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## Responses of soil nitrous oxide flux to soil environmental factors in a subtropical coniferous plantation: A boundary line analysis



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### ABSTRACT

Soil N<sub>2</sub>O responds differently to various soil variables; moreover these driving variables usually have low explanatory capacity of soil N<sub>2</sub>O flux variability. Anthropogenic nitrogen (N) deposition inputs to the subtropical plantation forests markedly increase N<sub>2</sub>O emission from soils, but the relative contribution of deposited NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> is not well understood. Based on a two-type (NH<sub>4</sub>Cl and NaNO<sub>3</sub>) and three-level (0, 40, 120 kg N ha<sup>-1</sup> yr<sup>-1</sup>) N addition experiment, we simultaneously determined soil N<sub>2</sub>O flux and related soil variables once a week in 2015. Boundary line analysis was used to describe the maximum responses of soil N<sub>2</sub>O fluxes to different soil variables. Four years of N addition led to significant accumulations of soil NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N, thereby accelerating soil acidification. Nitrogen addition significantly increased soil N<sub>2</sub>O fluxes by 4.7–19.3 folds relative to control; moreover, the promotions to soil N<sub>2</sub>O emission and soil acidification were greater with ammonium-N addition than with nitrate-N addition. The relationships between the maximum soil N<sub>2</sub>O fluxes and individual soil variables were well fitted to the Gaussian equations, and the optimum values of soil temperature, moisture, pH, and NO<sub>3</sub><sup>-</sup>-N content indicate that nitrification dominated soil N<sub>2</sub>O production. Overall, our results suggest that exogenous NH<sub>4</sub><sup>+</sup> input to the subtropical plantation appears to have greater negative effects on soil acidification and N<sub>2</sub>O emission compared with NO<sub>3</sub><sup>-</sup> input. Boundary line analysis well depicts the shape and magnitude of response functions of soil N<sub>2</sub>O fluxes against soil variables, which should be incorporated into terrestrial ecosystem process models.

### 1. Introduction

Nitrous oxide (N<sub>2</sub>O) is a potent greenhouse gas in the atmosphere after carbon dioxide (CO<sub>2</sub>) and methane (CH<sub>4</sub>). On a 100-year time-scale, the global warming potential (GWP) of single molecule N<sub>2</sub>O is 298 and 25 folds of CO<sub>2</sub> and CH<sub>4</sub>, respectively [1]. Also, N<sub>2</sub>O is the most important ozone-depleting substance and the primary source of stratospheric NO<sub>x</sub> [2]. Since the pre-industrial era, human activity has led to an increase in the atmospheric N<sub>2</sub>O concentration by 20% [3], and N<sub>2</sub>O emissions are expected to continue to increase in the next few decades [4]. The accumulation of atmospheric N<sub>2</sub>O contributes to the global warming by about 6% [5]. Soils are the main source and account for approximately 37% of global N<sub>2</sub>O emissions [3], and N<sub>2</sub>O emission from subtropical and tropical forest soils accounts for 14–23% of the global N<sub>2</sub>O budget [5].

Besides N<sub>2</sub>O, human activities such as fossil fuel combustion, fertilizer production and utilization, animal husbandry intensive have emitted a large amount of reactive N into the atmosphere, thereby leading to a dramatic increase in atmospheric N deposition. It is estimated that N deposition increased from 34 Tg N yr<sup>-1</sup> in 1860 to 100 Tg N yr<sup>-1</sup> in 1995 and is likely to reach 200 Tg N yr<sup>-1</sup> by 2050 [6,7]. As one of the major deposition centers in the world, the average rate of N deposition in China is between 12.9 and 21.1 kg N ha<sup>-1</sup> yr<sup>-1</sup> [8,9]. Exogenous N inputs to terrestrial ecosystems improve plant N uptake, elevate soil N transformation and loss, but slightly increase soil N storage, indicating that terrestrial ecosystems are leaking [10]. For a given terrestrial ecosystem, chronic N deposition or N addition can increase [11], not affect [12], and decrease [13] soil N<sub>2</sub>O emission, respectively. By performing a meta-analysis of global N addition experiment data, Liu and Greaver [14] reported that N additions up to

