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Response of CH₄ emission to moss removal and N addition in boreal peatland of Northeast China

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Abstract

Boreal peatlands are an important natural source of atmospheric methane (CH_4). Recently, boreal peatlands have been experiencing increased nitrogen (N) input and decreased moss production. However, little is known about the interactive effect of moss and N availability on CH_4 emission in boreal peatlands. In this study, the effects of moss removal and N addition ($6 \text{ gNm}^{-2} \text{ yr}^{-1}$) on CH_4 emission were examined during the growing seasons of 2011 to 2013 in a boreal peatland in the Great Hinggan Mountain of Northeast China. Notably, the response of CH_4 emission to moss removal and N addition varied with experimental duration. Moss removal and N addition did not affect CH_4 emission in 2011 and 2012, but respectively declined CH_4 emission by 50 % and 66 % in 2013. However, moss removal and N addition did not produce an interactive effect on CH_4 emission. Specifically, moss removal plus N addition had no effect on CH_4 emission in 2011 and 2012, but decreased CH_4 emission by 68 % in 2013. These results suggest that the effects of moss removal and N enrichment on CH_4 emission are time-dependent in boreal peatlands, and also imply that increased N loading and decreased moss growth would independently inhibit CH_4 emission in the boreal peatlands of Northeast China.

1 Introduction

Methane (CH_4), as the second most important greenhouse gas after carbon dioxide, contributes 18 % to the overall global radiative force and is predicated to play an important role in determining future climate change (IPCC, 2007). Boreal peatlands are recognized as a primary natural source of atmospheric CH_4 and contribute one-tenth of total CH_4 emission to the atmosphere, despite covering a small area of the earth's surface (Wahlen, 1993; Moore et al., 1998; Baird et al., 2009). In boreal peatlands, CH_4 is produced by methanogens in the anaerobic layer and is then consumed by methanotrophs in the aerobic layer (Whalen, 2005). The amount of CH_4 released from

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peat to the atmosphere depends on the difference of CH₄-producing and CH₄-oxidizing processes in peat (Sundh et al., 1994). In boreal peatlands, CH₄ flux dynamics are influenced by soil temperature, water table position, substrate quality, microtopography and vegetation distribution (Bubier et al., 1995; Bellisario et al., 1999).

5 The moss layer is usually dominant in peatland ecosystems and is probably the only aerobic layer for CH₄ consumption before it enters to the atmosphere (Basiliko et al., 2004). Moss provides a good thermal layer for the underlying soils and may play a role in controlling CH₄ oxidation (Basiliko et al., 2004; Turetsky, 2004). About 90 % of the CH₄ produced in peat could be consumed in the moss layer and the soil (Bubier and
10 Moore, 1994; Whalen, 2005). It has been reported that the rate of CH₄ oxidation was > 0.2 μL mol CH₄ g dryweight⁻¹ h⁻¹ by submerged brown moss (Liebner et al., 2011). However, climate change inhibits moss growth and decreases moss production in boreal peatlands (Rustad et al., 2001; Limpens et al., 2011). This could influence the CH₄ emission from the boreal peatlands, given the important role of moss in CH₄ oxidation.
15 Human activities have already increased nitrogen (N) input to boreal ecosystems (Vitousek et al., 1997; Kaiser, 2001) and climate warming would further stimulate the soil N mineralization rate and increase N availability in soils (Rustad et al., 2001). Previous studies regarding the effects of increased N availability on CH₄ emission have yielded inconsistent results; some studies showed that increased N input increases
20 CH₄ emission (Saarnio et al., 2000; Granberg et al., 2001), and other studies found that N enrichment either decreased CH₄ emission (Granberg et al., 2001) or had no effect on CH₄ production and oxidation (Saarnio and Silvola, 1999). To accurately develop the CH₄ budget in boreal peatlands, further studies are needed to examine the effect of N enrichment on CH₄ emission.

