

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

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5 W. Zhang<sup>1,2</sup>, X. Zhu<sup>1,3</sup>, Y. Luo<sup>2</sup>, R. Rafique<sup>2</sup>, H. Chen<sup>1,3</sup>, J. Huang<sup>1</sup>, and J. Mo<sup>1</sup>

6 <sup>1</sup> Key Laboratory of Vegetation Restoration and Management of Degraded  
7 Ecosystems, South China Botanical Garden, Chinese Academy of Sciences,  
8 Guangzhou 510650, China

9 <sup>2</sup> Department of Microbiology and Plant Biology, University of Oklahoma, Norman,  
10 OK, 73019, USA

11 <sup>3</sup> University of Chinese Academy of Sciences, Beijing 100039, China

## Abstract

Leguminous tree plantations at phosphorus (P) limited sites may result in excess nitrogen (N) and higher rates of nitrous oxide (N<sub>2</sub>O) emissions. However, the effects of N and P applications on soil N<sub>2</sub>O 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<sub>2</sub>O emissions from tropical plantations with N-fixing vs. non-N-fixing tree species. We found that the average N<sub>2</sub>O emission from control was greater in AA ( $2.3 \pm 0.1$  kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup>) than in EU plantation ( $1.9 \pm 0.1$  kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup>). For the AA plantation, N-addition stimulated N<sub>2</sub>O emission from the soil while P-addition did not. Applications of N with P together significantly decreased N<sub>2</sub>O emission compared to N-addition alone, especially in the high level treatments (decreased by 18%). In the EU plantation, N<sub>2</sub>O emissions significantly decreased in P-addition plots compared with the controls, however, N- and NP-additions did not. The different response of N<sub>2</sub>O 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<sub>2</sub>O emissions from leguminous tree plantations in the tropics, whereas P fertilization has the potential to mitigate N deposition-induced N<sub>2</sub>O emissions from such plantations.

## 1 Introduction

Nitrous oxide is a powerful greenhouse gas that is 298 times more potent than carbon dioxide (CO<sub>2</sub>) over a 100 yr lifespan (IPCC, 2007), and contributes to stratospheric ozone (O<sub>3</sub>) depletion (Ravishankara et al., 2009). Atmospheric N<sub>2</sub>O concentration has been increasing by 0.2-0.3% yr<sup>-1</sup> over the last 250 yr (Stocker et al., 2013). N<sub>2</sub>O 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<sub>2</sub>O emission, accounting for 14% to 23% of current global N<sub>2</sub>O budget (IPCC, 2007). The major factors of controlling N<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O than the N-limited forests after N fertilization. Hall and Matson (1999) measured N<sub>2</sub>O emission after adding N in two tropical rainforests in Hawaii (USA), and found that N<sub>2</sub>O emission from P-limited site was 54 times greater compared with that from N-limited site. Martinson et al. (2013) also

found lower N<sub>2</sub>O 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<sub>2</sub>O 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* (FAO, 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<sub>2</sub>O emissions, due to excess N easily promotes N<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O production. We hypothesized that: (i) the promotion effect of N-addition on N<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O 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 rainfall was  $43.1 \pm 3.9$  kg N ha<sup>-1</sup> yr<sup>-1</sup>, 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<sup>-3</sup> 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<sup>-1</sup> yr<sup>-1</sup>), high-N (HN, 100 kg N ha<sup>-1</sup> yr<sup>-1</sup>), medium-P (MP, 50 kg P ha<sup>-1</sup> yr<sup>-1</sup>), high-P (HP, 100 kg P ha<sup>-1</sup> yr<sup>-1</sup>), medium-NP (MNP, 50 kg N ha<sup>-1</sup> yr<sup>-1</sup> + 50 kg P ha<sup>-1</sup> yr<sup>-1</sup>), and high-NP (HNP, 100 kg N ha<sup>-1</sup> yr<sup>-1</sup> + 100 kg P ha<sup>-1</sup> yr<sup>-1</sup>). Ammonium

nitrate ( $\text{NH}_4\text{NO}_3$ ) and sodium biphosphate ( $\text{NaH}_2\text{PO}_4$ ) 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 with each treatment event.

