Effects of Nitrogen Deposition on CH_4 Uptake and CO_2 Emission from temperate forests in Northeastern China

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Abstract Little is known about how N deposition affects greenhouse gas balances in different temporal patterns (daytime and nighttime),and few studies have been conducted to quantify the effects of N deposition on soil CH_4 uptake and CO_2 emission in a typical temperate forests. To investigate the effects of N deposition on soil CH_4 uptake and CO_2 emission, simulated N deposition experiment was initiated in temperate forests in Northeastern China in May 2011. In this study, NH_4NO_3 fertilizer was applied throughout the growing season at four N treatment levels (three replicates): control (CK), no added N; low-N (T_L), 5 g N $m^{-2}{\cdot}yr^{-1}$; medium-N (T_M), 10 g N $m^{-2}{\cdot}yr^{-1}$; and high-N (T_H), 15 g N $m^{-2}{\cdot}yr^{-1}$. Diurnal soil CH_4 uptake and CO_2 emission rates were observed in August 2014 using Greenhouse Gas Analyzers. Our results showed that N deposition tended to restrict CH_4 uptake and significantly increased soil CO_2 emission (except in the T_H treatment). In addition, the CO_2 and CH_4 fluxes in daytime and nighttime were significantly different. These results indicate that N deposition controls the CH_4 uptake and suggest that alterations of the N cycle due to N deposition may convert sequestered C in forest soils into a C source. However, this paper is also to promote a better understanding of the impact of N deposition on soil C fluxes under the different temporal patterns.

Introduction

Since the onset of the industrial revolution, human activities have substantially altered the N pathway and drastically increased the amount of N transferred from the atmosphere to terrestrial ecosystems. Consequently, the trend of atmospheric N deposition is projected to continue in the coming decades due to the rapid expansion of industrial and agricultural activities [1]. Atmospheric N deposition has increased the amount of reactive N in the soil in some regions of the world [12]. The northeastern United States, and western and central Europe have attracted much attention for their considerably high rates of N deposition [3]. Simultaneously, China is experiencing intense

deposition of atmospheric N in terrestrial and aquatic ecosystems, and the average annual bulk deposition of N increased by approximately 0.8 g N m^{-2} between the 1980s (1.32 g N m^{-2}) and the 2000s (2.11 g N m^{-2}) [4]. Nitrogen deposition rates exceeding 4 g N $m^{-2}\cdot yr^{-1}$ may be due to large amounts of fossil fuel combustion and fertilizer application in some areas of southeast China [5]. Aseconomic development increases with human population growth, N deposition rates are likely to rise indefinitely. Global anthropogenic N deposition has increased ten-fold (from ~15 to ~160 Tg $N\cdot yr^{-1}$) over the past 150 years [6], and this suggests that many terrestrial ecosystems are converted from being naturally N limited to eutrophic, or even N saturated. More reactive N deposition in terrestrial ecosystems may affect climate change by its strong impact on greenhouse gas (such as CH_4 or CO_2) emission or uptake. Forests represent a major component of the terrestrial ecosystem, and forest soils are considered to be an important CO_2 source and CH_4 sink [7]. Diverse results have been presented in numerous studies with respect to the effect of N deposition on the carbon balance of forest ecosystems, particularly with respect to the most potent greenhouse gases, i.e., CO_2 and CH_4.

CO_2 and CH_4 are two important atmospheric greenhouse gases, and increases in the concentrations of these gases are predicted to cause global warming that will significantly impact the world's environment. Atmospheric CO_2 and CH_4 concentrations have increased from ~280 and ~0.715 ppm at the start of the industrial revolution to more than ~391 and ~1.803 ppm, respectively [8]. Enhanced concentrations of CO_2, CH_4, and other greenhouse gases in the atmosphere may result in an average temperature increase of 1.1-6.4℃ by 2100 [9]. However, forest ecosystems perform an important function in the process of regulating global change. Forest soils are important as natural sinks for carbon sequestration to mitigate climate change [10]. In addition, soil plays a major role in the global accounting of C due to the considerable amount of C stored in soil, and the amount of CO_2 emitted from soil respiration is 10-15 times that from fossil fuel burning [11]. It is estimated that 5-20% of CO_2 and 15-30% of CH_4 in the atmosphere originates from soil. Chronic N deposition input into terrestrial ecosystems can cause soil acidification and N saturation, altering plant physiology and soil microbial community and activities, and therebyaffecting the soil biogenic CH_4 and CO_2 fluxes [12].

