Large difference of inhibitive effect of nitrogen deposition on soil methane oxidation between plantations with N-fixing tree species and non-N-fixing tree species

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[1] The responses of soil methane (CH_4) net fluxes to nitrogen (N) addition in a N-fixing tree species (Acacia auriculiformis (AA)) and a non-N-fixing tree species (Eucalyptus citriodora (EU)) plantation were studied in southern China. Treatments were conducted at each plantation with three N levels (0, 50, and 100 kg N ha⁻¹ yr⁻¹ for control, medium-N, and high-N treatment, respectively, abbreviated as C, MN, and HN). From August 2010 to July 2011, CH₄ flux was measured biweekly using a static chamber and gas chromatography technique. The soils of both sites acted as sink of atmospheric CH_4 . The CH₄ uptake rate in control of the AA site (36.3 \pm 3.2 μ g CH₄-C m⁻² h⁻¹) was greater than that of the EU plantation (29.9 \pm 0.9 μ g CH₄-C m⁻² h⁻¹). In the AA plantation, the averaged rates of CH₄ uptake for the MN (28.6 \pm 2.3 μ g CH₄-C m⁻² h⁻¹) and HN treatment (23.8 \pm 2.8 μ g CH₄-C m⁻² h⁻¹) were decreased by 21% and 35%, respectively, compared to the control. However, there was no change of soil CH₄ uptake between N-treated plots and the controls in the EU site. Our results indicated that there might be large difference of inhibitive effect of N deposition on soil CH_4 oxidation between the AA and EU plantations. The projected increase of N deposition would weaken the capability of N-fixing tree species plantations for atmospheric CH₄ sink in tropical and subtropical regions.

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1. Introduction

[2] Humans have altered regional and global cycles of N more than any other element [*Vitousek et al.*, 1997]. Atmospheric N deposition rates have increased dramatically during recent decades because of intensive agricultural and industrial activities [*Gruber and Galloway*, 2008]. Globally, the quantity of N deposition increased from 41 Tg N yr⁻¹ in 1950 to 103 Tg N yr⁻¹ in 2000 [*Galloway et al.*, 2008]. Worldwide N deposition was projected to increase by 50%–100% by 2030 relative to 2000, with the greatest absolute increases occurring over East and South Asia [*Reay et al.*, 2008]. As a developing country, China has a dramatic increase in atmospheric N deposition since 1980s [*Liu et al.*,

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2011]. N deposition via precipitation in southern China has been reported to range from 30 to 73 kg N ha⁻¹ yr⁻¹ [*Xu* et al., 2001; *Zhou and Yan*, 2001; *Huang et al.*, 2010], and was projected to increase in the coming decades [*Galloway* et al., 2004; *Liu et al.*, 2011]. Excess N deposition has aroused concerns about its negative impacts on ecosystem health and services [*Galloway et al.*, 2008], such as loss of biodiversity [*Sala et al.*, 2000; *Lu et al.*, 2010], N saturation and soil acidification [*Aber et al.*, 1998; *Vogt et al.*, 2006], and inhibition of the capability of CH₄ oxidization in forest soils [*Steudler et al.*, 1989; *Aronson and Helliker*, 2010].

[3] Methane is an important greenhouse gas (GHG) with a global warming potential of 25 times more than carbon dioxide (CO₂) over a 100 year period, contributing about 17% to climate warming [Intergovernmental Panel on Climate Change, 2007]. The global atmospheric concentration of CH₄ has increased from a preindustrial value of about 0.715 ppm to 1.803 ppm in 2009 [World Meteorological Organization, 2010]. After a short-term period of stabilization during 1990–2006, the atmospheric CH₄ levels are increasing again since 2007 [Bloom et al., 2010], leading to intensified research efforts regarding variability in sources and sinks of atmospheric CH₄ (Bodelier, 2011]. Forest soils are an important CH₄ sink or source through the activity of methanotrophic and methanogenes bacteria [Castro et al.,

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	AA Pl	antation	EU Plantation			
	Acacia auriculiformis	Other Plants	Total	Eucalyptus citriodora	Other Plants	Total
Stem density (tree ha^{-2})	357	1719	2076	1186	776	1961
Mean height (m)	12.2	5.6		11.5	4	
Diameter at breast height (cm)	15	4.8		11.1	3.9	
Tree age (yr)	27			27		
Basal area $(m^2 ha^{-2})$	7.3	5.2	12.5	14.7	2	16.7
Percentage of basal area (%)	59	41	100	88	12	100

Table 1. Indices of the Tree Structure in AA and EU Plantations^a

^aThe survey was conducted in July 2010 (27 yr after tree planting) before the first N application.

