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Responses of nitrous oxide emissions to nitrogen and phosphorus additions in two tropical plantations with N-fixing vs. non-N-fixing tree species

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Abstract

Leguminous tree plantations at phosphorus (P) limited sites may result in higher rates of nitrous oxide (N_2O) emissions, however, the effects of nitrogen (N) and P applications on soil N_2O emissions from plantations with N-fixing vs. non-N-fixing tree species has rarely been studied in the field. We conducted an experimental manipulation of N and P additions in two tropical plantations with *Acacia auriculiformis* (*AA*) and *Eucalyptus urophylla* (*EU*) tree species in South China. The objective was to determine the effects of N- or P-addition alone, as well as NP application together on soil N_2O emissions from tropical plantations with N-fixing vs. non-N-fixing tree species. We found that the average N_2O emission from control was greater in *AA* (2.26 ± 0.06 kg N_2O-N ha^{-1} yr^{-1}) than in *EU* plantation (1.87 ± 0.05 kg N_2O-N ha^{-1} yr^{-1}). For the *AA* plantation, N-addition stimulated ~~the~~ N_2O emission from soil while P-addition did not. Applications of N with P together significantly decreased N_2O emission compared to N-addition alone, especially in high level treatment plots (decreased by 18 %). In the *EU* plantation, N_2O emissions significantly decreased in P-addition plots compared with the controls, however, N- and NP-additions did not. The differing response of N_2O emissions to N- or P-addition was attributed to the higher initial soil N status in the *AA* than that of the *EU* plantation, due to symbiotic N fixation in the former. Our results suggest that atmospheric N deposition potentially stimulates N_2O emissions from leguminous tree plantations in the tropics, whereas P fertilization has the potential to mitigate N deposition-induced N_2O emissions from such plantations.

1 Introduction

Nitrous oxide is a powerful greenhouse gas that is 298 times more potent than carbon dioxide (CO_2) over a 100 yr lifespan (IPCC, 2007), and contributes to stratospheric ozone (O_3) depletion (Ravishankara et al., 2009). Atmospheric N_2O concentration has been increasing by 0.2–0.3 % yr^{-1} over the last 250 yr (Stocker et al., 2013). N_2O is naturally produced by bacterial metabolism during nitrification and denitrification in many environments, particularly soils (Barnard et al., 2005). Tropical forest soils are an important source for N_2O emission, accounting for 14 to 23 % of current global N_2O budget (IPCC, 2007). The major factors of controlling N_2O emission are availability of soil inorganic N and dissolved organic carbon (DOC), soil temperature, moisture, and pH value (Rowlings et al., 2012).

Anthropogenic activities have great impact on global and regional N cycle, thereby enhancing the mobility of reactive N within ecosystems (Vitousek et al., 1997). Atmospheric N deposition ~~rate~~ has increased dramatically during recent decades due to intensive agricultural, fossil fuel combustion, and cultivation of N-fixing plants (Galloway et al., 2008). Worldwide N deposition is projected to increase by 50 to 100 % in 2030 relative to 2000, with the greatest increases occurring in tropical regions such as Southeast Asia and Latin America (Reay et al., 2008). In China, the rate of N deposition has increased since 1980s and is projected to increase in the coming decades (Liu et al., 2013). N_2O emissions have often been found to be elevated in the forests exposed to high N inputs including N deposition, fertilization, or biological N fixation via leguminous trees (Venterea et al., 2003; Zhang et al., 2008; Arai et al., 2008).

In contrast to temperate forests, primary production in many tropical forests is limited by P rather than by N availability (Vitousek et al., 2010). Previous studies found that P-limited forests could emit more N_2O than the N-limited forests after N fertilization (Hall and Matson, 1999, 2003). Hall and Matson (1999) measured N_2O emission after adding N in two tropical rainforests in Hawaii (USA), and found that N_2O emission from P-limited site was 54 times greater compared with that from N-limited site. Martinson et al. (2013) also found lower N_2O emissions when N and P were fertilized together compared to N application alone in tropical montane forests. This is because ~~that~~ poor P availability of tropical forests may decrease N uptake and immobilization and hence cause higher N_2O emission (Hall and Matson, 1999; Martinson et al., 2013). However, most studies have been carried out in natural forests while very few in tropical plantations (Martinson et al., 2013; Mori et al., 2013).

According to *Food and Agriculture Organization of the United Nations* (FAOUN, 2010), plantations occupy about 264 million ha worldwide. The total area of plantations in China is 61.7 million ha, accounting for approximately 32 % of the total forest area (available data from the seventh national forest resources inventory survey of China. http://www.forestry.gov.cn/main/65/content-326341.html). The percentage of forest land cover in South China increased from 26 % in 1979 to 56 % in 2005 (Peng et al., 2009). In this region, most ~~of~~ tree species are *Acacia* spp., *Eucalyptus* spp., and some native species (Chen et al., 2011). Because excess N may easily promote N_2O emission from P-limited soils, leguminous tree plantations at P-limited sites may result in higher rates of N_2O emissions (Arai et al., 2008; Konda et al., 2008). Fertilizations of N and/or P are common practices to improve forest productivity in plantation management in the tropical and subtropical regions. However, direct evidences of N- and P-addition on soil N_2O emissions in tropical forests are still rare (Hall and Matson, 1999; Koehler et al., 2009), especially from plantations with N-fixing vs. non-N-fixing tree species (Mori et al., 2013).

In this study, the main objective was to determine the different effects of N- or P-addition alone, and their interactions on N_2O emissions from tropical plantations with N-fixing (*Acacia auriculiformis, AA*) vs. non-N-fixing tree species (*Eucalyptus urophylla, EU*) and clarify the underlying mechanisms. We hypothesized that: (i) the promotion effect of N-addition on N_2O emissions would be higher in the *AA* plantation due to its relatively higher initial soil N status compared to the *EU* plantation, because of additional N input into the former via biological N fixation by leguminous trees; (ii) P-addition would decrease N_2O emissions in both plantations due to stimulated uptake and/or immobilization of N by the alleviation of P limitation; and (iii) N and P interaction could reduce N addition-induced N_2O emission from the soils of both plantations.