Soil respiration can be characterized by biological processes, including the combination of autotrophic respiration by live roots and their associated mycorrhizae, and heterotrophic respiration by microbes that oxidize plant detritus, root exudates and humified organic matter [13-14]. The exchange of CH_4 between the soil and atmosphere is regulated by two disparate groups of microorganisms, calledmethanotrophic bacteriaand methanogenic bacteria [15].The most of CH_4 emission from soil occurs largely due to the action of methanogenic bacteria in anaerobic conditions, and the most of the CH_4 consumption by methanotrophic bacteria in aerobic conditions [15]. Nitrogen deposition can result in changes in the N status and N cycling rate of forest soils, C sequestration rate and species composition (plant, animaland microorganism), and the rates of microbial N and C turnover, and thus affect CO_2 and CH_4 uptake or emission in forests ecosystem on the above- and belowground. In general, chronic N deposition will increase NH_4^+ and NO_3^- availability in soil of forest ecosystems, thereby affecting CH_4 uptake through a change in the activity and composition of themethanotrophic community [16]. Simultaneously, N deposition can increase soil respiration through increased microbial activities [17] and increased decomposition rates due to N deposition, which results in higher tissue N contents and lower tissue C/N ratios [18]. Previous experimental studies found that additions of N to soil typically resulted in increased CO_2 emission and decreased CH_4 uptake [7, 19]. In contrast, some studies found that an increased N input negatively affected soil CO_2 emission [20] or had no effect [16]. However, most of these

studies were carried out in temperate climates, where most forests are naturally N-limited [21]. Few studies have focused on determining the response of both CO_2 emission and CH_4 uptake to N deposition in China's boreal forest.

The cold region forest covers 13% of the earth's continental surface (Schultz 1995). However, these regions are N-limited ecosystems that exhibit slow decomposition rates due to cool climates, and consequently have a large capacity for C storage. Little attention has been paid to N deposition-driven changes in C fluxes and pools in the cold region forests of China, and few studies also have focused on assessing the different impact of both daytime and nighttime on CO_2 fluxes and CH_4 fluxes. Our aim was to examine the effects of elevated N deposition on soil CH_4 uptake and CO_2 emission ina *Picea*-fir Korean pine Forest of the Lesser HingganMountains in Northeast China. Furthermore, our study is to promote a better understanding of the effects of N deposition on soil C fluxes under the different temporal patterns (daytime and nighttime) from the cold region forest, and the subject is helpful for accurate assessment soil C fluxes. In addition, the experiment was designed to investigate the link between CH_4 and CO_2 fluxes as affected by changes in soil N. We hypothesized that N deposition would inhibit CH_4 uptake and promote CO_2 emission in these forests. Further, we hypothesized that N deposition would affect the link between CH_4 and CO_2 fluxes due to changes in the soil N status.

Materials and methods

Site description

The research was conducted in a *Picea*-fir Korean pine Forest, located in the Fenglin Biosphere Reserve of the the Lesser Hinggan Mountains in Heilongjiang Province, northeast China (128˚58' ~ 129˚15' E, 48˚02' ~ 48˚12' N). The terrain is fairly gentle with an average altitude of 300 m above sea level and an average slope of 10-25˚. The region is characterized by a continental monsoon climate, with dry, cold winters, and humid, warm summers. The mean annual air temperature is -0.5℃, and the lowest and highest monthly mean air temperatures are -24℃ in January and 21℃ in July, respectively. Mean annual precipitation ranges from 675 to 760 mm, and approximately 80% of precipitation is concentrated in July and August. The soil is classified as a dark brown forest soil according to the Chinese Soil Taxonomic System and has an average thickness of 60 cm. We established research sites in a mature *Picea*-fir Korean Pine Forest (the average age of the major canopy trees exceeded 200 years). The dominant species in the mature forest is *Pinuskoraiensi*. The major species in the canopy layer of the naturally mature Broadleaved Korean Pine Forest species are *Pinuskoraiensi, Abiesnephrolepis, Piceakoraiensis, Piceajezoensis var. microsperma, Larixgmelini, Betulaplatyphylla, Acer mono, Fraxinusmandshurica,* and *Betulacostata*.

