Nonlinear responses of soil nitrous oxide emission to multi-level nitrogen enrichment in a temperate needle-broadleaved mixed forest in Northeast China

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ABSTRACT

The responses of nitrous oxide (N₂O) emission from forest soils to increasing atmospheric nitrogen (N) deposition are controversial. In this study, our objectives were to explore the response curves of soil N₂O flux to multi-level N inputs, as well as to examine the key factors dominating the changes in soil N₂O emission caused by N enrichment in the temperate needle-broadleaved mixed forest, Northeast China. The study consists of nine levels of urea addition (0, 10, 20, 40, 60, 80, 100, 120, 140 kg N ha⁻¹ yr⁻¹) with 4 replicates for each treatment. Soil N₂O fluxes were monitored weekly using the static chamber and gas chromatograph technique. NH₄⁺-N, NO₃⁻-N, total dissolved N (TDN), dissolved organic N (DON), and auxiliary variables (soil temperature and moisture in 0–10 cm depth) were measured at the same frequency to examine the regulation of soil N₂O flux. The results showed that high rates of urea inputs (>60 kg N ha⁻¹ yr⁻¹) significantly increased soil NO₃⁻-N concentrations in litter layer and mineral layer (0–10 cm depth) by 120–180% and 56.4–84.6%, respectively. Soil N₂O flux increased exponentially with increase in the rates of urea addition by 194% to 334% for the 60 to 140 kg N ha⁻¹ yr⁻¹ treatments relative to the control. The critical level of N input for the significant alternation of soil N accumulation and N₂O emission was approximately 70 kg N ha⁻¹ yr⁻¹. The changes in soil N₂O flux elicited by N addition were positively related to those of soil NO₃⁻-N and soil moisture contents. These results suggest that exogenous N input at the rate below the critical load will not significantly promote soil N₂O emission over the short term, which is favorable to carbon sequestration of the temperate needle-broadleaved mixed forest, Northeast China.

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1. Introduction

Nitrous oxide (N₂O) is the third most important anthropogenic greenhouse gas and the largest remaining anthropogenic source of stratospheric ozone depleting substances (Stocker et al., 2013). The global source strength of N₂O is estimated to be 17.7 Tg N yr⁻¹, with agriculture and soils under natural vegetation contributing 53.1% (Hirsch et al., 2006; Solomon, 2007). N₂O is mainly removed from the atmosphere via a series of processes such as photolysis, chemical transformation, and wet and dry deposition. The global sink strength of N₂O is estimated to be 12.6 Tg N yr⁻¹ (Solomon, 2007). Over the past 250 years, anthropogenic N deposition has increased by about three times (Galloway et al., 2008), which leads to an increase in carbon (C) sequestration in terrestrial ecosystems (20–470 kg C kg⁻¹ N, Högberg, 2007; Magnani et al., 2007; Reay et al., 2008; Sutton et al., 2008; de

Vries et al., 2009; Thomas et al., 2010; Templer et al., 2012; Pinder et al., 2013). However, increasing N deposition also accelerates soil N₂O emission and inhibits soil methane (CH₄) uptake (Lu et al., 2011a, 2011b). If the effects on soil CH₄ uptake and N₂O emission are considered, the C sink caused by exogenous N inputs can be offset by 53% to 76% (Liu and Greaver, 2010). Thus, deep understanding of the responses of soil N₂O emission to increasing N deposition is crucial to reduce the uncertainty of C sink estimation in terrestrial ecosystems.

Soil N₂O is produced by denitrification under anaerobic conditions and by nitrification under aerobic conditions (Davidson et al., 2000). Soil N₂O emission is influenced by soil temperature, moisture, soil N availability, dissolved organic C (DOC), and pH (Florinsky et al., 2004; Rowlings et al., 2012). Theoretically, there is greater emissions of N₂O under elevated N deposition or N application due to increased availability of NH₄⁺ and NO₃⁻ for both nitrification and denitrification (Davidson, 2009). However, evidences from various forest ecosystems show that simulated N deposition increases (Kim et al., 2012), decreases (Skiba et al., 1999) or does not change (Borken and Beese, 2005) soil N₂O

