Response of CH_4 and CO_2 fluxes to simulated nitrogen deposition in a boreal forest in the Northeastern China

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Keywords: Nitrogen deposition; Soil properties; CH_4 uptake; CO_2 emission; boreal forest; Northeastern China.

Abstract. It is not clearly understand that the effects of changes in nitrogen (N) deposition on soil carbon (C) fluxes in boreal forests. Moreover, little is known about how the temporal pattern (daytime and nighttime) has impacted on soil carbon fluxes. To investigate these effects, 12 plots (20 m×20 m) were established in boreal forests in May 2012. Four NH_4NO_3 treatments (three replicates) were applied. The CH_4 uptake and CO_2 emission rates were measured daily and soil properties were simultaneously observed. The results showed that N deposition tended to restrain soil CH_4 uptake but significantly promoted soil CO_2 emission (except T_H). There were significant difference in soil CO_2 and CH_4 fluxes between daytime and nighttime. In addition, N deposition had significantly impacted on soil properties. These results suggest that a longer study period is needed to verify effects of increasing N deposition on soil C fluxes and soil properties.

Introduction

Carbon dioxide (CO_2) and methane (CH_4) affect the Earth's climate through the absorption of solar infrared radiation in the lower atmosphere. Since 1958, the annual mean concentration of CO_2 has increased from a pre-industrial value of 280-379 ppm in 2005, 390 in 2011, and to above 400 ppm in 2013 [1-3], and for the past decade (2005-2014), the average annual increase was 2.1 ppm per year. Thus, the atmospheric concentration of CO_2 has increased significantly since the Industrial Revolution, and it has been suggested that this increase has effect on climate variability [3-4]. Similarly, atmospheric CH_4 has monotonically increased due to anthropogenic input to the atmosphere. Since the Industrial Revolution, the concentration of CH_4 has increased from 715 ppb to 1803 ppb by 2011(Fig.1) [3, 5]. Although the concentration of CH_4 in the atmosphere is significantly lower than that of CO_2, CH_4 highly affects the climate system, reflecting a global warming potential of 25 times higher than that of CO_2. Thus, CO_2 and CH_4 make substantial contributions to global warning.

The forest ecological system regulates the concentration of greenhouse gases, and previous studies have shown that forest soil is a source for CO_2 and a sink for CH_4 [6-7]. CO_2 emission occurs through root respiration, plant decomposition, and microorganism respiration, and CH_4 is produced through methanogens and consumed through methanotrophs in the soil [8]. The emission of CO_2 and uptake of CH_4 in the soil are a complicated biological process, jointly influenced through various biotic and abiotic factors [9-10], such as species composition and diversity, soil temperature, N status, moisture and so on. Among the various factors affecting the CH_4 uptake and CO_2 emission rate, the effects of N deposition have been arisen concerns [11]. The N deposition in

terrestrial ecosystems has significantly increased, primarily reflecting anthropogenic activities, such as fertilizer application and fossil fuel use [12-13]. However, it remains unknown whether nitrogen deposition promotes or inhibits CH_4 uptake. Chronic N deposition increases NH_4^+ and NO_3^- availability in the forest soil, thereby changing the activity and composition of microorganisms that affect CH_4 uptake [14]. Increases in inorganic N input to forest ecosystems inhibit methanotrophic bacteria involved in CH_4 oxidation [11,15,16], and this mechanism could potentially compete with soil NH_3 with soil CH_4 for the use CH_4 monooxygense [17], and indirect effects of applied N and associated salt ions through osmotic stress could inhibit methanotrophic bacteria involved in CH_4 oxidation [18]. Not all studies support the observation that elevated atmospheric N deposition reduces forest soil CH_4 uptake [19]. However, N deposition could decrease, increase or have no effect on soil CH_4 uptake in forest ecosystems, due to forest types [20], N fertilizer types and doses [21], and multiple environmental factors. Soil respiration is the efflux of CO_2 from the Earth's surface, and this process is typically divided into autotrophic respiration by live roots and associated mycorrhizae, and heterotrophic respiration by microbes that oxidize plant detritus, root exudates and humified organic matter [22-23]. The increase of N availability exerts great influences on plant growth, species composition and diversity, thereby altering CO_2 fluxes. Bowden et al. (2004) [9] reported an immediate change in soil CO_2 flux following the addition of N. The addition of nitrogen to soil typically promotes CO_2 emission and decreases CH_4 uptake [24].