25 Although previous studies have independently examined the effects of moss and N availability on CH₄ emission (Ferenci et al., 1975; Conrad, 1999; Riutta et al., 2007; Larmola et al., 2010), there is little information about the interactive effect of moss and N addition on CH₄ emission in boreal peatlands. Given the wide co-occurrence of declined moss growth and increased N availability in boreal peatlands, determining the

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effects of moss and N availability on CH₄ emission would help to better understand CH₄ dynamics, especially in light of future climate change. In this study, a field experiment was established in a boreal peatland in the Great Hinggan Mountain in Northeast China, and a three year (2011 to 2013) continuous observation was conducted to assess the effects of moss removal and N addition on CH₄ emission during the growing season of a boreal peatland.

2 Materials and methods

2.1 Study site

The research was conducted in a boreal peatland ecosystem located in the north of the Great Hinggan Mountain (52°56′ N, 122°52′ E, 457 m a.s.l.) in Northeast China. The study site is located in the continuous permafrost zone, and belongs to the cool continental climate (Miao et al., 2012). The mean annual precipitation (1991–2010) is ~ 450 mm with 45% falling from July to August, and the mean annual air temperature is ~ -3.9°C with monthly mean ranging from -31.9°C in January to 19.8°C in July. The soil of the study site is a typical peat soil and the depth of the peat layer ranges from 40 to 100 cm, with a mean soil bulk density of 0.16 g cm⁻³, pH of 5.0, soil organic carbon of 371.68 g kg⁻¹, and total N content of 17.2 g kg⁻¹ at 0–20 cm depth (Sun, 2012). The dominant plant species are *Betula fruticosa*, *Ledum palustre*, *Chamaedaphne calyculata*, *Vaccinium uliginosum*, *Rhododendron parvifolium*, *Eriophorum vaginatum*, *Sphagnum moss* and *Aulacomnium androgynum*. Hummocks are covered by continuous moss with some shrubs and occupy ~ 50% of the ground surface. Moss biomass ranges from 190 to 400 g m⁻².

2.2 Experiment design

A complete randomized block design with control (CK), moss removal (MR), N addition at 6 g N m⁻² yr⁻¹ (N) and moss removal plus N addition (MR × N) treatments was used.

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Each treatment was replicated three times, resulting in 12 50 × 50 cm plots. Plots were separated from adjacent plots by ~ 1 m buffer zones, to avoid horizontal movement and lateral loss of the added N. In 2011, plots were placed on flat hummocks with a *Sphagnum* moss-dominated community. Moss was removed by cutting the green part of the moss layer (~ 10 cm) in May from 2011 to 2013. The N was added as urea and applied twice a year (mid-May and mid-July). The urea was dissolved in 1 L purified water and sprayed. The control treatments were sprayed with 1 L purified water without N fertilizer.

2.3 CH₄ flux measurement

CH₄ emission was measured by static chamber and gas chromatography at 7 day intervals between 9 a.m. and 11 a.m. during the growing periods of 2011 to 2013. The removable open-bottom chambers (stainless steel, two small fans fixed symmetrically inside, 50 × 50 × 50 cm) were put on the base flumes (stainless steel, 50 × 50 × 30 cm) during sample collecting, and immediately removed after collection. The grooves (2 cm wide) of the base collar were filled with water to ensure gas tightness. Gas samples were taken at 0, 10, 20 and 30 min from the chamber headspace following closure by 60 mL plastic syringes attached to three-way stopcocks. Immediately, the samples were stored in 100 mL vacuum Tedlar[®] air sample bags, and analyzed within a week in the laboratory by modified gas chromatography (Agilent HP-7820A, USA), which was modified by adding an independent sample injector by the Institute of Atmospheric Physics, Chinese Academy of Sciences and equipped with a flame ionization detector. Details and configurations of the measuring system for analyzing concentrations of CH₄ and the associated method for calculating the flux have been described by Wang and Wang (2003) and Song et al. (2009). Where the linear regression with coefficients of determination (R^2) were < 0.8, the samples were rejected for CH₄.

