Simulated Nitrogen Deposition Reduces CH$_4$ Uptake and Increases N$_2$O Emission from a Subtropical Plantation Forest Soil in Southern China

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Abstract
To date, few studies are conducted to quantify the effects of reduced ammonium (NH$_4$)$^+$ and oxidized nitrate (NO$_3^-$) on soil CH$_4$ uptake and N$_2$O emission in the subtropical forests. In this study, NH$_4$Cl and NaNO$_3$ fertilizers were applied at three rates: 0, 40 and 120 kg N ha$^{-1}$ yr$^{-1}$. Soil CH$_4$ and N$_2$O fluxes were determined twice a week using the static chamber technique and gas chromatography. Soil temperature and moisture were simultaneously measured. Soil dissolved N concentration in 0–20 cm depth was measured weekly to examine the regulation to soil CH$_4$ and N$_2$O fluxes. Our results showed that one year of N addition did not affect soil temperature, soil moisture, soil total dissolved N (TDN) and NH$_4$)$^+$-N concentrations, but high levels of applied NH$_4$Cl and NaNO$_3$ fertilizers significantly increased soil NO$_3^-$-N concentration by 124% and 157%, respectively. Nitrogen addition tended to inhibit soil CH$_4$ uptake, but significantly promoted soil N$_2$O emission by 403% to 762%. Furthermore, NH$_4$)$^+$-N fertilizer application had a stronger inhibition to soil CH$_4$ uptake and a stronger promotion to soil N$_2$O emission than NO$_3^-$-N application. Also, both soil CH$_4$ and N$_2$O fluxes were driven by soil temperature and moisture, but soil inorganic N availability was a key integrator of soil CH$_4$ uptake and N$_2$O emission. These results suggest that the subtropical plantation soil sensitively responds to atmospheric N deposition, and inorganic N rather than organic N is the regulator to soil CH$_4$ uptake and N$_2$O emission.

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Introduction
Humid tropical biome stores approximately 10% of global soil carbon (C) [1] and plays a vital role in the budget of ecosystem C and nitrogen (N) fluxes. The amount of nitrous oxide (N$_2$O) emission from the subtropical and tropical forest soils is estimated at 0.9–3.6 T g yr$^{-1}$, accounting for 14% to 23% of the global N$_2$O budget [2]. Simultaneously, well-aerated soils in the subtropical and tropical forests potentially function as a significant sink of atmospheric methane (CH$_4$) during the dry season [3–6]. The uptake of CH$_4$ from the subtropical and tropical forest soils is estimated to be 6.2 T g yr$^{-1}$, accounting for 28% of the global CH$_4$ sink [7]. Although the importance of the subtropical and tropical forest soils as atmospheric CH$_4$ sink and N$_2$O source is well understood, few observations can be available in this region [8–10]. Moreover, low-frequency measurement of gas fluxes in the few studies is unable to accurately estimate the annual amount of soil CH$_4$ uptake and N$_2$O emission, which leads to a high uncertainty in the budget of global soil CH$_4$ and N$_2$O fluxes.