Experimental design

An increasing N deposition experiment with a randomized block design was established in the boreal forest in 2011.Three blocks were established, each consisting of four plots measuring 20 m×20 m, and the plots were separated by 10-m wide buffer strips to avoid horizontal movement of the added N. Four N addition treatment levels (replicated three times) were used at the experimental site: control (without N addition, CK), low-N (5 g N $m^{-2}\cdot yr^{-1}$, T_L), medium-N (10 g N $m^{-2}\cdot yr^{-1}$, T_M), and high-N (15 g N $m^{-2}\cdot yr^{-1}$, T_H) [22]. The plots and treatments were laid out randomly. The N application was initiated in May 2011. The N was added an ammonium nitrate (NH_4NO_3) solution and was distributed on five occasions during each growing season, which began in May and ended in September. During each application, NH_4NO_3 was weighed, dissolved in 32 L of distilled water, and sprayed onto the surface soil and understory vegetation in fertilized plots using backpack sprayers. Each control plot was sprayed 32 L of distilled water simultaneously. The amounts of

solution applied did not impact the moisture conditions of the surface soil layers. The applications were made from May 2011 to the present, and will continue.

Measurements of CH_4 and CO_2 fluxes and environmental parameters

Soil CO_2 and CH_4 fluxes were measuredfrom1st to 23rd in August 2014, three years after the initial experimental N applications. For each of 12plots,three polyvinyl chloride (PVC) collars (i.e., soil collars) with a 19-cm inside diameter and 10cm in height were randomly installed for soil CH_4 and CO_2 flux measurements in each plot, and a total of 36 PVC collars were installed. The collars were inserted into the soil to a depth of 5 cm, just below the litter layer, taking care to avoid cutting fine roots. The collars were installed in each plot more than 24 hours before the measurements were collected to minimize the effects of the disturbance caused by inserting the collars into the soil. Living plants were removed from the soil collars before measurements were taken to minimize the effects of the disturbance caused by plant respiration. All fluxes of CO_2 and CH_4 were measured using a fast-response Greenhouse Gas Analyzers (GGA-24p, Los Gatos Research (LGR), United States). Soil gas flux was measured using the GGA and the PVC soil ring. A lead battery was installed to provide electrical power to the GGA and to allow continuous data collection. The CH_4 and CO_2 fluxes from each collar were measured continuously over a 24-hour period to understand the diurnal dynamics of these fluxes.

During the measurement of CH_4 fluxes and CO_2 fluxes, soil temperature and moisture at a depth of 10 cm below the soil surface were monitored at points adjacent to each soil collar. Soil temperature was measured using a portable temperature probe and soil moisture was measured using a moisture meter (Decagon Devices, Inc. USA).

Simultaneously, soil samples were collected nearby the soil collar. The cores of mineral soil were taken at soil depth of 0-10 cm using a stainless-steel corer with a 5 cm diameter. Three core sets were collected at each plot. Soils were immediately passed through a 2mm sieve, and any visible roots, gravel and stones were removed from the sieved soil. The each sieved soil sample was extracted to determine NH_4^+-N concentrations and NO_3^--N concentrations with K_2SO_4 from soil. Extractable NH_4^+ concentration was measured using the in dophenol blue method, followed by colorimetric analysis. The NO_3^-content was determined using the copper-cadmium reduction method. In addition, soil pH measured in a 1:2 soil: water suspension using a standard pH meter (SX7150, China).

Statistical analyses

All statistical analyses were performed using R 3.1.1 (R Development Core Team 2014). Pearson rank correlation was used to determine the relationships between CH_4 uptake rates, CO_2 emission rates and soil temperature, soil moisture content. One-way ANOVA with Tukey's HSD test was used to examine the difference in soil temperature, moisture, pH and total inorganic nitrogen (NH_4^+-N and NO_3^--N) between the different N treatments. A repeated measure ANOVA with Tukey's HSD test was used to examine the difference in soil CH_4 uptake rates and CO_2 emission rates between the N treatments. 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 using *p*-values <0.05 unless otherwise stated.

