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Low-Level Nitrogen Addition Promotes Net Methane Uptake in a Boreal Forest across the Great Xing'an Mountain Region, China

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Chronic nitrogen (N) deposition may alter the amount and direction of methane (CH_4) uptake in boreal forest soils, but the critical level of N deposition eliciting the alteration of soil CH_4 uptake remains unknown. In a cold-temperate coniferous forest in the Great Xing'an Mountain of northeastern China, NH_4NO_3 fertilizer was added at four rates: control (0 kg N ha^{-1} $year^{-1}$), low N (10 kg N ha^{-1} $year^{-1}$), medium N (20 kg N ha^{-1} $year^{-1}$), and high N (40 kg N ha^{-1} $year^{-1}$). Soil atmospheric CH_4 fluxes as well as soil temperature, soil moisture, mineral N, dissolved organic carbon (DOC), and pH values were determined to explore the main factors controlling soil CH_4 uptake fluxes under different N addition levels. Our results showed that N addition did not alter soil temperature, soil moisture, and soil NH_4^+-N concentrations. Low N rather than medium and high N significantly increased soil DOC and NO_3^--N concentrations in mineral horizon as well as soil CH_4 uptake fluxes. In addition, soil acidification occurred in the fertilized forest soils, because soil pH declined by 0.4 unit. CH_4 uptake in the cold-temperate forest soil was dominated by soil moisture followed by soil DOC and soil mineral N. Low N seemed to stimulate soil CH_4 uptake through increasing the contents of soil DOC and NO_3^--N. These results suggest that low-level N addition (< 20 kg N ha^{-1} $year^{-1}$) can promote CH_4 uptake in the cold-temperate coniferous forest soil, which is also conducive to carbon sequestration of the boreal forests.

Keywords: atmospheric N deposition, CH_4 uptake, soil mineral N, controlling factors, cold-temperate coniferous forest

Methane (CH_4) is the second most important greenhouse gas after CO_2 in the atmosphere and has a global warming potential more than 20 times greater than that of CO_2 across a 100-year time scale (Solomon 2007). The concentration of atmospheric CH_4 reached 1.774 ppm in 2005 and has increased at a rate of about 0.9% per year since the 1800s (Solomon 2007, Van Huissteden et al. 2008), contributing up to 15–20% to global warming effects (Dutaur and Verchot 2007). In general, aerobic soils are identified to be the only biological sink for atmospheric CH_4 and the annual uptake by soils is estimated to be 36 ± 23 Tg, which is equal to the annual increase in the atmospheric CH_4 pool (Borken and Brumme 2009). Aerobic forest soils have an ability to strongly oxidize atmospheric CH_4 and contribute ~7% to the global atmospheric CH_4 sink (Smith et al. 2000, Wang and Ineson 2003, Aronson and Helliker 2010).

Forest soil CH_4 uptake is a biologically mediated process. Soil methanogenic bacteria use small molecule carbon compounds to produce CH_4 under anaerobic conditions, which soil methanotrophs can oxidize into CO_2 (Mancinelli 1995). Soil atmospheric CH_4 uptake fluxes are determined by the balance between CH_4 production and oxidation within soil profiles. Soil properties including soil moisture, soil temperature, soil texture, soil acidity, and

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nitrogen (N) availability can affect the CH_4 uptake fluxes from forest soils (Borken and Brumme 2009). CH_4 uptake fluxes from forest soils are highly regulated by the availability of soil mineral N, including enhancement (Maljanen et al. 2006), no change (Whalen and Reeburgh 2000, Saari et al. 2004), and inhibition (Steudler et al. 1989, Adamsen and King 1993, Gulledge et al. 2004, Ambus and Robertson 2006, Galloway et al. 2008, Zhang et al. 2008). The increases in both soil NH_4^+ and NO_3^- concentrations induced by N deposition inhibits soil CH_4 oxidation and uptake (Steudler et al. 1989, Gulledge et al. 1997, Wang and Ineson 2003, Reay and Nedwell 2004). However, the relative contributions of soil NH_4^+ and NO_3^- accumulation to CH_4 uptake fluxes from forest soils are unclear, and the underlying mechanisms are not well understood (Bodelier and Laanbroek 2004).

The boreal forest, the second largest forest biome after the tropical forest, accounts for 14.5% of the earth's continental surface (Gower et al. 2001). Furthermore, well-drained boreal forest soils are important in atmospheric CH_4 uptake (Whalen et al. 1991). In the boreal forest soils, N is primarily bound in organic matter and mineral N content generally accounts for less than 1% of total soil N content (Saari et al. 2004, Fang et al. 2010). Moreover, rates of N mineralization are very slow in boreal forest soils as a result of low soil temperature and low soil pH as well as poor-quality litter (Fang et al. 2010). Whether chronic low-level N deposition will alter the CH_4 uptake rates in boreal forest soils remains controversial. For example, N input at a rate of 140 kg $(NH_4)_2SO_4$-N ha^{-1} had no impact on CH_4 sink strength in an Alaskan boreal forest soil, which was consistent with a similar observation from a 3-year NH_4NO_3 addition experiment at a rate of 60 kg N ha^{-1} $year^{-1}$ in a spruce forest (Gulledge et al. 1997, Whalen 2000). Conversely, continuous NH_4NO_3 addition in a spruce forest soil for three growing seasons led to an increased CH_4 uptake rate in situ of 16.3% (Maljanen et al. 2006). To some degree, these contrary results may reflect different responses of soil methanotrophs to low- and high-dose N fertilizer addition (Whalen and Reeburgh 2000). Therefore, it is necessary to better understand the responses of CH_4 uptake in boreal forest soils to chronic N deposition (Whalen and Reeburgh 2000, Galloway et al. 2008).

