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Effects of nitrogen and phosphorus additions on nitrous oxide emission in a nitrogen-rich and two nitrogen-limited tropical forests

M. H. Zheng[1,5,†], T. Zhang[2,†], L. Liu[3], W. X. Zhu[4], W. Zhang[1], and J. M. Mo[1,*]

[1]Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China

[2]School of Life Science and Technology, Lingnan Normal University, Zhanjiang 524048, China

[3]State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-Environmental Science, Chinese Academy of Sciences, Beijing 100085, China

[4]Department of Biological Science, State University of New York – Binghamton, Binghamton NY, 13902, USA

[5] University of Chinese Academy of Sciences, Beijing 100039, China

[†] These authors contributed equally to this work.

Correspondence to: J. M. Mo (mojm@scib.ac.cn)

Abstract

Nitrogen (N) deposition is generally considered to increase soil nitrous oxide (N_2O) emission in N-rich forests. In many tropical forests, however, elevated N deposition has caused soil N enrichment and further phosphorus (P) deficiency, and the interaction of N and P to control soil N_2O emission remains poorly understood, particularly in forests with different soil N status. In this study, we examined the effects of N and P additions on soil N_2O emission in an N-rich old-growth forest and two N-limited younger forests (a mixed and a pine forest) in southern China, to test the following hypotheses: (1) soil N_2O emission is the highest in old-growth forest due to the N-rich soil; (2) N addition increases N_2O emission more in the old-growth forest than in the two younger forests; (3) P addition decreases N_2O emission more in the old-growth forest than in the two younger forests; and (4) P addition alleviates the stimulation of N_2O emission by N addition. The following four treatments were established in each forest: Control, N addition (150 kg N ha^{-1} yr^{-1}), P addition (150 kg P ha^{-1} yr^{-1}), and NP addition (150 kg N ha^{-1} yr^{-1} plus 150 kg P ha^{-1} yr^{-1}). From February 2007 to October 2009,

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monthly quantification of soil N_2O emission was performed using static chamber and gas chromatography techniques. Mean N_2O emission was shown to be significantly higher in the old-growth forest (13.86 ± 0.71 μg N_2O-N m^{-2} h^{-1}) than in the mixed (9.86 ± 0.38 μg N_2O-N m^{-2} h^{-1}) or pine (10.83 ± 0.52 μg N_2O-N m^{-2} h^{-1}) forests, with no significant difference between the latter two. N addition significantly increased N_2O emission in the old-growth forest but not in the two younger forests. However, both P- and NP-addition had no significant effect on N_2O emission in all three forests, suggesting that P addition alleviated the stimulation of N_2O emission by N addition in the old-growth forest. Although P fertilization may alleviate the stimulated effects of atmospheric N deposition on N_2O emission in N-rich forests, we suggest future investigations to definitively assess this management strategy and the importance of P in regulating N cycles from regional to global scales.

1 Introduction

Nitrous oxide (N_2O) is a long-lived (approximately 114 years) greenhouse gas that has 298 times the ability of carbon dioxide (CO_2) to trap heat in the atmosphere (Cicerone, 1987; IPCC, 2007). It has been recognized as a major ozone-depleting substrate in the 21st century (Ravishankara et al., 2009). According to an estimation by the WMO (2012), atmospheric N_2O concentration increased from 270 ppb during pre-industrial periods, to 324.2 ppb in 2011. The average emission rate of N_2O increased by approximately 0.73−0.85 ppb yr^{-1} from 1999 to 2005 (Hirsch et al., 2006; IPCC, 2007), and is predicted to continue increasing during the following decades (Bouwman et al., 2013). Global estimations show that soils, including agricultural soils and soils under natural vegetation, are dominant sources of atmospheric N_2O (Hirsch et al., 2006; IPCC, 2007; Bouwman et al., 2013).