2 Materials and methods

2.1 Site description

This study was 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, South China. The region has a tropical monsoon climate with a distinct wet and dry season. The average annual precipitation and air temperature were 1295 mm and 21.7 °C, respectively (Chen et al., 2011). N deposition in precipitation was ~~about~~ 43.1 ± 3.9 $\text{kg N ha}^{-1}\,\text{yr}^{-1}$, with almost equal contributions from oxidized and reduced forms (no published data, measured from July 2010 to June 2012). ~~Both~~ plantations with N-fixing and non-N-fixing tree species (located 500 m apart) were used in this experiment. The dominant species in the canopy layer was *Acacia auriculiformis* in the *AA* plantation, and *Eucalyptus urophylla* in the *EU* plantation. Indices of the tree structure of both plantations are given in Table S1. The soils in both sites are classified as lateritic soils (Chen et al., 2011). Soil bulk density is 1.18 and 1.09 g cm^{-3} for the *AA* and *EU* stand, respectively.

2.2 Experimental design

An experimental manipulation of nutrient addition was conducted with a complete randomized block design. Three blocks were established (three replicates) per plantation in July 2010. Each block had seven treatments which were randomly assigned to 10 m × 10 m plots. Each plot was surrounded by a 10 m buffer strip. The treatments included control (C, without N and P addition), medium-N (MN, 50 $\text{kg N ha}^{-1}\,\text{yr}^{-1}$), high-N (HN, 100 $\text{kg N ha}^{-1}\,\text{yr}^{-1}$), medium-P (MP, 50 $\text{kg P ha}^{-1}\,\text{yr}^{-1}$), high-P (HP, 100 $\text{kg P ha}^{-1}\,\text{yr}^{-1}$), medium-NP (MNP, 50 $\text{kg N ha}^{-1}\,\text{yr}^{-1}$ + 50 $\text{kg P ha}^{-1}\,\text{yr}^{-1}$), and high-NP (HNP, 100 $\text{kg N ha}^{-1}\,\text{yr}^{-1}$ + 100 $\text{kg P ha}^{-1}\,\text{yr}^{-1}$). Ammonium nitrate ($NH_4NO_3$) and sodium biphosphate (NaH_2PO_4) were applied as N and P source, respectively. The additions were weighed and dissolved in 10 L water for each plot. The solutions were

sprayed monthly onto the forest floor using a backpack sprayer since August 2010. Each control plot received 10 L water simultaneously.

2.3 Field sampling and measurements

2.3.1 N_2O flux measurements

From August 2010 to July 2012, N_2O fluxes were measured bi-weekly using a static chamber method. The chamber design and the measurement procedure were adopted from Zhang et al. (2012). Gas samples were collected at 0, 15 and 30 min intervals after the chamber closure. N_2O concentrations were analyzed within 24 h using a gas chromatograph (Agilent 5890 D, USA) equipped with an electron capture detector (ECD). Fluxes were calculated from the linear rate of change in gas concentration, chamber volume, and soil surface area (Holland et al., 1999), and adjusted for the field-measured air temperature and atmospheric pressure.

2.3.2 Soil sampling and analyses

Soil samples were collected in July 2011 and July 2012 for analyzing properties. Three soil cores (3.5 cm diameter) were collected randomly from each plot at 0–10 cm depth and combined to one composite sample. The samples were passed through a 2 mm sieve and divided into two parts. One part of fresh soil was used for the analysis of ammonium (NH_4^+), nitrate (NO_3^-), microbial biomass C (MBC), and microbial biomass N (MBN) contents. The other part was air dried at room temperature (25 °C) for the estimation of other chemical parameters.

Soil NH_4^+ and NO_3^- contents were analyzed with a flow-injection autoanalyzer (Lachat Instruments, Milwaukee, USA). Total N content was determined by the micro-Kjeldahl digestion (Bremner and Mulvaney, 1982), followed by detection of NH_4^+ with a UV-8000 Spectrophotometer (Metash Instruments Corp., Shanghai, China). Soil organic carbon (SOC) was determined by wet digestion with a mixture of potassium dichromate and concentrated sulphuric acid (Liu et al., 1996). Soil pH was measured in a 1 : 2.5 soil : water suspension using a pH meter (HM-30G, TOA Corp., Japan). Available P was extracted with 0.03 M ammonium fluoride and 0.025 M hydrochloric acid and analyzed colorimetrically (Anderson and Ingram, 1989). Gravimetric water content was determined through oven drying at 105 °C for 48 h.

Both soil MBC and MBN were estimated by chloroform fumigation-extraction method (Vance et al., 1987). In brief, fresh soil samples were fumigated with Chloroform ($CHCl_3$) for 24 h at 25 °C then extracted with 0.5 M K_2SO_4. Simultaneously, subsamples for non-fumigated soil were also extracted with the same methodology. Soil MBC and MBN were calculated as the difference in extractable C, N between fumigated and non-fumigated soils. The conversion factors of 0.33 and 0.45 were used for calculating soil MBC and MBN, respectively (Cabrera and Beare, 1993; Tu et al., 2006).

From 1 to 31 July 2012, the in situ soil net N-mineralization and nitrification were measured using an intact core incubation (Zhu and Carreiro, 1999). Six soil cores (3.5 cm diameter) were sampled from each plot. Three of the cores were brought to the laboratory for extraction (2 M KCl) of inorganic N contents, and the others were returned to the plot for in situ incubation. Nitrification rate was calculated from the difference between extractable NO_3^- contents before and after incubation, and net N-mineralization rate was calculated as the accumulation of total inorganic N over the incubation (Zhu and Carreiro, 1999). The data were expressed as $mg\,N\,kg^{-1}$ dry weight soil $month^{-1}$.

2.3.3 Litterfall mass

Two litterfall traps (1.0 m × 1.0 m with a mesh size of 1 mm) were established in each plot. Litter was collected monthly. The samples were oven dried at 65 °C for 48 h and weighed to determine litter biomass. Subsamples of dried litter was grounded and analyzed for N and P concentrations using H_2SO_4-H_2O_2 digestion followed by colorimetric analysis (Dong et al., 1996).

2.3.4 Soil temperature and moisture

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

$$\mathrm{WFPS} = \mathrm{Vol}/(1 - \mathrm{SBD}/2.65) \qquad (1)$$

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 soil particle density ($g\,cm^{-3}$).