So far, the effects of N addition on CH_4 uptake from the boreal forest soils in Northeast China have not been well documented, and the relation of soil CH_4 flux to soil environmental variables under increasing N regimes is not well understood. In this study, we report the early responses of soil CH_4 uptake to low-level N addition in a cold-temperate coniferous forest. Our specific aims were to investigate the effects of different N addition levels on soil properties and CH_4 uptake and to explore the main factors controlling soil CH_4 uptake under different N addition levels.

Materials and Methods

Site Description

This study was conducted at the Genhe Boreal Forest Station in Inner Mongolia (50°49′−50°51′ N, 121°30′−121°31′ E). The station is located on the western slope of the Great Xing'an Mountains, with an average elevation of 826 m and an average slope of <3°. This site is characterized by its cold-temperate humid climate with a mean annual temperature of −5.4° C and mean annual precipitation (MAP) of 580 mm. Rain accounts for 80% of MAP and mainly occurs from May to September. Snowfall ranges from 20 to 40 cm, occurring from the end of September to early May, accounting for 20% of the total MAP. The vegetation type is cold-temperate coniferous forest with stand age of about 200 years, dominate species being *Larix gmelinii*, *Betula platyphylla*, *Ledum palustre*, *Rhododendron simsii*, and *Vaccinium vitis-idaea*. Soils are classified as a haplic podzol (International Union of Soil Science Working Group 2006) developed on granite parent material. The average thicknesses of the organic and mineral layers are 10 and 20 cm, respectively. Soil physical and chemical properties for the organic and mineral layers are summarized as follows: soil organic matter contents range from 10 to 30%, total N contents range from 2.9 to 4.7 g kg^{-1}, total phosphorus contents range from 0.5 to 1.1 g kg^{-1}, pH values range from 4.5 to 6.5, and soil bulk density ranges from 0.15 to 0.74 g cm^{-3} (Fang et al. 2010).

Experimental Design

The N addition experiment was a randomized complete block design with three replicates. Referring to the atmospheric N deposition rate (8.5 kg N ha^{-1} $year^{-1}$) (Lü and Tian 2007) in the Great Xing'an Mountain region, ammonium nitrate (NH_4NO_3) fertilizer was added as four rates: control (0 kg N ha^{-1} $year^{-1}$), low N (10 kg N ha^{-1} $year^{-1}$), medium N (20 kg N ha^{-1} $year^{-1}$), and high N (40 kg N ha^{-1} $year^{-1}$). The three treatments were set to simulate the changes in carbon (C) and N cycles in the boreal forest ecosystems under the scenarios of atmospheric N deposition being increased by 1, 2, and 4 times in the future. Each plot was 20 m by 10 m, with a 10-m buffer zone being set between plots. During the growing season (May−October), 95.2, 190.4, and 380.8 g of NH_4NO_3 fertilizers were weighed and dissolved in 40 L of water and then were evenly sprayed onto each low, medium, and high N plot at the first of each month, respectively. Control plots were treated with equal amounts of water, which is equivalent to an increase in annual precipitation of 1.2 mm.

Measurements of Soil Temperature, Moisture. and CH_4 Uptake Fluxes

The net exchange flux of soil CH_4 was measured 3 times per month using a static chamber and gas chromatograph technique. A chamber (50 cm in length, 50 cm in width, and 40 cm in height) and a collar (50 cm in length, 50 cm in width, and 10 cm in height) made from stainless steel was placed at each plot. The collar was inserted into the ground, to which an insulating polystyrene material was used to cover the chamber to avoid a rapid increase in chamber temperature. Soil CH_4 flux measurements started in early June, and all gas samples were collected between 9:00 and 11:00 am (China Standard Time) (Wang and Wang 2003). After the chamber was closed, gas samples were taken using a 100-ml plastic syringe at intervals of 0, 10, 20, and 30 min at each plot. All gas samples were then injected into a gas chromatograph (Agilent GC-7890A, equipped with a stainless steel column (⅛ outside diameter by 2 m long) packed with 13X molecular sieve (60/80 mesh) and a flame-ionization detector (detector temperature, 250° C; oven temperature, 55° C; carrier gas, N_2 at 30 ml min^{-1}; burning gas, purified H_2 at 30 ml min^{-1}, and synthetic air, 400 ml min^{-1}) to determine CH_4 concentrations within 24 hours. The soil CH_4 uptake rates were calculated according to Wang and Wang (2003). In addition to gas sampling, we also measured atmospheric temperature, both inside the chamber and 10 cm below ground using a JM624 electronic thermometer (JinMing Instrument Co., Ltd.), and measured soil water content in the upper 10-cm horizon using a TDR200 soil moisture meter (Spectrum Technologies).