Results

Soil properties

Soil temperature at a depth of 10 cm fluctuated little during the entire observation period. There was no significant difference in the soil temperature and soil moisture between the various treatments (table 1). Soil pH values was consistently decrease significant in treatment plots, but no

significant differences were detected between CK, T_L and T_M (p>0.05) (table 1). However, soil pH values was significant different between CK and T_H (p<0.05). The results suggest long-period N deposition and high-level N deposition were significantly effect on soil pH values.N deposition tended to alter soil inorganic N concentration.NO_3^--N concentration increased after N treatments, and NH_4^+-N concentration consistently increased (except T_H) (table 1). Furthermore, there was significant difference in NO_3^--N concentration between the control and T_M, T_H, respectively (Table 1). The NH_4^+-N concentrations was significant difference between the control and T_L (Table 1).

Table 1 Soil Temperature at 10 cm depth, Soil Moisture in 10cm depth, Soil inorganic N contents and pH Values in the different treatments. Data are shown as means with standard errors.

treatment	Temperature(10cm)	Moisture(10cm)	NO_3^--N (mg.kg^{-1})	NH_4^+-N(mg.kg^{-1})	pH
CK	14.66±0.19a	39.91±0.22a	2.64±0.07c	6.60±0.33bc	5.02±0.09ab
T_L	14.62±0.17a	40.11±0.13a	3.19±0.70bc	9.11±0.60a	4.65±0.12ab
T_M	14.60±0.17a	40.53±0.14a	4.34±0.08ab	7.65±0.13b	4.80±0.18a
T_H	14.62±0.17a	40.67±0.15a	4.64±0.20a	5.54±0.60c	4.57±0.12b

Different letters show significant differences between the different N treatments at the level of 0.05.

Soil CO_2 fluxes

Diurnal rates of soil CO_2 fluxes varied as a single-peak pattern, and the maximum occurred at 3 P.M. (Fig1a. tif). During the experimental period, there was significant difference in CO_2 emissions between the control and N deposition treatments. The effects of N deposition on CO_2 flux varied with the level of N added. The repeated measure ANOVA showed that CO_2 emission rates in the low- and medium-level N addition plots increased significantly, whereas CO_2 emission rates in the high-level N addition plots decreased significantly (p<0.05) (Table2) for daytime and nighttime, respectively. The mean daytime rates of CO_2emission were increased by 11.65％compared with nighttime. Significant differences in CO_2 fluxes between the daytime and nighttime were found in our study.

Soil CH_4 uptake

During the entire observation period, the soil at the boreal forest site at Fenglin was a sink for atmospheric CH_4 (Table 2). CH_4 uptake exhibited similar diurnal variations in all N deposition addition treatments, and there were significantly separate in curves of the different N addition levels (Fig1b. tif). The effects of N on CH_4 uptake varied depending on the N addition level. The mean daytime rates of CH_4 uptake were decreased by 6.71％ compared with the nighttime. Statistical analysis showed that increased N input to the boreal forest significantly decreased CH_4 uptake rates. With increasing N deposition levels, CH_4 uptake rates were inhibited more strongly, and the sink may switch to being a source for CH_4 in the boreal forest. However, the nighttime rates of CH_4uptake were significantly higher than the daytime rates of CH_4 uptake for the treatments, and the control has the opposite conclusion (Table 2).