2.4 Statistics

Repeated Measures Analysis of Variance (ANOVA) was used to examine the effect of nutrient additions on N_2O fluxes, soil temperature and WFPS, as well as soil properties from August 2010 to July 2012. Within each year, two-way ANOVA was performed to analyze the difference in mean N_2O emissions, soil properties, MBC, MBN, and literfall mass among treatments of each plantation. Linear regression analysis was performed to evaluate the relationships of N_2O emissions with soil temperature and WFPS. All statistical analyses were conducted using SPSS 16.0 for windows (SPSS Inc., Chicago, IL, USA). Statistically significant difference was set at $p \leq 0.05$ unless otherwise stated. Mean values ±1 standard error was reported in the text.

3 Results

3.1 Soil nutrients and pH

The variations of soil properties were depended on nutrient addition levels and plantation types. Soil available N (NO_3^- and NH_4^+), total N, and SOC contents were greater in the *AA* plantation than in *EU* stand (Table 1; *t* test, all $p < 0.05$). In contrast, soil pH value of *AA* was marginally significant lower than that of *EU* plantation (Table 2; $p =$ 0.06~~1 and 0.055 for the first and second year, respectively)~~.

During the two years, soil available N (NH_4^+ and NO_3^-) and TN contents of the *AA* plantation significantly increased following N treatment levels (Table 1). For the *EU* plantation, HN treatment significantly increased soil NO_3^- content (Table 1), while NH_4^+ and TN contents had no changes in the first year (Table 1). However, N-addition significantly increased soil NO_3^- and NH_4^+ contents in the second year (Table 1; all $p < 0.05$), but TN did not. N-addition did not change soil pH of the *EU* stand, however, a marginally significant decrease in pH value with N-additions was observed in the *AA* plantation (Table 2; $p = 0.074$ and 0.068, respectively for the first and second year). After two years of N application, there were no significant changes in SOC and available P of each plantation (Table 1). The soil C : N ratio significantly decreased following N treatment levels in the *AA* plantation, but did not in the *EU* site (Table 1).

There were significant increases of soil available P contents with the levels of P-addition in both plantations (Table 1; all $p < 0.05$). For the *AA* plantation, P-addition tended to slightly increase soil available N (NO_3^- and NH_4^+) contents in the first year, especially in HP treatment plots (Table 1). By contrary, for the *EU* plantation, P addition significantly decreased soil available N (NO_3^- and NH_4^+) contents in the second year (Table 1; all $p < 0.05$), while did not in the first year. Soil pH values of HP treatment plots were significantly higher than that of HN plots in both plantations, especially in the second year (Table 2; $p < 0.05$). There were no differences in soil TN, SOC, and C : N ratios with P-additions in each plantation (Table 1; all $p > 0.05$).

Application of NP together significantly increased soil available P in both plantations (Table 1, all $p < 0.05$). For the *AA* plantation, soil available N slightly increased following NP-addition. In both plantations, applications with N and P together tended to increased SOC contents in the second year, but there was no statistical difference (Table 1, all $p >$ 0.05). NP-addition significantly increased soil C : N ratio of *AA* plantation (Table 1, $p =$ 0.039). During two years of investigation period, soil TN and pH of both plantations had no significant change following NP treatments (Table 2; all $p > 0.05$). The interactive effects of N- × P-addition on soil available N (NO_3^- and NH_4^+) and TN were found in the *AA* plantation (Table 3). There was an interactive effect of N- × P-addition × year on soil NO_3^- in the *AA* plantation (Table 3; $p = 0.019$). For the *EU* plantation, the interactive effect of N- × P-addition on soil NO_3^- contents was also found (Table 3; $p = 0.001$).

3.2 Nitrification and net N-mineralization

In the *AA* plantation, N-addition significantly increased the rates of nitrification (Fig. 1a; $p = 0.033$), which were from 10.8 ± 3.5 in the controls to 18.1 ± 6.3 and 29.8 ± 4.2 mg N kg soil^{-1} month^{-1} in the MN and HN treatment plot, respectively. The rates of net N-mineralization also significantly increased following N treatment levels (Fig. 1a; $p = 0.041$). The average rates of net N-mineralization were from 14.5 ± 4.7 in the controls to 18.3 ± 4.3 and 27.0 ± 2.5 mg N kg soil^{-1} month^{-1} in the MN and HN treatment plot, respectively. However, P- or NP-addition did not significantly change the rates of nitrification and net N-mineralization (Fig. 1a; all $p > 0.05$).

For the *EU* plantation, N-addition slightly increased the rates of nitrification and net N-mineralization (Fig. 1b). By contrary, P-addition tended to marginally decrease the rates of nitrification and net N-mineralization (Fig. 1b, $p = 0.066$ and 0.058 respectively for nitrification and net N-mineralization rate). Accordingly, the rate of nitrification in HP treatment plots (5.1 ± 1.3) was significantly lower than that in HN (17.2 ± 5.6) and HNP (13.8 ± 4.4 mg N kg soil^{-1} month^{-1}) treatment plots (Fig. 1b; $p < 0.05$). Similarly, the significant difference of net N-mineralization rate between the HN and HP treatment plots was found in the field incubation experiment (Fig. 1b; $p < 0.05$).

3.3 Soil microbial biomass and litterfall mass

In the *AA* plantation, soil MBC tended to decrease with N application, but there was no significant difference between N-addition plots and the controls (Table 2; $p > 0.05$). Meanwhile, a marginally increase in soil MBN following N treatment levels was found (Table 2; $p = 0.071$). NP-addition increased soil MBC only in the first year, but did not change MBN (Table 2). P-addition neither change soil MBC nor MBN throughout the two years (Table 2). For the *EU* plantation, there were no changes in soil MBC and MBN following nutrient additions (Table 2).

There were no differences in annual total litter mass between the controls of both plantations (Table 2; *t* test, all $p > 0.05$). The quantity of litter mass among any nutrient treatment plots in each plantation was also not significantly different (Table 2). Leaf litter N concentrations were significantly increased by any nutrient additions in the *EU* plantation, especially in each high level treatment (Table 2; $p < 0.05$). In the *AA* plantation however, marginally increase in leaf litter N concentrations was found only in MN and HN treatment plots (Table 2; $p = 0.088$ and 0.071, respectively for MN and HN treatment). The fertilization with P alone, as well as NP interaction strongly increased P concentrations of leaf litter, especially for high treatment levels in both plantations (Table 2). For both plantations, N : P ratios of leaf litter significantly decreased by P-addition, as well as NP interactions (Table 2; all $p < 0.05$). The N : P ratio of leaf litter from the controls of *AA* was more than that of *EU* plantation (Table 2; *t* test, $p < 0.001$).