2.4 Precipitation, soil moisture and soil temperature

Precipitation was measured by a rain gauge located near the experimental area. Soil moisture at 5 and 10 cm depth were recorded using a portable Time Domain Reflectometry instrument (Field Scout TDR-100, Spectrum Technologies Inc., Plainfield, IL, USA) in each plot. Soil moisture data recorded as % volumetric moisture content was measured during gas sampling from 7 July to 17 October in 2011, from 17 May to 12 October in 2012 and from 14 May to 22 September in 2013. Soil temperatures at 5 cm below the peat surface were collected in the center of each plot using the portable digital thermometer (JM 624, Jinming Instrument CO., Ltd, Tianjin, China).

2.5 Statistical analysis

The seasonal mean values were calculated by averaging the monthly mean values from May to October, and then it was multiplied by the number of experimental days and the CH₄-C transformed was the seasonal carbon(C) budget. A *p* value of < 0.05 was considered significant unless otherwise stated. Dependent variables were tested for normality by the Kolmogorov–Smirnov test, and were log-transformed when data were not following the normal distribution. One-way ANOVA was used to examine the differences in seasonal C budget among treatments, followed by Tukey's or Tamhane's multiple comparison test. Repeated measures of ANOVAs were used to examine the effects of sampling date, moss removal and N addition on CH₄ flux and soil moisture. In each year, two-way ANOVAs were used to assess the effects of moss removal, N addition and their interactions on CH₄ budgets. Linear regression analysis was conducted to examine the relationship between CH₄ flux and soil moisture or soil temperatures. All the statistical analyses were tested using SPSS package 16.0 (SPSS Inc., Chicago, IL, USA), and figures were conducted by Origin 8.0 (Origin Lab Corporation, USA) and SigmaPlot 12.0 (Systat Software, Inc. USA) for Windows.

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3 Results

3.1 Precipitation, soil moisture and soil temperature

Precipitation showed great annual variations during the sampling periods. The precipitation during the growing seasons of 2011 (496.2 mm) and 2012 (347.1 mm) was 28.8% higher and 9.9% lower than the 20 yr (1991–2010) mean annual value (385.4 mm), respectively, whereas total precipitation during the growing season in 2013 (621.4 mm) was much higher than the 20 yr mean annual value. Annual fluctuations in precipitation resulted in the highest soil moisture in 2013 ($p < 0.001$). Moss removal significantly increased soil moisture at 5 cm ($p < 0.001$) in all years, whereas N addition significantly decreased soil moisture ($p < 0.001$) in 2012 and 2013. Both moss removal and N addition significantly increased soil moisture ($p < 0.01$) in 2011, but significantly decreased ($p < 0.05$) it in 2013 (Table 1, Fig. 1b, e, and h). Moss removal significantly increased soil moisture at 10 cm ($p < 0.001$) in 2012, but significantly decreased ($p < 0.01$) it in 2013 whereas N addition significantly decreased ($p < 0.001$) it in both 2012 and 2013. Addition of N interacted with moss removal significantly increased ($p < 0.05$) soil moisture in 2011, but significantly decreased ($p < 0.001$) it in 2013 (Table 1, Fig. 1c, f and i). Soil temperature at 5 cm significantly increased under moss removal in 2011 ($p < 0.05$), whereas N addition showed no effect ($p > 0.05$) in all years. Moss removal and N addition produced a significant interaction on soil temperatures ($p < 0.01$) in 2012 (Table 1, Fig. 1a, d and g).

3.2 Effects of moss removal and N addition on CH₄ flux

Moss removal significantly reduced CH₄ emission by 50.4% in 2013 ($p < 0.05$, Table 1, Fig. 2c), but had no significant effects in 2011 and 2012 ($p > 0.05$, Table 1, Fig. 2a and b). The N addition showed a significant negative effect on CH₄ emission in 2013 (65.8%, $p < 0.05$), but did not significantly affect it in 2011 and 2012 ($p > 0.05$). However, moss removal and N addition did not produce an interactive effect on CH₄

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emission during the whole sampling periods (Table 2). Moss removal and N addition decreased CH₄ emission by 68.5 % in 2013, but had no effect in 2011 and 2012.

