



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, Högborg, 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 Hettelingh, 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

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