In addition, latest studies have shown that altering multiple environmental conditions significantly changes soil CO_2 emission and CH_4 uptake [25]. Temperature and moisture are likely to play important roles in regulating CO_2 and CH_4 efflux rates. In temperate or cold regions, variations in CH_4 uptake have been correlated with soil temperature [26]. The soil moisture content also shows correlations between wetter sites and decreased CH_4 uptake or increased CH_4 release. The effect of soil moisture content has been studied in many incubation experiments [27-28], under both low and high soil water content, showing that CH_4 uptake and CO_2 emission might be suppressed through the restriction of diffusive CH_4 and root respiration and physiological water stress. Previous studies have shown that soil temperature was dominant environmental factor influencing variations in soil CO_2 and CH_4 fluxes [29-30], and soil moisture has been demonstrated as a second independent variable [31].

In recent decades, a great number of site-specific research studies on the effects of N deposition on CO_2 and CH_4 fluxes have been conducted in China. However, little is known about how the temporal pattern (daytime and nighttime) has impacted on soil CH_4 uptake and CO_2 emission. However, we hypothesized that low-levels of N addition promoted CO_2 emission and high-levels of N addition inhibited CO_2 emission, and CH_4 was significantly decreased with the N addition levels. We also hypothesized that the temporal pattern (daytime and nighttime) has impacted on soil CH_4 uptake and CO_2 emission, and daytime. The aim of the present study was to determine the impact of N deposition on atmospheric CH_4 uptake and soil CO_2 emission, and the impact of temporal pattern on CH_4 uptake and CO_2 emission in boreal forest soil.

Materials and Methods

Site description

The experiment was conducted in a Larch-birch forest, located in Nanwenghe National Natural Reserve (51° 05'-51° 39' N, 125° 07'-125° 50' E) in the Greater Hinggan Mountains of Northeastern China. This area has a cool continental climate with long, cold winters and short, warm summers. The mean annual temperature is -2.4 °C, with a mean maximum monthly temperature of 18.6°C in July and a mean minimum monthly temperature of -26.3 °C in January. The mean annual precipitation is 489.2 mm, distributed in a distinct seasonal pattern, with 80% annual precipitation falling from June to September. The soil type is stony-to-sandy loam, and the plant community is dominated with *Larix gmelini* and *Betula platyphylla Suk*.

Experimental design

To investigate changes in soil CH_4 and CO_2 fluxes following N application, we established three random blocks, each comprising four plots of 20 m×20 m separated by 10 m wide buffer strips to avoid the horizontal movement of the added N. The addition of N was initiated at the onset of the experiment, and included four treatments, control (CK, no added N), low N (T_L, 2.5 g $N.m^{-2}.yr^{-1}$), medium N (T_M, 5 g $N.m^{-2}.yr^{-1}$) and high N (T_H, 7.5 g $N.m^{-2}.yr^{-1}$), with three latticed replicates of each treatment [32-33], in total 12 experimental plots. Dilute ammonium nitrate (NH_4NO_3) solution was applied to the forest floor every two weeks during the growing season (from May to October) since May 15, 2012. In each plot, fertilizer was mixed with 32 L of water (equal to 0.08 mm annual precipitation) and applied below the canopy using a backpack sprayer. Two passes were made across each plot to ensure an even distribution of the fertilizer. The control plots received 32 L of water without N addition. Three polyvinyl chloride (PVC) collars (Soil collar) were randomly installed into each plot, and a total of 36 PVC soil collars were installed.

Measurement of soil CH_4 and CO_2 fluxes

CO_2 and CH_4 fluxes were measured using a greenhouse gas analyzer (CH_4, CO_2, H_2O) (GGA-24p, Los Gatos Research (LGR), 67 East Evelyn Ave, Mountain View, CA 94041-1529, United States), based on off-axis integrated-cavity output spectroscopy configured for high-flow operation and 10 Hz sampling rates. The PVC soil collars were inserted 5 cm from the ground surface into the soil, and the living plants were removed more than 24 hours before the measurements were taken to minimize the effects of the disturbance caused by plant respiration. To understand the dynamics of greenhouse gases, CH_4 and CO_2 were continuously measured at each soil collar from July 26, 2014 to August 16, 2014. A lead battery was installed to provide electrical power to the GGA and facilitate continuous data collection. Simultaneously, the soil temperature at 5 cm below the soil surface was monitored to continuously measure the hourly rate of CH_4 and CO_2 fluxes using an Em-50 data logger (Decagon Devices, Inc. USA). However, there were significant different in soil temperature and no significant different in soil moisture between daytime and nighttime in our test site, and soil moisture was not monitored.

