

## Effects of nitrogen form and dose on soil N<sub>2</sub>O emissions in cold-temperate coniferous forests: Postprint

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### Abstract

Atmospheric nitrogen deposition inputs increase nitrogen availability in forest ecosystems, thereby altering soil N<sub>2</sub>O production and emission; however, knowledge regarding the effects of deposition of different nitrogen ion forms (oxidized NO<sub>3</sub>-N versus reduced NH<sub>4</sub><sup>+</sup>-N) on soil N<sub>2</sub>O emission remains limited. Using a cold-temperate coniferous forest in the Greater Khingan Mountains as the study site, we established a nitrogen addition control experiment with three chemical types (NH<sub>4</sub>Cl, KNO<sub>3</sub>, NH<sub>4</sub>NO<sub>3</sub>) and four nitrogen addition rates (0, 10, 20, 40 kg N hm<sup>-2</sup>a<sup>-1</sup>). Soil inorganic nitrogen content in the litter and mineral layers, net N<sub>2</sub>O exchange flux at the soil-atmosphere interface, and related environmental factors were measured four times per month using a flow injection analyzer and the static chamber-gas chromatography method. We analyzed the effects of nitrogen addition type and rate on soil nitrogen availability and soil N<sub>2</sub>O flux, and explored the environmental driving mechanisms of soil N<sub>2</sub>O flux under nitrogen enrichment conditions. The results showed that both nitrogen addition type and rate significantly affected soil inorganic nitrogen content, with the accumulation effect of soil NH<sub>4</sub><sup>+</sup>-N being significantly higher than that of NO<sub>3</sub>-N. Nitrogen addition consistently increased soil N<sub>2</sub>O emission from the cold-temperate coniferous forest, with NH<sub>4</sub>NO<sub>3</sub> showing the most pronounced promotion effect, with increases ranging from 442% to 677%, which exceeds the global average level (134%). Soil N<sub>2</sub>O flux was positively correlated with soil temperature and litter layer NH<sub>4</sub><sup>+</sup>-N content, and increased with increasing nitrogen addition rates. The results indicate that atmospheric nitrogen deposition will not cause substantial NO<sub>3</sub>-N loss from cold-temperate coniferous forest soils in the short term, but will significantly promote soil N<sub>2</sub>O emission. Furthermore, exogenous NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> inputs exhibit a synergistic effect in promoting soil N<sub>2</sub>O emission, and should be treated separately in future studies on nitrogen cycling and nitrogen balance in forest ecosystems.

## Full Text

### Preamble

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### The Effects of Nitrogen Types and Doses on Soil N<sub>2</sub>O Emissions in a Cold-Temperate Coniferous Forest, Northern China

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### Abstract

Atmospheric nitrogen (N) deposition increases nitrogen availability in forest ecosystems, thereby altering soil N<sub>2</sub>O production and emissions. However, the contrasting effects of different nitrogen ions (i.e., oxidized NO<sub>3</sub><sup>-</sup>-N and reduced NH<sub>4</sub><sup>+</sup>-N) on N<sub>2</sub>O emissions remain poorly understood, particularly in boreal forests. This study investigated a cold-temperate coniferous forest in the Greater Khingan Mountains using a nitrogen addition control experiment. We employed flow chemistry analyzers and static chamber-gas chromatography methods to measure monthly inorganic nitrogen content in the litter and mineral soil layers, net N<sub>2</sub>O exchange fluxes, and related environmental factors. The experiment included three nitrogen fertilizer types (NH<sub>4</sub>Cl, KNO<sub>3</sub>, and NH<sub>4</sub>NO<sub>3</sub>) and four nitrogen addition rates (0, 10, 20, and 40 kg N hm<sup>-2</sup>).

