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Original article

Relationships between ammonia-oxidizing communities, soil methane uptake and nitrous oxide fluxes in a subtropical plantation soil with nitrogen enrichment

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ABSTRACT

Ammonia-oxidizers play an essential role in nitrogen (N) transformation and nitrous oxide (N_2O) emission in forest soils. It remains unclear if ammonia-oxidizers affect interaction between methane (CH_4) uptake and N_2O emission. Our specific goal was to test the impacts of changes in ammonia-oxidizing communities elicited by N enrichment on soil CH_4 uptake and N_2O emission. Based on a field experiment, two-forms (NH_4Cl and $NaNO_3$) and two levels (40 and 120 kg N ha^{-1} yr^{-1}) of N were applied in the subtropical plantation forest of southern China. Soil CH_4 and N_2O fluxes, the abundance and structure of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) communities were measured using static chamber-gas chromatography, quantitative PCR (qPCR), and terminal-restriction fragment length polymorphism (T-RFLP). Nitrogen addition tended to inhibit soil CH_4 uptake, but significantly promoted soil N_2O emission; moreover, these impacts were more significant with $NH_4^+ - N$ than with $NO_3^- - N$ addition. NH_4Cl addition significantly changed ammonia-oxidizer abundance with an increase in AOA and a decrease in AOB. Nitrogen additions significantly decreased the relative abundance of 329 bp and 421 bp of archaeal *amoA* gene. Negative relationships occurred between soil CH_4 uptake and AOA abundance and between soil CH_4 uptake and AOA/AOB ratio; however, a positive relationship was found between soil N_2O emission and AOA abundance. These results indicate that a shift in abundance and composition of ammonia-oxidizing communities is closely linked to changes in soil CH_4 uptake and N_2O emission under N enrichment. Furthermore, AOA communities play a contrasting role from AOB communities for regulating the fluctuation between soil CH_4 and N_2O fluxes.

1. Introduction

Methane (CH_4) and nitrous oxide (N_2O) are two potent greenhouse gases, and their global warming potential (GWP) is 25 and 298 times as high as carbon dioxide (CO_2), respectively [1]. The contribution of accumulated CH_4 and N_2O in the atmosphere to overall global warming is more than 25% of total CO_2 equivalent [1]. Global change strongly affects the capacity of undisturbed soils (i.e., forests, grasslands, shrubs) to act as atmospheric CH_4 sink and N_2O source [2]. Reactive nitrogen (N) content in the atmosphere and N deposition rate globally caused by human activities has increased by 11 fold and 2.5 fold since the 1860s, respectively [3]. Overall, the increased N deposition input to terrestrial ecosystems improves the net primary productivity, but inhibits CH_4 uptake and promotes N_2O emission in soils [4,5]. Considering the effects of N deposition on soil CH_4 uptake and N_2O emission, the carbon sequestration potential elicited by N deposition would be offset from 53% to 76% [2].

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Soil N_2O mainly originates from soil nitrification and denitrification processes [6]. NH_3 oxidation to NO_2^-, the first and rate-limiting step of nitrification, is catalyzed by two groups of prokaryotes, including ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) [7–9]. CH_4 in the soil can be oxidized by both methane monooxygenase (MMO) and ammonia monooxygenase (AMO) that are synthesized by methanotrophs and ammonia-oxidizers, respectively [10]. Due to the homology of MMO and AMO enzymes [11], the same habitats [12,13], and a variety of analog substrates [14], both methanotrophs and AOB are simultaneously able to oxidize CH_4 and NH_3 in soils [11]. Pure culture studies provide the direct evidence for nitrification by methanotrophs [15] and for CH_4 oxidation by nitrifiers [16,17]. Moreover, a negative relationship between CH_4 oxidation and NH_3 oxidation is found, which could be partly due to competition for O_2 in soils [18]. Nevertheless, the above mechanisms are rarely examined and verified in the field. It is essential to explore the links between ammonia-oxidizers and soil CH_4 consumption and N_2O production, so as to clarify the mechanism responsible for the trade-off between CH_4 uptake and N_2O emission under N enrichment.

In China, the forest plantations cover an area of 6.26×10^7 ha, accounting for 31.8% of China's forest area and ranking first in the world [19]. Approximately 63% of forest plantations are distributed in the subtropical region of southern China [20]. This region has a high atmospheric N deposition rate ranging from 30 to 73 kg N ha^{-1} yr^{-1} [21]. The humid subtropical forests are considered to be N-rich compared with the boreal and temperate forests, and respond differently to N enrichment [22]. Exogenous N inputs to the subtropical forests can significantly decrease soil CH_4 uptake [21,23] and increase N loss via NO_3^- – N leaching [24], as well as via gaseous N emission (N_2O, NO, and N_2) [25,26]. Experimental N deposition increases [27,28], decreases [29,30] or does not change [31–34] soil AOA abundance. The effects of N additions on soil AOB abundance are controversial, including positive [30,34,35], negative [36], and neutral effects [27,28]. Moreover, few studies on the contrasting effects of soil NH_4^+ – N and NO_3^- – N enrichment on ammonia-oxidizer communities are available [37]. Few studies have investigated the relationships between ammonia-oxidizers and N_2O emission in grassland [29,38] and agricultural soils [39]. Little information is available on links between ammonia-oxidizers and soil CH_4 uptake and N_2O emission in the subtropical forest plantation under N enrichment.