1995; Le Mer and Roger, 2001]. The diffusivity of CH_4 through the soil profile is the primary limiting factor upon CH_4 oxidation, which is influenced by soil moisture and bulk density [*Teh et al.*, 2005; *Kolb*, 2009]. Furthermore, soil available N contents (NH_4^+ and NO_3^-) can limit CH_4 oxidation directly by competing with the monooxygenase enzyme of methanotrophs [*Hanson and Hanson*, 1996; *Nyerges and Stein*, 2009].

[4] Increased N availability due to N deposition may inhibit the oxidation capacity of forest soils for atmospheric CH₄ [Aronson and Helliker, 2010]. Laboratory studies indicated that oxidation of CH₄ by a variety of methanotrophs was inhibited competitively by N input [Ferenci et al., 1975]. It has generally been accepted that the consumption of CH_4 in forest soil is inhibited by N addition [Steudler et al., 1989; Castro et al., 1994; Mochizuki et al., 2012]. However, not all studies support the general conclusion. Bradford et al. [2001a] found that N deposition had no significant effect on soil CH₄ oxidation in a temperate deciduous woodland in southwest England. Positive effects of N addition on soil CH₄ uptake rates were observed in severely N-limited forests [Börjesson and Nohrstedt, 2000; Steinkamp et al., 2001]. Further studies should be undertaken to better understand the mechanisms responsible for N deposition-induced suppression of CH₄ uptake in forest soils.

[5] According to Food and Agriculture Organization of the United Nations (FAOUN) [2010], forest plantations occupy about 264 million ha worldwide. The total area of plantations in China is 53.6 million ha, accounting for approximately 30% of the total forest area (available from the seventh national forest resources inventory of China [in Chinese], http://www.forestry.gov.cn/portal/main/s/65/content-326341.html). The percentage of forest land cover in the Pearl River Delta, Guangdong Province, increased from 26% in 1970 to 49.5% in 2010 [Peng et al., 2009; Zhang et al., 2012], most of which are Eucalyptus spp., Acacia spp., and some native species [Chen et al., 2011]. Afforestation may affect CH₄ fluxes by altering some key physical and chemical properties in soils, and soil microbial activity as well [Merino et al., 2004]. Most studies focused on the effect of plantation on mitigation of GHGs and C sequestration [Liu and Greaver, 2009; Peichl et al., 2010]. Few efforts have been made to investigate the effect of tree species on the fluxes of soil CH₄ uptake [H. Wang et al., 2010; Christiansen and Gundersen, 2011].

[6] In this study, we investigated the effect of N addition on soil CH_4 fluxes in an *Acacia auriculiformis* (*AA*) (Nfixing tree species) and a *Eucalyptus citriodora* (*EU*) (non-N-fixing tree species) plantation in southern China, where atmospheric N deposition has elevated dramatically in recent years [*Fang et al.*, 2011]. The main objective was to determine the differential effect of N addition on soil CH₄ uptake in the two plantations. We hypothesized that (1) the rate of soil CH₄ uptake in the *EU* plantation would be higher than that of the *AA* site, due to higher soil available N contents in the later; (2) N addition would significantly inhibit soil CH₄ uptake in both plantations because of elevated soil N availability; and (3) the inhibitive effect of N addition on soil CH₄ oxidation would be higher in the *AA* stand because of additional N input into the soil via biological N fixation for the *AA* plantation.