## **2.3 Field sampling and measurements**

### **2.3.1 $\text{N}_2\text{O}$ flux measurements**

From August 2010 to July 2012,  $\text{N}_2\text{O}$  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.  $\text{N}_2\text{O}$  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 ( $\text{NH}_4^+$ ), nitrate ( $\text{NO}_3^-$ ), 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  $\text{NH}_4^+$  and  $\text{NO}_3^-$  contents were determined by extraction with 2 M KCl solution followed by colorimetric analysis on 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  $\text{NH}_4^+$  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<sub>3</sub>) vapor for 24 h at 25 °C then extracted with 0.5 M K<sub>2</sub>SO<sub>4</sub>. 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<sub>3</sub><sup>-</sup> 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<sup>-1</sup> dry weight soil month<sup>-1</sup>.

### **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 °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<sub>2</sub>SO<sub>4</sub>-H<sub>2</sub>O<sub>2</sub> 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 ( $\text{g cm}^{-3}$ ), and 2.65 is the soil particle density ( $\text{g cm}^{-3}$ ).

### 2.4 Statistics

Repeated Measures Analysis of Variance (ANOVA) was used to examine the effect of nutrient additions on  $\text{N}_2\text{O}$  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  $\text{N}_2\text{O}$  emissions, soil properties, MBC, MBN, and litterfall mass among treatments of each plantation. Multiple regression analysis was performed to evaluate the relationships of  $\text{N}_2\text{O}$  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  $\pm 1$  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 ( $\text{NO}_3^-$  and  $\text{NH}_4^+$ ), 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 ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) contents of both plantations (Table 3). For the *EU* plantation, N-addition significantly increased soil  $\text{NO}_3^-$  content, while  $\text{NH}_4^+$  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 ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) 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  $\text{N}_2\text{O}$  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-  $\times$  P-addition on soil available N ( $\text{NO}_3^-$  and  $\text{NH}_4^+$ ) and TN were found in the AA plantation (Table 3). There was an interactive effect of N-  $\times$  P-addition  $\times$  year on soil  $\text{NO}_3^-$  in the AA plantation (Table 3). For the *EU* plantation, the interaction of N-  $\times$  P-addition on soil  $\text{NO}_3^-$  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  $11 \pm 3$  in the controls to  $23 \pm 3$  mg N kg soil<sup>-1</sup> month<sup>-1</sup> 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  $12 \pm 3$  in the controls to  $14 \pm 2$  and  $27 \pm 3$  mg N kg soil<sup>-1</sup> month<sup>-1</sup>, 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 ( $5 \pm 1$ ) was significantly lower than that in HN ( $17 \pm 6$ ) and HNP ( $14 \pm 4$  mg N kg soil<sup>-1</sup> month<sup>-1</sup>) 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<sub>2</sub>O 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<sub>2</sub>O emissions from the controls

During the two years of experimental period, the soils of both plantations were a net source of N<sub>2</sub>O (Fig. 2 a, b). Average N<sub>2</sub>O emission from the controls of the *AA* plantation ( $2.3 \pm 0.1$  kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup>) was significantly greater ( $t$ -test,  $p = 0.007$ ) than that of *EU* plantation ( $1.9 \pm 0.1$  kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup>). The *AA* plantation showed more and higher N<sub>2</sub>O peaks compared to the *EU* plantation (Fig. S1 a, b). Emissions of N<sub>2</sub>O 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<sub>2</sub>O fluxes

In the *AA* plantation, N<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O emissions (Table 3).

For the *EU* plantation, nutrient additions had no significant effects on soil N<sub>2</sub>O emissions during the two years (Table 3). However in the second year, soil N<sub>2</sub>O 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<sub>2</sub>O emission (Table 3).

## 4 Discussion

### 4.1 Comparisons of N<sub>2</sub>O emission

The rates of N<sub>2</sub>O emission observed from the controls of AA and *EU* plantations (1.9 to 2.3 kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup>) are comparable with previous reports in (sub)tropical regions of southern China (2.0 to 4.8 kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup>) (Zhang et al., 2008; Zhu et al., 2013a), and also within the range of published results (1.2-2.6 kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup>) from other tropical forests (Werner et al., 2007; Ghehi et al., 2012). The higher rates of N<sub>2</sub>O emissions (3.7-7.5 kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup>) 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<sub>2</sub>O emissions of 0.1 to 0.7 kg N<sub>2</sub>O-N ha<sup>-1</sup>yr<sup>-1</sup> 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<sub>2</sub>O 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<sub>2</sub>O (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<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O emission from the AA stand (Liu et al., 2010).

