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 excess nitrogen (N) and higher rates of nitrous oxide (N_2O) emissions. However, the effects of N and P applications on soil N_2O emissions from plantations with N-fixing vs. non-N-fixing tree species have rarely been studied in the field. We conducted an experimental manipulation of N and/or 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* (2.3 ± 0.1 kg N_2O-N ha^{-1} yr^{-1}) than in *EU* plantation (1.9 ± 0.1 kg N_2O-N ha^{-1} yr^{-1}). For the *AA* plantation, N-addition stimulated N_2O emission from the soil while P-addition did not. Applications of N with P together significantly decreased N_2O emission compared to N-addition alone, especially in the high level treatments (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 different response of N_2O emission to N- or P-addition was attributed to the higher initial soil N status in the *AA* than that of *EU* plantation, due to symbiotic N fixation in the former. Our result suggests 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 processes 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 soil N availability, dissolved organic C (DOC), soil temperature, moisture, and pH value (Rowlings et al., 2012).

Anthropogenic activities have great impact on the global and regional N cycles, thereby enhancing the mobility of reactive N within ecosystems (Vitousek et al., 1997). Atmospheric N deposition has increased dramatically during recent decades due to intensive agricultural production, 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 from the forest soils 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) 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 the 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 the *Food and Agriculture Organization of the United Nations* (FAOUN, 2010), plantation 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 planted tree species are *Acacia* spp., *Eucalyptus* spp., and some native species (Chen et al., 2011), especially on eroded and degraded lands. Leguminous tree plantations at P-limited sites may result in higher rates of N_2O emissions, due to excess N easily promotes N_2O emission from P-limited soils (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 of N_2O production. 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 availability 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 would 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 (112º50' E, 22º34' 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 ºC, respectively (Chen et al., 2011). N deposition in rainfall was 43.1 ± 3.9 kg N ha^{-1} yr^{-1}, with almost equal contributions from oxidized and reduced forms (unpublished data, measured from July 2010 to June 2012). 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. As a result of long-term disturbances, the soil in this area has eroded, leading to vast areas of degraded lands. The *AA* and *EU* plantations are commonly used for promoting forest restoration on the degraded lands in this region. 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), and soil bulk density is 1.2 and 1.1 g cm^{-3} for the *AA* and *EU* stand, respectively.

2.2 Experimental design

An experimental manipulation of nutrient additions 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 to the next plot. The treatments included control (C, without N and P addition), medium-N (MN, 50 kg N ha^{-1} yr^{-1}), high-N (HN, 100 kg N ha^{-1} yr^{-1}), medium-P (MP, 50 kg P ha^{-1} yr^{-1}), high-P (HP, 100 kg P ha^{-1} yr^{-1}), medium-NP (MNP, 50 kg N ha^{-1} yr^{-1} + 50 kg P ha^{-1} yr^{-1}), and high-NP (HNP, 100 kg N ha^{-1} yr^{-1} + 100 kg P ha^{-1} yr^{-1}). Ammonium

129 nitrate (NH_4NO_3) and sodium biphosphate (NaH_2PO_4) were applied as N and P source,
130 respectively. The additions were weighed and dissolved in 10 L water for each plot.
131 The solutions were sprayed monthly onto the forest floor using a backpack sprayer
132 since August 2010. Each control plot received 10 L water simultaneously with each
133 treatment event.

134

135 **2.3 Field sampling and measurements**

136 **2.3.1 N_2O flux measurements**

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

145

146 **2.3.2 Soil sampling and analyses**

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

154

155 Soil NH_4^+ and NO_3^- contents were determined by extraction with 2 M KCl solution
156 followed by colorometric analysis on a flow-injection autoanalyzer (Lachat
157 Instruments, Milwaukee, USA). Total N content was determined by the micro-
158 Kjeldahl digestion (Bremner and Mulvaney, 1982), followed by detection of NH_4^+
159 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 ^{o}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 ($CHCl_3$) vapor for 24 h at 25 ^{o}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 July 1 to 31, 2012, soil net N-mineralization and nitrification were measured using an intact core incubation. Six soil cores (3.5 cm diameter) were sampled from each plot. Three cores were brought to the lab 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

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 ^{o}C for 48 h and weighed to determine litter mass. 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:

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

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. 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. Multiple regression analysis was performed to evaluate the relationships of N_2O emissions with soil temperature, WFPS and soil parameters. All statistical analyses were conducted using SPSS 16.0 for windows (SPSS Inc., Chicago, IL, USA). Statistically significant difference was set at $p \leq 0.05$. 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, $p < 0.05$). In contrast,

soil pH value of *AA* was marginally significant lower than that of *EU* plantation (Table 2, $p = 0.06$ for both years).

During the two years, N-addition significantly influenced soil available N (NH_4^+ and NO_3^-) contents of both plantations (Table 3). For the *EU* plantation, N-addition significantly increased soil NO_3^- content, while NH_4^+ and TN contents had no changes in the first year (Table 1, 3). 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, $p = 0.07$ for the two experimental years). After two years of N application, there were no changes in SOC and available P of each plantation (Table 1, 3). 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 following P-addition in both plantations (Table 3). Soil available N (NH_4^+ and NO_3^-) contents in *EU* plantation significantly decreased following P-addition, while the *AA* stand did not (Table 1, 3). Soil pH values of HP treatment plots were significantly higher than that of HN plots in the *AA* plantation, while the *EU* site did not (Table 2, $p < 0.05$). There were no differences in soil TN, and SOC contents with P-additions in each plantation (Table 1). Multiple regression analysis indicated that there were no significant relationships between N_2O emissions and TN or SOC content.

