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 plantations with *Acacia auriculiformis* (*AA*, N-fixing) and *Eucalyptus urophylla* (*EU*, non-N-fixing) 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 these tropical plantations. We found that the average N_2O emission from control was greater in the *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 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 interaction 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 (three replicates) were established 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 nitrate (NH_4NO_3) and sodium biphosphate (NaH_2PO_4) were applied as N and P source, respectively. The additions were weighed and dissolved in 10 L water for each plot. The solutions were sprayed monthly onto the forest floor using a backpack sprayer since August 2010. Each control plot received 10 L water simultaneously with each treatment event.

2.3 Field sampling and measurements

2.3.1 N_2O flux measurements

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

2.3.2 Soil sampling and analyses

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

Soil NH_4^+ and 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 (TN) content was determined by the micro-Kjeldahl digestion (Bremner and Mulvaney, 1982), followed by detection of NH_4^+ with a UV-8000 Spectrophotometer (Metash Instruments Corp., Shanghai, China). Soil organic carbon (SOC) was determined by wet digestion with a mixture of potassium dichromate and concentrated sulphuric acid (Liu et al., 1996). Soil pH was measured in a 1:2.5 soil:water suspension using a pH meter (HM-30G, TOA Corp., Japan). Available P was extracted with 0.03 M ammonium fluoride and 0.025 M hydrochloric acid and analyzed colorimetrically (Anderson and Ingram, 1989). Gravimetric water content was determined through oven drying at 105 ^oC 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 ^oC then extracted with 0.5 M K_2SO_4. Simultaneously, subsamples for non-fumigated soil were also extracted with the same method. Soil MBC and MBN were

158 calculated as the difference in extractable C, N between fumigated and non-fumigated soils.
159 The conversion factors of 0.33 and 0.45 were used for calculating soil MBC and MBN,
160 respectively (Cabrera and Beare, 1993; Tu et al., 2006).

161

162 From July 1 to 31, 2012, soil net N-mineralization and nitrification were measured using an
163 intact core incubation. Six soil cores (3.5 cm diameter) were sampled from each plot. Three
164 cores were brought to the lab for extraction (2 M KCl) of inorganic N contents, and the others
165 were returned to the plot for in situ incubation. Nitrification rate was calculated from the
166 difference between extractable NO_3^- contents before and after incubation, and net N-
167 mineralization rate was calculated as the accumulation of total inorganic N over the
168 incubation (Zhu and Carreiro, 1999). The data were expressed as mg N kg^{-1} dry weight soil
169 $month^{-1}$.

170

171 **2.3.3 Litterfall**

172 Two litterfall traps (1.0 m × 1.0 m with a mesh size of 1 mm) were established in each plot.
173 Litter was collected monthly. The samples were oven dried at 65 ^oC for 48 h and weighed to
174 determine litter mass. Subsamples of dried litter was grounded and analyzed for N and P
175 concentrations using H_2SO_4-H_2O_2 digestion followed by colorimetric analysis (Dong et al.,
176 1996).

177

178 **2.3.4 Soil temperature and moisture**

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

186 $$WFPS = Vol/(1-SBD/2.65) \quad (1)$$

187 where *WFPS* is water filled pore space (%), *Vol* is volumetric water content (%), *SBD* is soil
188 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 litter 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 were 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^+), TN, and SOC contents of the control plots 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^-) and TN contents of the *AA* plantation (Table 1 and 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 and 3). N-addition did not change soil pH of the *EU* stand, however, a marginally significant decrease in pH value with N-addition 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 and 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). In the second experimental year, soil NO_3^- content decreased significantly following P-addition in the *EU* plantation ($p = 0.05$), but not significantly in the *AA* stand (Table 1 and 3; $p = 0.39$). Soil pH values of HP were significantly higher than that of HN treatments 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 contents of both plantations.

Applications of NP together significantly increased soil available P in both plantations (Table 1 and 3). For the *AA* plantation, soil available N slightly increased following NP-addition (Table 1 and 3). In the second year, NP-addition significantly increased soil C:N ratio of *AA* plantation ($p = 0.04$), while *EU* plantation did not (Table 1). The interactions of N- × P-addition on soil available N (NO_3^- and NH_4^+) 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. 1a; $p = 0.03$), which were from 11 ± 3 in the controls to 23 ± 3 mg N kg $soil^{-1}$ $month^{-1}$ in HN treatment plots. The rates of net N-mineralization also significantly increased following N treatment levels (Fig. 1a; $p = 0.04$). The average rates of net N-mineralization were from 12 ± 3 in the controls to 14 ± 2 and 19 ± 2 mg N kg $soil^{-1}$ $month^{-1}$, respectively for the MN and HN treatments. However, P- or NP-addition did not significantly change the rates of nitrification and net N-mineralization (Fig. 1a).