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emission. Overall, although N addition tends to promote soil N₂O emission (Lu et al., 2011a), the curves of soil N₂O flux responded to the rates of N addition are diverse including linear (Borken and Beese, 2005; Liu et al., 2012), exponential growth (Ambus et al., 2006; Liu et al., 2013b), and quadratic (McSwiney and Robertson, 2005), etc. These contradictory results may be attributed to the differences in the initial C and N status, the microbial community composition, and the type and rate of N application (Kim et al., 2013). Response of soil N₂O emission to N addition depends on the stages of N saturation (Aber et al., 1998), but the critical levels of N inputs or soil available N contents are not quantified for most terrestrial ecosystems.

Tropical forest soils are responsible for 14–23% of global N₂O emissions (Solomon, 2007), whereas the boreal and temperate forest soils are generally considered to contribute little to atmospheric N₂O accumulation (Werner et al., 2007; Koehler et al., 2009). However, both warming and chronic N deposition increase the N availability in the high-latitude forest ecosystems, thereby significantly promotes soil N₂O emission (Repo et al., 2009; Elberling et al., 2010). China is located at the center of the global N deposition, and the average N deposition rate is estimated to range between 12.9 and 21.1 kg N ha⁻¹ yr⁻¹ (Lü and Tian, 2007; Liu et al., 2013a; Jia et al., 2014). Inorganic N (NH₄⁺ and NO₃⁻) and organic N account for 73% and 27% of the total N deposition, respectively (Zhu et al., 2015). In the past decades, mineral N fertilizers (e.g., NH₄NO₃, NH₄Cl, or NaNO₃) are often applied to simulate the effects of N deposition on soil N₂O production and emission in some temperate and subtropical forests (Zhang et al., 2008; Wang et al., 2014; Bai et al., 2014). However, little information can be available about the effects of organic N input on soil N accumulation and soil N₂O emission in the N-poor temperate forests.

In this study, we defined the critical load as the level of N deposition (Bobbink and Hetteling, 2011), below which soil N₂O flux was not significantly impacted. Before the N input amount adequately supplied plant N demand, N addition would result in low soil N₂O emission; after that, soil N₂O flux could be greatly stimulated and might exhibit threshold responses to N addition rates. The different stages of soil N₂O flux response to N addition were closely related to the availability of nitrification and denitrification substrates. Thus, we hypothesized that organic N addition would not promote N₂O emission from the N-poor temperate forest soils until dissolved N content in soils or N addition rate exceeded their critical values. We also hypothesized that the changes in soil N₂O emission fluxes caused by N addition could be explained by the changes in soil dissolved N contents. Our objectives in this study were: (1) to investigate the changes in dissolved N contents, N₂O flux, soil temperature, and soil moisture among different experimental treatments; and (2) to elucidate those factors which are responsible for the changes in N₂O emission from the temperate forest soils induced by N addition.

2. Materials and methods

2.1. Study site and experimental design

This study was conducted in an old-growth broadleaf and Korean pine mixed forest, located at Changbaishan Forest Ecosystem Research Station (CFERS, 127°38' E, 41°42' N) in Jilin province, China. Long-term mean annual temperature and precipitation are 3.6 °C and 700 mm, respectively. The soil is classified as Eutric Cambisol (FAO classification) with bulk density of 0.53 g cm⁻³, total C of 156.6 g kg⁻¹, total N of 7.17 g kg⁻¹, total P of 0.97 g kg⁻¹, pH of 5.85, and C/N ratio of 21.84 in 0–20 cm layer (Wang et al., 2012). The dominant tree species are *Pinus koraiensis*, *Fraxinus mandshurica*, *Quercus mongolica*, and *Tilia amurensis*.

