net nitrification and CH_4 consumption was reported elsewhere (Hart 2006; Peterjohn et al. 1994). It was further shown that NH_4^+ accumulation in the soil significantly inhibited CH_4 oxidation in this alpine meadow (Fang et al. 2014; Fang et al. 2010; Jiang et al. 2010) while NO_3^-N either increased or showed no effect on CH_4 uptake (Corton et al. 2000; Dunfield et al. 1995; Fang et al. 2010), depending on the ecosystem type and biological climate zone. In our study, warming caused less accumulation of soil NH_4^+-N (average increase of 16 % compared to home plot) than cooling (average increase of 39 %). Therefore, cooling may have inhibited CH_4 uptake to a larger degree than warming. The increase in soil NO_3^-N (on average by 1.4 %) with warming was also less than with cooling (average increase of 44 %). Therefore, the inhibition on CH_4 uptake caused by the accumulation of soil mineral N with warming was probably less than with cooling, which may have contributed to the higher CH_4 temperature sensitivity to warming in this alpine meadow.

Conclusion

Generally, our results indicated that warming increased CH_4 uptake but that cooling had the opposite effect in this alpine meadow, although their effects varied depending on vegetation type, elevation and timescale (i.e., daily and seasonally). Soil temperature rather than soil moisture and soil mineral N content (i.e. NH_4^+-N and NO_3^-N) mainly controlled seasonal CH_4 uptake along the elevation gradients. Given the asymmetrical response of CH_4 uptake to warming and cooling, the effects of climate change on CH_4 uptake would be overestimated if we neglected cooling effects.

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