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10–562 kg N ha<sup>-1</sup> yr<sup>-1</sup> significantly increased soil N<sub>2</sub>O emission by 223%–838% across all ecosystems. Thus, there is great uncertainty as to the magnitude of soil N<sub>2</sub>O emission response to N addition. Soil N<sub>2</sub>O is mainly produced by nitrification and denitrification [4], and is regulated by multiple environmental factors such as soil temperature, moisture, pH, and available C and N contents, etc [15,16]. However, the above environmental factors have usually low explanatory capacity of soil N<sub>2</sub>O flux with the coefficient of determination (R<sup>2</sup>) of the regression equation less than 0.5 [17]. This indicates that the effects of environmental factors on soil N<sub>2</sub>O flux are extremely complex, and multiple factors interact and work together. Quantifying the relationships between soil N<sub>2</sub>O flux and environmental factors is essential to accurately predict N<sub>2</sub>O emissions from soil under different scenarios.

The boundary line analysis provides the maximum response of a biogeochemical process to a given environmental factor. It is based on the hypothesis that the line connecting data points at the outer margin of the data cloud defines the maximum possible dependent variable for the given value of each independent variable, i.e. where all other factors are non-limiting [18]. Therefore, the boundary line analysis is very suitable to identify soil N<sub>2</sub>O emission responses to multiple environmental factors [17,19,20]. Unfortunately, little information is available about the relationships between soil N<sub>2</sub>O flux and related environmental factors under ambient and N enrichment; moreover, we do not know whether there is an optimum value of environmental factors where soil N<sub>2</sub>O emission is the highest.

There is approximately 200 million ha of plantations in the world today, and these lands sequester atmospheric CO<sub>2</sub> at a rate of 0.18 Pg C yr<sup>-1</sup> [21]. China has the largest plantation area (62 million ha) in the world [22,23], approximately 63% of plantations in China distributed in the subtropical region where N deposition rate is the highest in the nation (> 30 kg N ha<sup>-1</sup> yr<sup>-1</sup> [24]). Furthermore, the subtropical plantation forests are vulnerable to increased N deposition due to its single community structure and poor soil fertility. Several studies in the subtropical plantations have shown that inorganic N addition significantly increases soil NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N contents [25], accelerates soil acidification [26], and promotes soil NO and N<sub>2</sub>O emissions [27,28]. Using <sup>15</sup>N-labeling incubation experiment with a Markov chain Monte Carlo Metropolis algorithm, Gao et al. [29,30] found that NH<sub>4</sub><sup>+</sup> addition did not affect heterotrophic, autotrophic and gross nitrification, whereas NO<sub>3</sub><sup>-</sup> addition significantly stimulated autotrophic nitrification. High rate of NH<sub>4</sub><sup>+</sup> input inhibits soil N mineralization and fungal activity, and NO<sub>3</sub><sup>-</sup> addition inhibits or promotes organic N mineralization [29,30]. Wang et al. [31] also documented that NH<sub>4</sub>Cl addition significantly changed ammonia-oxidizer abundance with an increase in ammonia-oxidizing archaea (AOA) and a decrease in ammonia-oxidizing bacteria (AOB), but the effects of NaNO<sub>3</sub> addition were not significant. Thus, the reduced NH<sub>4</sub><sup>+</sup> and oxidized NO<sub>3</sub><sup>-</sup> have contrasting influences on soil acidification, N transformation, and related functional microbial community. However, we do not know whether the effects of exogenous NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> inputs on soil N<sub>2</sub>O emissions are different.

We hypothesized that exogenous NH<sub>4</sub><sup>+</sup> input to the subtropical plantation would have a greater effect on soil N<sub>2</sub>O flux than NO<sub>3</sub><sup>-</sup> input due to stronger soil acidification from NH<sub>4</sub><sup>+</sup>-N addition. We also hypothesized that each environmental factor would have a given range at which has the maximal effect on soil N<sub>2</sub>O emission considering the optimal environmental conditions for both nitrifiers and denitrifiers. To test these two hypotheses, we conducted a field experiment with two N types and three application rates in the subtropical plantation of southern China. The specific purposes of this study were to (1) investigate the effects of N addition rates and types on soil N<sub>2</sub>O flux and N<sub>2</sub>O-associated soil properties (i.e. soil temperature, moisture, pH, and inorganic N contents), and (2) evaluate the dependency of soil N<sub>2</sub>O flux on given soil variables under ambient and N enrichment using the method of boundary line analysis.