Our results demonstrate that both nitrogen type and dose significantly altered soil inorganic nitrogen content, with NH<sub>4</sub><sup>+</sup>-N accumulation significantly exceeding that of NO<sub>3</sub><sup>-</sup>-N following nitrogen addition. Nitrogen addition consistently increased N<sub>2</sub>O emissions from the boreal forest soil, with NH<sub>4</sub>NO<sub>3</sub> treatments producing the highest fluxes (442%–677% increase), which substantially exceeds the global average (134%). Soil N<sub>2</sub>O fluxes showed positive correlations with soil temperature and with NH<sub>4</sub><sup>+</sup>-N content in the litter layer. Multiple regression analysis revealed that soil temperature was the primary driver of N<sub>2</sub>O fluxes, followed by NH<sub>4</sub><sup>+</sup>-N content in the litter layer.

These findings suggest that atmospheric nitrogen deposition will not cause significant NO<sub>3</sub><sup>-</sup>-N leaching from boreal forests in the short term, but can substantially promote soil N<sub>2</sub>O emissions. Furthermore, exogenous NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> inputs have synergistic effects on soil N<sub>2</sub>O emissions, a distinction that should

be considered in future studies of forest ecosystem nitrogen cycling and balance using nitrogen tracer methods.

**Keywords:** atmospheric nitrogen deposition; soil N<sub>2</sub>O flux; nitrogen availability; controlling factors; boreal forest

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## 1. Introduction

Nitrous oxide (N<sub>2</sub>O) is one of the three major greenhouse gases in Earth's atmosphere. On a century timescale, its global warming potential (GWP) per molecule is 298 times that of CO<sub>2</sub>, contributing approximately 6% to global warming. N<sub>2</sub>O also destroys the ozone layer by diffusing into the stratosphere, where it is photolyzed, ultimately forming nitric acid and nitrate, thereby increasing ground-level UV radiation. The IPCC Fifth Assessment Report indicates that atmospheric N<sub>2</sub>O concentrations in 2011 were 20% higher than pre-industrial levels, with a recent average annual increase of 0.25%. Natural vegetation soils represent the primary emission source, accounting for 17.7 Tg N/year (37.8% of total natural sources), while the atmospheric sink is estimated at 12.6 Tg N/year.

Reactive nitrogen reaches the surface through wet and dry deposition, with current global atmospheric nitrogen deposition reaching 105 Tg N/year. This significantly alters terrestrial ecosystem carbon and nitrogen cycles, affecting ecosystem structure and function. Soil N<sub>2</sub>O emissions mainly originate from nitrification and denitrification processes mediated by functional microbial communities including nitrifying bacteria, denitrifying bacteria, and some mycorrhizal fungi. Exogenous nitrogen inputs can substantially alter the activity and composition of N<sub>2</sub>O-producing microbial communities, thereby affecting soil N<sub>2</sub>O emissions. The effects of nitrogen addition on natural vegetation soil N<sub>2</sub>O emissions show varied responses including promotion, no change, or inhibition, depending on ecosystem type, initial nitrogen status, nitrogen form, and duration of application.

Previous studies on N<sub>2</sub>O emission responses to nitrogen deposition have yielded some general patterns, but two major deficiencies remain: (1) Most nitrogen addition experiments have used relatively high doses (>50 kg N hm<sup>-2</sup>), making it difficult to observe ecosystem responses to low-dose nitrogen input in the short term; and (2) Most experiments have employed single nitrogen fertilizer types, with few studies distinguishing the different effects of oxidized NO<sub>3</sub><sup>-</sup> and reduced NH<sub>4</sub><sup>+</sup> inputs on soil N<sub>2</sub>O emissions in nitrogen-limited natural vegetation.

Boreal forests, covering 14.5% of global land area with soil carbon density of 296 t C/hm<sup>2</sup>, represent the second largest forest biome after tropical forests. Due to low temperatures and slow soil nitrogen mineralization, these forests have extremely limited nitrogen availability and are highly sensitive to exogenous ni-

trogen inputs. Nitrogen deposition can significantly alter plant and microbial community composition, ecosystem productivity, and carbon sequestration potential. While tropical and subtropical forests with favorable hydrothermal conditions were previously thought to be major  $N_2O$  sources, recent studies indicate that warming and increased nitrogen deposition have enhanced carbon-nitrogen transformation in high-latitude forest soils, resulting in substantial  $N_2O$  emissions. This long-term underestimation of  $N_2O$  emissions from high-latitude natural vegetation may contribute to uncertainties in global  $N_2O$  budgets.