Soil variables

During the measurement of soil CH_4 and CO_2 fluxes, soil samples were synchronously collected at six randomly selected points from each experimental plot using a 5 cm diameter stainless-steel corer. The soil samples collected at depths of 0 to 10 cm and 10 to 20 cm comprised a mixture of six samples from the same layer of the same treatment, in total 72 soil samples. Samples were immediately sieved (<2 mm) to remove visible extraneous materials (roots, gravel and stones), and then divided into two parts. One part was maintained fresh for the measurement of soil inorganic-N concentrations (NH_4^+-N and NO_3^--N). NH_4^+ contents were analyzed using the indophenol blue method and NO_3^- contents were determined using the Phenol two sulfonic acid colorimetric method. The other was air-dried at ambient temperature, soil total organic C (TOC) and total N contents were measured using a N/C analyzer (Multi N/C 2100, Analytik Jena, Germany). Soil total P contents were determined using the sodium bicarbonate alkali digestion method and molybdenum antimony colorimetry [34]. Moreover, soil pH values were measured using a pH meter (SX7150, China).

Statistical analysis

All statistical analyses were performed using R 3.1.1 (R Development Core Team 2014). One-way ANOVA with Tukey's HSD test was used to examine the differences of soil NH_4^+-N, soil NO_3^--N, soil TOC, soil total N, soil pH, CH_4 uptake and CO_2 fluxes between control and treatments. Pearson's correlation coefficient was used to investigate the linear impact of 5 cm soil temperature on soil CH_4 uptake and CO_2 emission at each sampling occasion. Correlation analyses and linear regression analyses were used to examine the relationships between soil CO_2 emission rates and CH_4 uptake rates. Statistically significant differences were set at $p < 0.05$, unless stated otherwise.

Results

Soil properties

The mean soil temperature was not significantly different between the control and N deposition treatments in the same temporal pattern (Table 1). However, there was significant difference in soil temperature between daytime and nighttime (Table 1). Soil temperature in nighttime was significantly lower than that of daytime (Table 1). In measurement period, N deposition decreased slightly soil pH values in the levels of N deposition, and significant differences were detected between the control and the treatments in 0-10 cm soil depth (Table 2). Moreover, the soil pH values were increasing with soil depth, but no significant differences between the control and N deposition plots in 10-20 cm soil depth (Table 2).

Table 1, Comparison of the CO_2 and CH_4 fluxes (umol $m^{-2}s^{-1}$) in response to increasing N deposition. Values followed by the same letter in the same column or row are not significantly different at $p < 0.05$

Treatment	CO_2		CH_4		Soil temperature (5 cm)	
	daytime	nighttime	daytime	nighttime	daytime	nighttime
CK	6.59±0.12Ac	5.35±0.07Bc	2.13e-03±6.82e-05Aa	2.11e-03±1.35e-04Ba	12.99±0.26Aa	12.54±0.12Ba
T_L	12.28±0.14Aa	11.20±0.14Ba	1.97e-03±6.52e-05Ab	1.86e-03±1.26e-04Bb	12.99±0.17Aa	12.40±0.09Ba
T_M	8.48±0.08Ab	7.08±0.06Bb	1.93e-03±5.15e-05Ab	1.85e-03±7.76e-05Bb	12.15±0.11Ab	11.58±0.17Bb
T_H	6.18±0.09Ad	5.37±0.06Bc	1.64e-03±4.16e-05Ac	1.54e-03±1.54e-04Bc	12.10±0.17Ac	11.77±0.24Bb

Soil total N and TOC in T_L and T_M were consistently lower in the 0-10cm soil depth compared with the control, and soil total N was not significantly different and soil TOC intended to increase in T_H compared with the control (Table 2). Soil total P intended to decrease in all N treatments, but there was no significantly difference in 0-10 cm soil total P contents among various treatments (Table 2). Soil total N, TOC and total P concentrations were decreasing with soil depth from 0-10cm to 10-20cm soil depth.