There were substantial annual variations in CH₄ emission ($p < 0.001$). In the control plots, the average CH₄ emission rate during the growing season was 1.89 mgCH₄m⁻²h⁻¹ in 2013, which was 800% higher than those in 2011 (0.21 mgCH₄m⁻²h⁻¹) and 2012 (0.21 mgCH₄m⁻²h⁻¹) (Fig. 2). The CH₄ flux significantly varied with sampling date during the three growing seasons ($p < 0.001$, Table 1). The N addition interacted with the sampling date to significantly affect CH₄ flux in 2013 ($p < 0.05$). Similarly, N addition significantly interacted with moss removal or sampling date to affect CH₄ flux ($p < 0.05$). However, moss removal and sampling date did not produce an interaction on CH₄ flux ($p > 0.05$, Table 1).

Across the three growing seasons, CH₄ flux decreased linearly with increased soil temperatures ($p < 0.05$, Fig. 4a), and showed a positive linear dependence on soil moisture at 5 and 10 cm ($R^2 = 0.22$, $p < 0.01$, Fig. 4b; $R^2 = 0.39$, $p < 0.01$, Fig. 4c). In all 12 treatments, CH₄ flux was positively and linearly correlated with soil moisture at 5 cm in both 2012 ($R^2 = 0.32$, $p < 0.01$, Fig. 5e) and 2013 ($R^2 = 0.20$, $p < 0.05$, Fig. 5h), and soil moisture at 10 cm in both 2012 ($R^2 = 0.30$, $p < 0.01$, Fig. 5f) and 2013 ($R^2 = 0.54$, $p < 0.01$, Fig. 5i), but negatively and linearly with soil temperatures at 5 cm in 2012 ($R^2 = 0.33$, $p < 0.01$, Fig. 5d).

4 Discussion

Moss removal and N addition were found to have no effects on CH₄ emission in 2011 and 2012, but to significantly decrease CH₄ emission in 2013. These results imply that the effects of moss removal and N addition on CH₄ emission vary with experiment duration and suggest that long-term studies are needed to accurately develop the CH₄ budget in boreal peatlands.

In this study, moss removal had no effects on CH₄ emission in 2011 and 2012, and produced a negative effect in 2013. Methanogens produced CH₄ in strictly anaerobic

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condition and were limited by substrate availability (Yavitt et al., 2012). Moss removal not only decreased substrate availability for methanogens in soil and moss mats (Riutta et al., 2007), but also declined soil moisture status (Fig. 1), which may shift the peats from anaerobic to aerobic conditions (Amaral and Knowels, 1995). Moreover, Larmola et al. (2010) observed that methanotrophy were less frequent on high hummocks, and the oxidation rates were not detectable because of the dry moss layer for evaporation. Therefore, moss removal would decrease CH₄ emission in boreal peatlands. The result from this study showed that the effect of moss removal on CH₄ emission was time-lagged in boreal peatlands.

Similar to moss removal, N addition had no effects on CH₄ emission in 2011 and 2012, but inhibited CH₄ emission in 2013 in the boreal peatland of the study. Previous studies also found that N addition effects on CH₄ emission were inconsistent. Saarnio et al. (2000) and Granberg et al. (2001) reported that N input increased CH₄ emission in the peatland. In contrast, Granberg et al. (2001) showed that N enrichment decreased CH₄ emissions in a fen. In addition, Saarnio and Silvola (1999) found no response of CH₄ production and oxidation to N addition. Moss and vascular plants intercepted the added N in the initial two years, which made N unavailability to soil microbes (Nordin et al., 1998; Saarnio and Silvola, 1999; Bobbink et al., 2010). Hence, N addition did not affect CH₄ emission in 2011 and 2012 in the boreal peatland. The subsequent negative effect of N addition on CH₄ emission in 2013 was explained by the following mechanisms. Firstly, N addition reduced decomposition rate in peat soil, which may decrease substrates for methanogenesis (Williams and Silcock, 1997). Secondly, N addition inhibited methanogenesis, due to competition for hydrogen with some microbes and toxicity of denitrification products to the methanogens (Conrad, 1999). Thirdly, N addition promoted CH₄ oxidation by methanotrophs (Bodelier et al., 2000; Bodelier and Laanbroek, 2004).