This study addresses these knowledge gaps through a low-dose nitrogen addition experiment in a cold-temperate coniferous forest of the Greater Khingan Mountains. We tested two hypotheses: (1) Exogenous nitrogen input would significantly increase soil nitrogen availability, thereby promoting  $N_2O$  production and emissions; and (2) The stimulatory effect of  $NO_3^-$ -N on  $N_2O$  emissions would exceed that of  $NH_4^+$ -N, given that nitrification may dominate  $N_2O$  production in boreal forest soils.

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## 2. Materials and Methods

### 2.1 Study Area

The study site is located in the Kailaqi Forest Farm, east of the Inner Mongolia Greater Khingan Mountains Forest Ecosystem National Field Scientific Observation and Research Station (50°20' -50°30' N, 121°45' -122°00' E). This region represents the northwest slope of the Greater Khingan Mountains with a cold-temperate semi-humid climate. Winters are cold and long, with an average annual temperature of -5.4°C and annual precipitation of 800-1200 mm. The highest temperatures occur in August. The main species is Dahurian larch (*Larix gmelinii*), with associated species including white birch (*Betula platyphylla*), rhododendron (*Rhododendron simsii*), Labrador tea (*Ledum palustre*), and lingonberry (*Vaccinium vitis-idaea*). The vegetation type is characterized as *Ledum palustre*-*Larix gmelinii* forest. Soils are brown coniferous forest soils developed on granite residuum, with pH 4.5-6.5 and organic matter content of 10%-30%.

### 2.2 Experimental Design

Based on actual atmospheric nitrogen deposition fluxes in the Greater Khingan region, we established a nitrogen addition experiment with three fertilizer types ( $NH_4Cl$ ,  $KNO_3$ , and  $NH_4NO_3$ ) and four addition rates: control (0 kg N  $hm^{-2}$ ), low N (10 kg N  $hm^{-2}$ ), medium N (20 kg N  $hm^{-2}$ ), and high N (40 kg N  $hm^{-2}$ ). A split-plot design was used with nitrogen level as the main treatment and nitrogen form as the subplot treatment. Each nitrogen level included a control to enhance comparison among nitrogen types. To minimize effects of microtopographic heterogeneity, plot size was 10 m  $\times$  20 m with at least 10

m spacing between adjacent plots. The annual nitrogen addition was evenly distributed during the growing season (May–October), with fertilizers dissolved in water and sprayed uniformly across each plot at the beginning of each month.

### 2.3 Soil N<sub>2</sub>O Flux Measurement

Soil N<sub>2</sub>O emissions were measured using the static chamber-gas chromatography method. In each plot, permanent bases (50 cm × 50 cm × 10 cm) with grooves were installed. During measurement, a chamber (50 cm × 50 cm × 20 cm) equipped with a thermometer and small fan was carefully placed into the groove, which was then filled with water to ensure sealing. Gas samples (100 mL) were collected at 0, 10, 20, and 40 minutes using a syringe. Simultaneously, atmospheric temperature, chamber temperature, and soil temperature at 10 cm depth were recorded. Soil volumetric water content was measured using a TDR200 soil moisture probe (Spectrum Technologies, USA). N<sub>2</sub>O concentrations were analyzed using a 7890A gas chromatograph (Agilent, USA) with column oven temperature at 55°C, injector temperature at 250°C, and carrier gas (N<sub>2</sub>) flow rates of 30 mL/min for the main line, 300 mL/min for auxiliary, and 50 mL/min for makeup. Fluxes were calculated using standard equations.

### 2.4 Soil Sampling and Analysis

After gas sampling, organic and mineral soil layer samples were collected. Undecomposed litter (Oi horizon) was removed, and organic layer samples were collected using a 10 cm × 10 cm × 15 cm frame along plot diagonals. Mineral layer samples (0–10 cm) were collected using a 2.5 cm diameter corer. Five subsamples per plot were composited into one sample. Soil gravimetric water content was determined by oven-drying. Inorganic nitrogen (NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N) was extracted with 2 mol/L KCl (15 g fresh soil in 100 mL KCl, shaken for 1 hour, filtered) and analyzed using an AA3 flow chemistry analyzer (SEAL, Germany).