The experiment used a completely randomized design with 36 plots (15 m × 15 m each) arranged for nine N enrichment treatments (4 replications). Relatively flat underlying surface was selected to prepare plots, and each plot contained pine and other broadleaf species as

many as possible. The distance between any two adjacent plots was > 10 m. N was added as solid urea (CO(NH₂)₂) once a month, and the treatments started since 2013. Referring to the actual atmospheric N deposition of Changbai Mountain (10.79 kg N ha⁻¹ yr⁻¹, Zhu et al., 2015) and the highest levels of N deposition in China (117 kg N ha⁻¹ yr⁻¹, He et al., 2007), we fertilized at eight levels of N (10, 20, 40, 60, 80, 100, 120, and 140 kg N ha⁻¹ yr⁻¹), and a control treatment (0 kg N ha⁻¹ yr⁻¹). Hereafter, treatments will be denoted as: N₀, N₁₀, N₂₀, N₄₀, N₆₀, N₈₀, N₁₀₀, N₁₂₀, and N₁₄₀. At the first day of every month, 71.42, 142.85, 214.27, 285.69, 357.12, 428.54, 499.96, and 571.39 g of urea fertilizers (analytical reagent, N content of 46%) were weighed and dissolved in 40 L of water and then were evenly sprayed onto corresponding N addition plots, and the control ones received equivalent water.

2.2. Measurements of soil N₂O flux and auxiliary variables

Three permanent chamber bases (area 0.25 m², height 15 cm, ~10 cm inserted into the soil) were randomly installed on each plot. Over the growing seasons (from May to October) of 2013, the soil N₂O fluxes were determined using the static chamber and gas chromatography technique. Five gas samples (100 mL each) were removed at 0, 10, 20, 30, and 40 min after chamber closure and stored in pre-evacuated glass containers with a teflon-coated stopcock. Gas samples were analyzed using a gas chromatograph (Agilent 7890A, Santa Clara, California, USA) equipped with an electron capture detector and an autosampler. N₂O concentrations were determined by comparison of integrated peak areas of samples and five standard gases (320, 550, 1010, 1500, and 2300 ppb N₂O). N₂O fluxes were calculated from the linear increase of gas concentration in the chamber vs. time, and were adjusted for air temperature and atmospheric pressure measured at the time of sampling (Zheng et al., 2008). During the same time as the gas samples were collected, soil temperature and soil moisture at 10 cm below soil surface were monitored at each chamber using portable temperature probes (JM624 digital thermometer, Living-Jinming Ltd., China) and a moisture probe meter (TDR100, Spectrum, USA), respectively.

2.3. Measurements of soil dissolved N concentrations

After gas samples were collected, soils in litter layer (Oe/Oa) and 0–10 cm mineral layer (A) were taken using a 2.5 cm diameter auger. Five random cores were sampled at each plot, and then pooled for one sample. Soil samples were immediately passed through a 2-mm sieve to remove roots, gravel and stones, and then were transported to the laboratory in chilled polystyrene boxes. Soils were extracted using 2 M KCl for NH₄⁺, NO₃⁻, and total dissolved N (TDN) within a week to minimize storage effects on mineral N pools. Approximately 15 g fresh soil plus 100 mL of 2 M KCl were weighed into polyethylene bottles and shaken for 1 h. The soil solution was filtered using Whatman #1 filters, and the extracts were immediately frozen. Soil NH₄⁺-N, NO₃⁻-N, and TDN concentrations were determined by colorimetry on a continuous flow Auto Analyzer (AA3, SEAL, Germany). Because NO₂⁻ in soil extracts is unstable and easily changed to NO₃⁻, it was not determined in this study. Therefore, we approximately thought that dissolved organic nitrogen (DON) was equal to the difference between TDN and total inorganic N (NH₄⁺-N and NO₃⁻-N).

2.4. Statistical analyses

Repeated measures analysis of variance (ANOVA) was applied to examine the differences of soil temperature, soil moisture, dissolved N concentrations, and soil N₂O fluxes for different rates of N addition. Comparisons of the means were conducted using Tukey's honestly significant difference test. Stepwise regression analysis was used to examine the relationships between soil N₂O fluxes and other measured variables. Also, linear regression was used to elucidate the relationships between the changes in soil N₂O fluxes (Δ soil N₂O fluxes) and the

Table 1

Summary of repeated measures ANOVA evaluating the effects of date, N level and their interactions on soil N₂O fluxes and relevant soil properties in the litter layer (O) and mineral layer (M).