In this study, our objectives were (1) to examine the effects of NH_4^+ and NO_3^- fertilization on soil CH_4 and N_2O fluxes, as well as on the abundance and structure of ammonia-oxidizer communities in the subtropical plantation soils; and (2) to evaluate the relationships between the abundance of ammonia-oxidizer communities and soil CH_4 and N_2O fluxes. Since AOA adapts well to a lower pH environment and requires a lower NH_3 concentration compared with AOB [40], we hypothesized that (1) AOA dominated the soil ammonia oxidation process in the acid subtropical plantation forest soil, and NH_4^+ – N fertilization could have a greater effect on soil ammonia-oxidizer communities than NO_3^- – N fertilizations; and (2) N additions would inhibit CH_4 uptake and promote N_2O emissions through changing the abundance and structure of ammonia-oxidizer communities. To test these two hypotheses, we *in situ* investigated soil CH_4 and N_2O fluxes, and ammonia-oxidizer communities using field observation and a molecular biology method based on a N addition experiment.

2. Materials and methods

2.1. Site description

This experiment was conducted in a subtropical slash pine (*Pinus elliottii*) plantation at the Qianyanzhou Ecological Station (26°44′39″N, 115°03′33″E), located in Taihe city, Jiangxi province, China. The monsoon climate dominates the region with a mean annual temperature of 17.9 °C and a mean annual precipitation of 1505 mm. Little rainfall and high temperatures in late summer often result in seasonal droughts [41]. Slash pine was planted in 1985 with a mean diameter at breast height (DBH) of 16.0 cm and total living biomass of 104.13 t ha^{-1} [42]. Soils are typically red soils, classified as Cambosols (IUSS classification), which were derived from sandstone and sandy conglomerate [43]. Topsoil (0–20 cm) organic C is 20.44 g kg^{-1}, total N is 1.10 g kg^{-1}, total phosphorus is 1.12 g kg^{-1}, pH is 4.26, and soil bulk density is 1.54 g cm^{-3}.

2.2. Experimental design

The N addition experiment is a randomized block design with three replicates. To simulate the effects of deposited NH_4^+ and NO_3^- on soil microbial communities and biogeochemical cycles, two fertilizers (NH_4Cl and $NaNO_3$) were added at two rates: 40 and 120 kg N ha^{-1} yr^{-1}. There were five treatments, including a control, low-NH_4Cl, low-$NaNO_3$, high-NH_4Cl, and high-$NaNO_3$, per block respectively. A total of 15 plots (20 m × 20 m) were established, each surrounded by a 10-m-wide buffer strip. Each month, 509.6 g and 1528.8 g NH_4Cl, as well as 809.5 g and 2428.5 g $NaNO_3$ were weighted and dissolved in 40 L of water for low and high N treatments, respectively. N fertilizer solutions were sprayed below the canopy during the first week of every month, and the control plots only received equivalent amounts of water, which is equivalent to an increase in annual precipitation of 1.2 mm [23]. The experiment was carried out over 1.5 years beginning in May 2012 and ending in October 2013.

2.3. Measurement of soil CH_4 and N_2O fluxes

Soil CH_4 and N_2O fluxes were measured using a static opaque chamber and gas chromatography techniques [23]. In each plot, a stainless steel collar (50 cm × 50 cm × 10 cm) was permanently inserted into the soil to a depth of 10 cm and remained intact. Chambers (length × width × height = 50 cm × 50 cm × 15 cm) were temporarily mounted onto the frames for gas sampling. The soil CH_4 and N_2O fluxes were measured twice a week and conducted between 9:00 and 11:00 am (China Standard Time, CST). Five gas samples from the chamber headspace were collected at 10 min intervals using 100 ml plastic syringes during 40 min. The concentrations of CH_4 and N_2O in the gas samples were analyzed within 24 h using gas chromatography (Agilent 7890A, USA). Soil CH_4 and N_2O fluxes were calculated based on the rate of change in concentration within the chamber, which was estimated as the slope of linear or nonlinear regression between concentration and time [44]. All the coefficients of determination (r^2) of the regression were greater than 0.90 in this study. The flux measurements began in May 2012, but only the flux data from January to October 2013 were used to perform this study.

2.4. Soil sampling, DNA extraction, and quantitative PCR

In September 2013, soil samples (0–20 cm) were collected, and in five subsamples were pooled to make one composite sample from each plot. All soil samples were passed through a 2.0 mm sieve in the field, and then transported to the lab in a biological refrigerator. Soil samples were stored at −80 °C before analysis.

Soil DNA was extracted from a 0.5 g soil using the Fast DNA® SPIN Kit for soil (Qbiogen Inc., Carlsbad, CA, USA) following the manufacturer's instructions. The extracted DNA was checked on a 1% agarose gel and the DNA concentration was assessed using a