Application of NP together significantly increased soil available P in both plantations (Table 1). For the *AA* plantation, soil available N slightly increased following NP-addition (Table 1, 3). In the second year, NP-addition significantly increased soil C:N ratio of *AA* plantation ($p = 0.039$), while *EU* plantation did not (Table 1). 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). For the *EU* plantation, the interaction of N- × P-addition on soil NO_3^- contents was also found (Table 3).

3.2 Nitrification and net N-mineralization

In the *AA* plantation, N-addition significantly increased the rates of nitrification (Fig. 1 a, $p = 0.03$), which were from 11 ± 3 in the controls to 23 ± 3 mg N kg soil^{-1} month^{-1} in the HN treatment plot. The rates of net N-mineralization also significantly increased following N treatment levels (Fig. 1 a, $p = 0.04$). The average rates of net N-mineralization were from 12 ± 3 in the controls to 14 ± 2 and 27 ± 3 mg N kg soil^{-1} month^{-1}, respectively for the MN and HN treatment plot. However, P- or NP-addition did not significantly change the rates of nitrification and net N-mineralization (Fig. 1 a).

For the *EU* plantation, N-addition slightly increased the rates of nitrification and net N-mineralization (Fig. 1 b). By contrary, P-addition tended to marginally decrease the rates of nitrification and net N-mineralization (Fig. 1 b, $p = 0.07$ and 0.06 respectively for nitrification and net N-mineralization rate). Accordingly, the rate of nitrification in HP treatment plots (5 ± 1) was significantly lower than that in HN (17 ± 6) and HNP (14 ± 4 mg N kg soil^{-1} month^{-1}) treatment plots (Fig. 1 b, $p < 0.05$). Similarly, the significant differences of net N-mineralization rate between the HP and HN, HNP treatment plots were found in the field incubation experiment (Fig. 1 b, $p < 0.05$).

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). Meanwhile, a marginally increase in soil MBN following N treatment levels was found (Table 2, $p = 0.07$). 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). Multiple regression analysis showed that there was a weak relationship between litterfall mass and N_2O emission. Leaf litter N concentrations were significantly increased by any nutrient additions in the *EU* plantation, especially in each high level treatment (Table 2). In the *AA* plantation however, marginally increase in leaf litter N concentrations was found only in MN and HN treatment plots (Table 2, $p = 0.09$ and 0.07, respectively for MN and HN treatment). The fertilization with P alone, as well as NP interaction strongly increased P concentrations of leaf litter, especially in high level treatments for both plantations (Table 2). 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, $p < 0.01$).

3.4 N_2O emissions from the controls

During the two years of experimental period, the soils of both plantations were a net source of N_2O (Fig. 2 a, b). Average N_2O emission from the controls of the *AA* plantation (2.3 ± 0.1 kg N_2O-N ha^{-1} yr^{-1}) was significantly greater (*t*-test, $p = 0.007$) than that of *EU* plantation (1.9 ± 0.1 kg N_2O-N ha^{-1} yr^{-1}). The *AA* plantation showed more and higher N_2O peaks compared to the *EU* plantation (Fig. S1 a, b). Emissions of N_2O tended to be higher in summer (June to August) than in winter (November to January of next year) (Fig. S1 a, b, $p = 0.04$ and 0.05, respectively for *AA* and *EU* plantation).

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 change following P-addition relative to the controls (Fig. 2 a, Table 3). During the two years of experimental period, the MN and HN treatments significantly increased soil N_2O emissions by 16% and 36%, respectively (Fig. 2 a, $p = 0.05$ and 0.04, respectively for the MN and HN treatment). The NP-addition significantly increased N_2O emission in the first year, especially for HNP treatments (by 33%) compared with the controls (Fig. 2 a, $p = 0.04$), but did not in the second. 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. 2 a, $p = 0.041$).

Repeated Measures Analysis indicated that there was a significant interaction of N- × P-addition on N_2O emissions (Table 3).

For the *EU* plantation, nutrient additions had no significant effects on soil N_2O emissions during the two years (Table 3). 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. 2 b, $p = 0.05$ and 0.04, respectively for the MP and HP treatment). There was a significant interactive effect of P-addition × year on N_2O emission (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 previous 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). The higher rates of N_2O emissions (3.7-7.5 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 0.1 to 0.7 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 these pine forest soils.

The *AA* plantation had significantly higher N_2O emissions than that of the *EU* stand, which was consistent with our expectation. Our result supports the notion that leguminous tree plantations in tropics and subtropics may potentially emit more N_2O (Arai et al., 2008; Konda et al., 2008). The presence of leguminous trees resulting in higher soil N availability, including higher rates of net N-mineralization and nitrification which was considered to be the main reason for the higher rate of N_2O emission from the *AA* plantation, and 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

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 more comparable to the findings from related N-poor forests (Matson et al., 1992; Zhang et al., 2008), which showed that N addition did not enhance N_2O emissions.