### 2.5 Statistical Analysis

Repeated measures ANOVA was used to compare effects of nitrogen level and form on soil temperature, moisture, inorganic nitrogen content, and N<sub>2</sub>O fluxes. Tukey's HSD test was applied for multiple comparisons. Simple and multiple stepwise regression analyses examined relationships between N<sub>2</sub>O fluxes and soil variables. All analyses were conducted using SPSS 16.0, with figures prepared in SigmaPlot 12.5.

### 3. Results

#### 3.1 Soil Temperature and Moisture

Throughout the growing season, soil temperature showed significant seasonal variation with a unimodal pattern, peaking in August. Soil temperature ranged from 0.70°C to 15.03°C and was not significantly affected by nitrogen addition. Soil volumetric water content in the 0–10 cm layer exhibited clear seasonal fluctuations, gradually decreasing from May to October. May through July had relatively high moisture content, with the highest values in early May (27.48% in control plots) and lowest in late October (5.86%). Both nitrogen level and form significantly altered soil moisture content ( $P = 0.02$  and  $P = 0.03$ , respectively).

#### 3.2 Soil N<sub>2</sub>O Fluxes

Soil N<sub>2</sub>O fluxes in control plots ranged from -1.19 to 5.13 g N m<sup>-2</sup> h<sup>-1</sup>, showing significant seasonal variation ( $P < 0.001$ ) with emission peaks in August. Nitrogen addition dramatically increased N<sub>2</sub>O emissions, with both nitrogen level and form having highly significant effects ( $P < 0.001$ ). Emissions increased progressively with nitrogen addition rate. Among nitrogen forms, NH<sub>4</sub>NO<sub>3</sub> showed the strongest stimulatory effect, with flux increases of 442%–677% compared to control—substantially higher than the global average of 134%. The effect of nitrogen dose was more pronounced than nitrogen form, with NH<sub>4</sub>NO<sub>3</sub> and KNO<sub>3</sub> showing significantly greater promotion than NH<sub>4</sub>Cl.

#### 3.3 Soil Inorganic Nitrogen Content

Throughout the growing season, organic layer NH<sub>4</sub><sup>+</sup>-N content showed little seasonal variation but exhibited two peaks in June and September, ranging from 0.21 to 9.09 mg/kg in control plots. Mineral layer NH<sub>4</sub><sup>+</sup>-N content was lower than in the organic layer and showed no significant accumulation except at high nitrogen doses. Nitrogen addition level significantly affected NH<sub>4</sub><sup>+</sup>-N accumulation, with low-dose treatments showing higher accumulation than medium and high doses. For NO<sub>3</sub><sup>-</sup>-N, both organic and mineral layers showed significant seasonal variation ( $P < 0.001$ ), with organic layer content (5.4–21.84 mg/kg) approximately 30 times higher than mineral layer content. Nitrogen form significantly affected organic layer NO<sub>3</sub><sup>-</sup>-N ( $P = 0.003$ ), while nitrogen level marginally affected mineral layer NO<sub>3</sub><sup>-</sup>-N ( $P = 0.09$ ).

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## 4. Discussion

### 4.1 Effects of Nitrogen Type and Dose on Soil Inorganic Nitrogen Accumulation

Soil inorganic nitrogen accumulation depends on the balance of nitrogen mineralization, microbial immobilization, and adsorption by soil organic matter

and minerals. Our findings that nitrogen type and dose significantly altered soil inorganic nitrogen content support our first hypothesis. The accumulation of  $\text{NH}_4^+$ -N was significantly greater than  $\text{NO}_3^-$ -N following nitrogen addition, consistent with results from nitrogen-poor boreal and temperate forests. Only ammonium-based fertilizers ( $\text{NH}_4\text{Cl}$  and  $\text{NH}_4\text{NO}_3$ ) caused significant  $\text{NH}_4^+$ -N accumulation in mineral soils, indicating that added nitrogen first adsorbs to litter layer organic matter. In the Greater Khingan region, plants and microorganisms are ammonium-preferring, with plants being superior competitors for nitrogen. This selective uptake, combined with the soil's sandy texture (51.76% sand, 27.55% silt, 9.53% clay) and high moisture content, results in significant vertical leaching and denitrification losses, preventing  $\text{NO}_3^-$ -N accumulation except at high deposition rates.