	Ts ^a	Ms ^a	N ₂ O flux	NH ₄ ⁺ -N		NO ₃ ⁻ -N		DON ^a		TDN ^a	
				O	M	O	M	O	M	O	M
<i>Between subjects</i>											
N level	0.99	<0.001	0.001	0.029	0.30	<0.001	<0.001	0.005	0.73	<0.001	0.38
<i>Within subjects</i>											
Date	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Date × N level	0.99	0.45	0.49	0.020	0.98	0.58	0.39	0.98	0.99	0.074	0.94

^a Ts, Ms, DON, TDN are 10 cm soil temperature, 10 cm soil moisture, dissolved organic nitrogen, and total dissolved nitrogen, respectively.

changes in soil variables (Δ soil variables) among the treatments in two soil layers. All statistical analyses were conducted using the SPSS software package (version 16.0) and graphics were drawn using the SigmaPlot software (version 12.5). Statistical significant differences were set with P values = 0.05.

3. Results

3.1. Soil temperature, moisture, and N₂O flux

Soil temperature in 0–10 cm depth showed a single-peak variation, with the peak occurring in early August ($P < 0.001$, Table 1, Fig. 1a). Soil temperature in 0–10 cm depth, ranging from 5.9 °C to 18.7 °C, was not influenced by N addition (Fig. 1b, Table 1). Soil moisture in 0–10 cm depth exhibited significant “event” characteristics three peaks in soil moisture content in early June, early September, and later October, corresponding to the period of snowmelt in June, and rain and snow events in September and October, respectively ($P < 0.001$, Table 1, Fig. 1c). Soil moisture averaged 35.4% in the control plots, and N addition at the rates of $>80 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (N₈₀) significantly increased soil

moisture by 17.75% to 28.49% (Fig. 1d). Also, there was significant seasonality for soil N₂O flux with a peak occurring in early August ($P < 0.001$, Table 1, Fig. 1e). Soil N₂O flux averaged $13.75 \mu\text{g N ha}^{-1} \text{ yr}^{-1}$ at the control plots, and N addition significantly increased soil N₂O flux by 194%, 297%, and 334% at the N₆₀, N₁₂₀, and N₁₄₀, respectively (Fig. 1f). Overall, soil N₂O flux grew exponentially with increase in the rates of N addition ($R^2 = 0.89$, $P = 0.001$, Fig. 1f).

3.2. Soil dissolved N concentrations

The seasonal variations of soil NO₃⁻-N, NH₄⁺-N, DON, and TDN in litter layer were significant ($P < 0.001$, Table 1, Fig. 2). The peaks of soil NO₃⁻-N, NH₄⁺-N, DON, and TDN in litter layer occurred in later October, early August, mid-June, and later October, respectively (Fig. 2). In the control plots, soil NO₃⁻-N, NH₄⁺-N, and DON concentration averaged 52.99 mg kg^{-1} , 275.0 mg kg^{-1} , and $118.89 \text{ mg kg}^{-1}$, accounting for 12.13%, 62.95%, and 27.22% of soil TDN content, respectively (Fig. 2b, d, and f). High rates of N addition ($>80 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) consistently and significantly increased soil NO₃⁻-N and TDN concentrations in litter layer by 120%–180% and 33.4%–51.3%, respectively (Fig. 2b and h).

Fig. 1. The monthly variations of soil temperature, soil moisture at 0–10 cm depth and soil N₂O flux under nine rates of N addition. Asterisk (*) means significant difference between N treatments and control at the level of 0.05.

Fig. 2. The monthly variations of soil NO_3^- -N, NH_4^+ -N, dissolved organic N (DON), and total dissolved N (TDN) concentrations at the litter layer under nine rates of N addition. Asterisk (*) means significant difference between N treatments and control at the level of 0.05.

However, N addition slightly increased soil NH_4^+ -N concentrations and did not change DON concentrations in litter layer (Table 1, Fig. 2d, and f).