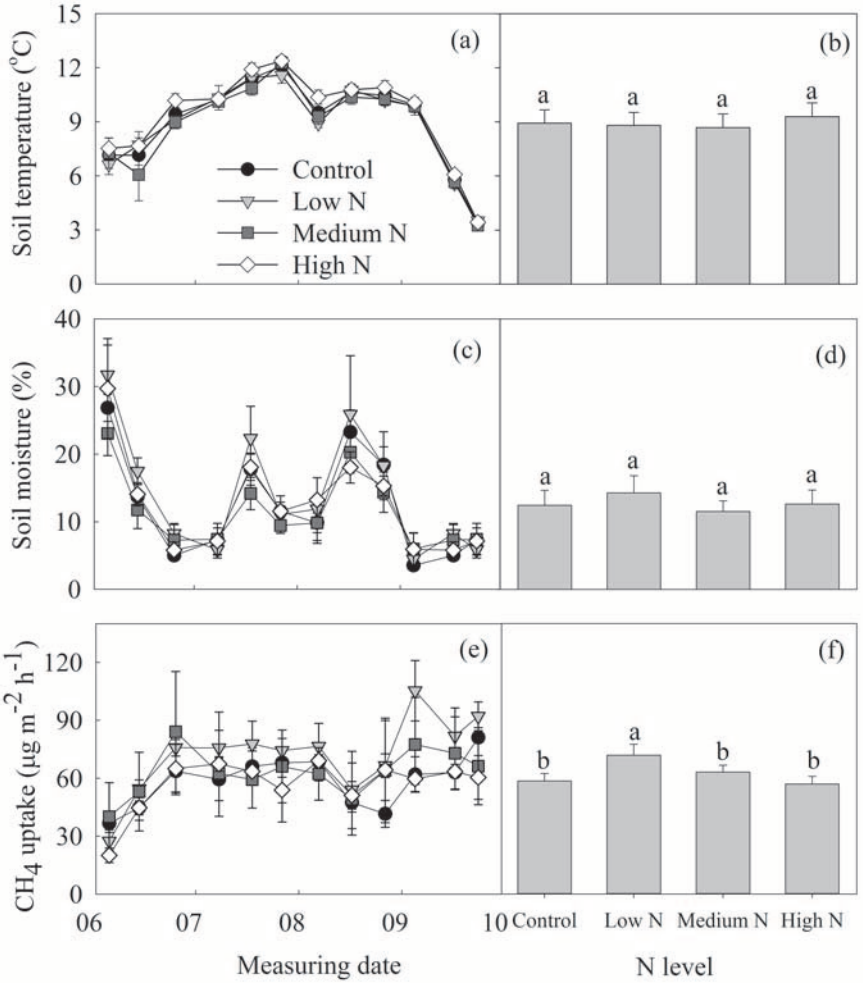

Figure 1. Monthly variations in soil temperature (a and b), soil moisture (c and d), and soil CH_4 fluxes (e and f) and their responses to N addition levels. The data in columns represent the means and SEs of the whole growing season. Different letters above the columns indicate significant differences between N levels.

Soil Sampling and Analysis

On the 15th day of each month during the growing season of 2010, soil samples in organic (Oe/Oa) and mineral horizons (A) were collected. After the surface organic horizon was removed, mineral soil samples were taken using a 2.5-cm diameter corer to the depth of 10 cm at each plot and were transported to the laboratory in chilled polystyrene boxes. In the laboratory, soil samples were sieved through a 2-mm sieve to remove stones and roots and were then stored at 4° C before analysis. Soil NH_4^+-N and NO_3^--N concentrations were determined by colorimetry on a continuous flow AA3 AutoAnalyzer (Bran Luebbe) (Fang et al. 2012). Soil dissolved organic matter was extracted as described previously by Jang et al. (2011), and then its C concentration was measured using the FlashEA 1112 total organic carbon analyzer (Elementa). Soil pH values in the 0–10 cm mineral horizon were determined using a pH meter (S20K; Mettler Toledo) with the soil/water ratio of 1:2.5.

Statistical Analyses

Repeated-measures multivariate analysis of variance (ANOVA) was applied to examine the difference in soil properties and soil CH_4 uptake fluxes for different levels of N addition. Comparisons of the means were conducted using Tukey's honestly significant difference test. Standardized regression analysis was used to analyze the relationships between soil CH_4 uptake fluxes and soil properties including soil temperature, moisture, mineral N, dissolved organic carbon (DOC), and pH with the N addition levels as categorical variables (Fang et al. 2012). All statistical analyses were performed using SPSS software (version 16.0; SPSS, Inc.). In addition, SigmaPlot software (version 10.0) was applied for statistical graphics. Statistical significant differences were accepted at $P < 0.05$ unless otherwise stated.