Table 2, Effects of N deposition on soil properties. Values followed by the same letter in the same row are not significantly different at $p < 0.05$.

Treatment	Soil depth	CK	T_L	T_M	T_H
Total N content ($g.kg^{-1}$)	0-10 cm	2.51±0.54a	2.16±0.43a	2.27±0.25a	2.42±0.37a
	10-20 cm	1.84±0.30a	1.71±0.43a	1.88±0.34	1.79±0.46a
TOC content ($g.kg^{-1}$)	0-10 cm	33.17±2.06a	24.25±2.29a	27.98±1.86a	36.78±2.75a
	10-20 cm	17.18±1.37a	17.80±1.85a	14.49±2.11a	15.71±2.11a
Total P content ($mg.g^{-1}$)	0-10 cm	0.67±0.10a	0.57±0.17a	0.60±0.13a	0.59±0.08a
	10-20 cm`	0.54±0.12a	0.50±0.17a	0.53±0.13a	0.48±0.11a
NO_3^--N content (mg/kg)	0-10 cm	7.52±0.55a	6.75±0.48a	5.80±0.87a	5.75±0.35a
	10-20 cm	2.96±0.80a	3.85±0.24a	3.25±0.15a	2.52±0.47a
NH_4^+-N content (mg/kg)	0-10 cm	29.35±0.92c	30.62±0.92b	32.12±0.44b	35.33±0.78a
	10-20 cm	25.70±0.52a	27.82±0.58a	21.20±0.79a	25.15±0.72a
pH value	0-10 cm	5.15±0.17a	5.04±0.12b	4.95±0.13c	4.94±0.13c
	10-20 cm	5.34±0.10a	5.11±0.13a	5.25±0.24a	5.33±0.20a

In study site, NO_3^--N contents decreased with N deposition, but NO_3^--N concentrations did not significantly different between the control and N treatments plots in 0-10cm soil depth (Table 2). However, N-addition increased NO_3^--N contents in the mineral soil (10-20cm soil depth) in the T_L and T_M treatments, and contrary to T_H (Table 2). N deposition significantly increased soil NH_4^+-N contents with increasing levels of N addition in 0-10cm soil depth, and different levels of N deposition did not significantly impact on NH_4^+-N concentrations in mineral layers (10-20cm soil depth). Otherwise, a significantly variations in soil pH values were found in the control and treatments plots (Table 2). T_L treatments significantly decreased soil pH values, while decrease of soil pH values by T_M and T_H treatments was more significant in 0-10cm soil depth. The effects of N addition on pH values of 10-20cm soil depth were no significant in various N deposition levels.