For the *EU* plantation, N-addition slightly increased the rates of nitrification and net N-mineralization (Fig. 1b). By contrary, P-addition tended to marginally decrease the rates of nitrification and net N-mineralization (Fig. 1b; $p = 0.07$ 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. 1b; $p < 0.05$). Similarly, the significant differences of net N-mineralization rate between the HP and HN or HNP treatments were found in the field incubation experiment (Fig. 1b; $p < 0.05$).

3.3 Soil microbial biomass and litterfall mass

In the *AA* plantation, soil MBC tended to decrease with N application, but there was no significant difference between N-addition plots and the controls (Table 2). Meanwhile, a marginally increase in soil MBN following N treatment levels was found in the first year (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 nutrient treatment plots in each plantation was also not significantly different (Table 2). Multiple regression analysis showed that there was a weak relationship between litter 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, there was no changes in leaf litter N concentrations following nutrient additions (Table 2). 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; all $p < 0.05$). 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 significantly higher 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). 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 the *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). N_2O emissions of both plantations tended to be higher in summer (June to August) than in winter (November to January of next year) (Fig. S1; $p < 0.05$ for both plantations).

3.5 Effects of nutrient additions on N_2O fluxes

In the *AA* plantation, N_2O emissions significantly increased following N applications (Fig. 2a; all $p < 0.05$), however, did not change following P-addition relative to the controls (Fig. 2a; all $p > 0.05$). During the two years of experimental period, the MN and HN treatments significantly increased soil N_2O emissions by 16% and 36%, respectively (Fig. 2a; $p = 0.05$ 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 (increased by 33%) compared with the controls (Fig. 2a; $p = 0.04$), 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. 2a; $p = 0.04$). Repeated Measures Analysis indicated that there was a significant interaction of N- × P-addition on N_2O emissions from *AA* plantation soil (Table 3).

For the *EU* plantation, nutrient additions had no significant effects on soil N_2O emissions in the first year (Fig. 2b; all $p > 0.05$). However in the second year, soil N_2O emissions significantly decreased by 23% and 27% for MP and HP treatments compared with the controls (Fig. 2b; $p = 0.05$ 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

313 Butterbach-Bahl, 2002). However, our result is above the reported average N_2O emissions of
314 0.1 to 0.7 kg N_2O-N $ha^{-1}yr^{-1}$ for pine forests in the southwestern China (Wang et al., 2010),
315 probably due to the higher pH values of these pine forest soils.

316

317 The *AA* plantation had significantly higher N_2O emissions than that of the *EU* stand, which
318 was consistent with our expectation. Our result supports the notion that leguminous tree
319 plantations in tropics and subtropics may potentially emit more N_2O (Arai et al., 2008; Konda
320 et al., 2008). The presence of leguminous trees resulting in higher soil N availability,
321 including higher rates of net N-mineralization and nitrification which was considered to be
322 the main reason for the higher rate of N_2O emission from the *AA* plantation, and supported by
323 the study of Dick et al. (2006). Leguminous trees can not only supply N via their unique
324 ability of N-fixing, but also increase soil C content (Li et al., 2012). The higher SOC and
325 fertility in the *AA* plantation compared to *EU* plantation may also partly explain the higher
326 N_2O emission from the *AA* plantation. Additionally, soil pH of the *AA* plantation was 0.5-0.7
327 lower than that of the *EU* site, which might directly or indirectly increase N_2O emission from
328 the *AA* stand (Liu et al., 2010).

329

330 **4.2 Effects of N application on N_2O emission**

331 Consistent with our hypothesis, the soil of *AA* plantation responded to N-addition greater than
332 the *EU* stand, with a large and immediate loss of N_2O emission. The increase of soil N_2O
333 emissions following NH_4^+ or NO_3^- addition was observed in many N-rich ecosystems
334 (Butterbach-Bahl et al., 1998; Hall and Matson, 1999; Koehler et al., 2009). In the present
335 study, the result from *AA* plantation is consistent with the reported results that N additions
336 could increase N_2O emissions from N-rich forest soils (Venterea et al., 2003; Zhang et al.,
337 2008). Whereas the result from the *EU* site is more comparable to the findings from related N-
338 poor forests (Matson et al., 1992; Zhang et al., 2008), which showed that N addition did not
339 enhance N_2O emissions.

340

341 There are several factors causing the different responses of soil N_2O emissions to N-addition
342 between the *AA* and *EU* plantations. The initial soil N status between both plantations
343 contributed to the different responses of N_2O emissions to N-addition. For the *AA* plantation
344 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 an 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-addition did not change N_2O emission from the *AA* plantation soil. The reason for this is currently not clear. Further study is necessary to identify causal relationships between N_2O emission, N availability of leguminous trees plantation and nutrient additions.