Chronic N deposition input into terrestrial ecosystems alters plant physiology and soil microbial community, thereby changes the soil biogenic CH$_4$ and N$_2$O fluxes [11–13]. Based on a meta-analysis of N addition experimental data in globe, Liu and Greaver [14] concluded that N addition reduced CH$_4$ uptake by 38% and increased N$_2$O emission by 216%. In general, chronic N deposition will increase NH$_4^+$ and NO$_3^-$ availability in the forest ecosystems, thereby affects CH$_4$ uptake from the forest soils through changing the activity and composition of methanotrophic community [15–17]. Soil NH$_4^+$ accumulation can decrease, increase or have no effects on soil CH$_4$ uptake in the forest ecosystems, depending on forest types [5], duration of N application [18], and N fertilizer types and doses [19]. Three potential mechanisms have been proposed to clarify the inhibition of NH$_4^+$ accumulation to soil CH$_4$ uptake: (1) the competition of soil NH$_4^+$ to use CH$_4$ monoxygenase with soil CH$_4$ [20], (2) the toxic inhibition of hydroxylamine and nitrite produced during soil NH$_4^+$ oxidation [21], and (3) the indirect effects of applied N and associated salt ions through osmotic stress [22]. On the contrary, elevated soil NH$_4^+$ availability can increase soil CH$_4$ uptake, which is related to the increase in the quantity of soil ammonia-oxidizing microorganisms [23]. Soil NO$_3^-$ accumulation can also decrease or increase soil CH$_4$ uptake [18,24]. Osmotic stress caused by NO$_3^-$-N fertilizer-associated salts [22] and anaerobic
'microsites' produced by NO$_3^-$ reduction [25] are toxic to CH$_4$-oxidizing bacteria. The mechanism responsible for the promotion of NO$_3^-$ addition to soil CH$_4$ uptake is still unclear and need a number of experimental evidences to support [26]. A positive relationship between the amount of N addition and N$_2$O fluxes from the subtropical forest soils is mainly attributed to the promotion of soil nitrification or/and denitrification rates caused by increased N availability [27,28]. Some studies reported that denitrification was the main source of N$_2$O emission from the subtropical forest soils [29–31], whereas other studies claimed that nitrification dominated soil N$_2$O fluxes [32]. To date, single N fertilizer (i.e., NH$_4$NO$_3$) is widely used to simulate the effects of N deposition in all N manipulative experiments of subtropical forests in China [8,33,34]. The above studies have not evaluated the relative contributions of the deposited N ions (NH$_4^+$ and NO$_3^-$) to soil CH$_4$ uptake and N$_2$O emission. Moreover, most of soil CH$_4$ and N$_2$O fluxes are measured by low-frequency sampling over the short term, which is difficult to accurately assess the budget of soil CH$_4$ and N$_2$O fluxes and leads to great uncertainty.

In China, the plantations cover an area of 6.2 x 10$^7$ ha, accounting for 31.8% of China's forest area and ranking first in the world [35]. Approximately 63% of plantations concentrate in the subtropical region of southern China [36]. Meanwhile, the southern China is the most economically developed regions with high population density, and plantations, cities and farmlands are interspersed. Because a number of reactive N originated from fossil fuel combustion and fertilizer use is emitted to atmosphere, the forests in this region are receiving a high level of anthropogenic N deposition, mostly as ammonium [37]. Atmospheric N deposition rate via precipitation in southern China has been reported and ranges from 30 to 73 kg N ha$^{-1}$ yr$^{-1}$ [8]. So far, few studies are conducted to examine the effects of N deposition on CH$_4$ uptake and N$_2$O emission from the plantation of this region [33,34].

Humid subtropical forest soils are generally characterized by high N availability and high N turnover [38]. Therefore, we hypothesize that increased NH$_4^+$ and NO$_3^-$ availability via experimental N deposition will inhibit soil CH$_4$ uptake and promote N$_2$O emissions from the subtropical plantation. Furthermore, NH$_4^+$-N fertilizer addition will decrease CH$_4$ uptake and increase N$_2$O emission due to soil NH$_4^+$-N accumulation. In contrast, the effects of NO$_3^-$-N fertilizer addition on soil CH$_4$ uptake and N$_2$O emission depend on the concentration of soil NO$_3^-$-N as well as associated salt ions. Our objectives were (1) to quantify the effects of NH$_4^+$-N and NO$_3^-$-N fertilizer application on soil CH$_4$ uptake and N$_2$O fluxes and soil variables in the subtropical plantation; (2) to examine the relationships between soil CH$_4$ and N$_2$O fluxes and the relevant soil properties.

### Materials and Methods

#### Site description

This study was conducted in a subtropical slash pine plantation at the Qianyanzhou Ecological Station (QYZ, 26°44′39″N, 115°03′33″E) in southern China. The station belongs to the Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences. All necessary permits were obtained for this field study. The field study did not involve endangered or protected species. According to local climate records from 1989 to 2008, mean temperature of QYZ site varies between 17 and 19°C. Mean annual precipitation ranges from 945 to 2145 mm, of which 24%, 41%, 23% and 12% occurs in four quarters in turn. The rainfall scarcity and high temperature in late summer often result in seasonal drought [39]. The exotic slash pine plantation was established in 1985. Mean tree height, diameter at breast height, stand basal area, and leaf area index were 12.0 m, 15.8 cm, 35 m$^2$ ha$^{-1}$, and 4.5 m$^2$ m$^{-2}$, respectively [40]. The main understory and midstory species are Woodwardia japonica (L.f.) Sm., Dicksonia dichotoma (Thunb) Bernh., Liriopeformi...
The red soil is weathered from red sand rock, and soil texture is divided into 2.0-0.05 mm (17%), 0.05-0.002 mm (68%), and <0.002 mm (15%) [39].