3.4 N_2O emissions from the control plots

During two years of experiment period, the soils of both plantations were a net source of N_2O (Fig. 2a and b). Average N_2O emission from the controls of the *AA* plantation (2.26 ± 0.06 kg N_2O-N $ha^{-1} yr^{-1}$) was significantly greater ($p = 0.007$) than that of *EU* plantation (1.87 ± 0.05 kg N_2O-N $ha^{-1} yr^{-1}$). The *AA* plantation showed higher and more N_2O peaks compared to the *EU* plantation (Fig. S1a and b). ~~Variability in~~ N_2O ~~emissions was observed which~~ tended to be higher in summer (June to August) ~~and lower~~

in winter (November to January of next year) (Fig. S1a and b; *p* ~~= 0.044 and 0.048 for *AA* and *EU* plantation, respectively).~~

3.5 Effects of nutrient additions on N_2O fluxes

In the *AA* plantation, N_2O emissions significantly increased following N applications, however, did not significantly changed following P- or NP-addition relative to the controls (Fig. 2a; all $p > 0.05$). During two years of experiment period, the MN and HN treatments significantly increased soil N_2O emissions by 16 %, and 36 %, respectively (Fig. 2a; $p = 0.047$ and 0.035, respectively for MN and HN treatment). The NP-addition significantly increased N_2O emission in the first year, especially in HNP treatment plots (by 33 %) compared with the controls (Fig. 2a; $p = 0.041$). However, there was no statistically difference between NP-addition plots and the controls in the second year (Fig. 2a). The average N_2O emission rates of HNP plots was significantly decreased by 18 % compared to that of HN treatments in the second year (Fig. 2a; $p = 0.041$). Repeated Measures Analysis indicated significant interactive effects between N and P addition treatments on N_2O emissions (Table 3).

For the *EU* plantation, nutrient additions had no significant effects on soil N_2O emissions in the first year (Fig. 2b; all $p > 0.05$). However in the second year, soil N_2O emissions significantly decreased by 23 % and 27 % for MP and HP treatments compared with the controls (Fig. 2b; $p = 0.047$ and 0.043, respectively for MP and HP treatments). There was a significant interactive effect between P addition and time (Table 3).

4 Discussion

4.1 Comparisons of N_2O emission

The rates of N_2O emission observed from the controls of *AA* and *EU* plantations (1.9 to 2.3 kg N_2O-N $ha^{-1} yr^{-1}$) are comparable with the reports in (sub)tropical regions of southern China (2.0 to 4.8 kg N_2O-N ha^{-1} yr^{-1}) (Zhang et al., 2008; Zhu et al., 2013a), and also within the range of published results (1.2–2.6 kg N_2O-N ha^{-1} yr^{-1}) from other tropical forests (Werner et al., 2007; Ghehi et al., 2012). Some higher rates of N_2O emission (3.7~~4~~–7.4~~5~~ kg N_2O-N ha^{-1} yr^{-1}) than our study were also reported in tropical forests (Keller and Reiners, 1994; Kiese and Butterbach-Bahl, 2002). However, our result is above the reported average N_2O emissions of 0.13 to 0.71 kg N_2O-N ha^{-1} yr^{-1} for pine forests in the southwestern China (Wang et al., 2010), probably due to the higher pH values of th~~e~~ pine forest soil.

The *AA* plantation had significantly higher average N_2O emissions than that of the *EU* stand, which was in accordance with our expectation. The result supported the notion that ~~potentially higher~~ ~~N_2O~~ ~~emissions may emit from~~ leguminous tree plantations in tropics and subtropics (Arai et al., 2008; Konda et al., 2008). The presence of leguminous trees resulting in higher ~~initial soil N contents~~, which was considered to be the main reason for the higher rate of N_2O emission from the *AA* plantation. ~~Another cause might be~~ higher rates of net N-mineralization and nitrification ~~in the *AA* plantation, which was also~~ supported by the study of Dick et al. (2006). Leguminous trees can not only supply N via their unique ability of N-fixing, but also increase soil C content (Li et al., 2012). The higher SOC and fertility in the *AA* plantation compared to *EU* plantation may also partly explain the higher N_2O emission from the *AA* plantation. Additionally, soil pH of the *AA* plantation was 0.5–0.7 lower than that of *EU* site, which might directly or indirectly increase N_2O emission from the *AA* stand (Liu et al., 2010).

4.2 Effects of N application on N_2O emission

~~In~~ consistent with our hypothesis, the soil of *AA* plantation responded to N-addition greater than the *EU* stand, with a large and immediate loss of N_2O emission. The increase of soil N_2O emissions following NH_4^+ or NO_3^- addition was observed in many N-rich ecosystems (Butterbach-Bahl et al., 1998; Hall and Matson, 1999; Koehler et al., 2009). In the present study, the result from *AA* plantation is consistent with

the reported results that N additions could increase N_2O emissions from N-rich forest soils (Venterea et al., 2003; Zhang et al., 2008). Whereas the result from *EU* site is comparable to the findings from related N-poor forests (Matson et al., 1992; Zhang et al., 2008), which showed that N addition did not significantly enhance N_2O emissions. There are several factors caus~~ed~~ the different responses of soil N_2O emissions to N-addition between the *AA* and *EU* plantations.

The initial soil N status between these two plantations contributed to the difference in responses of N_2O emissions to N-addition. For the *AA* plantation abundant in symbiotic N-fixers (*Azotobacteria*), which act to incorporate large amounts of N into the soil (Hedin et al., 2009). Therefore, the *AA* plantation presents an initial N-rich soil, while the *EU* plantation dominated by *Eucalyptus* spp. did not. Moreover, the rates of net N-mineralization and nitrification in the *AA* plantation were significantly increased following N applications. This might be a potential cause for the different response of soil N_2O emissions to N-additions between both plantations. For the *EU* plantation, the fast growing trees of *Eucalyptus* spp. may have strong competition with microbes (e.g., nitrifying and denitrifying bacteria) for N uptake (Forrester et al., 2006), which was proved by the increase in N concentrations of leaf litter following N-addition. The changes of soil MBC and MBN contents following N applications were not found in the *EU* plantation, so, the vegetation sink for N input would be a buffer and provide the resistance in preventing N losses as N_2O emission (Attiwill et al., 2001). There was also no evidence for the changes in soil MBC and MBN of the *AA* plantation, which might be caused by adequate N ~~using~~ for plants and microbes in this ecosystem.