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 the soil of *AA* plantation in the first year. The result was in line with the report 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 added N 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 tree 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 control 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 are 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). There were no differences between treatments and the controls in each plantation, in terms of soil temperature (p = 0.7 and 0.6, respectively for the *AA* and *EU* plantation) 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 0.3% 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 yr), and the plantations planted on eroded soils are relatively poor in nutrients compared with natural forest soils. Compared to HN treatment, HNP-addition significantly decreased the N_2O emission factor by 50% in the *AA* plantation (Table 5; p = 0.04). 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 leguminous tree plantations.

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 N was absorbed and utilized by the vegetation with P application in these tropical plantations. 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.

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623 **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)a	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)a	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

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

628 **Table 2.** Soil pH, MBC, MBN, LM 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)	914(39)	14(0.3)b	1.1(0.3)b	15(5)ab

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

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

		N_2O	NO_3^-	NH_4^+	TN	SOC	C:N	Av. P	MBC	MBN	pH
AA	**N**	**<0.01**	**<0.001**	**<0.001**	0.45	0.80	**0.07**	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.

641 **Table 4.** Regression analysis between N_2O fluxes and soil temperature and WFPS in the
642 controls of *AA* and *EU* plantations

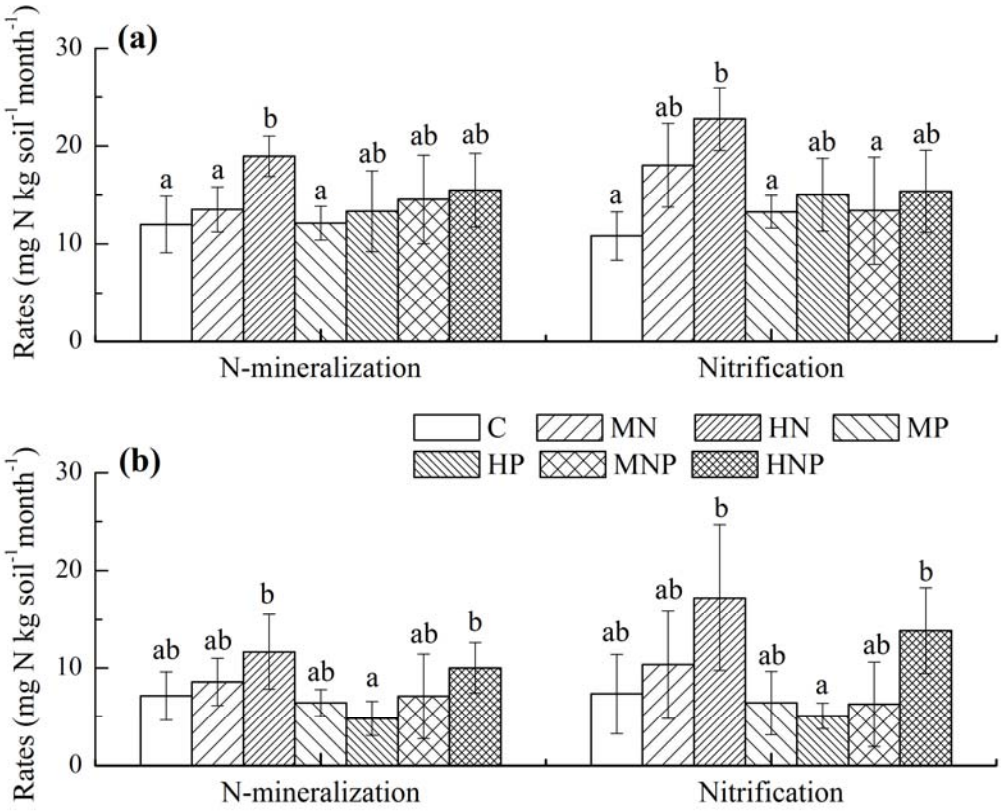
	AA (n = 108)	***EU*** (n = 108)	***AA* + *EU*** (n = 216)
Soil temperature (T, ^oC)			
R^2	0.32***	0.35***	0.30***
p	< 0.001	< 0.001	< 0.001
$f(T)$	1.34T + 2.28	1.43T + 7.44	1.34T - 2.05
Soil moisture (M, WFPS, %)			
R^2	0.19***	0.26***	0.23***
p	< 0.001	< 0.001	< 0.001
$f(M)$	0.49M + 3.70	0.56M - 5.58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

643 **Notes**: Gas samples, soil temperature and soil moisture were collected simultaneously. * $p <$
644 0.05; ** $p < 0.01$; *** $p < 0.001$. *AA, Acacia auriculiformis* plantation; *EU, Eucalyptus*
645 *urophylla* plantation; f, N_2O flux; T, soil temperature; M, soil moisture (water filled pore
646 space, WFPS).