Experimental design

The N addition experiment is a randomized block design. In May 1, 2012, two N fertilizers (NH₄Cl and NaNO₃) were used to simulate the effects of deposited NH₄⁺ and NO₃⁻ on ecosystem processes and functions. According to the level of atmospheric N deposition at the QYZ site, two levels referred to as low N (40 kg N ha⁻¹ yr⁻¹) and high N (120 kg N ha⁻¹ yr⁻¹) were used to simulate a future increase in the atmospheric N deposition by 1-, and 3-fold. A control treatment was designed at each block to calculate the net effect of N addition. Each N treatment was replicated three times, and a total of 15 plots were included. Each plot with 20 m x 20 m was divided into four subplots with 5 m x 5 m, and the plots were separated by 10 m wide buffer strips. Three subplots were used to measure soil CH₄ and N₂O fluxes, and the other one was used to investigate aboveground biomass and diversity. N fertilizer solutions were sprayed on the plots once a month in 12 equal applications over the entire year, and the control plots received equivalent deionized water only.

Measurement of soil CH₄ and N₂O fluxes

Flux measurements of soil CH₄ and N₂O fluxes were performed by using a static opaque chamber and gas chromatography method [41]. The static chambers were made of stainless steel and consisted of two parts: a square base frame (length x width x height = 50 cm x 50 cm x 10 cm) and a removable top (length x width x height = 50 cm x 50 cm x 15 cm). The installed equipments on the static chambers were detailed by Fang et al. [42]. The frames were inserted directly into the soil to a depth of 10 cm and remained intact during the entire observation period. To assess the spatial heterogeneity of soil C and N fluxes, a pre-experiment was conducted to examine the difference of CH₄ and N₂O fluxes among the three subplots of each plot before N addition. No significant difference of CH₄ and N₂O fluxes among the three subplots was found during the observation, suggesting a negligible effect of soil heterogeneity. Considering the practical reasons such as high labor intensity, we collected gas samples through changing the subplots within a month. The soil CH₄ and
N<sub>2</sub>O fluxes were measured twice a week and conducted between 9:00 and 11:00 am (China Standard Time, CST). Five gas samples were taken using 100 ml plastic syringes at intervals of 0, 10, 20, 30, and 40 min after closing the chambers. CH<sub>4</sub> and N<sub>2</sub>O concentrations of gas samples were analyzed within 24 h with a gas chromatography (Agilent 7890A, USA) equipped with an electron capture detector (ECD) for N<sub>2</sub>O analysis and a flame ionization detector (FID) for CH<sub>4</sub> analysis. The high purity N<sub>2</sub> and H<sub>2</sub> were used as carrier gas and fuel gas, respectively. The ECD and FID were heated to 350°C and 200°C, respectively, and the column oven was kept at 55°C. The soil fluxes were calculated based on their rate of concentration change within the chamber, which was estimated as the slope of linear or nonlinear regression between concentration and time [41]. All the coefficients of determination ($r^2$) of the regression were greater than 0.90 in our study.

Measurements of soil temperature and moisture

Simultaneously, soil temperature at 5 cm (T<sub>s</sub>) and soil moisture at 10 cm below soil surface (SM) were monitored at each chamber. Soil temperature was measured using a portable temperature probes (JM624 digital thermometer, Living-Jinming Ltd., China). Volumetric soil moisture ($m^3 \text{m}^{-3}$) was measured using a moisture probe meter (TDR100, Spectrum, USA).

Soil sampling and mineral N analysis

During the measurement of soil CH<sub>4</sub> and N<sub>2</sub>O fluxes, soil samples were collected weekly nearby the static chambers from a depth of 0–20 cm using an auger (2.5 cm in diameter). Five soils were collected and were pooled to one composite sample for each soil layer at each plot. Soils were immediately passed through a 2 mm sieve to remove roots, gravel and stones. Soil samples were extracted in 1.0 M KCl solution (soil: water = 1:10) and shaken for 1 h. The soil suspension was subsequently filtered through Whatman No. 40 filter papers to remove NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N, and total dissolved nitrogen (TDN) determination on a continuous-flow autoanalyzer (Seal AA3, Germany). Dissolved organic nitrogen (DON) concentration was calculated as the difference between TDN and total inorganic nitrogen (NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N).