Results

Soil Temperature, Moisture, and CH_4 Uptake Fluxes

There were significant seasonal variations in 0–10 cm soil temperature and soil moisture ($P < 0.001$) (Figure 1a–d; Table 1). The peak soil temperature in 0–10 cm depth occurred at the end of July (Figure 1a). N addition did not alter the average soil temperature, which ranged from 8.7° C at low N plots to 9.3° C at high N plots ($P = 0.15$) (Figure 1b; Table 1). Three peaks in soil moisture content occurred in early June, mid-July, and mid-August, corresponding to the periods of snowmelt in June and rain events in July and August, respectively (Figure 1c). There was no significant difference in soil moisture among N addition treatments ($P = 0.46$) (Table 1).

The cold-temperate coniferous forest soil was a net sink for atmospheric CH_4 during the growing season, with the measured CH_4 uptake fluxes ranging from 20.0 to 105.3 μg CH_4 m^{-2} h^{-1}

Table 1. Results of repeated-measures ANOVAs on the effects of month, N level, and their interactions on soil CH_4 flux and soil variables.

	P value									
					Soil NH_4^+-N		Soil NO_3^--N		Soil DOC	
Source of variation	CH_4 flux	Soil temperature	Soil moisture	Soil pH	Original	Minimal	Original	Minimal	Original	Minimal
Month	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	0.17	0.001	<0.001	0.72
N level	0.032	0.15	0.46	<0.001	0.86	0.14	0.31	0.005	0.001	0.02
Month × N level	0.66	0.99	0.94	<0.001	0.43	0.73	0.67	0.99	0.37	0.98

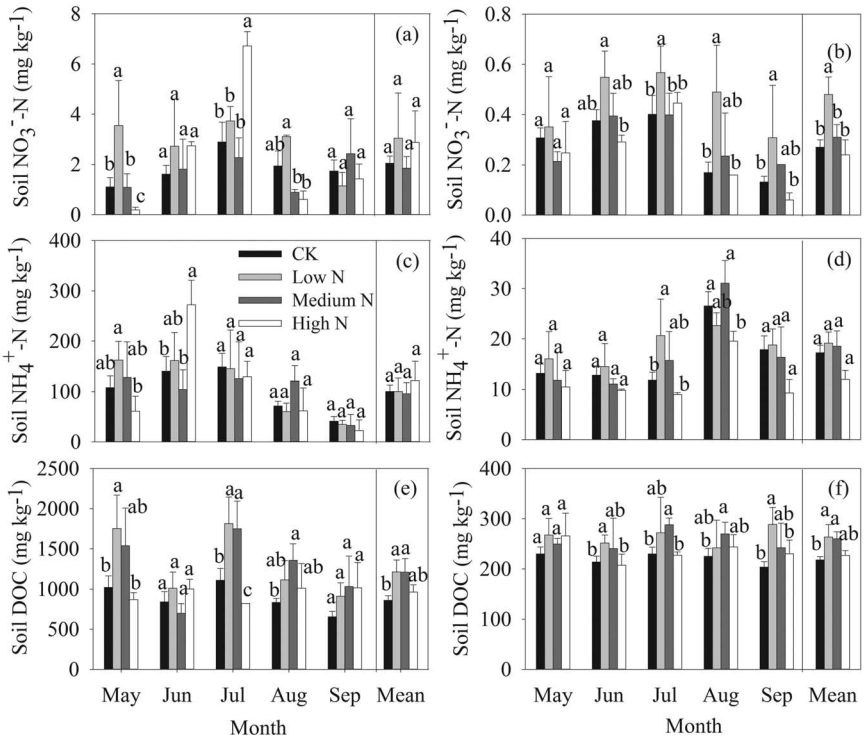

Figure 2. Monthly variations in soil mineral N and DOC concentrations at the organic (a, c, and e) and mineral horizons (b, d, and f) and their responses to N addition levels. Different letters above the columns indicate significant differences between N levels.

(Figure 1e). The seasonal variation in soil CH_4 uptake fluxes was significant ($P < 0.001$) (Table 1), and the maximum and minimum fluxes occurred in early September and early June, respectively (Figure 1e). In addition, a significant difference was detected in the soil CH_4 uptake fluxes among the N addition treatments ($P = 0.032$) (Table 1), with the greatest CH_4 uptake flux in the low N treatment (Figure 1f).

Soil Mineral N and DOC Concentrations

With the exception of soil NO_3^--N concentrations in organic horizon, soil NO_3^--N and NH_4^+-N concentrations exhibited significant monthly variations ($P = 0.001$, $P < 0.001$, and $P = 0.001$) (Figure 2a–d; Table 1) in both soil horizons. Furthermore, mineral N concentrations spiked in July or August, time points corresponding to high soil temperature. In the control plots, the averages of soil NO_3^--N and NH_4^+-N concentrations were 2.1 and 100.4 mg kg^{-1} in the organic horizon and 0.3 and 17.2 mg kg^{-1} in the mineral horizons, respectively (Figure 2a–d). More than 97% of mineral N in the boreal soil was NH_4^+-N. In the first growing season of N addition, low N rather than medium or high N significantly increased soil NO_3^--N concentrations in the mineral horizon by 78% ($P = 0.005$) (Figure 2b; Table 1). However, there was no difference in NH_4^+-N concentration for all N treatments ($P = 0.86$ and $P = 0.14$) (Figure 2c–d; Table 1).