Table 5. N_2O emission factor

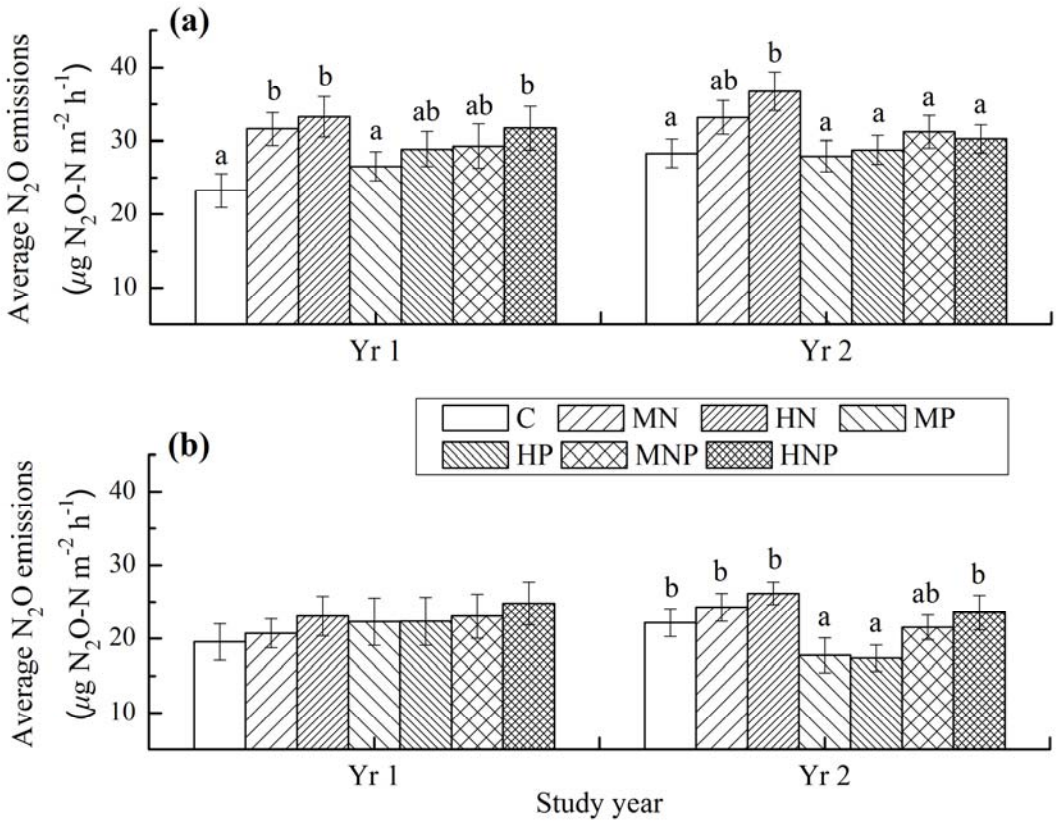
Plantation type	Treatments	N_2O emission (kg N ha^{-1} yr^{-1})	N addition (kg N ha^{-1} yr^{-1})	N_2O emission factor (%)
	C	2.3(0.1) a	0	
	MN	2.6(0.2) ab	50	0.72 (0.17) ab
AA	HN	3.1(0.1) b	100	0.81 (0.09) b
	MNP	2.6(0.0) ab	50	0.64 (0.11) ab
	HNP	2.7(0.1) ab	100	0.41 (0.04) a
	C	1.9(0.1)	0	
	MN	1.9(0.1)	50	0.11 (0.03)
EU	HN	2.0(0.2)	100	0.15 (0.04)
	MNP	2.1(0.1)	50	0.34 (0.07)
	HNP	2.1(0.0)	100	0.23 (0.04)

Notes: Gas samples were collected from August 2010 to July 2012. Values are presented as means with SE in parentheses (n = 3). Different letters in the same column indicate significantly different mean values among treatments of each stand (Tukey's HSD test, $p \leq 0.05$). N_2O emission factor of a block was calculated as (annual N_2O-N emission of N treatment plot – annual N_2O-N emission of the control plot)/(total N applied in each year). *AA*, *Acacia auriculiformis* plantation; *EU*, *Eucalyptus urophylla* plantation.



654

655 **Fig. 1.** The rates of net N-mineralization and nitrification in the 0-10 cm mineral soil of (a)
656 *Acacia auriculiformis* and (b) *Eucalyptus urophylla* plantation. The field incubation was
657 conducted in July 2012 (the second year after nutrient additions). The error bars denote ±1 SE.
658 Different letters represent statistically significant differences at $p < 0.05$.



659

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