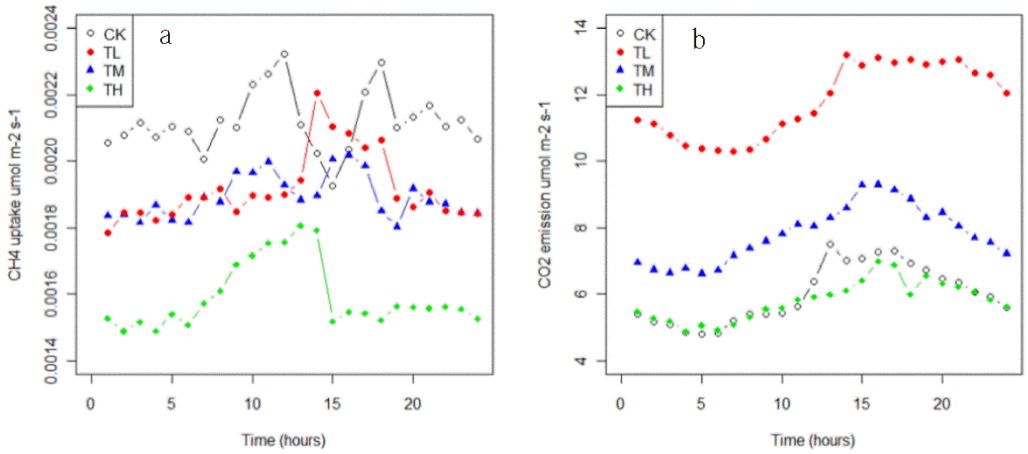


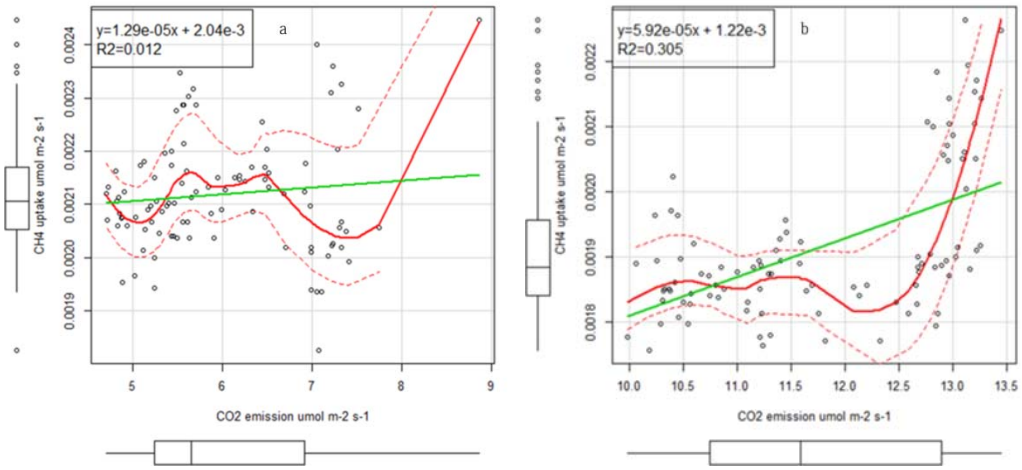
Figure 1, Daily variations in soil CH_4 (a) and CO_2 (b) fluxes (Nanwenghe Reserve) during the growing season in August 2014. Monthly applications of NH_4NO_3 were initiated in August 2012 and continued thereafter. The error bars are eliminated for clarity.

Soil CH_4 fluxes

Throughout the measurement period, daily variations in the CH_4 flux (Fig. 1a) were observed in a boreal forest during the growing period in August 2014. Significant differences in CH_4 fluxes between control and N-treatment soils were detected. In the treatments plots, the CH_4 flux varied as a single-peak pattern, and the maximum CH_4 uptake occurred at approximately 2:00 p.m (Fig. 1a). The N addition inhibited soil CH_4 uptake, particularly in treatments with high levels of N. Repeated measures ANOVA showed a significant negative effect of N on soil CH_4 uptake between control and treatment conditions, but no significant difference was observed between T_L and T_M. Moreover, the rates of CH_4 uptake were significant difference between daytime and nighttime (Table 1).

Soil CO_2 fluxes

N fertilization significantly decreased CH_4 uptake and increased CO_2 emission, except T_H (Fig. 1b). Daily variations in CO_2 fluxes showed single peak during the measurement period (Fig. 1b). Significantly differences in daytime soil CO_2 effluxes between control and treatments were observed, showing 6.59±0.12, 12.28±0.14, 8.48±0.08, and 6.18±0.09 umol CO_2 m^{-1} s^{-1} for CK, T_L, T_M and T_H, respectively. Moreover, there were significantly different between the control and treatments in nighttime soil CO_2 fluxes (Table 1). However, the rates of soil CO_2 emission were significantly difference between daytime and nighttime (Table 1). In addition, treatment with NH_4NO_3 at 2.5 and 5 g $N.m^{-2}.yr^{-1}$ increased soil CO_2 emission 186% and 129% compared with the control in the daytime, respectively (Table 1). However,