There are several factors causing the different responses of soil N_2O emissions to N-addition between the *AA* and *EU* plantations. The initial soil N status between both plantations contributed to the difference in response 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 another potential cause for the different responses. 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 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 availability 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. Multiple regression analysis indicated the variations of C:N had a potential contribution to N_2O fluxes. 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, soil 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 works should be conducted to determine whether such a link exists.

4.3 Effects of P application on N_2O emissions

P-addition promoted uptake of N by plants (Hall and Matson, 1999), which could reduce N_2O emission by decreasing N substrate. Higher plant N uptake could lead to decrease N availability for microbial nitrification and denitrification that would be lost as N_2O from the soil of *EU* plantation. 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 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* spp. forest in Australian, but almost the entire mineral N utilized by the vegetation. For our *EU* plantation, the significant increases in P concentrations and decreases in N:P ratios of leaf litter proved that P-addition increased P uptake, as well as leading to faster N uptake by plants. P fertilization did not change N_2O emission from the *AA* plantation soil, which mechanism is currently

unclear. Further study is necessary to identify causal relationships between N_2O emission, N availability of leguminous trees plantation and nutrient addition.

Additionally, 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 nitrifying and denitrifying bacteria. 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 Interaction of N and P on N_2O emission

Application of N and P together tended to increase N_2O emissions from soils of the *AA* plantation in the first year. The 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 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 nutrient competition between soil microorganisms and plants growth after nutrient applications in tropical leguminous trees plantations.

4.5 Effects 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). There is a covariation between soil temperature and WFPS in the monsoon climate zone of southern China. The interaction of soil temperature and WFPS may constrain the processes of nitrification and denitrification, which mainly controlled the production of N_2O emission (Barnard et al., 2005). In our study, N_2O fluxes showed

positive linear relationships with soil temperatures (R^2 = 0.32 and 0.35) and WFPS (R^2 = 0.19 and 0.26, respectively for *AA* and *EU* plantation) (Table 4), which were consistent with tropical and subtropical forests (Butterbach-Bahl et al., 2004; Zhang et al., 2008; Zhu et al., 2013a). Stepwise multiple linear regression analysis indicated that soil temperature and WFPS were the significant variables explaining the variability of N_2O emissions (Table 4). Increasing soil moisture would increase soil microbial activities and therefore N_2O production (Rowlings et al., 2012). On the other hand, increased soil moisture under warm conditions could exponentially increase denitrification (Arah and Smith, 1989). Most of the N_2O peaks were observed in response to rainfall events at suitable temperature. There were no differences between treatment plots and the controls in each plantation, in terms of soil temperature (p = 0.7 and 0.6, respectively for *AA* and *EU*) and WFPS (p = 0.9 for both plantations). Accordingly, nutrients additions did not change the relationships of N_2O fluxes with soil temperature or WFPS.

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 0.7% to 0.8% and 0.1% to 0.2% for treatment level in *AA* and *EU* plantation, respectively (Table 5). The N_2O emission factor of *AA* plantation was similar to the average of 0.9% 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 (1-9%) (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. In our study, the lower N_2O emission factor might be due to a short-term of the experiment (2 years), and the plantations planted on eroded soils are relatively poor in nutrients compared with natural forest soils. Compared to HN treatment, HNP-addition decreased the N_2O emission factor by 50% at the *AA* plantation (Table 5). 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 leguminous tree plantations in the study regions may potentially emit more N_2O after N addition, due to its high initial soil N availability. Application of N and P together decreased the rate of N_2O emission compared to N treatment alone in N-fixing trees plantation, while application of P alone significantly reduced N_2O emission 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, this study is among the first to investigate the effect of nutrient additions on soil N_2O emissions 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.

Acknowledgments

This research was financially supported by the National Key Basic Research 973 Program (2010CB833502), and the National Natural Science Foundation of China (Nos. 31000236 and 31370011). The authors wish to acknowledge Shengxing Fu for gas sampling, Dr. Frank S. Gilliam and Dr. Dejun Li for their constructive comments that improved the quality of the manuscript.

References

Anderson, J. M. and Ingram, J. S. I.: Tropical Soil Biology and Fertility, in: A Handbook of Methods, Wallingford, UK, CAB International, 1989.

Arah, J. R. M. and Smith, K. A. Steady-state denitrification in aggregated soils: a mathematical model. J. Soil Sci., 40:139-149, 1989.

Arai, S., Ishizuka, S., Ohta, S., Ansori, S., Tokuchi, N., Tanaka, N., and Hardjono, A.: Potential N_2O emissions from leguminous tree plantation soils in the humid tropics,

503 Global Biogeochem. Cy., 22, GB2028, doi:10.1029/2007GB002965, 2008.

504 Attiwill, P. M. and May, B. M.: Does nitrogen limit the growth of native *eucalypt*
505 forests: some observations for mountain ash (*Eucalyptus regnans*), Mar.
506 Freshwater Res., 52: 111-117, doi:10.1071/MF00046, 2001.

507 Barnard, R., Leadley, P. W., and Hungate, B. A.: Global change, nitrification, and
508 denitrification: A review, Global Biogeochem. Cy., 19, GB1007, doi:
509 10.1029/2004GB002282, 2005.

510 Bremner, J. M. and Mulvaney, C. S.: Total nitrogen, in: Methods of Soil Analysis,
511 edited by: Page, A. L., Miller, R. H., and Keeny, D. R., American Society of
512 Agronomy and Soil Science Society of America, Madison, 1119-1123, 1982.