Soil DOC concentrations in the organic horizon showed a significant monthly variation with the maximum occurring in July ($P < 0.001$) (Figure 2e–f; Table 1). At the control plots, the averages of soil DOC concentrations were 859.2 and 218.0 mg kg^{-1} in organic and mineral horizons, respectively (Figure 2e–f). N addition significantly increased soil DOC concentration in both organic and mineral horizons, and the differences between low N, medium N, and control were significant ($P = 0.001$ and $P = 0.02$, respectively) (Figure 2e–f; Table 1). The increases in DOC induced by N addition ranged from 11.9 to 41.1% in the organic horizon and from 4.1 to 20.9% in the mineral horizon (Figure 2e–f).

Soil pH Values

Multivariate ANOVA results indicated that both month and N addition level significantly affected soil pH values, with a significant interaction between month and N level ($P < 0.001$) (Table 1). Soil pH values in the 0–10 cm mineral horizon ranged from 4.9 to 5.2, with an average of 5.0. Interestingly, we found that N addition led to significant soil acidification, with medium and high N reducing

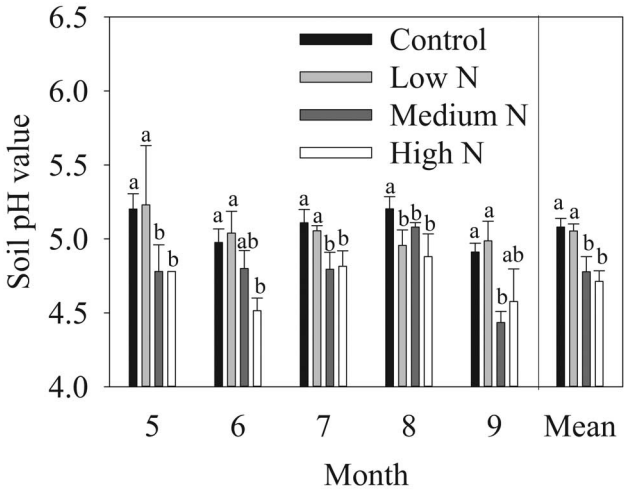

Figure 3. Monthly variations in soil pH values at 0–10 cm mineral horizon and their responses to N addition levels. Different letters above the columns indicate significant differences between N levels.

Table 2. Regression models between soil CH_4 fluxes and environmental variables based on data pooled by all N addition levels.

Environmental variable	Equation	r^2	*P* value
10 cm Ts	$F_{CH4} = 222.57 - 37.05Ts + 1.99Ts^2$	0.301	<0.001
10 cm Ms	$F_{CH4} = 78.41 - 1.69Ms$	0.393	<0.001
Soil NH_4^+-N content	$F_{CH4} = 64.23 - 0.10NH_4^+$	0.236	0.001
Soil NO_3^--N content	$F_{CH4} = 56.60 + 1.58NO_3^-$	0.213	0.003
Soil DOC content	$F_{CH4} = 52.19 + 0.012DOC$	0.204	0.004
Soil pH value	$F_{CH4} = 196.73 - 27.62$ pH	0.244	<0.001
Combined	$F_{CH4} = -0.44Ms - 0.36NH_4^+ + 0.24NO_3^- + 0.37DOC + 0.29$low N	0.543	<0.001

Ts soil temperature; Ms, soil moisture.

soil pH values in mineral horizons ($P < 0.001$) (Figure 3). The mean soil pH value at high N plots decreased by about 0.4 unit compared with the control (Figure 3).

Relationships Between Soil CH_4 Uptake Fluxes, and Soil Properties

The correlation between soil CH_4 uptake fluxes and soil temperature could be characterized using a quadratic equation; the critical temperature at the negative apex of the regression was about 9° C ($r^2 = 0.301$, $P < 0.001$) (Table 2; Figure 4a). Soil CH_4 fluxes were negatively correlated with soil moisture, pH, and NH_4^+-N contents ($r^2 = 0.393$, 0.244, and 0.236, respectively, $P < 0.001$) (Table 2; Figure 4b–d), and positively associated with soil DOC and soil NO_3^--N concentrations ($r^2 = 0.213$ and 0.204, respectively, $P < 0.01$) (Figure 4e–f). All significant continuous variables and categorical variables explained more than 54.3% of the variation in soil CH_4 uptake fluxes (Table 2). Multiple stepwise regression analysis revealed that soil CH_4 uptake fluxes were mainly controlled by soil moisture followed by DOC and mineral N (Table 2). Taking N levels as categorical variables, we found that soil CH_4 uptake fluxes were positively correlated with low N and not related to medium and high N.