A lower soil C : N ratio of *AA* plantation with N-addition was likely the other cause for the different response. The rich in initial soil N of the *AA* plantation, while as decrease in soil C : N ratio following N-addition, which are likely a "hotspot" for nitrification and/or denitrification and sensitive in response to increased N inputs (Barnard et al., 2005). Additionally, acidity has been reported to support high N_2O emissions by denitrification (Liu et al., 2010). A lower soil pH after N application might contribute to the increase in N_2O emission from the *AA* plantation. Further work would be needed to establish whether such a link exists.

4.3 Effects of P application on N_2O emissions

Higher plant N uptake could lead to decrease N availability for microbial nitrification and denitrification that would be lost as N_2O from the *EU* plantation soil. P-addition promoted uptake of N by plants (Hall and Matson, 1999), which could reduce N_2O emission by decreasing N substrate. Alleviation of P limitation resulting from P-addition might increase the stress of N limitation in the soil of *EU* plantation, due to increasing N immobilization. Sundareshwar et al. (2003) also reported that P addition to sediment from a coastal salt marsh in South Carolina decreased N_2O emissions by increasing N immobilization. On contrary, in a soil incubation experiment (excluded plant), Mori et al. (2010) found that P-addition increased N_2O emissions from soil underneath an *Acacia mangium* plantation. They pointed that the possible mechanism might be P addition stimulated N cycling and relieved the P shortage for nitrifying and/or denitrifying bacteria, however, the competition for N by plants was ignored. Falkiner et al. (1993) reported that application of P increased soil net N-mineralization of a *Eucalyptus* species forest in Australian, but almost the entire mineral N utilized by the vegetation. For the *EU* plantation, the significant increases in P concentrations and decreases in N : P ratios of leaf litter proved that P-addition increased P uptake (Table 2), as well as led to faster N uptake by plants. In our study, P fertilization did not change N_2O emission from the *AA* plantation soil. The mechanism is currently not clear. Further study is necessary to identify clear causal relationships between soil N_2O emissions, N availability of leguminous trees plantations and nutrient additions.

Mori et al. (2010) reported that P-addition decreasing N_2O emission could be associated with increased other microbe immobilization of N after P addition, decreasing the N substrate for nitrification and denitrification. In the present study, net N-mineralization and nitrification rates, as well as soil MBC and MBN contents did not change following P applications. Therefore, it is unlikely that microbial immobilization mechanism would explain the trend in our results.

4.4 ~~Interactive effects~~ of N and P on N_2O emission

Application of N and P together tended to increase N_2O emissions from soils of both plantations. Our result was in line with the reports that addition of NO_3^- with P together stimulated soil N_2O emissions from *Acacia mangium* plantation soil (Mori et al., 2013). The increase in N_2O emission was possibly attributed to the fact that the added N increased substrates (Xu et al., 2012), and the added P stimulated nitrification and denitrification by relieving P shortage for nitrifying and denitrifying bacteria (Minami and Fukushi, 1983). However, NP-addition decreased N_2O emission compared to N-addition in the *AA* plantation. The main cause of this might be that most of N added was absorbed and utilized by the vegetation after relieving the P shortage by applied P together. Further study is necessary to identify clear nutrient competition between soil microorganisms and plants growth after nutrient applications in tropical leguminous trees plantations.

4.5 Effect of soil temperature and WFPS on N_2O emission

There were clear seasonal patterns of soil temperature and WFPS in the controls of both plantations, which followed the seasonal patterns of air temperature and rainfall (Fig. S2). N_2O fluxes showed significantly positive linear relationship with soil temperatures and WFPS (Fig. 3a and b), which were consistent with (sub)tropical forests (Butterbach-Bahl et al., 2004; Zhang et al., 2008; Zhu et al., 2013a). Most of the N_2O peaks were observed in response to rainfall events at suitable temperature. Soil water availability and temperature ~~strongly~~ constrain~~ed~~ the processes of nitrification and denitrification, which mainly controlled the production of N_2O emission (Barnard et al., 2005). There were no differences between treatment plots and the controls in each plantation, in terms of soil temperature ($p = 0.65$ and 0.57, for *AA* and *EU*) and

WFPS ($p = 0.97$ and 0.96, for *AA* and *EU*, respectively). Accordingly, nutrients additions did not change the relationships of N_2O fluxes with soil temperature or WFPS of each plantation.

4.6 N_2O emission factors

According to N- and NP-addition plots, N_2O emission factor based on percentage of applied N ranged between 0.72 % to 0.81 % and 0.11 % to 0.15 % for the *AA* and *EU* plantation, respectively (Table 4). The N_2O emission factor of *AA* plantation is similar to the average of 0.87 % for forest ecosystems (Liu and Greaver, 2009), and the IPCC default factor (1 %) (IPCC, 2007). It is among the lowest range of data from other tropical forests (1–8.6 %) (Hall and Matson, 1999; Steudler et al., 2002). In contrary, Zhu et al. (2013b) reported that emission factors amounted to 8–10 % of N deposition in subtropical forests of southern China. The lower N_2O emission factor might be due to a short-term of the experiment (2 yr), and the plantations used in our study are relatively poor nutrient compared with natural forests. Compared with application of N alone, and NP-addition decreased the N_2O emission factor by 8.3 % and 49 % for MN and HN treatment plots, respectively, at the *AA* plantation (Table 4). This result suggests that the combined application of N and P together may probably mitigate N_2O emission in comparison with N fertilization alone in tropical plantations with leguminous trees.

5 Conclusions

The responses of soil N_2O emissions to nutrients additions were studied in two tropical plantations with N-fixing and non-N-fixing tree species. We found that application of N and P together decreased the rate of soil N_2O emission compared to N treatment alone in N-fixing trees plantation, while application of P alone significantly reduced N_2O emissions from non-N-fixing trees plantation. The main cause of these might be that

most of soil N added was absorbed and utilized by the vegetation with P application together in these tropical forests. As far as we known, the study is among the first to investigate the effect of nutrient additions on soil N_2O emission from tropical plantations with N-fixing vs. non-N-fixing tree species. The results indicate that the projected increase of atmospheric N deposition would potentially increase soil N_2O emissions from leguminous tree plantations. Our findings also suggest that moderate fertilization of P might eventually reduce N deposition-induced N_2O emissions from leguminous tree plantations in the tropical and subtropical regions.