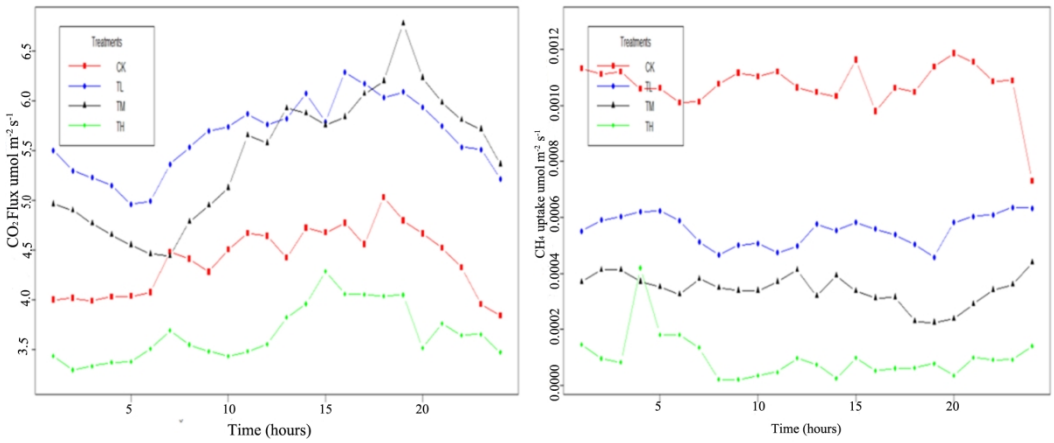


Fig.1 Day-rates of soil CO_2 (a) and CH_4 (b) fluxes in situ (Fenglin) in the growing reason during the August 2014. Monthly applications of NH_4NO_3 began in August 2011 and since then.

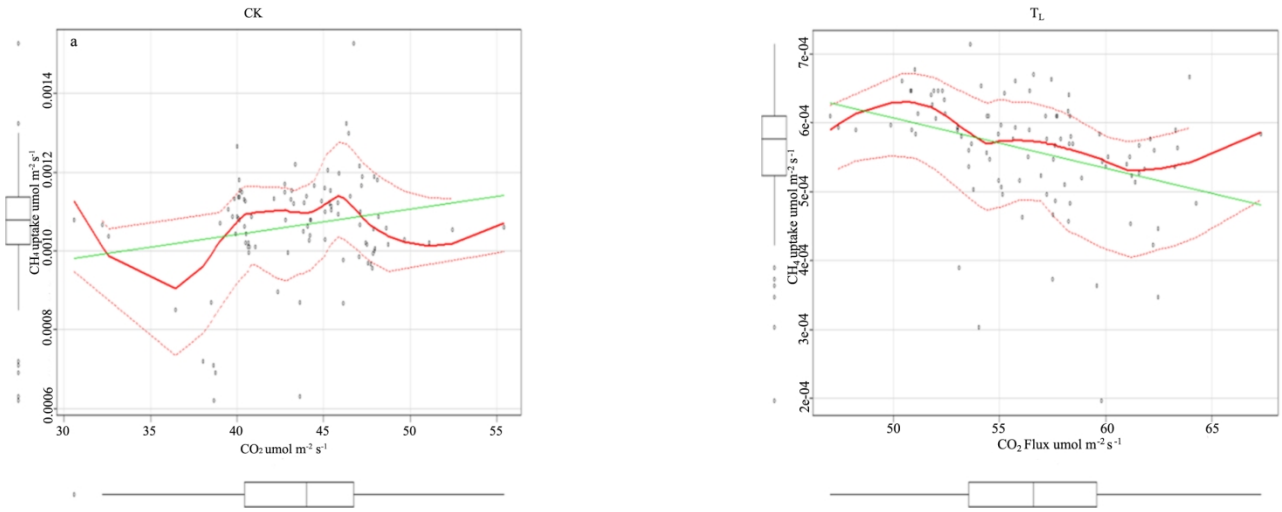
Table 2 daytime and nighttime average of CH_4 uptake rates (mean±standard error, umol CH_4 $m^{-2}s^{-1}$) and CO_2 emission rates (mean± standard error, umol CO_2 $m^{-2}s^{-1}$) in the different treatments.

Treatment	CO_2 flux		CH_4 uptake	
	daytime	nighttime	daytime	nighttime
CK	4.58±0.06cA	4.12±0.03cB	1.10e-03±1.61e-05aA	1.03e-03±2.16e-05aB
T_L	5.99±0.04aA	5.31±0.03aB	5.25e-04±1.26e-05bB	5.91e-04±1.05e-05bA
T_M	5.82±0.07bA	5.00±0.07bB	3.11e-04±1.27e-05cB	3.72e-04±8.74e-06cA
T_H	3.64±0.05dA	3.51±0.03dB	5.49e-05±5.70e-07dB	1.41e-04±2.00e-05dA

Values followed by the same letter in the same column and same row are not significant (P<0.05)

Relationships between soil CH_4 uptake and CO_2 emission

In our experiment, CH_4 uptake rates and CO_2 emission rates were measured simultaneously in the same soil collar. There was a weak positive correlation between soil CH_4 uptake and CO_2 emission rates in the control plots (Fig 2-CK. tif). However, N deposition altered this relationship. Soil CH_4 uptake rates were significantly negatively correlated with soil CO_2 fluxes in the Low- and Medium-N treatments (Fig 2-T_L. tif and Fig 2-T_M. tif), but not in the High-N treatment (Fig2-T_H. tif). The relationships between soil CH_4 uptake and CO_2 emission rates were well fitted using linear equations (Table 3). Therefore, our results suggest that CH_4 oxidation in the elevated CO_2 soil was restricted in the Low-level N addition treatment.