Discussion

Effects of N Addition on Soil CH_4 Uptake Fluxes

The rate of atmospheric N deposition in the boreal forest area of the Great Xin'an Mountain was estimated to be 8.5 kg N ha^{-1} $year^{-1}$, which was slightly higher than the rates of boreal forests in Scandinavia, Canada, and Alaska (Lü and Tian 2007). Low-level N deposition input increased the N availability of the boreal forest and then affected other soil properties and soil CH_4 uptake. During the growing season, the cold-temperate coniferous forest soil in the Great Xing'an Mountain region was a significant sink for atmospheric CH_4 with the average magnitude of 58.49 μg CH_4 m^{-2} h^{-1}. We did not measure soil CH_4 fluxes in the nongrowing season because of very low temperature and soil freezing. One of our previous studies had shown that gas exchange between soil and atmosphere was weak in winter (Fang et al. 2010). Most studies reported that elevated atmospheric N deposition or fertilization inhibited atmospheric CH_4 oxidation and decreased net CH_4 uptake in forest soils (Castro et al. 1995, Sitaula et al. 1995, Steinkamp et al. 2001, Gulledge et al. 2004, Chan et al. 2005, Maljanen et al. 2006, Zhang et al. 2008, Basiliko et al. 2009). Three mechanisms have been proposed for the partial inhibition of CH_4 uptake by well-drained soils in response to increased N inputs: competitive inhibition of CH_4 monooxygenase; toxic inhibition by hydroxylamine (NH_2OH) and nitrite (NO_2^-) produced via NH_4^+ oxidation; and osmotic stress due to high concentrations of NO_3^- and/or NH_4^+ (Schnell and King 1994, Bradford et al. 2001, Bodelier and Laanbroek 2004, Reay and Nedwell 2004). However, in our study N addition slightly promoted soil CH_4 uptake fluxes. Our results were consistent with findings of some other studies from boreal forests (Whalen and Reeburgh 2000, Saari et al. 2004, Maljanen et al. 2006, Basiliko et al. 2009). For example, Whalen and Reeburgh (2000) reported that high doses of N fertilization application (140–580 kg NH_4^+-N ha^{-1} $year^{-1}$) did not alter CH_4 uptake rates in the boreal forest soil in Alaska, USA. A mechanism proposed to explain this is that the available N concentration in the ecosystem did not meet the threshold of atmospheric N deposition in the high latitudes of the northern hemisphere (Whalen and Reeburgh 2000). Saari et al. (2004) found that 27 years of continuous N addition did not reduce the CH_4 uptake fluxes from the Norway spruce forest soil. Both the in situ measurements of soil CH_4 uptake fluxes and ex situ laboratory incubation showed that N addition with NH_4NO_3 led to increased CH_4 oxidation and uptake in an N-poor boreal forest soil by more than 16.3% (Maljanen et al. 2006). Therefore, we expected that chronic N deposition in boreal forests in the Great Xing'an Mountain region was conductive to increasing CH_4 uptake. Unfortunately, the inherent mechanisms responsible for promoting CH_4 uptake in N-poor boreal forest soils remain unclear.

Recent evidence shows that soil NH_4^+-N contents were negatively correlated with soil CH_4 uptake fluxes (Zhang et al. 2008, Kim et al. 2012), whereas soil NO_3^--N content had either positive or negative effects on soil CH_4 uptake (Jang et al. 2006, Fang et al. 2010). In our study, N addition did not significantly accumulate soil NH_4^+-N, but significantly increased soil NO_3^--N. It was likely that soil NH_4^+ was assimilated by plants (preferred N form) and microbes and oxidized to NO_3^-. Furthermore, soil CH_4 uptake fluxes were negatively and positively correlated with soil NH_4^+-N and NO_3^--N contents, respectively. The results suggested that the promotion of low N and inhibition of NH_4^+ of soil CH_4 uptake fluxes were partly attributed to the accumulation of soil NO_3^--N.