## 4.2 Effects of N application on N<sub>2</sub>O 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<sub>2</sub>O emission. The increase of soil N<sub>2</sub>O emissions following NH<sub>4</sub><sup>+</sup> or NO<sub>3</sub><sup>-</sup> 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<sub>2</sub>O 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<sub>2</sub>O emissions.

There are several factors causing the different responses of soil N<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O emissions by denitrification (Liu et al., 2010). A lower soil pH after N application might contribute to the increase in N<sub>2</sub>O 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<sub>2</sub>O emissions**

P-addition promoted uptake of N by plants (Hall and Matson, 1999), which could reduce N<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O emissions by increasing N immobilization. On contrary, in a incubation experiment (excluded plant), Mori et al. (2010) found that P-addition increased N<sub>2</sub>O 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<sub>2</sub>O 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).  $N_2O$  fluxes showed positive linear

relationships with soil temperatures and WFPS (Fig. 3 a, b), which were consistent with tropical and subtropical forests (Butterbach-Bahl et al., 2004; Zhang et al., 2008; Zhu et al., 2013a). Increasing soil moisture would increase soil microbial activities and therefore N<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O fluxes with soil temperature or WFPS.

#### 4.6 N<sub>2</sub>O emission factors

According to N- and NP-addition plots, N<sub>2</sub>O 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 4). The N<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O emission factor by 50% at the AA plantation (Table 4). This result suggests that the combined application of N and P together may probably mitigate N<sub>2</sub>O emission in comparison with N fertilization alone in tropical plantations with leguminous trees.

## 5 Conclusions

The responses of soil N<sub>2</sub>O 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<sub>2</sub>O after N addition, due to its high initial soil N availability. Application of N and P together decreased the rate of N<sub>2</sub>O emission compared to N treatment alone in N-fixing trees plantation, while application of P alone significantly reduced N<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O emissions from leguminous tree plantations. Our findings also suggest that moderate fertilization of P might eventually reduce N deposition-induced N<sub>2</sub>O emissions from leguminous tree plantations in the tropical and subtropical regions.

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

Plantation	Treatment	July 2011						July 2012					
		NO <sub>3</sub> <sup>-</sup> -N (mg kg <sup>-1</sup> )	NH <sub>4</sub> <sup>+</sup> -N (mg kg <sup>-1</sup> )	TN (g kg <sup>-1</sup> )	SOC (g kg <sup>-1</sup> )	C:N ratio	Av. P (mg kg <sup>-1</sup> )	NO <sub>3</sub> <sup>-</sup> -N (mg kg <sup>-1</sup> )	NH <sub>4</sub> <sup>+</sup> -N (mg kg <sup>-1</sup> )	TN (g kg <sup>-1</sup> )	SOC (g kg <sup>-1</sup> )	C:N ratio	Av. P (mg kg <sup>-1</sup> )
AA	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
	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
EU	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
	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

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669 **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  
670 the same column indicate significantly different mean values among treatments of each plantation (Tukey's HSD test,  $p \leq 0.05$ ). AA: *Acacia*  
671 *auriculiformis* plantation; EU: *Eucalyptus urophylla* plantation. TN, total nitrogen; SOC, soil organic C; C:N ratio, SOC:TN ratio; Av. P, soil  
672 available P.