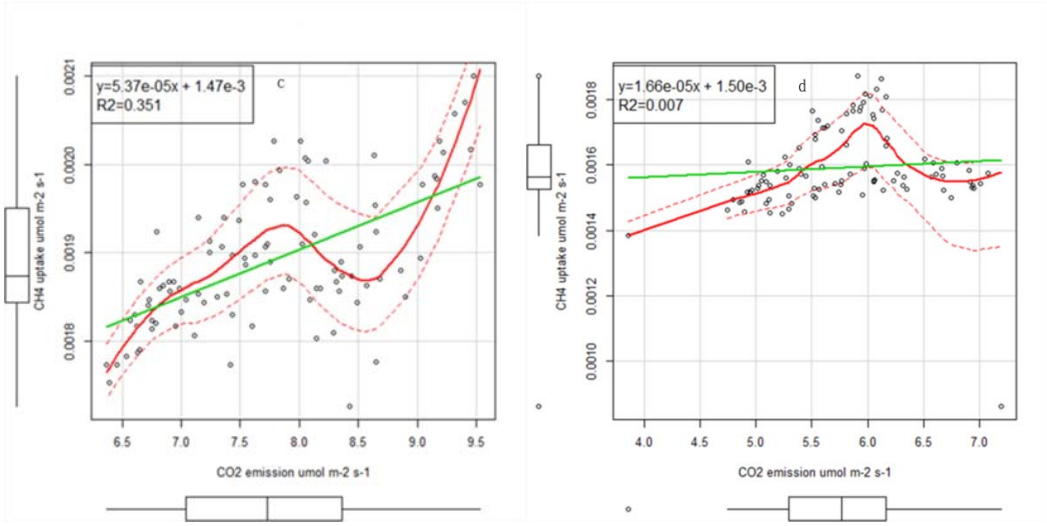


Figure 2. Relationship between soil CH_4 uptake and CO_2 emission. (a): the correlation between rates of soil CH_4 uptake and rates of soil CO_2 emission in CK; (b): in T_L; (c): in T_M; (d): in T_H.

no significant difference in the rate of CO_2 flux was observed in control plots after treatment with NH_4NO_3 at 7.5 g $N.m^{-2}.yr^{-1}$ in the nighttime, and contrary to daytime (Table 1). These results indicated that low-level N deposition might significantly promote soil CO_2 emission and high-level N deposition might inhibit CO_2 emission in boreal forest soils. However, we observed significant differences in CO_2 emission between the treatments.

Relationships between soil CH_4 and CO_2 fluxes

In the present study, soil CH_4 uptake rates were positively correlated with soil CO_2 emission rates, particularly for treatments at 2.5 g $N.m^{-2}.yr^{-1}$ (T_L) and 5 g $N.m^{-2}.yr^{-1}$ (T_M) (Fig. 2). The effects of N addition on soil CH_4 uptake and CO_2 emission dynamics in boreal forests are not well understood. Therefore, we simultaneously measured the CH_4 and CO_2 fluxes at the same points and observed a negative correlation between CH_4 and CO_2 fluxes; however, CH_4 uptake rates were positively correlated with soil CO_2 emission rates (Fig. 2). Thus, it is crucial to understand the effects of N addition on soil CH_4 uptake and soil CO_2 emission to have a better understanding of the carbon dynamics in boreal forest ecosystems.

Discussion

Effects of N deposition on soil properties

N deposition did not significantly alter soil TOC and total N in different soil depth of cold-temperate coniferous forest. In the cold-temperate coniferous forest, soil available N usually limit plant growth [35-36]. Therefore, N deposition could change soil N status and N cycling rate of forest soils, and plant roots strongly absorb exogenous N and microbe mediate soil N immobilization and transformation. That's reason why soil total N concentrations did not show an obvious increase in treatments plots. The phenomenon indicated N-addition would improve the net primary productivity of plant, and increased in NPP under N-deposition had been verified in many researches [37-38]. However, N-deposition could transfer the excessive underground labile C to aboveground, and decrease the quantity of soil labile C in forest soils, then the soil TOC under N addition tended to decrease compared with the control. This was consistent with our conclusions. Moreover, soil total P intended to decrease under N treatments, suggesting that N deposition could promote soil P absorption and transformation. Zhao et al. (2010) [39] indicated that N deposition could increase the P concentrations in the branch, stem and roots of larch plantation.

During the entire study periods, soil inorganic N inconsistently responded to N deposition in different treatments. Three years of N depositions caused significant change in soil NH_4^+-N, but not NO_3^--N. Consistently with our results, Hu et al. (2009) [40] indicated high N-addition enhanced soil NH_4^+-N and NO_3^--N concentrations in the temperate forest. Contrary to our conclusions, Gunderson and Rasmussen (1995) [41] found soil NH_4^+-N contents changed very slightly while NO_3^--N

concentrations significant increase at different soil layers. The inconsistency may be attributed to N status and levels of forest soils. Moreover, N deposition significantly decreased soil pH values of 0-10 cm soil depth in the boreal forest. The key factors have effect on soil pH values are microbial decomposition of organic matter and soil nitrification. N deposition could increase rates of decomposition [42], especially for N restricted soil in boreal forests. Otherwise, N deposition significantly increased NH_4^+-N contents, and NH_4^+-N accumulation may lead to decrease in soil pH values. However, soil pH decrease caused by N additions is the comprehensive consequences of soil inorganic N dynamics [43].