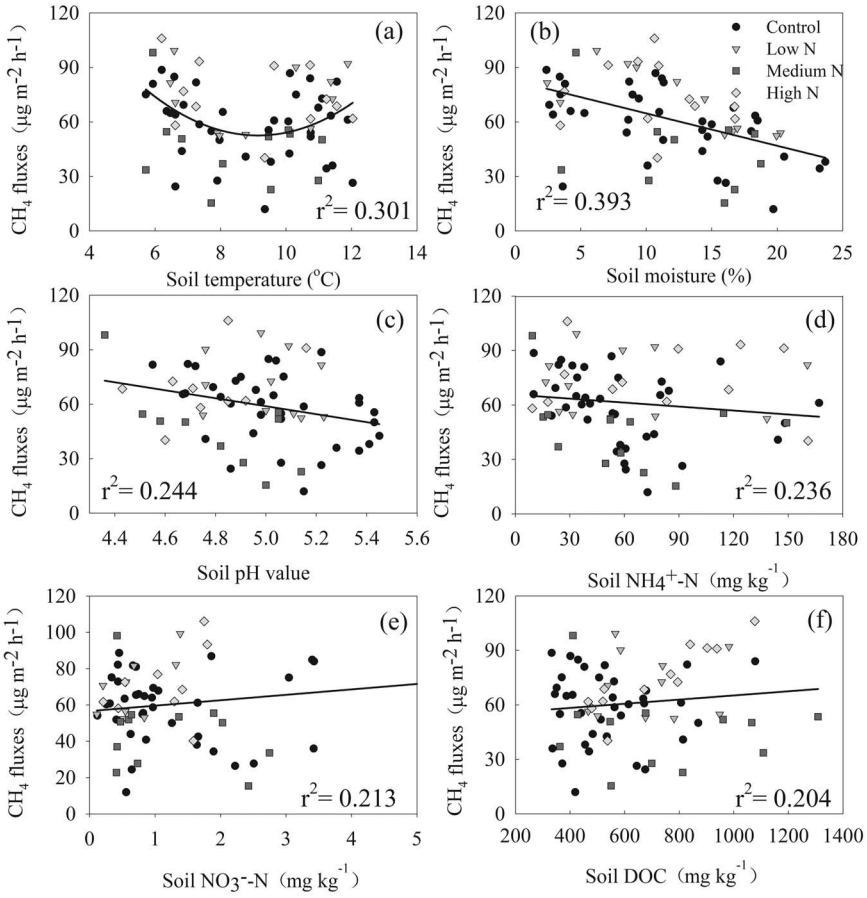

Figure 4. Correlations between soil CH_4 uptake fluxes and environmental variables.

Plant root uptake and soil microbial utilization of NH_4^+-N prevented significant accumulation of soil NH_4^+-N, and inhibition of NH_4^+ on soil CH_4 uptake did not occur. Whalen and Reeburgh (2000) also concluded that N inputs did not influence CH_4 uptake until they significantly increased soil N availability in boreal forest soils. Unfortunately, few studies to explore the promotion of soil NO_3^--N accumulation on the soil CH_4 uptake in N-limiting boreal forests have been conducted. The possible mechanisms responsible for it could be attributed to two aspects (Bodelier and Laanbroek 2004, Bodelier 2011): the NO_3^--N relieves N limitation of cell growth and subsequently increases the activity of the methanotrophic community; and NO_3^--N accumulation interferes more directly with the synthesis of involved enzymes in the CH_4 oxidation pathway of nitrogen-starved cells. In general, NO_3^--N has been found to be inhibitory only at very high concentrations, which probably gives rise to osmotic effects (Bodelier and Laanbroek 2004). Overall, we suggest that the promotion of soil nitrate accumulation on soil CH_4 uptake was indirect. Soil nitrification consumes soil NH_4^+-N and subsequently accumulates soil NO_3^--N in mesic condition, and subsequently no NH_4^+-N accumulation favors soil CH_4 uptake in boreal forests (Fang et al. 2010).

Other Factors Controlling Soil CH_4 Fluxes

Forest soil CH_4 uptake combines soil CH_4 production and consumption processes and is influenced by many factors such as substrate availability, soil temperature, soil moisture, soil pH, soil nutrients, and vegetation type (Whalen and Reeburgh 2000). Several studies show that soil moisture content is a major factor controlling CH_4 production and oxidation (Steudler et al. 1989, Koschorreck and Conrad 1993, Van den Pol-van Dasselaar et al. 1998, Stein and Hettiaratchi 2001, Dijkstra et al. 2010, Kim et al. 2012) and is negatively correlated with soil CH_4 uptake in boreal, temperate, and subtropical forest soils (Bowden et al. 1998, Zhang et al. 2008, Fang et al. 2010). N fertilizer application generally increases plant primary production and ecosystem evapotranspiration (Sonnleitner et al. 2001) and can increase rather than decrease soil moisture, depending on ecosystem types (Inouye 2006, Fang et al. 2012). Soil moisture alters soil CH_4 production and oxidation rates through changing soil aeration, redox potential, and diffusion properties of CH_4 and O_2 (Hütsch et al. 1994). In addition, soil moisture can indirectly affect soil CH_4 uptake through altering the composition of soil methane oxidizing bacteria (MOB). When CH_4 is high during a period of high soil moisture, the MOB can grow on the higher CH_4 and are likely to be stimulated by higher N availability. When CH_4 returns to atmospheric levels, the number of MOB utilizing atmospheric CH_4 is higher, resulting in higher rates (Bodelier 2011). However, N addition did not change soil moisture in our study. Although soil CH_4 fluxes in the boreal forest were dominated by the effects of soil moisture, the changes in soil CH_4 uptake elicited by N addition could not be caused by changes in soil moisture.