Supplementary material related to this article is available online at http://www.biogeosciences-discuss.net/11/1413/2014/bgd-11-1413-2014-supplement.pdf.

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Table 1. Soil properties (0–10 cm depth) of *Acacia auriculiformis* and *Eucalyptus urophylla* plantations.

		Jul 2011						Jul 2012					
Plantation	Treatment	NO_3^--N	NH_4^+-N	TN	SOC	C : N	Av. P	NO_3^--N	NH_4^+-N	TN	SOC	C : N	Av. P ($mg\,kg^{-1}$)
		($mg\,kg^{-1}$)	($mg\,kg^{-1}$)	($g\,kg^{-1}$)	($g\,kg^{-1}$)	ratio	($mg\,kg^{-1}$)	($mg\,kg^{-1}$)	($mg\,kg^{-1}$)	($g\,kg^{-1}$)	($g\,kg^{-1}$)	ratio	
AA	C	17.8(0.4)a	16.1(0.4)a	1.6(0.1)a	22.1(2)	13.6(2)b	1.8(0.2)a	14.1(1.6)a	11.1(0.6)a	2.2(0.1)a	40.7(3)	18.8(1)b	2.9(0.3)a
	MN	27.3(1.0)b	20.3(0.5)ab	1.8(0.3)ab	19.0(2)	11.7(2)ab	1.9(0.2)a	21.7(2.5)ab	13.8(0.3)ab	2.5(0.1)ab	38.0(2)	15.1(1)ab	2.8(0.1)a
	HN	33.1(1.0)b	25.1(1.1)b	2.2(0.1)b	21.5(1)	9.8(1)a	1.9(0.6)a	24.5(2.2)b	18.0(1.7)b	2.7(0.2)b	32.7(3)	12.5(2)a	3.0(0.2)a
	MP	21.3(1.8)ab	18.7(1.9)a	1.3(0.3)a	18.4(1)	15.6(3)b	3.3(1.2)ab	12.1(2.2)a	11.5(2.1)a	2.2(0.2)ab	38.5(3)	17.7(2)b	3.3(0.5)ab
	HP	22.7(1.4)ab	19.7(2.5)ab	1.5(0.2)a	19.7(3)	12.9(2)ab	8.9(0.4)c	12.0(0.8)a	14.0(0.8)ab	2.2(0.2)ab	45.3(4)	19.4(3)bc	4.1(0.5)b
	MNP	26.1(2.3)b	22.7(1.8)ab	1.6(0.2)a	21.5(1)	14.1(3)b	3.3(0.8)ab	19.8(2.4)ab	12.4(1.4)a	2.1(0.4)a	49.1(5)	26.1(4)c	3.6(0.3)ab
	HNP	21.3(1.2)ab	22.1(1.6)ab	1.5(0.1)a	22.6(2)	15.6(1)b	5.8(1.4)b	20.5(1.9)ab	14.4(0.9)ab	2.0(0.2)a	55.8(4)	28.5(3)c	4.0(0.1)b
EU	C	13.6(1.4)a	13.4(2.0)	1.4(0.02)	15.5(2)	10.6(1)	1.6(0.3)a	10.2(0.9)b	7.9(0.2)b	1.6(0.1)	20.9(3)	14.2(2)	2.6(0.1)a
	MN	21.1(1.3)ab	13.9(2.7)	1.5(0.3)	15.8(2)	10.6(1)	1.1(0.3)a	13.5(0.8)b	10.2(0.8)bc	1.4(0.2)	25.8(3)	18.7(3)	2.8(0.2)a
	HN	23.6(1.3)b	14.3(1.8)	1.8(0.2)	16.1(1)	9.0(1)	2.0(0.3)a	22.4(1.0)c	16.4(0.2)c	1.7(0.2)	28.9(2)	18.9(3)	3.4(0.1)ab
	MP	17.9(1.0)ab	13.8(1.8)	1.5(0.1)	17.2(1)	11.4(0)	2.1(0.7)a	6.6(0.7)a	4.6(0.5)a	1.5(0.1)	26.3(3)	20.5(3)	3.8(0.1)b
	HP	17.3(1.9)ab	13.2(1.8)	1.6(0.04)	18.8(2)	10.7(1)	5.3(1.1)b	7.7(1.0)a	6.1(0.9)a	1.6(0.3)	33.9(2)	19.7(2)	4.1(0.4)b
	MNP	19.1(0.9)ab	16.4(1.8)	1.8(0.1)	18.9(2)	10.6(2)	2.8(0.6)ab	10.4(2.5)b	7.1(1.6)ab	1.8 (0.2)	31.8(3)	19.2(1)	3.4(0.3)ab
	HNP	17.7(2.0)ab	15.3(1.4)	1.7(0.3)	17.3(3)	9.9(2)	6.3(1.3)b	10.9(0.7)b	8.1(0.8)b	1.7(0.1)	33.6(3)	16.8(1)	4.0(0.5)b

Notes: Soil samples were collected in July 2011 and July 2012. Values are presented as means with SE in parentheses ($n = 3$). Different letters in the same column indicate significantly different mean values among treatments of each plantation (Tukey's HSD test, $p \leq 0.05$). *AA*: *Acacia auriculiformis* plantation; *EU*: *Eucalyptus urophylla* plantation. TN, total nitrogen; SOC, soil organic C; C : N ratio, SOC : TN ratio; Av. P, soil available P.

Table 2. Soil pH, MBC, MBN, litterfall mass and N, P concentrations of leaf litter at *Acacia auriculiformis* and *Eucalyptus urophylla* plantations.