Effects of N addition on the soil CH_4 uptake

In the present study, the application of four N treatments at 2.5, 5, 7.5 g $N.m^{-2}.yr^{-1}$ decreased CH_4 uptake rates 7.5%, 11.3%, and 23.0% in the daytime and 11.8%, 12.3%, and 27.0% in the nighttime, respectively, compared with the control in boreal forest soil plots in northeastern China. Zhang et al. (2008) [20] observed that fertilization decreased soil CH_4 uptake in the Dinghushan Biosphere Reserve in southern China, consistent with a similar observation for a fertilized boreal forest. Numerous studies have shown that N deposition influences CH_4 uptake. In the boreal forest, N deposition significantly inhibited CH_4 uptake, and the inhibitory effects increased with increasing N treatment levels. These results were consistent with previous findings reporting the inhibition of CH_4 uptake in N-fertilized forest soils [16, 44, 45]. These authors reported a Meta-analysis revealing that soil CH_4 uptake was significantly reduced 38% across all ecosystems under N application.

The effects of N on CH_4 uptake in the soil environment are extraordinarily complex, and multiple mechanisms could contribute to the effect of N addition on CH_4 uptake. The soil exchange of CH_4 with the atmosphere is regulated through methanogens and methanotrophs. N deposition could increase the concentrations of NH_4^+, and NH_4^+ could inhibit soil CH_4 uptake through competition with CH_4 for the same active site on the CH_4 monooxygenase of methanotrophs [46]. Methanotrophic bacteria are optimally active under low osmotic stress. The amount of N deposition would increase the concentration of nitrogenous salt in the soil; thus, osmotic pressure would suppress the activity of methanotrophic bacteria [18, 47]. The results of a previous study showed that NO_3^- might have direct inhibitory effects on CH_4 oxidation [21]. In addition, N deposition significantly decreased soil pH values (Table 2), and there was a general trend of decreasing CH_4 uptake with lower soil pH values. Bradford et al. (2001) [19] found low soil pH inhibited methanotrophic bacteria activities. Moreover, soil pH values had become low leading to Al toxicity, and Al toxicity appear to limit CH_4 uptake in forest soils.

However, in the present study, soil CH_4 uptake was positively correlated with the soil temperature (except T_H) at a 5 cm depth. This phenomenon is consistent with the activities of methanogenic archaea under anaerobic conditions and methanotrophic bacteria under aerobic conditions. CH_4 oxidation could be associated with soil diffusivity at a 5-10 cm depth [48]. Atmospheric O_2 can easily diffuse into the soil at a 5 cm depth. However, high levels of N (7.5 g $N.m^{-2}.yr^{-1}$) are negatively correlated with soil temperature at a 5 cm depth, likely reflecting the stronger methanogenic archaea activity compared with *methanotrophic* bacteria under the conditions of high N inhibition. Thus, the effect of temperature on CH_4 absorption is changed at a 5 cm soil depth.

Effects of N addition on soil CO_2 emission

The impact of N addition on CO_2 emission widely varies with the level of N addition. However, the data obtained in the present study implied that the highest soil CO_2 effluxes occurred under low N conditions (T_L, 2.5 g $N.m^{-2}.yr^{-1}$), followed by medium N conditions (T_M, 5 g $N.m^{-2}.yr^{-1}$), whereas the lowest soil CO_2 efflux was observed under high N conditions (T_H 7.5 g $N.m^{-2}.yr^{-1}$). Thus, the low-level N deposition might significantly promote CO_2 emission and high-level N deposition might inhibit CO_2 emission in boreal forest soils, consistent with the results of a previous study [49]. The low-level N deposition might also promote fine root growth and accelerate the decomposition rate of soil organic matter [50-51], leading to increased autotrophic soil respiration and the