Soil CH_4 uptake was related to the optimal soil temperature when the diffusion rates of CH_4 and O_2 from the atmosphere into the soil are equal to soil CH_4 and O_2 consumption (Cai and Yan 1999, Fang et al. 2010). The optimal temperature for soil CH_4 oxidation varies with latitude: about 20–30° C in low-latitude

regions (Boeckx and VanCleemput 1996, Cai and Yan 1999), 5–25° C in middle-latitude regions (Castro et al. 1995), and >10° C in high-latitude regions (Van den Pol-van Dasselaar et al. 1998). On the contrary, our results showed that soil CH_4 uptake fluxes were positively and negatively related to soil temperature before and after the critical value, respectively, and the soil CH_4 uptake was lowest when the temperature was 9° C. Similarly, Castro et al. (1995) observed that temperature was a strong controlling factor of soil CH_4 consumption at low temperatures, but that CH_4 consumption became independent of soil temperature between 10 and 20° C. This may be explained by the shift in the CH_4 and O_2 diffusion potentials or the competition between soil methanotrophs and soil nitrifiers in different soil temperature ranges (Maljanen et al. 2003, Fang et al. 2010). King and Adamsen (1992) convincingly point out that atmospheric CH_4 uptake by soils is controlled by substrate supply rather than enzyme limitation; hence, a limited temperature effect is noted. In our study, the multiple regression analyses suggested that soil temperature was not the main factor controlling CH_4 uptake in the boreal forest soil.

The soil DOC content may affect soil CH_4 uptake and depends on the input from litter decomposition and rhizo-deposition and the output from leaching and microbial utilization (Park and Matzner 2003). DOC is an important organic substrate for methanogens, and the CH_4 they produce provides the substrate for methanotrophs. Therefore, DOC can both negatively and positively affect soil CH_4 uptake. Our results showed that low and medium N significantly increased soil DOC contents in organic and mineral horizons, which was consistent with some results from N manipulation experiments across temperate and subtropical forests (Fang et al. 2009, Xu and Inubushi 2009). For the N-limited boreal forest, N addition largely stimulated the litter decomposition and microbial C substrate utilization, which had been confirmed by the changes in soil CO_2 fluxes resulting from N addition (Wendu et al. 2012). In addition, we found that the soil DOC concentration had positive effects on CH_4 uptake in the well-drained boreal forest soil. Similarly, Jacinthe and Lal (2006) reported that the oxidation rates of CH_4 in the reclaimed grassland soils were positively correlated with both water-extracted organic C and NH_4^+. Using stepwise linear regression, Kim et al. (2012) also found that soil organic carbon content was the second most important factor controlling soil CH_4 uptake after soil moisture. The accumulation of soil DOC induced by N addition could increase substrate availability for methanogens to produce more CH_4 (Segers and Kengen 1998, Bradford et al. 2001). Because the soil CH_4 oxidation rate is generally dominated by the CH_4 concentration in soil profile, the increasing CH_4 concentration will lead to an increased rate of CH_4 oxidation (Dalal et al. 2008).

Finally, soil acidification could also be a driving factor for the changes in soil CH_4 uptake under N addition. According to previous studies, forest soil methanotrophs have an optimal pH ranging from 5.0 to 6.5, and the ability of soil methanotrophs to oxidize atmospheric CH_4 is significantly reduced when pH is <4.0 (Le Mer and Roger 2001, Semenov et al. 2004, Xu and Inubushi 2009). In this study, although medium and high N significantly reduced soil pH values by 0.2 and 0.4 unit, respectively, the pH range for soil CH_4 oxidation was still within the optimal pH range. Therefore, the contribution of soil pH to soil CH_4 uptake was less than those of other factors, and pH variation was not the main factor causing changes in soil CH_4 uptake that resulted from N addition.

Based on the multiple regression results, soil moisture, DOC, and inorganic N were the dominant factors that controlled soil CH_4 uptake fluxes. These factors only explain about half of the variability in soil CH_4 uptake. Other factors such as soil porosity, soil compactness, and thickness of the organic layer could also affect CH_4 uptake in the boreal forest soil (Borken and Brumme 2009). Under the scenarios of increasing N deposition, the availability of soil dissolved C and mineral N played more important roles in regulating soil CH_4 uptake in the boreal forest. Furthermore, the molecular mechanisms involved in regulating the effects of experimental N deposition on soil methanotrophic and methanogenic communities should be explored in the future.

Conclusions

A low-level N addition experiment was conducted to evaluate the effects of atmospheric N deposition on soil properties and soil CH_4 uptake fluxes in the boreal forest of the Great Xing'an Mountain region. During the first growing season, we found that N addition with NH_4NO_3 did not significantly lead to soil NH_4^+-N accumulation, because low N rather than high N tended to increase soil NO_3^--N and DOC contents. The critical level of N input for the alteration of the soil properties and soil CH_4 uptake is about 20 kg N ha^{-1} $year^{-1}$ when the ambient atmospheric N deposition rate is considered. In addition, CH_4 uptake in the cold-temperate coniferous forest soil was dominated by soil moisture content followed by soil DOC and mineral N concentrations. It seemed that low-level N addition promoted soil CH_4 uptake fluxes through increasing soil NO_3^--N and DOC concentrations. The fact that N addition promotes CH_4 uptake implies that the boreal forest may remove more CH_4 from the atmosphere under future increased nitrogen deposition conditions.

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