Notably, moss removal and N additions did not produce an interactive effect on CH₄ emission in the boreal peatland of this study. CH₄ emission depended on the balance among methanogenesis, CH₄ oxidation and CH₄ transport. In peatlands, methanogen-

esis and CH₄ oxidation were controlled mainly by soil temperature, soil moisture (or water table below the surface) and substrates (Yavitt et al., 2012). In this study, the mechanisms that controlled the combined effects of moss removal and increased N input on CH₄ emission in boreal peatland ecosystem have not been fully elucidated.

5 Nevertheless, the results suggest that moss removal and N addition independently affect CH₄ emission in boreal peatlands.

The mean CH₄ budget in the control plots ranged from 0.39 gCm⁻² in 2011 to 4.49 gCm⁻² in 2013 (Fig. 3), and varied substantially with annual precipitation over the study period in boreal peatlands. These results imply that altered precipitation regimes and increased extreme weather would exert profound influences on CH₄ emission in boreal peatlands in the context of global climate change.

5 Conclusion

In this study, we simultaneously assessed the impact of moss removal and N enrichment on CH₄ emission were simultaneously assessed during the growing seasons of 2011 to 2013 in a boreal peatland of Northeast China. Both moss removal and N addition did not affect CH₄ emission in 2011 and 2012, but suppressed it in 2013. Moreover, moss removal and N addition did not produce an interactive effect on CH₄ emission. These results suggest that the effects of moss removal and N addition on CH₄ emission are time-dependent, and long-term studies are needed to accurately develop knowledge of CH₄ emission in boreal peatlands in the context of global climate change. Meanwhile, these results also imply that moss removal and N addition independently suppressed CH₄ emission in boreal peatlands in Northeast China.

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Table 1. Results (*F* values) of Repeated measures ANOVAs on the effects of N addition (N), moss removal (MR), sample times and their interactions on CH₄ flux (gCH₄m⁻²h⁻¹), soil temperature (°C) at 5 cm depth, soil moisture (%) at 5 cm depth and soil moisture (%) at 10 cm depth.

	CH ₄ fluxes			Soil T at 5 cm depth			Soil M at 5 cm depth			Soil M at 10 cm depth		
	2011	2012	2013	2011	2012	2013	2011	2012	2013	2011	2012	2013
N	2.62	1.54	7.59 ^a	1.69	0.34	4.39	4.89	93.98 ^c	250.60 ^c	0.39	52.66 ^c	306.82 ^c
MR	3.24	1.94	5.42 ^a	5.91 ^a	1.20	4.05	89.02 ^c	70.13 ^c	120.58 ^c	4.58	37.91 ^c	27.82 ^b
MR × N	0.03	0.001	2.57	0.35	18.00 ^b	1.07	17.94 ^b	0.16	10.23 ^a	5.39 ^a	1.39	54.69 ^c
Time	6.13 ^c	5.88 ^c	36.62 ^c	608.31 ^c	393.16 ^c	21.65 ^b	3.73 ^a	18.80 ^c	52.31 ^c	5.40 ^b	16.95 ^c	59.72 ^c
Time × N	1.61	0.53	7.46 ^a	2.28	1.10	9.19 ^a	0.81	7.31 ^c	6.68 ^c	2.47	4.96 ^b	10.48 ^c
Time × MR	1.47	0.81	3.61	2.87 ^a	4.83 ^b	0.51	2.29	4.74 ^b	24.61 ^c	0.74	4.67 ^b	8.02 ^b
Time × N × MR	0.54	0.20	5.01 ^a	1.35	4.42 ^b	0.59	1.34	2.00	6.62 ^c	3.16	1.42	4.27 ^a

^a, ^b, and ^c represent significant at $p < 0.05$, 0.01, and 0.001, respectively.