#### 4.2 Effects of Nitrogen Type and Dose on Soil $\text{N}_2\text{O}$ Fluxes

Our results demonstrate that nitrogen addition consistently increased  $\text{N}_2\text{O}$  emissions from cold-temperate coniferous forest soils, with annual emissions of  $(1.23 \pm 0.42)$  kg N  $\text{hm}^{-2}$  in control plots. The 442%-677% increase under  $\text{NH}_4\text{NO}_3$  addition significantly exceeds the global average of 134%, indicating that nitrogen-poor boreal forest soils are highly sensitive to exogenous nitrogen input. The stronger response to nitrate-based fertilizers ( $\text{KNO}_3$  and  $\text{NH}_4\text{NO}_3$ ) suggests that denitrification may dominate  $\text{N}_2\text{O}$  production processes in these soils. The synergistic effect of combined  $\text{NH}_4^+$  and  $\text{NO}_3^-$  inputs ( $\text{NH}_4\text{NO}_3$ ) had an additive effect on emissions, likely because it simultaneously provides substrates for both nitrification and denitrification while increasing denitrifying bacterial activity.

Soil  $\text{N}_2\text{O}$  fluxes were primarily driven by temperature, followed by  $\text{NH}_4^+$ -N content in the litter layer, together explaining 27% of seasonal variation. While  $\text{N}_2\text{O}$  production is biologically mediated and temperature-dependent, emission rates also depend on substrate availability. The positive correlation with litter layer  $\text{NH}_4^+$ -N content suggests that nitrogen transformation processes in the organic horizon dominate soil  $\text{N}_2\text{O}$  emissions. However, the complexity of  $\text{N}_2\text{O}$  production and consumption processes, combined with our inability to quantify relative contributions from nitrification and denitrification, represents a limitation. Future research should integrate  $^{15}\text{N}$  tracer techniques and molecular biological methods to precisely determine nitrogen transformation rates and functional microbial community dynamics.

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## 5. Conclusion

This study examined the effects of nitrogen form and dose on soil  $\text{N}_2\text{O}$  emissions in a cold-temperate coniferous forest of the Greater Khingan Mountains through a low-dose nitrogen addition experiment. We monitored soil inorganic nitrogen content,  $\text{N}_2\text{O}$  fluxes, and hydrothermal environmental factors during the growing season to explore the environmental drivers of  $\text{N}_2\text{O}$  emissions under

nitrogen enrichment. Three main conclusions emerge:

1. Nitrogen type and dose significantly altered soil inorganic nitrogen content, with  $\text{NH}_4^+$ -N accumulation effects exceeding those of  $\text{NO}_3^-$ -N. Short-term atmospheric nitrogen deposition (<3 years) did not cause substantial nitrogen loss through leaching.
2. Nitrogen addition consistently increased  $\text{N}_2\text{O}$  emissions from cold-temperate coniferous forest soils, with the magnitude of increase (442%-677%) exceeding global averages. The stimulatory effects of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  inputs showed additive properties.
3. Soil  $\text{N}_2\text{O}$  fluxes were primarily temperature-driven, with litter layer  $\text{NH}_4^+$ -N content as a secondary control factor. Emissions increased with nitrogen addition level.

Limitations include the inability to quantify relative contributions of nitrification and denitrification to  $\text{N}_2\text{O}$  production. Future research should employ  $^{15}\text{N}$  tracer techniques and molecular ecological approaches to elucidate functional microbial community dynamics and their coupling with nitrogen transformation processes under nitrogen enrichment.

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