2. Materials and Methods

2.1. Site Description

[7] This study is conducted at the Heshan National Field Research Station of Forest Ecosystems (112°50'E, 22°34'N), which is located in the middle of Guangdong Province, Southern China. The region has a tropical monsoon climate with a distinct wet and dry season. The mean annual temperature and precipitation are 22.5°C and 1534 mm from 2004 to 2009 [Wu et al., 2011]. Atmospheric N deposition in precipitation is about 43.1 \pm 3.9 kg N ha⁻¹ yr⁻¹, with 1:1 ratio for NH_4^+ to NO_3^- (unpublished data, measured from July 2010 to June 2012). We selected two plantations, one with a N-fixing and one with a non-N-fixing tree species, for experimental sites. The two sites were located 500 m apart. The survey conducted in July 2010 (before the start of N addition) showed that the main species in the canopy layer of the AA plantation was Acacia auriculiformis, and the EU plantation was Eucalyptus citriodora. Indices of the tree structure of both plantations are given in Table 1. The soils in both sites are classified as Acrisol [FAOUN, 2006]. Soil bulk density (SBD) is 1.3 and 1.2 g cm⁻³ for AA and EU site, respectively. A pretreatment survey of the general soil properties showed no differences among the treated plots in each plantation before N treatment (data not shown).

2.2. Experimental Treatment

[8] Two N treatments and a reference control were established within each plantation in July 2010: control (C, without N addition), medium-N (MN, 50 kg N ha⁻¹ yr⁻¹) and high-N (HN, 100 kg N ha⁻¹ yr⁻¹). The experiment was a randomized complete block design: each of the two N treatments and a control were randomly located in three blocks per plantation type, with a total of 9 plots (10 × 10 m). Each plot was surrounded by a 10 m wide buffer strip. Ammonium nitrate (NH₄NO₃) solutions were sprayed monthly to the floor with a backpack sprayer starting from August 2010 to July 2011. NH_4NO_3 was weighed and dissolved in 10 L water for each plot. Each control plot received 10 L water simultaneously.

2.3. Field Sampling and Measurements

[9] Soil CH_4 fluxes (summation the rate of CH_4 production and consummation) were measured biweekly from August 2010 to July 2011 using a static chamber method, and thus represent the balance between CH₄ production and consummation. Two static chambers were installed in each plot in June 2010, two months before the first gas sampling campaign. The chambers were made of polyvinylchloride (PVC) pipe with an internal diameter of 25 cm and included two parts: (1) permanent collar and (2) chamber. The collars were permanently installed in the field to a depth of 5 cm. The headspace height of the chamber was 30 cm and hence the headspace volume was 14,719 mL. During gas collecting, the chamber was fitted tightly to the collar with a rubber band. Gas samples were collected from each chamber from 09:00 to 10:00 A.M. (local time). Diurnal studies in tropical forests of southern China showed that soil CH₄ flux measured during the midmorning (09:00-10:00 LT) was close to the daily mean [Tang et al., 2006]. Gas samples were collected with 60 mL plastic syringes (Double-Dove group CO., LTD, Shanghai, China) at 0, 15 and 30 min intervals after the chamber closure. Before each sampling, syringe was flushed 2 or 3 times with chamber gas to mix the headspace. CH₄ concentrations were analyzed within 24 h using a gas chromatograph (Agilent 5890 D, USA) equipped with a flame ionization detector (FID) Calibration gas (CH₄ at 3.52 ppm) was obtained from the Institute of Atmospheric Physics, Chinese Academy of Sciences (Beijing, China). The calculation of CH₄ flux followed that described by Zhang et al. [2008], by a linear regression of chamber gas concentration versus the incubation time [International Atomic Energy Agency, 1992; Holland et al., 1999].

[10] Atmospheric pressure was measured at sampling site using an air pressure gauge (Model THOMMEN 2000, Switzerland). Air temperature (inside chamber), soil temperature (5 cm depth) and moisture (0–10 cm depth) were measured simultaneously in each gas sampling. Temperature was measured using a digital thermometer (TES-1310, Ltd., China). Soil moisture content (0–10 cm depth) was detected using an ADR probe (Amplitude Domain Reflectometry, Model Top TZS-I, China), and converted to water-filled pore space (WFPS) as the following formula:

$$WFPS = Vol/(1 - SBD/2.65)$$
(1)

where *WFPS* is water-filled pore space (%), *Vol* is volumetric water content (%), *SBD* is soil bulk density (g cm⁻³), and 2.65 is the density of quartz (g cm⁻³).