Statistical analyses

Repeated measures analysis of variance (AVOVA) with Duncan test was applied to examine the differences of soil temperature, soil moisture, soil dissolved N, and soil CH<sub>4</sub> and N<sub>2</sub>O fluxes between control and N addition plots. Experimental treatments were set as factors of between-subjects and measurement date was selected as a variable of within-subjects. Linear and nonlinear regression analyses were used to examine the relationships between soil CH<sub>4</sub> and N<sub>2</sub>O fluxes and the measured soil variables in monthly scale. All statistical analyses were conducted using the SPSS software package (version 16.0), and statistical significant differences were set with $P$ values $<0.05$ unless otherwise stated. All figures were drawn using the Sigmaplot software package (version 10.0).

Results

Soil temperature, moisture and precipitation

During the whole observation period, soil temperature at 5 cm depth fluctuated greatly, which correlated with the weather condition. Soil temperature varied as a single-peak and single-sink curve, i.e. temperature was the highest in early July, gradually reached the lowest value in early January, and then increased (Fig. 1a). There was no significant difference in surface temperature among various treatments (Fig. 1b).
Soil moisture at 10 cm depth behaved as significant seasonal variation, with the maximum occurred in May and June and the minimum occurred in August and October (Fig. 1c). The seasonality of soil moisture was well consistent with that of precipitation (Fig. 1c). Similar to surface temperature, no significant difference in soil moisture was found among various treatments (Fig. 1d).

Soil dissolved N concentrations

Soil NO$_3$-N concentration showed significant seasonal variation, with the minimum and maximum occurring in May and August (Fig. 2a, Table 1, $P=0.016$). In the control, the concentration of soil NO$_3$-N ranged from 0.06 to 2.19 mg kg$^{-1}$, with an average of 1.25 mg kg$^{-1}$ (Fig. 2a–b). N addition tended to alter soil NO$_3$-N concentration, and the difference was significant among five experimental treatments (Table 1, $P=0.026$). Compared with the control, high level of NaNO$_3$ addition tended to increase soil NO$_3$-N concentration, while an opposite pattern was found in the low level of NaNO$_3$ addition treatment (Fig. 2b). Furthermore, the promotion of high level of NH$_4$Cl addition to soil NO$_3$-N concentration seemed to be stronger than that of low level of NaNO$_3$ addition (Fig. 2f). However, N addition did not change soil DON concentration at the level of 0.05 (Table 1, $P=0.203$).

Soil CH$_4$ and N$_2$O fluxes

Soil CH$_4$ fluxes showed a significant seasonal pattern (Table 2, $P=0.008$). We observed both soil CH$_4$ uptake and emission in the control plots, ranging from $-34.9$ to $17.9$ mg CH$_4$ m$^{-2}$ h$^{-1}$, with an average of $-5.36$ mg CH$_4$ m$^{-2}$ h$^{-1}$ (Fig. 3a). A weak interaction between measurement date and treatment was found (Table 2, $P=0.079$). Significant differences in CH$_4$ fluxes between the control and N addition treatments were only found in July and September (Fig. 3a). For the same level of N addition, NH$_4$Cl fertilizer exhibited a higher inhibition to soil CH$_4$ uptake than NaNO$_3$ fertilizer. However, there was no significant difference in soil CH$_4$ fluxes between the control and N addition treatments (Fig. 3b).

Soil N$_2$O fluxes also showed a significant seasonality with the minimum occurring from early October to March next year (Fig. 4a, Table 2, $P<0.001$). In the control, soil DON concentration ranged from 5.30 to 14.11 mg kg$^{-1}$, with an average of 8.18 mg kg$^{-1}$ (Fig. 2e-f). Low level of N addition tended to increase the concentration of soil DON, while high level of N addition tended to reduce the concentration of soil DON (Fig. 2f). However, N addition did not change soil DON concentration at the level of 0.05 (Table 1, $P=0.026$).

The seasonal variation of soil TDN concentration was consistent with that of soil NH$_4^+$-N concentration, dramatically decreasing from May to December (Fig. 2c and Fig. 2g). The seasonal variation of soil TDN concentration was significant (Table 1, $P=0.002$). N addition tended to increase soil TDN concentration; moreover, the promotion of NH$_4$Cl application to soil TDN concentration was slightly higher than that of NaNO$_3$ addition (Fig. 2h). However, the difference of soil TDN concentration among the five experimental treatments was not significant (Table 1, $P=0.273$).