There were significant seasonal variations in the three soil dissolved N in mineral layer with an obvious peak occurring in mid-July ($P < 0.001$, Table 1, Fig. 3). Under nature conditions, the soil NH_4^+ -N, NO_3^- -N, DON and TDN concentrations were lower in mineral layer than in litter layer, the former were 6.9–14.5 times as high as the latter (Figs. 2 and 3). N addition only led to significant accumulations of soil NO_3^- -N contents ($P < 0.001$, Table 1, Fig. 3b). No significant differences were observed in another three soil dissolved N contents across different N addition levels (Table 1, Fig. 3d, f, and h).

3.3. Relationships between soil N_2O fluxes and soil variables

The relationship between soil N_2O fluxes and soil temperature could be characterized by an exponential growth equation (Table 2, Fig. 4a). The relationships between soil N_2O fluxes, soil moisture, soil DON, and TDN concentrations could be well fitted by modified Gaussian equations (Fig. 4b, d, and f), and the critical values at the peak of the regression equation were 45.55%, 91.42 mg kg^{-1} , and 399.60 mg kg^{-1} , respectively (Table 2). Also, soil N_2O fluxes were positively and linearly correlated with soil moisture, soil NH_4^+ -N and NO_3^- -N concentrations (Table 2, Fig. 4c, and e). Multiple stepwise regression analysis revealed that soil N_2O fluxes were mainly controlled by soil temperature, followed by soil

NO_3^- -N and moisture (Table 2). They together explained >36% of the temporal variation in soil N_2O flux (Table 2).

To evaluate the relative contribution of each soil variable to the change in soil N_2O flux elicited by N addition, we performed a simple regression analysis of the data. The net change in soil N_2O flux (Δ soil N_2O flux, N addition treatments relative to the control) was positively correlated with the changes of soil NO_3^- -N and soil moisture (Δ soil NO_3^- -N content and Δ soil moisture); moreover, the correlation coefficient of the former was higher than that of the latter (Fig. 5).

4. Discussion

4.1. The responses of soil N_2O flux to N addition

Under natural conditions, the temperate broadleaf and mixed forest soil acted as a weak source for N_2O ($13.75 \pm 1.97 \mu\text{g N m}^{-2} \text{h}^{-1}$, Fig. 1f), which is higher than the measurements in the temperate pine and hardwood forests of North America ($0.27\text{--}1.10 \mu\text{g N m}^{-2} \text{h}^{-1}$, Bowden et al., 1991), as well as in European hardwood forests ($3\text{--}11 \mu\text{g N m}^{-2} \text{h}^{-1}$) (Schmidt et al., 1988). The reason for the observed differences might be ascribed to the N status of the forests and the capacity for nitrification between the stands. In this study, we found that N addition at levels of $<60 \text{ kg N ha}^{-1} \text{yr}^{-1}$ did not influence soil N_2O flux, while high N (N_{60} , N_{120} , N_{140}) significantly increased soil N_2O flux (Fig. 1e–f). These results, to a certain extent, supported our first hypothesis that organic N

Fig. 3. The monthly variations of soil NO_3^- -N, NH_4^+ -N, dissolved organic N (DON), and total dissolved N (TDN) concentrations at the mineral layer under nine rates of N addition. Asterisk (*) means significant difference between N treatments and control at the level of 0.05.

addition did not promote N_2O emission from the N-poor temperate forest soil until N addition rate exceeded its critical level. Soil N_2O flux grew exponentially with the rates of N addition, which was consistent with several studies in the forest, grassland, and cropland ecosystems (Ambus et al., 2006; Liu et al., 2013a, 2013b; Liu et al., 2015). The critical level of N input for the significant promotion of soil N_2O emission was approximately $70 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ while taking the ambient N deposition rate of $10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ into account (Zhu et al., 2015). Because soil NO_3^- -N content largely dominated the change of soil N_2O emission caused by N enrichment (Fig. 5a), the critical value of soil NO_3^- -N

content was estimated to be 6.89 g m^{-2} if the average soil bulk density of 0.53 g m^{-3} was used for calculating (Wang et al., 2012). Therefore, we can assume that the exogenous N input at the level of $<70 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ will not significantly stimulate soil N_2O emission, but it is favorable to C sequestration of the temperate needle-broadleaved mixed forest, Northeast China.