513 Butterbach-Bahl, K., Gasche, R., Huber, C., Kreutzer, K., Papen, H.: Impact of N-
514 input by wet deposition on N-trace gas fluxes and CH_4-oxidation in spruce forest
515 ecosystems of the temperate zone in Europe, Atmos. Environ., 32, 559-564, 1998.

516 Butterbach-Bahl, K., Kock, M., Willibald, G., Hewett, B., Buhagiar, S., Papen, H., and
517 Kiese, R.: Temporal variations of fluxes of NO, NO_2, N_2O, CO_2, and CH_4 in a
518 tropical rain forest ecosystem, Global Biogeochem. Cy., 18, GB3012, doi:
519 10.1029/2004GB002243, 2004.

520 Cabrera, M. L. and Beare, M. H.: Alkaline persulfate oxidation for determining total
521 nitrogen in microbial biomass extracts, Soil Sci. Soc. Am. J., 57, 1007-1012, 1993.

522 Chen, D. M., Zhang, C. L., Wu, J. P., Zhou, L. X., Lin, Y. B., and Fu, S. L.:
523 Subtropical plantations are large carbon sinks: evidence from two monoculture
524 plantations in South China, Agr. Forest Meteorol., 151, 1214-1225, 2011.

525 Dick, J., Skiba, U., Munro, R., and Deans, D.: Effect of N-fixing and non N-fixing
526 trees and crops on NO and N_2O emissions from Senegalese soils, J. Biogeogr., 33,
527 416-423, 2006.

528 Dong, M., Wang, Y. F., Kong, F. Z., Jiang, G. M., and Zhang, Z. B.: Standard methods
529 for observation and analysis in Chinese Ecosystem Research Network: survey,
530 observation and analysis of terrestrial biocommunities, Standards Press of China,
531 Beijing, 1-80, 1996 (in Chinese with English abstract).

532 Falkiner, R. A., Khanna, P. K., and Raison, R. J.: Effect of Superphosphate Addition
533 on N-Mineralization in Some Australian Forest Soils, Aust. J. Soil Res., 31, 285-

296, 1993.

FAOUN, Food and Agriculture Organization of the United Nations, Global Forest Resources Assessment 2010: Main Report, FAO Forestry Paper, vol. 163, Food and Agric. Org. of the UN, Rome, 2010.

Forrester, D. I., Bauhus, J., Cowie, A. L., and Vanclay, J. K.: Mixed-species plantations of *Eucalyptus* with nitrogen-fixing trees: a review, Forest Ecol. Manag., 233, 211-230, 2006.

Galloway, J. N., Townsend, A. R., Erisman, J. W., Bekunda, M., Cai, Z., Freney, J. R., Martinelli, L. A., Seitzinger, S. P., and Sutton, M. A.: Transformation of the nitrogen cycle: recent trends, questions, and potential solutions, Science, 320, 889-892, 2008.

Ghehi, N. G., Werner, C., Ntaboba, L. C., Muhinda, J. J. M., Van, R. E., Butterbach-Bahl, K., Kiese, R., and Boeckx, P.: Spatial variations of nitrogen trace gas emissions from tropical mountain forests in Nyungwe, Rwanda, Biogeosciences, 9, 1451-1463, 2012.

Hall, S. J. and Matson, P. A.: Nitrogen oxide emissions after nitrogen additions in tropical forests, Nature, 400, 152-155, 1999.

Hall, S. J. and Matson, P. A.: Nutrient status of tropical rain forests influences soil N dynamics after N additions. Ecol. Monogr., 73, 107-129, 2003.

Hedin, L. O., Brookshire, E. N. J., Menge, D. N. L., and Barron, A.: The nitrogen paradox in tropical forest ecosystems, Annu. Rev. Ecol. Evol. S., 40, 613-635, 2009.

Holland, E. A., Robertson, G. P., Greenberg, J., Groffman, P. M., Boone, R. D., and Gosz, J. R.: Soil CO_2, N_2O, and CH_4 exchange, in: Standard Soil Methods for Long-Term Ecological Research, edited by: Robertson G. P., Oxford University Press, New York, NY, USA, 185-201, 1999.

IPCC: Changes in Atmospheric Constituents and in Radiative Forcing. Climate Change 2007: The Physical Science Basis, Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, edited by: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K. B., Tignor, M., and Miller, H. L., Cambridge University Press, Cambridge, UK and

New York, NY, USA, 210-215, 2007.

Keller, M. and Reiners, W. A.: Soil atmosphere exchange of nitrous-oxide, nitric-oxide, and methane under secondary succession of pasture to forest in the Atlantic lowlands of Costa-Rica, Global Biogeochem. Cy., 8, 399-409, 1994.

Kiese, R. and Butterbach-Bahl, K.: N_2O and CO_2 emissions from three different tropical forest sites in the wet tropics of Queensland, Australia, Soil Biol. Biochem., 34, 975-987, 2002.

Koehler, B., Corre, M. D., Veldkamp, E., Wullaert, H., Wright, S. J.: Immediate and long-term nitrogen oxide emissions from tropical forest soils exposed to elevated nitrogen input, Glob. Change Biol., 15, 2049-2066, 2009.

Konda, R., Ohta, S., Ishizuka, S., Arai, S., Ansori, S., Tanaka, N., and Hardjono, A.: Spatial structures of N_2O, CO_2, and CH_4 fluxes from *Acacia mangium* plantation soils during a relatively dry season in Indonesia, Soil Biol. Biochem., 40, 3021-3030, 2008.