### Table 2. Results of repeated-measures ANOVAs on effects of experimental treatment, month and their interaction on soil CH$_4$ and N$_2$O fluxes.

| Source of variation | CH$_4$ flux | N$_2$O flux |
|---------------------|-------------|-------------|
|                     | F           | p           | F            | p           |
| Month               | 2.34        | 0.008       | 23.83        | <0.001      |
| Treatment           | 0.66        | 0.064       | 2.35         | <0.001      |
| Month x Treatment   | 1.37        | 0.079       |              |             |

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15.26 to 559.30 μg N₂O m⁻² h⁻¹, with an average of 10.60 μg N₂O m⁻² h⁻¹ (Fig. 4b). Nitrogen addition produced obvious peaks of soil N₂O emission, which was detected within one week after N addition (Fig. 4a). Soil N₂O fluxes positively responded to N addition, and the promotion increased with the levels of N addition (Fig. 4b). In addition, there was a significant interaction between month and N treatment in the entire observation period (Table 2, \(P < 0.001\)). For the same level of N addition, NH₄Cl fertilizer had a higher promotion to soil N₂O emission than NaNO₃ fertilizer (Fig. 4b).

**Relationships between soil CH₄ and N₂O fluxes and soil properties**

Both soil CH₄ and N₂O fluxes were positively correlated with soil temperature at 5 cm depth and soil moisture at 10 cm depth (Fig. 5, Table 3). The relationships between soil CH₄ fluxes and soil temperature and between soil CH₄ fluxes and soil moisture could be well fitted with quadratic and linear equations, respectively (Fig. 5a–b, Table 3). Similarly, soil N₂O fluxes were linearly correlated with soil temperature and soil moisture (Fig. 5c–d, Table 3).

Soil CH₄ fluxes were positively correlated with soil NO₃⁻-N concentrations, whereas no significant correlations between soil CH₄ fluxes and other dissolved N species were found (Fig. 6a–d, Table 3). Soil N₂O fluxes were linearly correlated with soil NO₃⁻-N and TDN concentrations (Fig. 6e, Fig. 6h, Table 3), and the relationship between soil N₂O fluxes and soil NH₄⁺-N concentrations was well fitted with a logarithm equation (Fig. 6f, Table 3).

**Discussion**

**Effects of N addition on soil CH₄ fluxes**

The subtropical plantation soils can act as a sink of atmospheric CH₄. The mean annual soil CH₄ uptake in the control (0.49 kg CH₄ ha⁻¹ yr⁻¹) was lower than those of subtropical plantation in Pingxiang (3.84 kg CH₄ ha⁻¹ yr⁻¹) and Dinghushan of southern China (1.34 kg CH₄ ha⁻¹ yr⁻¹) [5,6] as well as that of subtropical rainforest in Australia (3.13 kg CH₄ ha⁻¹ yr⁻¹) [3]. Except low level of NaNO₃ treatment, the other three treatments decreased, on average, the rates of soil CH₄ uptake by 18.38% to 41.04% relative to control (Fig. 3). The decrease in soil CH₄ uptake caused by N addition in our site was higher than those of plantations in Dinghushan [5] and Heshan stations of southern China [8], despite the levels of N addition are similar in the three forest sites (120 vs. 150 kg ha⁻¹ yr⁻¹). This result indicated that the response of soil CH₄ uptake to N addition was more sensitive in the northern subtropical plantations than in the southern subtropical...
### Table 3. Regression models between soil CH$_4$ and N$_2$O fluxes and soil properties.

| Flux   | Soil variables* | Regression equation                  | $R^2$ | $P$ value |
|--------|-----------------|--------------------------------------|-------|-----------|
| CH$_4$ | $T_s$           | $y = 0.028 T_s^2 - 0.90 T_s + 2.02$  | 0.94  | 0.044     |
|        | $M_s$           | $y = 0.15 M_s - 8.84$                | 0.123 | 0.006     |
|        | NO$_3^-$        | $y = -1.28$ NO$_3^-$ - 1.77         | 0.21  | 0.004     |
|        | Combined        | $y = 0.17 M_s - 10.66$               | 0.21  | 0.003     |
| N$_2$O | $T_s$           | $y = 4.67 T_s - 40.11$               | 0.25  | <0.0001   |
|        | $M_s$           | $y = 1.99 M_s - 29.76$               | 0.10  | 0.001     |
|        | NO$_3^-$        | $y = 12.29$ NO$_3^-$ + 10.58        | 0.22  | 0.005     |
|        | Combined        | $y = 81.52$ ln (NH$_4^+$) - 85.34    | 0.37  | <0.0001   |
|        | NO$_3^-$        | $y = 12.29$ NO$_3^-$ + 10.58        | 0.22  | 0.005     |
|        | NH$_4^+$        | $y = 81.52$ ln (NH$_4^+$) - 85.34    | 0.37  | <0.0001   |
|        | Combined        | $y = 9.26$ TDN - 93.50               | 0.17  | 0.008     |
|        | Combined        | $y = 0.01$ NH$_4^+$ + 0.013 NO$_3^-$ - 0.041 | 0.50  | <0.0001   |