Plantation	Treatment	Jul 2011				Jul 2012						
		pH value	MBC ($mg\,kg^{-1}$)	MBN ($mg\,kg^{-1}$)	LM ($g\,m^{-2}\,yr^{-1}$)	pH value	MBC ($mg\,kg^{-1}$)	MBN ($mg\,kg^{-1}$)	LM ($g\,m^{-2}\,yr^{-1}$)	Litter N ($mg\,g^{-1}$)	Litter P ($mg\,g^{-1}$)	N : P ratio
AA	C	3.83(0.02)ab	254(14)a	41.4(3.6)ab	749(85)	3.79(0.01)ab	330(31)a	66.6(11.7)	841(58)	12.4(0.5)a	0.16(0.0)a	76.9(1.6)c
	MN	3.81(0.03)ab	215(10)a	51.5(5.7)ab	712(57)	3.77(0.03)a	350(33)a	73.5(14.6)	704(59)	13.9(1.1)ab	0.20(0.0)a	71.8(9.9)c
	HN	3.73(0.02)a	204(15)a	59.9(6.5)b	800(23)	3.74(0.01)a	292(31)a	78.7(9.8)	846(72)	14.3(0.3)ab	0.19(0.0)a	84.5(3.2)c
	MP	3.85(0.04)ab	237(45)a	40.1(18.4)ab	964(96)	3.89(0.08)b	298(35)a	61.3(17.5)	864(64)	12.9(0.5)a	0.30(0.0)ab	44.6(6.7)b
	HP	3.90(0.05)b	234(27)a	28.3(4.4)a	715(54)	3.86(0.04)ab	634(38)b	85.9(16.7)	780(77)	14.0(0.5)ab	1.38(0.3)c	10.4(2.1)a
	MNP	3.84(0.02)ab	316(36)b	31.8(6.1)ab	751(66)	3.85(0.02)ab	414(32)ab	93.9(11.9)	744(59)	14.2(0.9)ab	0.43(0.1)ab	34.6(6.5)ab
	HNP	3.84(0.05)ab	426(32)b	50.6(7.8)ab	738(50)	3.86(0.02)ab	446(34)ab	51.6(13.9)	783(56)	14.5(1.2)ab	0.69(0.1)b	22.7(4.9)ab
EU	C	3.91(0.05)	288(21)	43.9(5.6)	644(28)	3.94(0.02)	378(33)	78.3(7.9)	870(67)ab	11.5(0.4)a	0.38(0.1)ab	33.3(7.2)b
	MN	3.90(0.04)	279(24)	31.1(0.4)	517(10)	3.90(0.03)	333(34)	60.1(13.2)	697(55)a	13.1(0.4)b	0.31(0.0)a	42.8(2.2)c
	HN	3.81(0.02)	246(23)	38.9(6.7)	520(61)	3.97(0.05)	326(26)	69.2(9.6)	674(58)a	13.2(0.4)b	0.31(0.0)a	44.2(4.9)c
	MP	3.88(0.04)	258(27)	40.2(7.4)	690(46)	3.94(0.01)	286(24)	72.8(8.6)	914(29)ab	12.3(0.8)ab	0.54(0.2)ab	22.7(5.5)ab
	HP	3.84(0.01)	328(36)	48.6(10.9)	574(59)	4.01(0.03)	359(26)	47.1(11.7)	826(57)ab	12. 9(0.3)b	1.43(0.2)c	9.1(0.7)a
	MNP	3.85(0.05)	293(18)	50.8(11.7)	486(54)	3.98(0.05)	361(16)	74.1(10.5)	817(45)ab	12.3(0.4)ab	0.85(0.1)ab	14.5(0.9)ab
	HNP	3.86(0.04)	285(16)	34.7(3.7)	634(13)	3.92(0.04)	350(20)	80.0(10.2)	1003(39)b	13.5(0.3)b	1.14(0.3)b	14.6(4.9)ab

Notes: Soil samples were collected in July 2011 and July 2012. Values are presented as means with SE in parentheses ($n = 3$). Different letters in the same column indicate significantly different mean values among treatments of each stand (Tukey's HSD test, $p \leq 0.05$). *AA*, *Acacia auriculiformis* plantation; *EU*, *Eucalyptus urophylla* plantation. MBC, microbial biomass C; MBN, microbial biomass N; LM, litter mass; N : P ratio, leaf litter N : leaf litter P.

Table 3. Results of repeated measures ANOVA for responses of N_2O fluxes, soil properties, soil MBC and MBN to N-, P-addition and year.

		N_2O	NO_3^-	NH_4^+	TN	SOC	C : N	Av. P	MBC	MBN	pH
AA	N	**0.002**	**<0.001**	**<0.001**	0.447	0.802	0.772	0.193	0.520	0.668	0.268
	P	0.746	0.155	0.981	**0.024**	0.350	**0.032**	**<0.001**	**0.010**	0.931	**0.021**
	Y	0.843	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.018**	0.165	**0.006**	**0.020**	0.627
	N × P	**0.046**	**0.044**	**0.012**	*0.098*	0.468	*0.079*	*0.082*	0.660	0.564	0.802
	N × Y	*0.059*	0.407	0.515	0.785	0.864	0.734	0.344	0.114	0.570	0.167
	P × Y	*0.056*	0.790	0.475	0.989	0.392	0.559	**0.001**	0.120	0.931	*0.074*
	N × P × Y	0.169	**0.019**	0.949	0.481	0.794	0.630	0.334	0.163	0.467	0.943
EU	N	*0.076*	**<0.001**	**0.042**	0.107	0.529	0.932	0.382	**0.063**	0.831	0.863
	P	0.857	**0.002**	**0.032**	0.223	*0.068*	0.638	**<0.001**	*0.090*	0.624	0.767
	Y	0.103	**<0.001**	**<0.001**	0.448	**<0.001**	**<0.01**	0.677	0.102	**0.008**	0.488
	N × P	0.352	**0.001**	0.544	*0.081*	0.515	0.487	0.603	0.233	0.466	0.524
	N × Y	0.820	0.301	0.449	0.660	0.658	0.894	0.734	0.959	0.682	**0.032**
	P × Y	**0.036**	**0.037**	0.103	0.917	0.469	0.861	**0.002**	0.984	0.818	0.214
	N × P × Y	0.571	0.325	0.513	0.334	0.855	0.547	0.575	0.747	0.535	*0.062*

Notes: The data were from High N and P treatment (HN, HP, HNP additions) plots. *p* values smaller than 0.05 and 0.10 are in bold and italic, respectively. N, N-addition; P, P-addition; Y, year, the first year (from August 2010 to July 2011) and the second year (from August 2011 to July 2012) after nutrient additions. *AA*, *Acacia auriculiformis* plantation; *EU*, *Eucalyptus urophylla* plantation. TN, total nitrogen; SOC, soil organic carbon; C : N, SOC : TN ratio; Av. P, soil available P; MBC, soil microbial biomass C; MBN, soil microbial biomass N.