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Table 2. Results (F values) of two-way ANOVAs on the effects of N addition (N), moss removal (MR) and their interactions on CH₄ flux ($\text{gCH}_4 \text{m}^{-2} \text{h}^{-1}$).

	2011	2012	2013	average
N	2.16	1.22	18.34 ^b	11.78 ^b
MR	3.87	1.65	5.64 ^a	5.56 ^a
MR × N	0.01	0.001	4.02	2.10

^a and ^b represent significant at $p < 0.05$ and 0.01 respectively.

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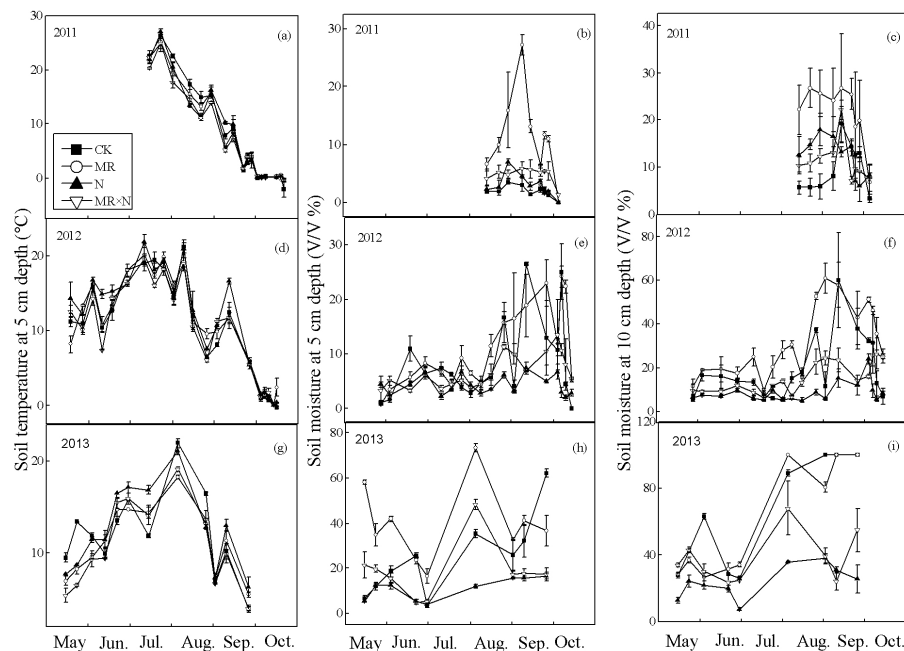


Fig. 1. Temporal dynamics of soil temperature at 5 cm depth (**a, d, g**), soil moisture at 5 cm depth (**b, e, h**) and soil moisture at 10 cm depth (**c, f, i**) during the growing seasons in 2011, 2012 and 2013. Data are daily averages for per treatment (\pm SE, $n = 3$). CK, control; MR, moss removal; N, N addition; MR \times N, moss removal plus N addition.