heterotrophic respiration, and consequently soil CO_2 emission would also increase. However, low-level N deposition could also increase microbial biomass and activity, and influence the rates of soil CO_2 emission [52-53]. In contrast, high-level N deposition would increase osmotic pressure, inhibit microbial activity and suppress fine root growth [54]. However, in the present study, we did not observe a decrease in CO_2 emission with the highest-level N treatment (7.5 g $N.m^{-2}.yr$) in the nighttime, and there was significant difference in soil CO_2 flux between the control and the highest-level N treatment (7.5 g $N.m^{-2}.yr^{-1}$) in the daytime. These observations suggest that low-level N deposition stimulates CO_2 emission, while high-level N deposition inhibits CO_2 emission, and this switch might occur at 5-7.5 g $N.m^{-2}.yr^{-1}$. In addition, the soil temperature is a key factor regulating the rate of soil CO_2 emission [55]. Indeed, we observed significant and strong exponential relationships between soil respiration and temperature with each treatment.

Effects of N addition on the relationship between soil CH_4 and CO_2 fluxes

In the present study, we observed a weak correlation between CH_4 uptake and CO_2 emission in the control plots (Fig. 2a). However, in different plots treated at 5 and 5 g $N.m^{-2}$ yr^{-1}, the rates of CH_4 uptake showed a strong correlation with rates of CO_2 emission (Fig. 2b, Fig. 2c). Notably, low-level N deposition (T_L, T_M) could increase the fine root biomass [56], which might increase air-filled porosity and promote rates of diffusion and soil CH_4 oxidation. Thus, rates of CH_4 uptake and CO_2 emission are positively correlation. In contrast high-level N deposition (T_H) does not show this strong positive correlation because high levels of N might not only inhibit soil respiration but also inhibit CH_4 uptake (Fig. 2d).

Effects of temporal pattern (daytime and nighttime) on soil C fluxes

Our result showed that soil CO_2 emission and CH_4 uptake were significantly difference between daytime and nighttime, and the daytime rates of CO_2 emission and CH_4 uptake were significantly higher than the nighttime. The different responses of soil CO_2 and CH_4 fluxes to the temporal pattern (daytime and nighttime) could be attributed to changes of various biotic and abiotic factors. Soil temperature are recognized as the main abiotic factors in controlling the temporal variability of soil CO_2 fluxes [57-58] and soil CH_4 uptake, and the results were consistent with our conclusions found the different of daytime and nighttime in boreal forest. In addition, abiotic factors such as illumination could contribute to variation in the temporal pattern of soil C cycling. However, plant photosynthesis in the daytime could lead to allocation photosynthates to roots, and increase the root activities. Moreover, plant roots in the daytime could have a higher absorb rates of salt ions. These biological processes could increase root respiration and associated mycorrhizae respiration. Significant difference between soil C dynamic of daytime and nighttime suggest that soil chemical and physical properties could interactively affect the temporal pattern of soil C fluxes. A few studies have elucidated the complex mechanisms by which temporal (daytime and nighttime) affects soil CH_4 uptake, and so further investigation should be carried out in the future. The accurately estimating soil C fluxes has been questioned only using the daytime rates of soil C flux, because there are significantly difference between daytime and nighttime.

Conclusions

The daily rates of CH_4 uptake and CO_2 emission were observed in a boreal forest in northeastern China. The results showed that increased N deposition decreased CH_4 uptake and increased CO_2 emission during the short-term experimental period. Otherwise, soil C fluxes were significantly difference between daytime and nighttime. Overall, the results of the present study demonstrated that N deposition is an important contribution to TOC loss in the soil. A longer study period is needed to verify the cumulative effects of increasing N deposition on CH_4 uptake and CO_2 emission to ensure the accurate evaluation of soil C loss in boreal forests.

Acknowledgments

The authors would like to thank Ligong Wang (Academy of Agriculture and Forestry Sciences

of the Greater Hinggan Mountains, China) for managing the experimental plots, Professor Junhui Zhang (Institute of Applied Ecology, Chinese Academy of Sciences) for valuable comments and suggestions on earlier versions of the manuscript, and improving the language of the revised manuscript. This research was supported through funding from the National Natural Science Foundation of China (31370494, 41575137, 31170421, 31070406 and 31200341), the key projects of Heilongjiang Province Natural Science Foundation (ZD201406), and the National Basic Research Priorities Program of the Ministry of Science and Technology of China (2014FY110600).

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