* $T_s$ is soil temperature at 5 cm depth, $M_s$ is soil moisture at 10 cm depth, NH$_4^+$, NO$_3^-$, and TDN are the concentrations of soil NH$_4^+$, NO$_3^-$, and TDN at 20 cm depth.

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**Figure 6.** Relationships between soil CH$_4$ and N$_2$O fluxes and soil dissolved N concentrations (n = 40).
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plantsations. This could be attributed to the lower soil N availability, lower atmospheric deposition rate, and the shorter duration of N application in QYZ, compared with the southern subtropical plantations [5,6]. Furthermore, the subsurface mineral soils generally have higher capacity of oxidizing CH$_4$ than surface litter layer [22,24]. In our site, exogenous N input would directly affect the soil methanotrophic community as well as the amount of CH$_4$ oxidation due to the lacking of litter layer.

Generally, atmospheric N deposition increases NH$_4^+$ accumulation and thereby inhibits CH$_4$ uptake in the well-drained forest soils [8,12,43], despite contrasting results such as promotion and no effect have also been documented [44,45]. In this study, we found that various levels and forms of N addition did not significantly change soil CH$_4$ uptake over one year (Fig. 3). This could be related to the following third aspects. First, the short-term N fertilizers application did not significantly lead to soil NH$_4^+$-N accumulation (Fig. 2b), and no significant relationship between soil CH$_4$ fluxes and soil NH$_4^+$-N concentrations was found (Fig. 6b). Whalen and Rreeburgh [46] also concluded that N inputs did not influence CH$_4$ uptake until they significantly increased soil NH$_4^+$ availability in the boreal forest soils. Although an inhibitory trend of soil CH$_4$ uptake under the NH$_4^+$-N addition treatments was found, the competition and toxic inhibition of accumulated NH$_4^+$ did not occur over the short term. Second, N addition enhances the availability of NH$_4^+$ to soil nitrifiers, which will accordingly decrease the extent to which CH$_4$ consumers are exposed to NH$_4^+$ [20]. A slight accumulation in soil NO$_3^-$-N concentration under NH$_4^+$-N fertilizer treatments indirectly supported our deduction (Fig. 3a). Third, we also found that soil NO$_3^-$-N accumulation could significantly promote soil CH$_4$ uptake (Fig. 6a), which had been documented in the subtropical plantations of southern China [6]. Especially, the low level of NaNO$_3$ treatment tended to reduce soil NO$_3^-$-N concentration, and thereby it slightly stimulated soil CH$_4$ uptake (Fig. 2b, Fig. 3b). Moreover, stronger relationships were found between soil CH$_4$ fluxes and soil NO$_3^-$-N concentrations than between soil CH$_4$ fluxes and other soil dissolved N concentrations (Fig. 6), suggesting that soil NO$_3^-$ played a more important role in soil CH$_4$ uptake than other soil dissolved N species in the subtropical plantation.

Soil CH$_4$ flux is controlled by methanogens operating at anaerobic conditions and methanotrophs taking oxygen as a terminal electron acceptor [47]. Activities and population sizes of these microbial communities depend on a series of soil factors, including soil temperature, moisture, pH, substrate availability, and aeration of soil profile [19,48,49]. Soil CH$_4$ uptake is dominated by an optimal soil temperature [50]. In our study, the optimal soil temperature was about 15°C (Table 3), and the capacity of soil methanotrophs to oxidize CH$_4$ would decline when soil temperature was lower or higher than the threshold [51]. Also, soil moisture controls the mass flow of air and diffusion of atmospheric CH$_4$ into the soil by altering the water filled pore space [WFPS] of soils [52]. We also found that soil CH$_4$ uptake was less affected by soil moisture (Table 3). Because N addition did not change soil moisture (Fig. 1), we reasonably deduced that the variation of CH$_4$ uptake elicited by N treatments was mainly attributed to the change in soil N availability.