Table 4. N_2O emission factor.

Plantation type	*AA* plantation					*EU* plantation				
Treatments	C	MN	HN	MNP	HNP	C	MN	HN	MNP	HNP
N_2O emissions (kg N ha^{-1} yr^{-1}) [a]	2.26	2.62	3.07	2.59	2.67	1.87	1.93	2.02	2.04	2.11
Total N applications (kg N ha^{-1} yr^{-1})	0	50	100	50	100	0	50	100	50	100
N_2O emission factor (%) [b]		0.72	0.81	0.66	0.41		0.11	0.15	0.34	0.23

Notes:
[a] The average rates of N_2O emissions, data from August 2010 to July 2012;
[b] The N_2O emission factor was calculated as (annual N_2O-N emission of N treatment plot – annual N_2O-N emission of the control plot)/(total N applied in each year).
AA: *Acacia auriculiformis*; *EU*: *Eucalyptus urophylla*.

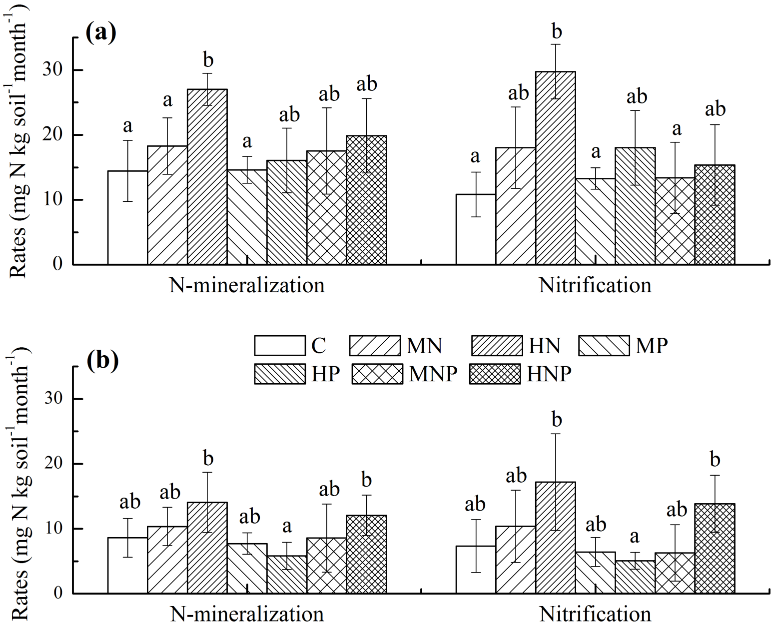

Fig. 1. The rates of net N-mineralization and nitrification in the 0–10 cm mineral soil of **(a)** *Acacia auriculiformis* and **(b)** *Eucalyptus urophylla* plantation. The error bars denote 1 SE. Different letters represent statistically significant differences at $p < 0.05$.

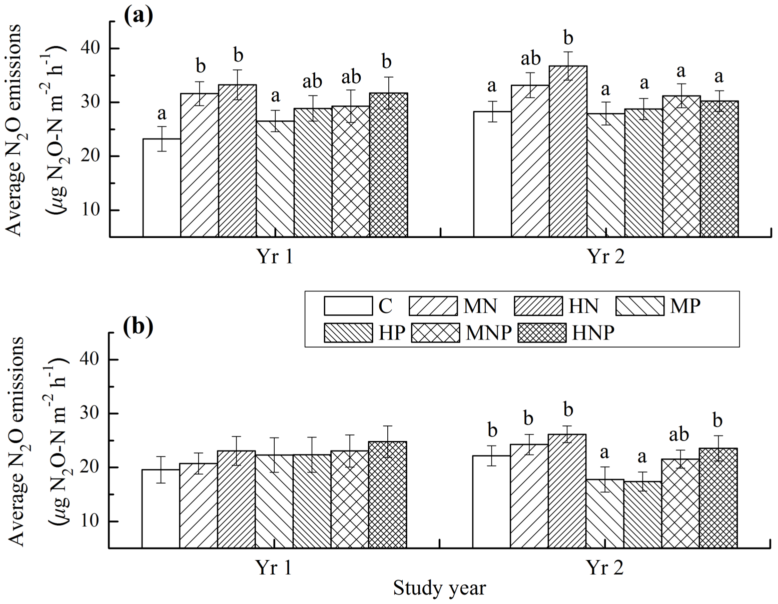

Fig. 2. Average N_2O emission rates for each treatment of **(a)** *Acacia auriculiformis* and **(b)** *Eucalyptus urophylla* plantations in the first and second year after nutrient additions. The error bars denote 1 SE. Different letters represent significant difference at $p < 0.05$. Yr 1: from August 2010 to July 2011; Yr 2: from August 2011 to July 2012.

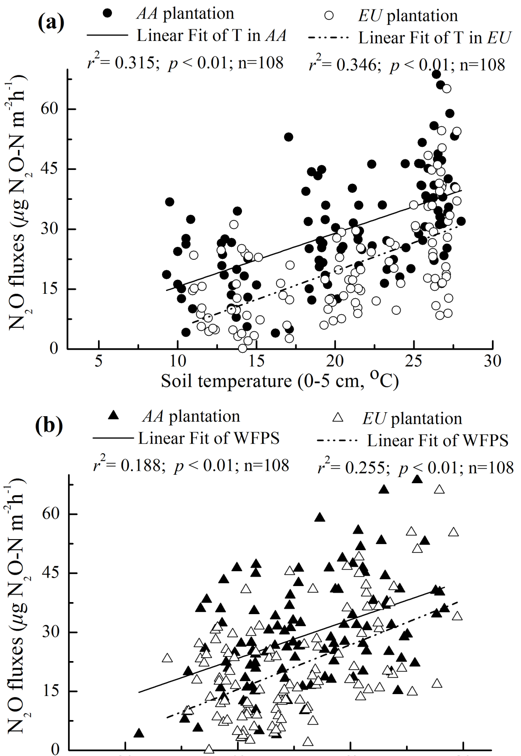

Fig. 3. Relationships of N_2O flux with **(a)** soil temperature and **(b)** WFPS for the control plots of both plantations. Each data is the average of three replications at each sampling date. *AA, Acacia auriculiformis* plantation; *EU, Eucalyptus urophylla* plantation.