[11] Soil samples were collected in July 2011 (after 1 yr of N application) for analyzing chemical properties. Five soil cores (3.5 cm diameter) were collected randomly from each plot at 0–10 cm depth and combined to one composite sample, resulting in three samples for each treatment per plantation type. After removing stones and coarse roots, the samples were passed through a 2 mm mesh and divided into two parts. One part of fresh soil was used for the analysis of NH_4^+ -N, NO_3^- -N, microbial biomass C (MBC), and

microbial biomass N (MBN) concentrations. The other part was air-dried at room temperature (25°C) for the estimation of other chemical parameters. Soil moisture contents and pH values (1:2.5 soil/water suspension) were determined immediately after sieving. Gravimetric water content was determined through oven drying at 105°C for 48 h. Soil pH value was measured using a pH meter (HM-30 G, TOA Corp., Japan). Soil NH_4^+ and NO_3^- contents were analyzed colorimetrically after being extracted using 2 M KCl. Soil organic carbon (SOC) was determined by wet digestion with a mixture of potassium dichromate and concentrated sulphuric acid [Liu et al., 1996]. Total N concentration was determined by the micro-Kjeldahl digestion [Bremner and Mulvaney, 1982], followed by detection of ammonium with a UV-8000 Spectrophotometer (Metash Instruments Corp., Shanghai, China). Soil MBC was estimated by chloroform fumigation-extraction [Vance et al., 1987], and MBN by applying the procedure of *Brookes et al.* [1985]. Available P was extracted with 0.03 M ammonium fluoride and 0.025 M hydrochloric acid and analyzed colorimetrically [Anderson and Ingram, 1989].

[12] Two litterfall traps $(1.0 \times 1.0 \text{ m})$ with a mesh size of 1 mm) were established in each plot. Litterfall was collected monthly and sorted into categories of leaf, seed, branch and miscellaneous material. The samples were oven-dried at 65°C for 48 h and weighed to determine biomass. Soil coring method was used to investigate fine root ($\leq 2 \text{ mm}$) biomass [*Vogt and Persson*, 1991]. A total of three soil cores (0–20 cm depth) were collected using an 8.7 cm diameter stainless steel core from each plot. Fine root samples were oven-dried at 65°C for 48 h and weighed.

2.4. Statistical Analysis

[13] Two-way Repeated Measures Analysis of Variance was used to examine the effect of N treatments on soil CH₄ fluxes from August 2010 to July 2011. One-way ANOVA was used to examine the difference in soil pH, NH_4^+ , NO_3^- , and available P among treatments, as well as the difference in CH₄ uptake rate between sites. Linear regression analysis was performed to quantify the relationship between CH₄ fluxes and soil temperature or WFPS. Repeated measures ANOVA analysis was performed using the MIXED procedure; ANOVA and linear regression were performed using the GLM procedure in SAS (SAS Institute Inc., Cary NC, USA). Graphic illustrations were generated using Origin 8.0 software (Origin Lab Corporation, Northampton, MA, USA). Statistically significant difference was set at p = 0.05level unless otherwise stated. Mean values ± 1 standard error are reported in the text.

3. Results

3.1. Soil Temperature and Water-Filled Pore Space

[14] Soil temperature at 5 cm depth and WFPS (0–10 cm) exhibited clear seasonal patterns in the controls of both plantations, and followed the seasonal patterns of air temperature and rainfall, respectively, at Heshan Station (Figure 1). The soils were warmer and wetter from April to September (growing season), and became cooler and dryer from November to March of next year (dormant season). The annual mean soil temperatures (5 cm depth) were 20.3 \pm



Figure 1. Weather and microclimate conditions at the experiment site. (a) Monthly rainfall and air temperature from August 2010 to July 2011, (b) seasonal patterns of soil temperature at 5 cm depth, and (c) soil water-filled pore space at 0–10 cm depth. Error bars represent standard error of means (n = 3).

 0.7° C and $20.7 \pm 0.9^{\circ}$ C for the controls of AA and EU plantations, respectively. The average of WFPS (0–10 cm) were 46.6% and 44.9% for the controls in the AA and EU sites, respectively. Monthly means of WFPS and temperature were similar between AA and EU plantations (One-way ANOVA test, p > 0.05). There was no difference between the N-treated plots and controls at each plantation in terms of soil temperature (p = 0.57 and 0.61 for AA and EU, respectively) and WFPS (p = 0.63 and 0.70 for AA and EU, respectively).