Li, D. J., Niu, S. L., and Luo, Y. Q.: Global patterns of the dynamics of soil carbon and nitrogen stocks following afforestation: a meta-analysis, New Phytol., 195, 172-181, 2012.

Liu, B. B., Morkved, P. T., Frostegard, A., and Bakken, L. R.: Denitrification gene pools, transcription and kinetics of NO, N_2O and N_2 production as affected by soil pH, FEMS Microbiol. Ecol., 72, 407-417, 2010.

Liu, G. S., Jiang, N. H., Zhang, L. D., and Liu, Z. L.: Soil Physical and Chemical Analysis and Description of Soil Profiles, Stands Press of China, Beijing, 1996 (in Chinese with English abstract).

Liu, L. L. and Greaver, T. L.: A review of nitrogen enrichment effects on three biogenic GHGs: the CO_2 sink may be largely offset by stimulated N_2O and CH_4 emission, Ecol. Lett., 12, 1103-1117, 2009.

Liu, X. J., Zhang, Y., Han, W. X., Tang, A. H., Shen, J. L., Cui, Z. L., Vitousek, P., Erisman, J. W., Goulding, K., Christie, P., Fangmeier, A., and Zhang, F. S.: Enhanced nitrogen deposition over China, Nature, 494, 459-462, 2013.

Martinson, G. O., Corre, M. D., and Veldkamp, E.: Responses of nitrous oxide fluxes and soil nitrogen cycling to nutrient additions in montane forests along an elevation

gradient in southern Ecuador, Biogeochemistry, 112, 625-636, 2013.

Matson, P. A., Gower, S. T., Volkmann, C., Billow, C., and Grier, C. C.: Soil-nitrogen cycling and nitrous-oxide flux in a rocky-mountain Douglas-Fir Forest - effects of fertilization, irrigation and carbon addition, Biogeochemistry, 18, 101-117, 1992.

Minami, K. and Fukushi, S.: Effects of phosphate and calcium-carbonate application on emission of N_2O from soils under aerobic conditions, Soil Sci. Plant Nutr., 29, 517-524, 1983.

Mori, T., Ohta, S., Ishizuka, S., Konda, R., Wicaksono, A., Heriyanto, J., and Hardjono, A.: Effects of phosphorus addition on N_2O and NO emissions from soils of an *Acacia mangium* plantation. Soil Sci, Plant Nutr., 56, 782-788, 2010.

Mori, T., Ohta, S., Ishizuka, S., Konda, R., Wicaksono, A., Heriyanto, J., and Hardjono, A.: Effects of phosphorus addition with and without ammonium, nitrate, or glucose on N_2O and NO emissions from soil sampled under *Acacia mangium* plantation and incubated at 100 % of the water-filled pore space, Biol. Fert. Soils, 49, 13-21, 2013.

Peng, S. L., Hou, Y. P., and Chen, B. M.: Vegetation restoration and its effects on carbon balance in Guangdong Province, China, Restor. Ecol., 17(4), 487-494, 2009.

Ravishankara, A. R., Daniel, J. S., and Portmann, R. W.: Nitrous oxide (N_2O): the dominant ozone-depleting substance emitted in the 21st century, Science, 326, 123-125, 2009.

Reay, D. S., Dentener, F., Smith, P., Grace, J., and Feely, R. A.: Global nitrogen deposition and carbon sinks, Nat. Geosci., 1, 430-437, 2008.

Rowlings, D. W., Grace, P. R., Kiese, R., and Weier, K. L.: Environmental factors controlling temporal and spatial variability in the soil-atmosphere exchange of CO_2, CH_4 and N_2O from an Australian subtropical rainforest, Glob. Change Biol., 18, 726-738, 2012.

Steudler, P. A., Garcia-Montiel, D. C., Piccolo, M. C., Neill, C., Melillo, J. M., Feigl, B. J., and Cerri, C. C.: Trace gas responses of tropical forest and pasture soils to N and P fertilization, Global Biogeochem. Cy., 16, 1023, doi: 10.1029/2001GB001394, 2002.

626 Stocker, B. D., Roth, R., Joos, F., Spahni, R., Steinacher, M., Zaehle, S., Bouwman, L.,
627 Xu, R., and Prentice, I. C.: Multiple greenhouse-gas feedbacks from the land
628 biosphere under future climate change scenarios, Nat. Clim. Change, 3, 666-672,
629 2013.

630 Sundareshwar, P. V., Morris, J. T., Koepfler, E. K., and Fornwalt, B.: Phosphorus
631 limitation of coastal ecosystem processes, Science, 299. 563-565, 2003.

632 Tu, C., Louws, F. J., Creamer, N. G., Mueller, J. P., Brownie, C., Fager, K., Bell, M.,
633 and Hu, S. J.: Responses of soil microbial biomass and N availability to transition
634 strategies from conventional to organic farming systems, Agr. Ecosyst. Environ.,
635 113, 206-215, 2006.

636 Vance, E. D., Brookes, P. C., and Jenkinson, D. S.: An extraction method for
637 measuring soil microbial biomass-C, Soil Biol. Biochem., 19, 703-707, 1987.