Effects of N addition on soil N$_2$O fluxes

Our result showed that the subtropical slash pine plantation in QYZ exhibited a source of atmospheric N$_2$O under natural conditions. The average soil N$_2$O flux in the control (0.93 kg N$_2$O ha$^{-1}$ yr$^{-1}$) was comparable with that of Heshengqiao station in Hubei province (0.71 kg N$_2$O ha$^{-1}$ yr$^{-1}$) [53], but lower than that of Dinghushan station in South China (2.11 kg N$_2$O ha$^{-1}$ yr$^{-1}$) [35]. In our study, NH$_4$Cl and NaNO$_3$ addition at rates of 40 and 120 kg N ha$^{-1}$ yr$^{-1}$ increased soil N$_2$O emission by 403% to 762%. On the contrary, in the pine, mixed and evergreen broadleaved forests of Dinghushan station, NH$_4$NO$_3$ addition at rates of 50, 100 and 150 kg N ha$^{-1}$ yr$^{-1}$ only increased soil N$_2$O fluxes by 38% to 58% [35]. These results indicated that the subtropical plantation had high turnover rates of soil N and sensitively responded to increased N deposition. The potential reasons include that the optimal hydrothermal conditions [54], low soil pH [55], and high clay content [39], which favor both soil nitrification and denitrification as well as soil N$_2$O emission.

Except soil DON concentration, soil N$_2$O fluxes were significantly correlated with concentrations of soil NH$_4^+$, NO$_3^-$, and TDN (Fig. 6), suggesting soil N$_2$O flux was dominated by both soil nitrification and denitrification processes. Furthermore, the promotion of NaNO$_3$ addition to N$_2$O emission was slightly lower than that of NH$_4$Cl addition (Fig. 4). Two potential mechanisms can be responsible for this phenomenon: (1) the high rates of NO$_3^-$ immobilization and nitrification [38], and the low denitrification potential are found in the same type of subtropical plantations [56]; and (2) temperature regulates soil N$_2$O flux through influencing soil N$_2$O-producing microorganisms, such as nitrifiers and denitrifiers [57]. Soil moisture effects on soil N$_2$O fluxes are a result of the limitation of O$_2$ diffusion into the soil and the expansion of soil anaerobic microbial community [58]. The relatively high temperature in wet season was benefit for soil nitrifiers and denitrifiers activities, which partly explained the seasonal variation of soil N$_2$O fluxes with maximum occurring in between May and June (Fig. 4a). Because N addition did not change soil temperature and soil moisture (Fig. 1), the changes in soil N$_2$O emission under N addition treatments were unlikely to be caused by the changes in soil temperature and soil moisture. Therefore, soil NH$_4^+$-N and NO$_3^-$-N concentrations were the dominant factors controlling soil N$_2$O emission in our study, and could explain 49.9% of the temporal variability of soil N$_2$O fluxes (Table 3).

Conclusions

This study emphasizes the contrasting effects of oxidized NO$_3^-$ and reduced NH$_4^+$ inputs on the fluxes of CH$_4$ uptake and N$_2$O emission from a subtropical plantation soil based on high frequency observations. We found that N addition tended to inhibit soil CH$_4$ uptake, and dramatically promoted soil N$_2$O emission. Compared with NO$_3^-$-N fertilizer application, NH$_4^+$-N fertilizer application had a stronger inhibition to soil CH$_4$ uptake and a stronger promotion to soil N$_2$O emission. Also, both soil CH$_4$ and N$_2$O fluxes were driven by soil moisture and temperature, but soil inorganic N availability was a key integrator of soil CH$_4$ uptake and N$_2$O emission. Overall, short-term N addition has already changed soil CH$_4$ and N$_2$O fluxes, which indicated that the subtropical plantation soil was sensitive to N deposition input. In the future, the long-term observation of soil fluxes and the measurement of key microbial functional groups are necessary to clarify the mechanisms responsible for the coupling between soil CH4 and N2O fluxes.

Author Contributions

Conceived and designed the experiments: YW HF SC GY. Performed the experiments: YW MX XD LL LW. Analyzed the data: YW HF SC. Wrote the paper: YW HF SC.
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