3.2. Soil CH₄ Fluxes

[15] The soils of both AA and EU plantations were net sink of atmospheric CH₄ during the study period (Figures 2a and 2b). The averaged soil CH₄ uptake rate in controls of AA (36.3 ± 3.2 µg CH₄-C m⁻² h⁻¹) was greater than that of EU plantation (29.9 ± 0.9 µg CH₄-C m⁻² h⁻¹; p = 0.046). In AA plantation, the annual mean rates of CH₄ uptake were significantly decreased by 21%, and 35% in MN (28.6 ± 2.3 µg CH₄-C m⁻² h⁻¹), and HN treatments (23.8 ± 2.8 µg CH₄-C m⁻² h⁻¹), respectively, relative to the controls (Figure 2a; p = 0.011). However, no significant difference was found among the treatments of the EU site (Figure 2b). [16] The rates of CH₄ uptake and soil WFPS were positively correlated in the controls of both plantations (p < 0.01, $R^2 = 0.23$ and 0.19 for the AA and EU site, respectively) (Figure 3). Under similar soil WFPS condition, CH₄ uptake rate was generally greater in AA than in EU site when WFPS < 70%. Soil CH₄ uptake rates were not correlated to soil temperatures (5 cm depth) in both plantations during the study period (p > 0.05).

3.3. Seasonal Patterns of CH₄ Uptake

[17] Soil CH₄ uptake rates in the N-treated plots showed a similar seasonal pattern to the controls of each plantation (Figures 4a and 4b). The rates of CH₄ uptake of both plantation types were greater in fall and lower in spring (Figures 4a and 4b). Two-way repeated measures ANOVA test showed that CH₄ uptake rates in N-treated plots of the *AA* plantation significantly decreased (p = 0.034) following N inputs compared with the controls (Figure 4a).

3.4. Other Soil Properties

[18] The soils showed pronounced variations with N treatment levels (Table 2). HN treatments increased contents of soil NH_4^+ and NO_3^- in AA plantation, while only increased NO_3^- contents in the EU site (Table 2). Soil CH₄ uptake negatively related to soil NO_3^- or NH_4^+ contents in the AA plantation (Figures 5a and 5b), while only negatively related to NO₃⁻ contents (p < 0.01, $R^2 = 0.28$) in the EU plantation (Figures 5c). After one year of N inputs, there were no changes of SOC and soil available P among plots in each plantation (p > 0.05, Table 2). In AA plantation, soil total N (TN) concentrations increased following N addition, and the difference between the controls and the HN plots was significant (Table 2). Accordingly, N addition significantly decreased C/N ratio in AA plantation (Table 2). For the EU plantation, soil TN concentrations and C/N ratio had no change following N addition (Table 2; p > 0.05). Soil MBC decreased in the same way in MN- and HN-treated plots of AA plantation, compared to their controls (Table 2). There was no response of soil MBN to N addition at both plantations (Table 2). Soil pH value decreased significantly only in the HN-treated plots of AA plantation (Table 2). Fine root biomass (to a depth of 20 cm, diameter ≤2 mm) was significantly decreased by N addition at the AA plantation (Table 2). The annual total litterfall mass of the controls at the AA site was greater than that at the EU plantation (p = 0.02, Table 2). There was no N treatment effect on litterfall mass at each plantation (Table 2).

4. Discussion

4.1. Effect of Soil Temperature and Water-Filled Pore Space

[19] Soil CH₄ uptake was correlated negatively with WFPS in our study, which was consistent with previous studies [*Castro et al.*, 1995; *Kiese et al.*, 2003; *Davidson and Nepstad*, 2004; *Christiansen and Gundersen*, 2011]. We found that CH₄ uptake in the two plantation soils was increased mainly in fall of 2010, which coincided with lower soil water content. Methanotrophs use CH₄ as the only C and energy source and oxygen availability is the main factor limiting their activity [*Le Mer and Roger*, 2001]. Lower WFPS might lead to greater soil aeration, which favors



Figure 2. Comparisons of mean soil CH₄ fluxes between N treatment plots and the controls at (a) AA and (b) EU plantations. Error bars indicate ± 1 SE (n = 3). Different letters "a, b" denote significant difference ($p \le 0.05$) between treatments.

diffusion of atmospheric CH_4 and O_2 into soil profile for methanotrophs bacteria [*Ball et al.*, 1997; *Kolb*, 2009].