638 Venterea, R. T., Groffman, P. M., Verchot, L. V., Magill, A. H., Aber, J. D., and
639 Steudler, P. A..: Nitrogen oxide gas emissions from temperate forest soils receiving
640 long-term nitrogen inputs, Glob. Change Biol., 9, 346-357, 2003.

641 Vitousek, P. M., Aber, J. D., Howarth, R. W., Likens, G. E., Matson, P. A., Schindler,
642 D. W., Schlesinger, W. H., and Tilman, D.: Human alteration of the global nitrogen
643 cycle: sources and consequences, Ecol. Appl., 7, 737-750, 1997.

644 Vitousek, P. M., Porder, S., Houlton, B. Z., and Chadwick, O. A.: Terrestrial
645 phosphorus limitation: mechanisms, implications, and nitrogen-phosphorus
646 interactions, Ecol. Appl., 20, 5-15, 2010.

647 Wang, H., Liu, S. R., Mo, J. M., and Zhang, T.: Soil-atmosphere exchange of
648 greenhouse gases in subtropical plantations of indigenous tree species, Plant Soil,
649 335, 213-227, 2010.

650 Werner, C., Kiese, R., and Butterbach-Bahl, K.: Soil-atmosphere exchange of N_2O,
651 CH_4, and CO_2 and controlling environmental factors for tropical rain forest sites in
652 western Kenya, J. Geophys. Res., 112, D03308, doi:10.1029/2006JD007388, 2007.

653 Xu, R., Prentice, I. C., Spahni, R., and Niu, H. S.: Modelling terrestrial nitrous oxide
654 emissions and implications for climate feedback, New Phytol., 196, 472-488, 2012.

655 Zhang, W., Mo, J. M., Yu, G. R., Fang, Y. T., Li, D. J., Lu, X. K., and Wang, H.:
656 Emissions of nitrous oxide from three tropical forests in Southern China in

response to simulated nitrogen deposition, Plant Soil, 306, 221-236, 2008.

Zhang, W., Zhu, X. M., Liu, L., Fu, S. L., Chen, H., Huang, J., Lu, X. K., Liu, Z. F., and Mo, J. M.: 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, J. Geophys. Res., 117, G00N16, doi: 10.1029/2012JG002094, 2012.

Zhu, J., Mulder, J., Wu, L. P., Meng, X. X., Wang, Y. H., and Dörsch, P.: Spatial and temporal variability of N_2O emissions in a subtropical forest catchment in China, Biogeosciences, 10, 1309-1321, doi:10.519/bg-10-1309-2013, 2013a.

Zhu, J., Mulder, J., Solheimslid, S. O., and Dörsch, P.: Functional traits of denitrification in a subtropical forest catchment in China with high atmogenic N deposition, Soil Biol. Biochem., 57, 577-586, 2013b.

Zhu, W. X. and Carreiro, M. M.: Chemoautotrophic nitrification in acidic forest soils along an urban-to-rural transect, Soil Biol. Biochem., 31, 1091-1100, 1999.

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

		July 2011						July 2012					
Site	**Treatment**	**NO_3^--N (mg kg^{-1})**	**NH_4^+-N (mg kg^{-1})**	**TN (g kg^{-1})**	**SOC (g kg^{-1})**	**C:N ratio**	**Av. P (mg kg^{-1})**	**NO_3^--N (mg kg^{-1})**	**NH_4^+-N (mg kg^{-1})**	**TN (g kg^{-1})**	**SOC (g kg^{-1})**	**C:N ratio**	**Av. P (mg kg^{-1})**
	C	8.1(0.2)a	10.5(0.3)a	1.6(0.1)a	22.1(2)	13.8(2)b	1.8(0.2)a	7.7(0.9)a	9.4(0.5)a	2.2(0.1)a	40.7(3)	18.5(1)b	2.9(0.3)a
	MN	12.3(0.5)b	13.2(0.4)ab	1.8(0.3)ab	19.0(2)	11.7(2)ab	1.9(0.2)a	11.9(1.4)ab	11.7(0.3)ab	2.5(0.1)ab	38.0(2)	15.2(1)ab	2.8(0.1)a
	HN	14.9(0.6)b	16.3(0.7)b	2.2(0.1)b	21.5(1)	9.8(1)a	1.9(0.6)a	13.5(1.2)b	15.3(1.4)b	2.7(0.2)b	32.7(3)	12.5(2)a	3.0(0.2)a
AA	MP	9.6(0.8)ab	12.2(1.2)a	1.3(0.3)a	18.4(1)	14.2(3)b	3.3(1.2)ab	6.7(1.1)a	9.8(1.8)a	2.2(0.2)ab	38.5(3)	17.5(2)b	3.3(0.5)ab
	HP	10.2(0.6)ab	12.8(1.6)a	1.5(0.2)a	19.7(3)	13.1(2)ab	8.9(0.4)c	6.6(0.4)a	11.9(0.7)ab	2.2(0.2)ab	45.3(4)	18.9(3)bc	4.1(0.5)b
	MNP	11.7(1.0)b	14.8(1.2)ab	1.6(0.2)a	21.5(1)	13.4(3)b	3.3(0.8)ab	10.9(1.3)ab	10.5(1.2)a	2.1(0.4)a	49.1(5)	23.4(4)c	3.6(0.3)ab
	HNP	9.6(0.5)ab	14.4(1.0)ab	1.5(0.1)a	22.6(2)	15.1(1)b	5.8(1.4)b	11.3(1.0)ab	12.2(0.8)ab	2.0(0.2)a	55.8(4)	27.9(3)c	4.0(0.1)b
	C	6.1(0.6)a	8.7(1.3)	1.4(0.0)	15.5(2)	11.1(1)	1.6(0.3)a	5.6(0.5)b	6.7(0.2)a	1.6(0.1)	20.9(3)	13.1(2)	2.6(0.1)a
	MN	9.5(0.7)ab	9.0(1.8)	1.5(0.3)	15.8(2)	10.5(1)	1.1(0.3)a	7.4(0.4)b	8.7(0.7)ab	1.4(0.2)	25.8(3)	18.4(3)	2.8(0.2)a
	HN	10.6(0.5)b	9.3(1.2)	1.8(0.2)	16.1(1)	9.0(1)	2.0(0.3)a	12.3(0.6)c	13.9(0.2)b	1.7(0.2)	28.9(2)	17.9(3)	3.4(0.1)ab
EU	MP	8.1(0.5)ab	9.1(0.9)	1.5(0.1)	17.2(1)	11.5(0)	2.1(0.7)a	3.6(0.4)a	6.6(0.4)a	1.5(0.1)	26.3(3)	17.5(3)	3.8(0.1)b
	HP	7.8(0.9)ab	8.6(1.2)	1.6(0.1)	18.8(2)	11.8(1)	5.3(1.1)b	4.2(0.7)a	5.2(0.8)a	1.6(0.3)	33.9(2)	21.2(2)	4.1(0.4)b