The responses of soil N_2O flux to N addition rates depend on the competitive interactions for soil available N between plants and soil microorganisms, and this competition includes three stages. First, plants are the winners for N acquisition in the long run compared with soil microbes (Kuzakov and Xu, 2013). So most of exogenous N could be absorbed by plants at low N concentrations and the activities of soil nitrifiers and denitrifiers are limited by available N. N addition does not change or reduces soil available N contents and soil N_2O emission (Aber et al., 1998; Borken and Beese, 2005). Second, after plant's N requirements are met, remaining available N is used by soil N nitrifying and denitrifying bacteria. Soil N_2O emission linearly increases with the increasing rate of N addition (Repo et al., 2009). Third, with the further accumulation of soil available N elicited by exogenous N input, N_2O -producing microbes can be stimulated and a higher percentage of available N is released in the form of N_2O (McSwiney and Robertson, 2005). At this time, soil N_2O emission grows exponentially with the increasing rate of N addition. For a given ecosystem, the observed response curve of soil N_2O flux to N addition should demonstrate one stage of the three.

Table 2
Regression models between soil N_2O fluxes and soil properties.

Soil variables ^a	Regression equation	R ²	P value
Ts	$F_{\text{N}_2\text{O}} = 1.25 \exp(0.23\text{Ts})$	0.20	<0.001
Ms	$F_{\text{N}_2\text{O}} = 48.47 \exp[-0.5 * \text{abs}(\text{Ms}-45.55) / 7.50]$	0.12	<0.001
NO_3^- -N	$F_{\text{N}_2\text{O}} = 7.37 + 0.49\text{NO}_3^-$ -N	0.11	<0.001
NH_4^+ -N	$F_{\text{N}_2\text{O}} = -5.11 + 0.23\text{NH}_4^+$ -N	0.099	<0.001
DON	$F_{\text{N}_2\text{O}} = 54.21 \exp[-0.5 * \text{abs}(\text{DON}-91.42) / 26.55]$	0.052	0.012
TDN	$F_{\text{N}_2\text{O}} = 58.74 \exp[-0.5 * \text{abs}(\text{TDN}-399.6) / 86.87]$	0.10	<0.001
Combined	$F_{\text{N}_2\text{O}} = -96.30 + 4.43\text{Ts} + 0.67 \text{NO}_3^-$ -N + 0.82Ms	0.36	<0.001

^a Ts is soil temperature at 10 cm depth, Ms is soil moisture at 10 cm depth, NH_4^+ , NO_3^- , and TDN are the average concentrations of soil NH_4^+ , NO_3^- , and TDN at 20 cm depth.

Fig. 4. The relationships between soil N_2O fluxes and related soil properties (soil temperature, soil moisture, and dissolved N concentrations).

4.2. The main processes controlling soil N_2O production

An exponential growth equation and a linear equation could be used to fit the relationships between the soil N_2O fluxes and soil temperature and between the soil N_2O fluxes and soil moisture, respectively (Fig. 4). Soil water content has a direct effect on oxygen availability due to its

effect on oxygen diffusion whereas soil temperature indirectly controls the oxygen status through its effect on microbial activity and thus, oxygen consumption (Ding et al., 2007). Soil moisture and temperature are often reported to explain most of the variation in N_2O fluxes in European forests (e.g., Schindlbacher et al., 2004; Pilegaard et al., 2006) even in forests highly enriched in N (Brumme et al., 1999). Also, nitrifying and denitrifying bacteria are quite sensitive to soil temperature and moisture, which fundamentally determines the nitrification and denitrification rates (Butterbach-Bahl et al., 2013). The cold and moist climate in the temperate needle-broadleaved mixed forest may favor nitrification and denitrification, and consequently N_2O emissions, regardless of abundant NH_4^+ and NO_3^- substrates (Eickenscheidt and Brumme, 2012). However, soil hydrothermal factors and soil dissolved N contents only explained 36% of the variation of soil N_2O flux, indicating that other environmental variables such as soil redox potential (Eh), water-filled pore space, pH, and labile C content could have greater effects on soil N_2O emission (Weier et al., 1993; Pilegaard et al., 2006). These factors often determine the abundance and composition of soil nitrifier and denitrifier communities (Wallenstein et al., 2006).