[20] Soil temperature variation had no effect on soil CH₄ uptake in the present study, which was consistent with previous results from tropical forests in southern China [*Tang et al.*, 2006; *H. Wang et al.*, 2010; *Zhang et al.*, 2011]. In contrast, soil CH₄ oxidation in temperate or boreal regions was generally positively correlated with soil temperature [*Maljanen et al.*, 2006]. In our study, almost all of soil temperatures were between 15 and 30°C, within the optimum temperature (22 to 38°C) for CH₄ oxidation [*de Visscher et al.*, 2007]. Therefore, we concluded that soil

temperature might not be a primary factor controlling CH₄ oxidization in tropical plantations of Southern China.

4.2. Methane Uptake

[21] The CH₄ uptake rates observed in the present study are similar to those found in some other tropical forests within the Pearl River Delta region (2.5 to 4.3 kg CH₄-C ha⁻¹ yr⁻¹) [*Tang et al.*, 2006; *Zhang et al.*, 2008; *Zhang et al.*, 2011], or other tropical plantations in southern China (2.3 to 3.4 kg CH₄-C ha⁻¹ yr⁻¹) [*Werner et al.*, 2006; *Yan et al.*, 2008; *H. Wang et al.*, 2010]. The CH₄ uptake rates in the present study were also within the range (0.8 to



Figure 3. Correlations between CH_4 flux and soil water-filled pore space (WFPS) for the controls in both stands.



Figure 4. Seasonal pattern of soil CH₄ uptake in (a) *AA* and (b) *EU* plantation. Asterisk (*) indicates significant differences between control and at least one of N addition treatments at p = 0.05 level. Error bars indicate ± 1 SE (n = 3).

4.7 kg CH₄-C ha⁻¹ yr⁻¹) reported for tropical forests in other regions [*Steudler et al.*, 1991; *Kiese et al.*, 2003; *Davidson and Nepstad*, 2004; *Werner et al.*, 2007; *Davidson et al.*, 2008].

[22] Contrary to our expectation, the AA plantation had higher CH_4 uptake rate than the EU site, although soil NH_4^+

and NO₃⁻ concentrations in the AA were approximately 20% and 31% higher, respectively. This seems in contrast with the fact that higher soil NH₄⁺ or NO₃⁻ level could inhibit the activity of methanotroph bacteria. Owing to their ability to fix atmospheric N₂ through microbial symbiosis, Acacia spp. can improve plant growth and thereby increase soil C and N cycling and stocks in soils [Macedo et al., 2008; Voigtlaender et al., 2012]. In terms of the present study, the litterfall input, fine root biomass and SOC content were higher in the AA than the EU site (Table 2). As a consequence, the structural stability and macropores, all of which favor the diffusiveness of CH₄ and O₂ into the soil profile [Merino et al., 2004], are greater in the AA site. Therefore CH₄ oxidation in the AA site was higher than the EU site.

[23] Contrary to the present study, some previous studies did not find a positive influence of *Acacia* spp. [*Fest et al.*, 2009] or *Acacia mangium* [*Konda et al.*, 2010] on the magnitude of CH₄ uptake. This might be due to the young growing stage of the plantations studied. For example, *Konda et al.* [2008] made the measurements at the plantation of 7 yr old. In our study, the improved SOC and soil fertility of the *AA* compared to that of the *EU* plantation might be a result of 27 yr restoration [*F. Wang et al.*, 2010]. If these plantations result in higher CH₄ uptake as observed in our study, there would be a greater potential of atmospheric CH₄ sink, since globally there are 8.3 million hectares of *Acacia* spp. plantation, with 96% in Asia [*FAOUN*, 2010].