MNP	8.6(0.4)ab	10.7(0.7)	1.8(0.1)	18.9(2)	10.6(2)	2.8(0.6)ab	5.7(1.4)b	6.0(1.4)a	1.8 (0.2)	31.8(3)	17.7(1)	3.4(0.3)ab
HNP	8.0(0.7)ab	9.9(0.8)	1.7(0.3)	17.3(3)	10.2(2)	6.3(1.3)b	6.0(0.6)b	6.9(0.7)a	1.7(0.1)	33.6(3)	19.8(1)	4.0(0.5)b

672 **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
673 the same column indicate significantly different mean values among treatments of each plantation (Tukey's HSD test, $p \leq 0.05$). *AA*: *Acacia*
674 *auriculiformis* plantation; *EU*: *Eucalyptus urophylla* plantation. TN, total nitrogen; SOC, soil organic C; C:N ratio, SOC:TN ratio; Av. P, soil
675 available P.

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

Site	Treatment	July 2011				July 2012						
		pH value	MBC (mg kg^{-1})	MBN (mg kg^{-1})	LM ($gm^{-2}yr^{-1}$)	pH value	MBC (mg kg^{-1})	MBN (mg kg^{-1})	LM ($gm^{-2}yr^{-1}$)	Litter N (mg g^{-1})	Litter P (mg g^{-1})	N:P ratio
	C	3.8(0.02)ab	254(14)a	41(4)ab	749(85)	3.8(0.01)ab	330(31)a	67(12)	841(58)	12(0.5)	0.2(0.0)a	77(2)c
	MN	3.8(0.03)ab	215(10)a	52(6)ab	712(57)	3.8(0.03)ab	350(33)a	74(15)	704(59)	14(1.1)	0.2(0.0)a	72(9)c
	HN	3.7(0.02)a	204(15)a	60(7)b	800(23)	3.7(0.01)a	292(31)a	79(10)	846(72)	14(0.3)	0.2(0.0)a	85(3)c
AA	**MP**	3.9(0.04)b	237(45)a	40(18)ab	964(96)	3.9(0.03)b	298(35)a	61(18)	864(64)	13(0.5)	0.3(0.0)ab	45(7)b
	HP	3.9(0.05)b	234(27)a	28(4)a	715(54)	3.9(0.04)b	634(38)b	86(17)	780(77)	12(0.5)	1.4(0.3)c	10(2)a
	MNP	3.8(0.02)ab	316(36)b	32(6)ab	751(66)	3.9(0.02)b	414(32)ab	94(12)	744(59)	13(0.9)	0.4(0.1)ab	35(7)ab
	HNP	3.8(0.05)ab	426(32)b	51(8)ab	738(50)	3.9(0.02)b	446(34)ab	52(14)	783(56)	14(1.6)	0.7(0.1)b	23(5)ab
	C	3.9(0.05)	288(21)	44(6)	644(28)	3.9(0.02)	378(33)	78(8)	870(67)	11(0.4)a	0.4(0.1)ab	33(7)b
	MN	3.9(0.04)	279(24)	31(1)	517(10)	3.9(0.03)	333(34)	60(13)	697(55)	13(0.4)b	0.3(0.0)a	43(2)c
	HN	3.8(0.02)	246(23)	39(7)	520(61)	4.0(0.05)	326(26)	69(10)	674(58)	13(0.4)b	0.3(0.0)a	44(5)c
EU	**MP**	3.9(0.04)	258(27)	40(7)	690(46)	3.9(0.01)	286(24)	73(9)	714(29)	12(0.8)ab	0.5(0.2)ab	23(6)ab
	HP	3.8(0.01)	328(36)	49(11)	574(59)	4.0(0.03)	359(26)	47(12)	826(57)	13(0.3)b	1.4(0.2)c	9(1)a