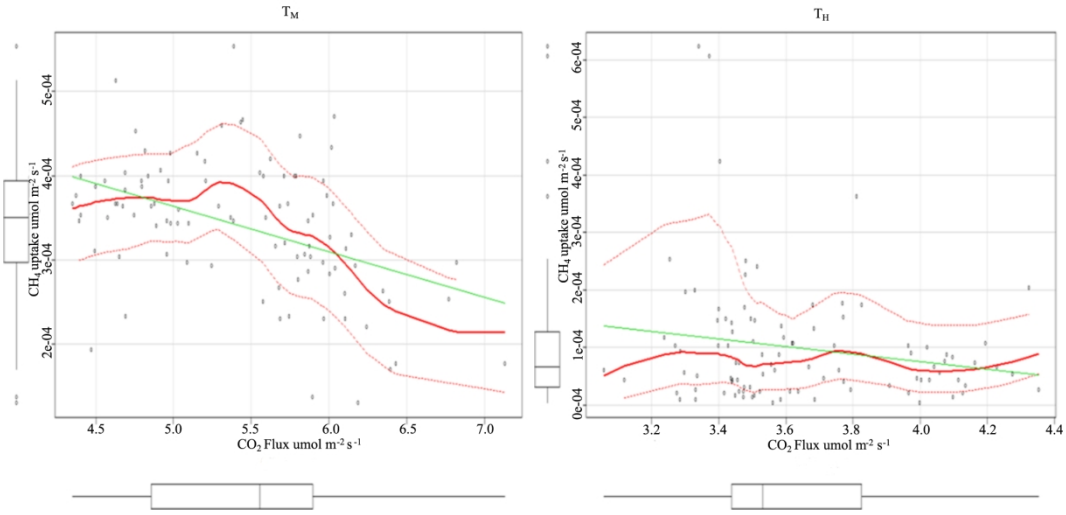


Fig.2 Relationships between soil CH_4 uptakes and CO_2 fluxes

Table3 Regression models between soil CH_4 uptakes and CO_2 fluxes

CH_4 uptake	CO_2 Fluxes	Regression equation	R^2	*p*-value	F
CK	CK	y= 6.4e-05x + 7.8e-04	0.039	0.052	3.87
T_L	T_L	y=-7.3e-05x + 9.7e-04	0.127	3.39e-04***	13.83
T_M	T_M	y=-5.4e-05x + 6.3e-04	0.187	7.89e-06***	22.35
T_H	T_H	y=-6.5e-05x + 3.4e-04	0.034	0.073	3.29

The level of significance of correlations and regression models are, **P<0.05 and ***P<0.001

Relationships between soil CH_4 uptake and CO_2 emission and soil properties

The CH_4 uptake rates were negatively correlated with soil temperature and moisture measured at a depth of 10 cm (Table 4). The CO_2 fluxes were positively correlated with soil temperature and moisture. These results are similar to soil warming experiments conducted in previous studies. Soil temperature and moisture were the dominant environmental variable that controlled the diurnal patterns of CO_2 flux and CH_4 uptake.

Table 4Relationship between CH_4uptake and CO_2 flux and soil variables measured. Pearson correlation coefficients are presented.