4.3. Effect of N Addition

[24] The inhibitive effect of N addition on CH₄ uptake in the AA was greater than that of EU plantation. The observations may be due to differences in soil N saturation thresholds between the two sites. There was no impact of adding N in the EU plantation probably because there was less N to begin with. Symbiotic N fixers (Azotobacteria) are abundant in the AA plantation (but not in the EU stand), which act to incorporate large amounts of new N into soil because of legumes plants fixing more atmospheric N₂ [Hedin et al., 2009]. Therefore, the AA plantation presents an initial N-rich soil, and the EU plantation dominated by a fast-growing tree species (Eucalyptus spp., non-N-fixing tree species) did not. The threshold for N addition to decrease consumption needed less additional N in AA than

Table 2. Soil Properties (0-10 cm Depth), Fine Root and Litter Mass at the Two Plantations^a

	AA Plantation				EU Plantation			
	Control	Medium N	High N	P Values	Control	Medium N	High N	P Values
$\overline{\text{SOC}(\text{g kg}^{-1})}$	22.1 (2.3)	19.0 (1.6)	21.4 (0.2)	p > 0.05	15.5 (0.9)	15.6 (2.1)	16.1 (0.8)	p > 0.05
NH_4^+ (mg kg ⁻¹)	16.0 (1.0) b	19.5 (1.4) b	23.5 (1.2) a	p = 0.04	13.4 (2.0)	13.9 (2.7)	14.3 (2.9)	p > 0.05
NO_{3}^{-} (mg kg ⁻¹)	17.8 (1.4) b	27.3 (3.5) ab	33.1 (2.0) a	p = 0.01	13.6 (2.6) b	21.1 (2.3) a	23.6 (2.3) a	p = 0.03
Total N (g kg ^{-1})	1.6 (0.1) b	1.8 (0.3) ab	2.2 (0.10) a	p = 0.04	1.5 (0.1)	1.5 (0.29)	1.8 (0.19)	p > 0.05
C/N ratio	13.8 (1.7) a	11.4 (1.7) ab	9.6 (0.24) b	p = 0.02	10.3 (0.8)	10.5 (1.0)	9.0 (0.8)	p > 0.05
Available P (mg kg $^{-1}$)	1.8 (0.2)	1.9 (0.2)	1.9 (0.6)	p > 0.05	1.6 (0.3)	1.1 (0.3)	2.0 (0.3)	p > 0.05
MBC (mg kg $^{-1}$)	254 (13.7) a	215 (9.7) b	204 (14.6) b	p = 0.03	288 (21.3) a	279 (24.4) a	206 (22.7) b	p = 0.04
MBN (mg kg $^{-1}$)	41.4 (3.6)	51.5 (5.7)	46.1 (6.5)	p > 0.05	43.9 (5.6)	31.1 (0.4)	38.9 (6.7)	p > 0.05
pН	3.83(0.03) a	3.80 (0.04) a	3.73 (0.03) b	p = 0.03	3.91 (0.05)	3.90 (0.04)	3.81 (0.02)	p > 0.05
Fine root $(g m^{-2})$	92.9 (13.6) a	62.8 (6.4) b	63.2 (6.3) b	p = 0.03	74.4 (3.2)	87.0 (7.2)	85.8 (6.4)	p > 0.05
Litter mass $(g m^{-2} yr^{-1})$	694.1 (25)	668.5 (56)	745.0 (24)	p > 0.05	590.2 (14)	477.4 (14)	483.9 (44)	<i>p</i> > 0.05

^aSoil samples were collected in July 2011 after 1 yr of N additions. Values are presented as means with SE in parentheses (n = 3). Different letters between treatments denote significant difference (Tukey's HSD test, $p \le 0.05$). Fine root (<2 mm) was sampled to a depth of 0–20 cm. The *p* values are given in boldface when p = 0.05. AA, Acacia auriculiformis; EU, Eucalyptus citriodora; SOC, soil organic carbon; C/N ratio, SOC/total N ratio; MBN, microbial biomass N.



Figure 5. Relationships between CH_4 fluxes and soil NH_4^+ and NO_3^- contents in the (a, b) AA and (c, d) EU plantation. CH_4 fluxes were measured in field at the same day of soil sampling (17 July 2011).