Tropical forest soils are important sources of N_2O, which is mainly produced by nitrification and denitrification (IPCC, 2007; Bouwman et al., 2013). At global scales, over half of the N_2O emissions occur in the tropics (D'Amelio et al., 2009), of which tropical forests account for approximately 14−23% (IPCC, 2007). Compared with temperate and boreal forests, tropical forests have shown a great increase in soil N_2O emissions (Matson

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and Vitousek, 1990). Although soil N_2O emission is suggested to be regulated by soil temperature, moisture, pH, and availability of nutrients (Werner et al., 2007; Rowlings et al., 2012), current knowledge on the factors controlling N_2O emission in tropical forests is poor. This is because tropical forests have complicated structures and functions, as well as great temporal and spatial variations of N_2O fluxes (D'Amelio et al., 2009; Zhu et al., 2013b).

During recent decades, elevated atmospheric N deposition caused by anthropogenic activities has greatly altered terrestrial N cycles, reducing N input via biological N fixation and increasing N losses via NO_3^- leaching and N_2O emission (Vitousek et al., 1997; Galloway et al., 2004). It is estimated that reactive N deposition increased from 34 Tg N yr^{-1} in 1860, to 100 Tg N yr^{-1} in 1995, and is expected to reach 200 Tg N yr^{-1} by 2050 globally (Galloway et al., 2008). Tropical forests are often rich in N, and thus N deposition into such ecosystems will exceed their capacity for N retention (Aber et al., 1989), leading to rapid N losses via N_2O emission. For example, Hall and Matson (1999) reported significant increases in soil N_2O emission after both short-term and long-term N addition in two Hawaiian forests. Zhang et al. (2008) suggested that N addition elevated soil N_2O emission more readily in N-rich than N-limited forest. In a secondary tropical forest, Wang et al. (2014) also found a significant increase in N_2O emission after 3 years of N fertilization. A meta-analysis by Liu and Greaver (2009) showed that N addition (10−562 kg N ha^{-1} yr^{-1}) significantly increased N_2O emission by approximately 216% across all ecosystems, among which tropical forests emitted the most.

In contrast to typically N-limited temperate forests, many tropical forests on highly weathered soils are rich in N but limited by phosphorus (P) (Vitousek and Matson, 1988; Vitousek et al., 2010). Hall and Matson (1999) reported that P-limited soils could emit more N_2O than N-limited soils after N addition, suggesting an important role of P in controlling soil N_2O emission. However, to date, studies on P-addition effects on soil N_2O emission have mainly relied on incubation experiments (Sundareshwar et al., 2003; Mori et al., 2010, 2013; Baral et al., 2014), or have been limited to two tropical plantations (Mori et al., 2014; Zhang et al., 2014) and a secondary

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forest (Wang et al., 2014). Generally, these studies reported a decrease in soil N_2O emission following P fertilization given the consequent increases in plant N uptake and/or microbial N immobilization, and thus reduced soil N availability for N_2O production (Sundareshwar et al., 2003; Baral et al., 2014; Mori et al., 2014; Zhang et al., 2014). Only Mori et al. (2010, 2013) found a positive response of N_2O emission to P addition, suggesting that P addition may stimulate soil N cycles and alleviate P limitation on nitrifying and denitrifying bacteria. Other than the studies above, similar work has not been carried out in other natural tropical forests. Moreover, in tropical forests with N-rich and P-limited conditions, the interaction of N and P to control soil N_2O emission remains poorly understood (Hall and Matson, 2003; Wang et al., 2014).

We hypothesize that P addition may reduce soil N_2O emission in tropical forests based on two lines of evidences. First, in several P-limited tropical forests or plantations, P addition significantly increased root N uptake capacity (Treseder and Vitousek, 2001) and aboveground plant N contents (Fernandez et al., 2000; Pampolina et al., 2002; Graciano et al., 2006). Second, our previous study found that P addition significantly increased soil microbial communities (Liu et al., 2012) and marginally increased microbial biomass N (Liu et al., 2013) in a N-rich tropical forest. Such findings indicate the potential capacity of P to increase N uptake and immobilization, thus decreasing N losses in tropical forests. Based on this evidence and considering current knowledge gaps regarding nutrient (N and P) control of N_2O emission in tropical forests, we conducted a randomized factorial design experiment to investigate the effects of N and P addition on soil N_2O emission in three tropical forests in southern China: a N-rich old-growth forest, and two N-limited younger forests (a mixed and a pine forest). We hypothesized that: (1) soil N_2O emission is the highest in old-growth forest due to the N-rich soil; (2) N addition increases N_2O emission more in the old-growth forest than in the two younger forests; (3) P addition decreases N_2O emission more in the old-growth forest than in the two younger forests; and (4) P addition alleviates the stimulation of N_2O emission by N addition.