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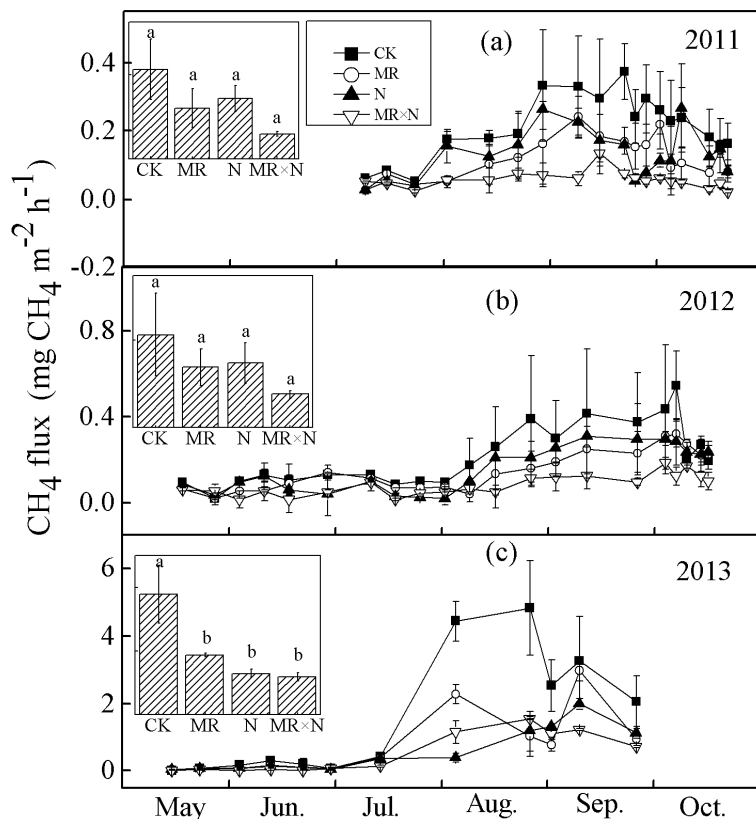


Fig. 2. Temporal dynamics of CH₄ flux during the growing seasons in 2011, 2012 and 2013. Data are daily means (\pm SE, $n = 3$). Insets represent the seasonal means. CK, control; MR, moss removal; N, N addition; MR \times N, moss removal plus N addition. Different lowercase letters indicate significant differences among treatments ($p < 0.05$).

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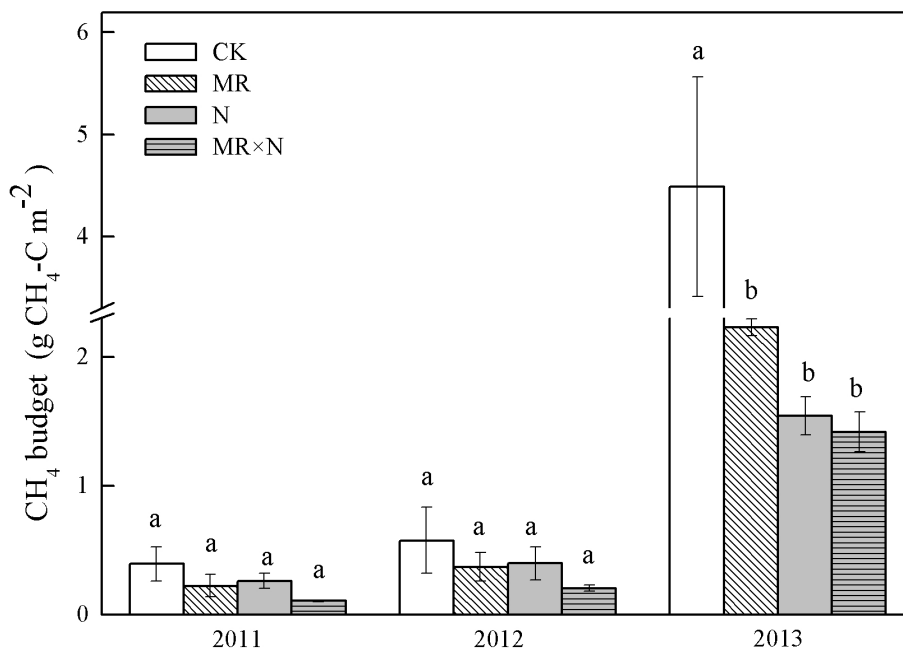


Fig. 3. Annual CH₄ budget in 2011, 2012 and 2013. Error bars represent standard errors ($n = 3$). CK, control; MR, moss removal; N, N addition; MR × N, moss removal plus N addition. Different lowercase letters indicate significant differences among treatments ($p < 0.05$).