MNP	3.9(0.05)	293(18)	51(12)	486(54)	4.0(0.05)	361(16)	74(11)	817(45)	12(0.4)ab	0.9(0.1)ab	15(1)ab
HNP	3.9(0.04)	285(16)	35(4)	634(13)	3.9(0.04)	350(20)	80(10)	803(39)	14(0.3)b	1.1(0.3)b	15(5)ab

677 **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
678 the same column indicate significantly different mean values among treatments of each stand (Tukey's HSD test, $p \leq 0.05$). *AA, Acacia*
679 *auriculiformis* plantation; *EU, Eucalyptus urophylla* plantation. MBC, microbial biomass C; MBN, microbial biomass N; N:P ratio, litter N:litter
680 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**	**<0.01**	**<0.001**	**<0.001**	0.45	0.80	0.77	0.19	0.52	0.67	0.27
	P	0.75	0.16	0.98	**0.02**	0.35	**0.03**	**<0.001**	**0.01**	0.93	**0.02**
	Y	0.843	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.02**	0.17	**0.01**	**0.02**	0.63
	N×P	**0.05**	**0.04**	**0.01**	0.10	0.47	*0.08*	*0.08*	0.66	0.56	0.80
	N×Y	*0.06*	0.41	0.52	0.79	0.86	0.73	0.34	0.11	0.57	0.17
	P×Y	*0.06*	0.79	0.46	0.99	0.39	0.56	**0.001**	0.12	0.93	*0.07*
	N×P×Y	0.17	**0.02**	0.95	0.48	0.79	0.63	0.33	0.16	0.47	0.94
EU	**N**	*0.08*	**<0.001**	**0.04**	0.11	0.53	0.93	0.38	**0.06**	0.83	0.86
	P	0.86	**<0.01**	**0.03**	0.22	*0.07*	0.64	**<0.001**	*0.09*	0.62	0.77
	Y	0.11	**<0.001**	**<0.001**	0.45	**<0.001**	**<0.01**	0.68	0.10	**<0.01**	0.49
	N×P	0.35	**0.001**	0.54	*0.08*	0.52	0.49	0.60	0.23	0.47	0.52
	N×Y	0.82	0.30	0.45	0.66	0.66	0.89	0.73	0.96	0.68	**0.03**
	P×Y	**0.04**	**0.04**	0.10	0.92	0.47	0.86	**<0.01**	0.98	0.82	0.21
	N×P×Y	0.57	0.33	0.51	0.33	0.86	0.55	0.58	0.75	0.54	*0.06*

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.

690 **Table 4** Regression analysis between N_2O fluxes and soil temperature and WFPS in
691 the controls of *AA* and *EU* plantations

	AA (n = 108)	***EU*** (n = 108)	***AA* + *EU*** (n = 216)
Soil temperature (T, ^oC)			
R^2	0.32***	0.35***	0.30***
***p* value**	< 0.001	< 0.001	< 0.001
***f* (*T*)**	1.34T + 2.28	1.43T + 7.44	1.34T - 2.05
Soil moisture (M, WFPS, %)			
R^2	0.19***	0.26***	0.23***
***p* value**	< 0.001	< 0.001	< 0.001
***f* (*M*)**	0.49M + 3.70	0.56M - 5.58	0.55M - 2.38
Multiple linear regression analysis (T and M)			
R^2	0.38***	0.43***	0.39***
***p* value**	< 0.001	< 0.001	< 0.001
***f* (*T, M*)**	1.11T + 0.31M - 9.56	1.12T + 0.35M - 18.50	1.06T + 0.38M - 15.05

692 **Notes**: Gas samples, soil temperature and soil moisture were collected simultaneously.
693 * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. *AA, Acacia auriculiformis* plantation; *EU,*
694 *Eucalyptus urophylla* plantation; *f*, flux of N_2O; T, soil temperature; M, soil moisture
695 (water filled pore space, WFPS).

696 **Table 5** N_2O emission factor

Plantation type	***AA* plantation**					***EU* plantation**				
Treatments	C	MN	HN	MNP	HNP	C	MN	HN	MNP	HNP
N_2O emission (kg N $ha^{-1}yr^{-1}$) [a]	2.3	2.6	3.1	2.6	2.7	1.9	1.9	2.0	2.1	2.1
N addition (kg N $ha^{-1}yr^{-1}$)	0	50	100	50	100	0	50	100	50	100
N_2O emission factor (%) [b]		0.7	0.8	0.6	0.4		0.1	0.2	0.3	0.2

697 **Notes:** [a] The average rates of N_2O emissions, data from August 2010 to July 2012;

698 [b] The N_2O emission factor was calculated as (annual N_2O-N emission of N treatment
699 plot – annual N_2O-N emission of the control plot)/(total N applied in each year).

700 *AA*: *Acacia auriculiformis*; *EU*: *Eucalyptus urophylla*.

Figures:

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 differences at $p < 0.05$. Yr 1: from August 2010 to July 2011; Yr 2: from August 2011 to July 2012.

Supporting Information:

Table S1 Indices of tree structure in *Acacia auriculiformis* and *Eucalyptus urophylla* plantations.

Fig. S1 Seasonal patterns of soil N_2O emissions from (a) *Acacia auriculiformis* and (b) *Eucalyptus urophylla* plantations.

Fig. S2 Temporal variation of climate factors, soil temperature and WFPS from August 2010 to July 2012.