## 2. Materials and methods

### 2.1. Study site and experimental design

This study was conducted at the Qianyanzhou Ecological Station of Chinese Academy of Sciences (26°44'39"N, 115°03'33"E) in Jiangxi province of China. This site is located in a typical red soil hilly area. It is a subtropical monsoon climate, with the total annual rainfall ranging between 945 mm and 2145 mm and mean annual temperature ranging between 17 °C and 19 °C in 1989–2008 [25]. Original vegetation is a subtropical evergreen broad-leaved forest, but it has now transformed into coniferous plantation forest resulting from deforestation and land use change. The dominant tree species is slash pine, and its height, diameter breast high, basal area, leaf area index are 12.0 m, 15.8 cm, 35 m<sup>2</sup> ha<sup>-1</sup> and 4.5 m<sup>2</sup> m<sup>-2</sup>, respectively [25]. Soils are typically red soils, classified as Cambosols (IUSS classification), which were derived from sandstone and sandy conglomerate. Topsoil (0–20 cm) organic C content is 20.44 g kg<sup>-1</sup>, total N is 1.10 g kg<sup>-1</sup>, total phosphorus is 1.12 g kg<sup>-1</sup>, pH is 4.26, and soil bulk density is 1.54 g cm<sup>-3</sup> [31].

The measured atmospheric N deposition rate in the study area was 32.6 kg N ha<sup>-1</sup> yr<sup>-1</sup> in 2013, and the ratio of NH<sub>4</sub><sup>+</sup> to NO<sub>3</sub><sup>-</sup> was 6/5 [24]. The N addition experiment was set up as a randomized block design. To assess the impacts of deposited NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> on soil N biogeochemical cycles, two types of N fertilizer, NH<sub>4</sub>Cl and NaNO<sub>3</sub>, were applied to the surface. Each N fertilizer was added at rates of 40 and 120 kg N ha<sup>-1</sup> yr<sup>-1</sup> to simulate possible atmospheric N deposition increasing. A control was set up at each block. Thereafter, five experimental treatments were referred to as control (CK), low NH<sub>4</sub>Cl (A40), low NaNO<sub>3</sub> (N40), high NH<sub>4</sub>Cl (A120), and high NaNO<sub>3</sub> (N120), respectively. There were 15 plots (20 m × 20 m) surrounded by a 10 m-wide buffer strip. We sprayed N fertilizer solutions under the canopy in the first week of each month. The control plots were sprayed the same amounts of water. The N addition experiment started in May 1, 2012 and ended at January 31, 2016.

### 2.2. Measurements of soil N<sub>2</sub>O flux and auxiliary variables

Soil N<sub>2</sub>O flux was measured using static opaque chamber and gas chromatography techniques [32]. At each plot, a stainless steel box, made up of one chamber (length × width × height = 50 cm × 50 cm × 15 cm) and one collar (length × width × height = 50 cm × 50 cm × 10 cm), was installed. Gas samples were collected twice a week between 9: 00 a.m. and 11: 00 a.m. (China Standard Time, CST). Five air samples were taken at 10-min intervals over a 40-min period using a 100 ml plastic syringe. N<sub>2</sub>O concentrations were determined on a gas chromatograph (Agilent 7890A, Santa Clara, California, USA), equipped with an ECD using pure N<sub>2</sub> (99.999%) and H<sub>2</sub> as the carrier gas and fuel gas, respectively. We calculated N<sub>2</sub>O fluxes from the linear or nonlinear increase of gas concentration in the chamber vs. time, and adjusted them for air temperature and atmospheric pressure measured at the time of sampling [32].

During the same time as the gas samples were collected, soil temperature and moisture at 0–10 cm depth were measured near each chamber. They were measured using a portable temperature probes (JM624 digital thermometer, Living-Jinming Ltd., China) and a moisture probe meter (TDR100, Spectrum, USA), respectively. A total of 10 readings were recorded and the average of these 10 data was used to represent the content of soil moisture at each plot.

### 2.3. Measurements of soil inorganic N concentration and pH

After gas samples were collected, soil samples in 0–10 cm depth were taken nearby the flux chambers using an auger (2.5 cm in diameter). Soils were passed through a 2 mm sieve to remove roots, gravel and stones and then stored at 4 °C before analysis. Soil samples were extracted using 2 M KCl solution (soil: water = 1: 10) and shaken for

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