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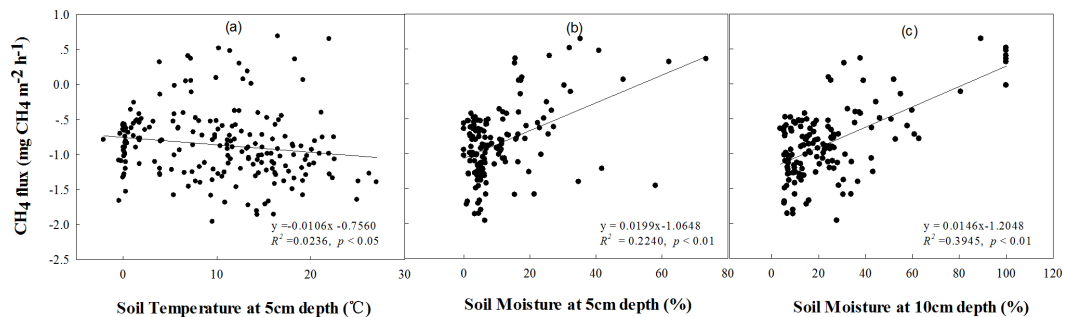


Fig. 4. Temporal dependence of CH₄ flux on soil temperature (a) and soil moisture (b, c) across the three growing seasons.

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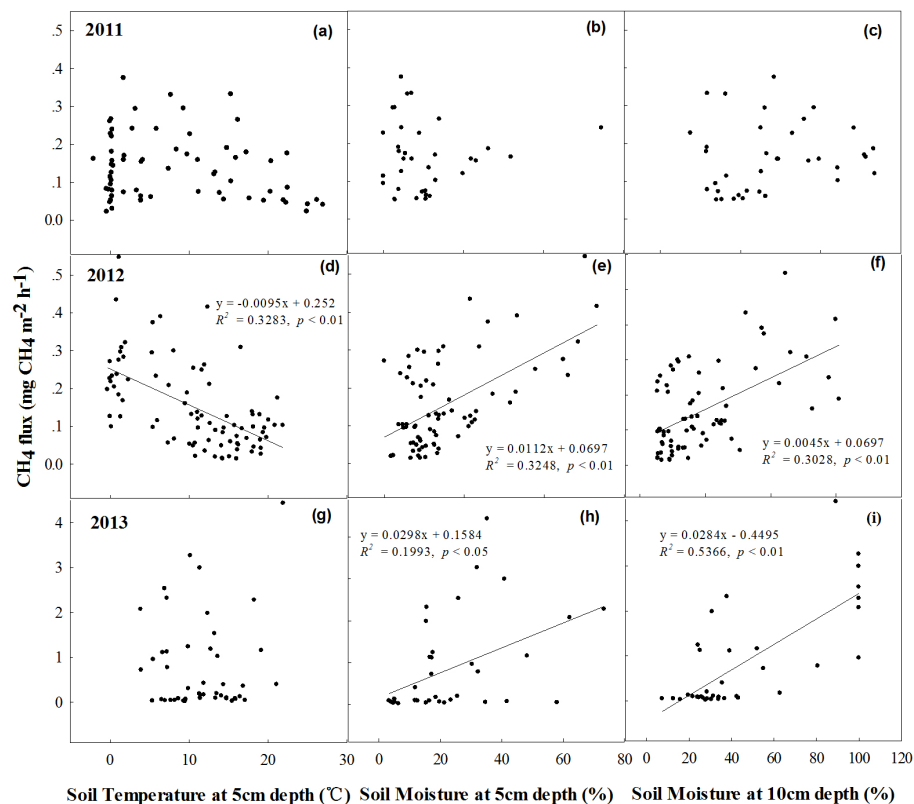


Fig. 5. Spatial dependence of seasonal mean CH₄ flux and soil temperature at 5 cm depth (a, d, g), soil moisture at 5 cm depth (b, e, h) and soil moisture at 10 cm depth (c, f, i), respectively.

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