	Treatments	Correlation analysis (Pearson)	
		$T_{(10cm)}$	$M_{(10cm)}$
CH4 uptake	CK	-0.74***	-0.51**
	T_L	-0.66***	-0.26
	T_M	-0.20	-0.69***
	T_H	-0.54***	-0.39
CO2 flux	CK	0.13	0.12
	T_L	0.45**	0.40**
	T_M	0.44**	0.31
	T_H	0.41**	0.20

The level of significance of correlations are, **P<0.05 and ***P <0.01

Discussion

Difference of CO2 and CH4 fluxes between daytime and nighttime

The research indicates that daytime and nighttime rates of CO_2 emission and CH_4 uptake were significantly difference. The temporal (daytime and nighttime) variation in soil CO_2 fluxes is related to biotic factors and abiotic factors, biotic factors mainly including root respiration and microbial

respiration, abiotic factors mainly including temperature. In the daytime, abiotic factors including temperature and illumination availability enhance physical and physiological effects on root metabolisms and microbial metabolisms lead to raise CO_2 fluxes compared with the nighttime. However, no clear and significant relationships between daytime CO_2 fluxes and nighttime CO_2 fluxes. In the daytime, the CH_4 uptake capacities in the N deposition plots were always smaller than that in nighttime, and the control (CK) has the opposite conclusion. In this study, it seems that increased CH_4 uptake in the nighttime in the N treatment plots is likely to be related to impact of N deposition on soil physical and chemical properties. To elucidate the complex mechanisms by which temporal (daytime and nighttime) affects soil CH_4 uptake, further investigation should be carried out. The estimated annual total soil C fluxes only by the daytime C fluxes were overestimated due to the difference of C fluxes between daytime and nighttime.

Effect of N deposition on soil CH_4 uptake

The *Picea*-fir Korean pine Forest plantation soils act as a sink for atmospheric CH_4. However, CH_4 uptake at our site was significantly inhibited by N addition, and the decrease in soil CH_4 uptake was higher than that reported for forest soils in South China [22-23] despite similar N addition levels. This result indicates that different responses of soil CH_4 uptake to N addition may be due to climate conditions, vegetation, soil type etc. In addition, this result indicates that boreal forest soils are more sensitive to N addition than southern forest soils. This may be due to the lower soil N availability in boreal forest soil, which strongly suggests that the historical N status of soil is the most important predictor of how soil will respond to future inputs of N.

In our study, we found that soil CH_4 uptake in the low- and medium-level N addition plots decreased by 48.08％ and 67.93％ compared with the control. Two mechanisms have been posited for the partial inhibition of CH_4 uptake in response to increased N inputs. First, N input addition may result in a significant accumulation of soil NH_4^+-N (Table 1). Aerts and Caluwe [24] linked the reduction in methane consumption to the competitive inhibition of methane monooxygenase (MMO) by NH_4^+. This theory has been widely accredited [25-26]. Methanotrophic bacteriaand ammonia oxidizing bacteria have the ability of mutual conversion of substrate, which is believed to be responsible for that soil exposed to high concentrations of NH_4^+inhibited CH_4 absorption [27]. Second, the elevated N deposition in boreal forests that result in a rapid reduction in CH_4 uptake may be due to a change in soil pH value (Table 1). Methanotrophic bacteria are sensitive to acidic environments, methanogenic bacteria are less sensitive to acidic environments [28], and there may be a few of potential mechanism by which pH could regulate CH_4 uptake or CH_4 emission. However, low soil pH could results in lower methanotrophic bacteria activity and lower CH_4 uptake rates in the site.

The CH_4 uptake in the high-level N deposition plots was 90.80％ lower than the control plots at our site. At high concentrations, N is strongly inhibitory, which likely affects osmotic conditions. Methanotrophic bacteria may be more sensitive to osmotic stress and pH values compared with methanogenic bacteria. Other authors suggest that salts have an inhibitory effort on methanotrophic bacteria due to osmotic stress [29]. Most studies have reported an inhibition of CH_4 uptake in response to N input in forest soils [30-31], although inverse effects have also been reported. The fact that N deposition inhibits CH_4 uptake implies that boreal forests may remove less CH_4 under future increased N deposition conditions in China.