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

Plantation	Treatment	July 2011				July 2012						
		pH value	MBC (mg kg <sup>-1</sup> )	MBN (mg kg <sup>-1</sup> )	LM (gm <sup>-2</sup> yr <sup>-1</sup> )	pH value	MBC (mg kg <sup>-1</sup> )	MBN (mg kg <sup>-1</sup> )	LM (gm <sup>-2</sup> yr <sup>-1</sup> )	Litter N (mg g <sup>-1</sup> )	Litter P (mg g <sup>-1</sup> )	N:P ratio
AA	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
	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
EU	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
	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

<b>MNP</b>	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
<b>HNP</b>	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

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674 **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  
675 the same column indicate significantly different mean values among treatments of each stand (Tukey's HSD test,  $p \leq 0.05$ ). AA, *Acacia*  
676 *auriculiformis* plantation; EU, *Eucalyptus urophylla* plantation. MBC, microbial biomass C; MBN, microbial biomass N; N:P ratio, litter N:litter  
677 P.

**Table 3** Results of repeated measures ANOVA for responses of N<sub>2</sub>O fluxes, soil properties, soil MBC and MBN to N-, P-addition and year.

		N <sub>2</sub> O	NO <sub>3</sub> <sup>-</sup>	NH <sub>4</sub> <sup>+</sup>	TN	SOC	C:N	Av. P	MBC	MBN	pH
AA	N	<b>&lt;0.01</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.45	0.80	0.77	0.19	0.52	0.67	0.27
	P	0.75	0.16	0.98	<b>0.02</b>	0.35	<b>0.03</b>	<b>&lt;0.001</b>	<b>0.01</b>	0.93	<b>0.02</b>
	Y	0.843	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>0.02</b>	0.17	<b>0.01</b>	<b>0.02</b>	0.63
	N×P	<b>0.05</b>	<b>0.04</b>	<b>0.01</b>	0.10	0.47	<i>0.08</i>	<i>0.08</i>	0.66	0.56	0.80
	N×Y	<i>0.06</i>	0.41	0.52	0.79	0.86	0.73	0.34	0.11	0.57	0.17
	P×Y	<i>0.06</i>	0.79	0.46	0.99	0.39	0.56	<b>0.001</b>	0.12	0.93	<i>0.07</i>
	N×P×Y	0.17	<b>0.02</b>	0.95	0.48	0.79	0.63	0.33	0.16	0.47	0.94
EU	N	<i>0.08</i>	<b>&lt;0.001</b>	<b>0.04</b>	0.11	0.53	0.93	0.38	<b>0.06</b>	0.83	0.86
	P	0.86	<b>&lt;0.01</b>	<b>0.03</b>	0.22	<i>0.07</i>	0.64	<b>&lt;0.001</b>	<i>0.09</i>	0.62	0.77
	Y	0.11	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.45	<b>&lt;0.001</b>	<b>&lt;0.01</b>	0.68	0.10	<b>&lt;0.01</b>	0.49
	N×P	0.35	<b>0.001</b>	0.54	<i>0.08</i>	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	<b>0.03</b>
	P×Y	<b>0.04</b>	<b>0.04</b>	0.10	0.92	0.47	0.86	<b>&lt;0.01</b>	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	<i>0.06</i>

**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.

687 **Table 4** N<sub>2</sub>O emission factor

Plantation type	AA plantation					EU plantation				
	C	MN	HN	MNP	HNP	C	MN	HN	MNP	HNP
<b>N<sub>2</sub>O emissions</b> (kg N ha <sup>-1</sup> yr <sup>-1</sup> ) <sup>a</sup>	2.3	2.6	3.1	2.6	2.7	1.9	1.9	2.0	2.1	2.1
<b>Total N applications</b> (kg N ha <sup>-1</sup> yr <sup>-1</sup> )	0	50	100	50	100	0	50	100	50	100
<b>N<sub>2</sub>O emission factor</b> (%) <sup>b</sup>		0.7	0.8	0.6	0.4		0.1	0.2	0.3	0.2

688 **Notes:** <sup>a</sup> The average rates of N<sub>2</sub>O emissions, data from August 2010 to July 2012;

689 <sup>b</sup> The N<sub>2</sub>O emission factor was calculated as (annual N<sub>2</sub>O-N emission of N treatment  
690 plot – annual N<sub>2</sub>O-N emission of the control plot)/(total N applied in each year).

691 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<sub>2</sub>O 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.

**Fig. 3** Relationships of N<sub>2</sub>O 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*; EU, *Eucalyptus urophylla*.

**Supporting Information:**

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

**Fig. S1** Seasonal patterns of soil N<sub>2</sub>O 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.