EU, because of initial higher soil N content of AA plantation. Several factors, which caused the large difference of inhibitive effect of N addition on soil CH₄ oxidation between the two plantation types, are described below.

[25] First, high soil inorganic N concentrations in N treatments may lower the rates of CH₄ oxidation. Exceptionally high soil NH_4^+ and NO_3^- contents significantly suppressed CH₄ oxidation at AA plantation, as was originally hypothesized. The results were consistent with the previous findings [Steudler et al., 1989; King and Schnell, 1994; Castro et al., 1995; Kim et al., 2012]. Methanotrophic bacteria have the ability to oxidize NH⁺₄ in favorable conditions [Hanson and *Hanson*, 1996]. The existence of high NH_4^+ contents could act as competitive substrates for CH₄ oxidation organisms [Bédard and Knowles, 1989], or enzymatic activity of CH₄ monooxygenase [Ferenci et al., 1975]. Furthermore, NO₃ and/or NO_2^- produced from the NO_3^- reduction were perhaps toxic to CH₄-oxidizing microbes [Dunfield et al., 1995; Reav and Nedwell, 2004]. We did not observe any change of soil NH_4^+ concentration in the EU plantation after N treatments. This was probably due to the tight nutrient cycling in the EU site, whereby N inputs were rapidly immobilized [Attiwill et al., 1996].

[26] Second, the suppressive effect of N on the activation of CH₄ oxidation bacteria is another potential mechanism [*Castro et al.*, 1994]. A significant decrease of MBC following N input was an indirect evidence for the lower CH₄ oxidation in HN plots at the *AA* plantation, since the decrease of MBC may incur corresponding shrinkage of the organism pool responsible for CH₄ oxidation. In contrast, MBN was not changed in HN plots of *AA* plantation relative to the control. The potential reason might be abundance N absorbed by other preferable N microbes, which were easier subject to environmental factor changes (such as pH, nutrient availability, etc.) than methanotrophic bacteria [*Hanson and Hanson*, 1996]. Therefore, N addition might stimulate population growth of the other microbes in soil of AA plantation, which could result in outcompeting methanotrophic bacteria for N, O₂ and/or other nutrient.

[27] Third, the lower pH after N addition might contribute to the decrease in CH₄ uptake rate [Hütsch, 1998]. Most of the soils which pose substantial CH₄ oxidation capacity have pH values within the range of $3 \sim 7.5$ [Born et al., 1990], with the optimal values of 5.0 \sim 6.5 [Le Mer and Roger, 2001; Kolb, 2009]. Soil pH values of the two plantations were below 4 (Table 2). A significant decrease of pH value was associated with an increase of N treatment level in the AA plantation, and thus might lead to decreased CH_4 oxidation rate. Similarly, Bradford et al. [2001b] reported that the decrease of pH in a beech forest in Southwest England contributed to a decrease in soil CH₄ uptake. In addition, aluminum (Al³⁺) release from exchangeable cations exchange in soils might increase as pH values decrease [Bradford et al., 2001b]. Al³⁺ toxicity after N input might also inhibit soil CH_4 uptake in HN treatments of the AA plantation. Furthermore, work would be needed to establish whether such a link exists.

5. Conclusions

[28] In the present study, soil CH₄ fluxes following N addition were measured in two plantations either with N-fixing tree species (*AA* plantation) or non-N-fixing tree species (*EU* plantation) in the tropical region. Soils of the two plantations were all net sink of atmospheric CH₄. There were differential effects of N addition on soil CH₄ uptake in the two plantations. Nitrogen addition significantly lowered soil CH₄ uptake in the *AA* plantation, however, there was no effect of N addition in the *EU* site. Alterations in CH₄ uptake might be resulted from N addition-induced changes in soil available N contents (NH₄⁺, NO₃⁻) and pH value, which affected the activities of bacteria responsible for CH₄ oxidation. As far as we known, our study is among the first to

investigate the effect of N deposition on soil CH_4 fluxes between reforestation with N-fixing species and non-Nfixing species. Our results implied that the projected increase of N deposition would potentially decrease the capacity of AA plantation as a sink of atmospheric CH_4 considering the large area of AA plantation in tropical/subtropical regions.

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