Effect of N deposition on soil CO_2 emission

The rate of CO_2 emission from soil depends on CO_2 production, transport and interactions between physical and biological processes in the soil. Elevated N deposition can affect soil CO_2 emission through changes in litter decomposition, root respiration and soil microbial respiration

rates [32]. N deposition can alleviate N-limitation and accelerate microbial activities. A previous report showed that N deposition accelerated soil CO_2 emission [33]. Similar to previous studies, our results indicated that low-level N deposition significantly increased CO_2 emission, and high-level N deposition significantly decreased CO_2 emission. The C/N stoichiometry of plants affects N cycling in terrestrial ecosystems. Increased low-level N deposition leads to lower tissue C/N ratios, and subsequent decomposition rates may increase [16], and thus elevating soil CO_2emission. Reich et al (2008) [34] stated that maintenance respiration is positively correlated with tissue N content, and litter with higher N content decomposes faster [35]. In addition, a previous study suggested that increased N supply significantly stimulated CO_2 emission, and these conditions generally promoted autotrophic plant respiration of above- and belowground parts [36], as well as rhizosphere respiration by microbes due to the accelerated decomposition of soil organic matter [37]. Wang et al. (2014) [38] showed that the root biomass of C. *angustifolia*was significantly enhanced by N deposition and the increased apportioning of biomass to root was of major significance. Plant respiration rates would increase with increasing tissue N and root biomass under N addition [39]. Therefore, elevated N deposition may result in higher C loss by increasing both autotrophic and heterotrophic respiration. However, in our study, respiration rates were considerably lower in the high N plots compared with the control plots. Janssen et al (2010) [40] demonstrated that the negative effect of N on soil respiration is widespread but not universal in temperate forest ecosystems. In addition, Zhang et al (2012) [41] showed that N deposition may decrease heterotrophic respiration by decreasing microbial activity. Decreases in soil pH under high N addition may have negative effect on the soil microbial community (Zhang et al. 2008) [42]. The production and emission of CO_2 in soil is a complicated biological process and is influenced by various biotic and abiotic factors. This result implies that N deposition affects soil CO_2 fluxes, and can affect the carbon balance in natural ecosystems. Further research is needed to determine the long-term effect of N enrichment on ecosystem C sequestration and ecosystem C allocation.

Effect of N deposition on the relationships between soil CH_4 uptake and CO_2 emission

The fluxes and balances between CO_2 and CH_4 are tightly interconnected in the biospheric carbon cycle [43]. Increasing low-level N deposition decreased CH_4 uptake and increased CO_2 emission at our site. Soil CH4 uptake rates were significantly negatively correlated with soil CO_2 fluxes in the Low- and Medium-N treatments. The increase in CH_4 emission or decrease in CH_4 uptake may be caused by elevated CO_2[44].An ^{15}N tracing study using soil collected from the Giessen FACE study showed that under elevated CO_2, the turnover of N changed to a high N cycling speed [45]. In N-limited forest ecosystems, N inputs could decrease soil interspaces through increased root biomass [46] and therefore also decrease CH_4 uptake capacity [47].To understand how the changing relationship between CO_2 emission and CH_4 uptake may beneficial to the understanding of the carbon cycle, it is necessary to further analyze the fluxes and examine the physicochemical and biological processes that affect them. However, it is necessary to study the concurrent fluxes of CO_2 and CH_4 under multi-factor global changes, especially increased N deposition. It is crucial to understand the effects of N addition on soil CH_4 uptake and CO_2 emission to develop a better comprehension of carbon dynamics of boreal forest ecosystems.

Conclusions

This study has provided the first simultaneous estimation of CO_2 and CH_4 fluxes in response to different levels of N deposition in a *Picea*-fir Korean pine forest of Northeast China. N deposition can alter the rates of microbial N- and C-turnover, and can thus affect CO_2 and CH_4 fluxes from boreal forest soils. We found that low-levels of N deposition tended to promote soil CO_2 emissions

and inhibit soil CH_4 uptake, and high-levels of N deposition tended to inhibit soil CO_2 emission and dramatically inhibit CH_4 uptake. The effects of N deposition on CO_2 emission and CH_4 uptake varied depending on the N addition levels and N status of the soil. In addition, the CO_2 emission rates and CH_4 uptake rates in daytime and nighttime were significantly different..In major ecosystem types in China, indications of N saturation have been revealed in recent decades, and the potentially detrimental effect of excessive N deposition, such as soil degradation, and the inhibited uptake of CH_4, a potent greenhouse gas, should be given more attention. In the future, long-term observations of soil fluxes and measurements of key microbial functional groups are necessary to clarify the mechanisms responsible for the coupling between soil CO_2 and CH_4 fluxes.

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