Moreover, N_2O emissions are byproducts of nitrification ($\text{NH}_4^+ \rightarrow \text{NO}_3^-$) and denitrification ($\text{NO}_3^- \rightarrow \text{N}_2$), which rely on the availability of soil NH_4^+ and NO_3^- . In our study, we also found significant and positive relationships between soil N_2O fluxes and soil NO_3^- -N contents, and between soil N_2O fluxes and soil NH_4^+ -N contents, respectively (Fig. 4c and e), which indicated the increase in substrate availability of autotrophic nitrification and biological denitrification. The nonlinear relationships between soil DON contents and N_2O emission suggested that there was an optimal substrate concentration (ca. 90 mg kg^{-1}) for soil heterotrophic nitrification (Fig. 4d). Using ^{15}N paired labeling and tracing model, Zhang et al. (2011) documents that heterotrophic nitrification of organic N is the predominant NO_3^- production pathway in the temperate forest soils in Northeast China. Therefore, heterotrophic nitrification could have a greater contribution to soil N_2O production than

Fig. 5. The relationships between the changes in soil N_2O fluxes and the changes in soil NO_3^- -N concentrations and soil moisture contents caused by N addition.

autotrophic nitrification under aerobic conditions (Zhu et al., 2011). Stepwise linear regression analysis showed that soil temperature, soil NO_3^- -N content, and soil moisture content were the dominant factors that controlled soil N_2O emissions (Table 2). Ambus et al. (2006) reports that NO_3^- -N is the major substrate for soil N_2O production in European temperate forests with the average contribution of 62%, whereas soil NH_4^+ -N can explain 34% of the variation of soil N_2O flux. Since NO_3^- -N was either produced by net or by gross nitrification, NO_3^- -N accumulated in soils may have become the exclusive source for denitrification and N_2O production. In our study, the net changes in soil N_2O fluxes caused by N addition were related to the net changes in soil NO_3^- -N content and soil moisture content (Fig. 5), which indicated that the increase in soil N_2O emission in the temperate needle-broadleaved forest under N enrichment could be mainly attributed to the increase in soil nitrification and denitrification. Similarly, based on the ^{15}N -tracing experiment, Cheng et al. (2014) suggests that the increase in N_2O emission following N fertilizer application is attributed to increased heterotrophic and autotrophic nitrification rates and an increased ratio of N_2O emission from denitrification.

5. Conclusions

The responses of soil N_2O fluxes as well as soil dissolved N to N addition in a broadleaf and mixed forest were examined in the growing season of 2013 with an urea addition trail, including nine N rates (0, 10, 20, 40, 60, 80, 100, 120 and 140 $\text{kg N ha}^{-1} \text{yr}^{-1}$). Low N addition did not change but high N addition significantly led to the accumulation of soil NO_3^- -N in both the litter layer and surface mineral layer. Soil N_2O flux grew exponentially with increase in the rates of N addition. Taking the ambient N deposition rate of 10 $\text{kg N ha}^{-1} \text{yr}^{-1}$ into account, we found that the critical level of N input for the significant alternation of soil dissolved N and soil N_2O emission in the studied forest was around 70 $\text{kg N ha}^{-1} \text{yr}^{-1}$. The increase in soil N_2O emission in the temperate needle-broadleaved mixed forest under N enrichment was mainly attributed to the increases in soil heterotrophic nitrification and biological denitrification. Unfortunately, we were unable to quantify the relative contributions of soil nitrification and denitrification to soil N_2O flux due to some technical limitations such as ^{15}N labeling, product isolation, measurement accuracy, etc.; moreover, the potential mechanisms involved in the formation of critical load for N retention are not clear. Further study is needed to simultaneously measure the rates of gross N transformation, and the abundance and composition of producing- N_2O microbial community in ambient and N enrichment soils. This would help to understand the mechanisms involved in the nonlinear responses of soil N_2O emission to increased N deposition.

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