2 Materials and Methods

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2.1 Site description

This study was conducted in the Dinghushan Biosphere Reserve (DHSBR), located in the center of Guangdong Province, southern China (112°10' E, 23°10' N). The reserve occupies an area of approximately 1200 ha and includes three forests: an old-growth forest and two younger forests (a mixed broadleaf/pine forest and a pine forest). The old-growth forest has been well protected from human disturbance for over 400 years, with major species such as *Castanopsis chinensis* Hance, *Schima superba* Chardn. & Champ., *Cryptocarya chinensis* (Hance) Hemsl., *Cryptocarya concinna* Hance, *Machilus chinensis* (Champ. Ex Benth.) Hemsl., and *Syzygium rehderianum* Merr. & Perry in the tree layer and *Calamus rhabdicladus* Burret, *Ardisia quinquegona* Bl., and *Hemigramma decurrens* (Hook.) Copel. in the understory layer (Wang et al., 1982). The two younger forests both originated from a 1930s clear-cut and subsequent pine plantation establishment (Mo et al., 2006, 2007). They experienced continuous human disturbance (the harvesting of understory and litter) from 1930 to 1956 (mixed forest) and 1998 (pine forest). Because of the colonization from natural dispersal of regional broadleaf species, the mixed forest contains both pine- and broadleaf-tree species (Mo et al., 2003, 2007). The mixed forest is dominated by *Pinus* (P) *massoniana*, *Schima superba* Chardn. & Champ., *Castanopsis chinensis* Hance, *Craibiodendron kwangtungense* S. Y. Hu, *Lindera metcalfiana* Allen, and *Cryptocarya concinna* Hance, while the pine forest is dominated by *P. massoniana*.

Earlier studies demonstrated net retention of 21−28 kg N $ha^{-1}yr^{-1}$ in the two younger forests but net loss of 8−16 kg N $ha^{-1}yr^{-1}$ from the soil in the old-growth forest (Fang et al., 2008). This indicates N saturation in the old-growth forest but N limitation in the two younger ones. Different soil N status is also supported by different litter decomposition rates, with negative N effects in the old-growth forest but positive effects in the two younger forests (Mo et al., 2006). The N-rich status of the old-growth forest is also directly supported by its higher foliar N:P ratios (20.6−36.8) compared with the two younger forests (13.8 in pine forest and 17.8−24.4 in mixed forest) (Huang et al., 2013). However, soil P is deficient in the old-growth forest, as evidenced by the

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positive responses of soil CH_4 uptake (Zhang et al., 2011), microbial biomass (Liu et al., 2012) and live fine root biomass (Zhu et al., 2013a) to P addition.

The reserve has a typical humid monsoon climate with an average annual precipitation of 1927 mm, 75% of which falls from March to August and only 6% from December to February (Huang and Fan, 1982). The mean annual temperature is 21 ºC with a January mean temperature of 12.6 ºC and July mean temperature of 28.0 ºC; annual mean relative humidity is 80% (Huang and Fan, 1982). Inorganic N deposition was 34, 24, and 26 kg N $ha^{-1}yr^{-1}$ in 2004 and 2005 for the old-growth, mixed, and pine forests, respectively, with an additional input of 15−20 kg N $ha^{-1}yr^{-1}$ as dissolved organic N (Fang et al., 2008). All forest soils are lateritic red earth formed from sandstone, and soil depth is < 30cm, 30−60cm, and >60cm in the old-growth, mixed, and pine forests, respectively (Mo et al., 2003). General soil properties are listed in Table 1.

2.2 Experimental design

The experiment was established in 2007 with five replicates of each four treatments in each forest: Control (no fertilization), N addition (150 kg N ha^{-1} yr^{-1}), P addition (150 kg P ha^{-1} yr^{-1}), and NP addition (150 kg N ha^{-1} yr^{-1} plus 150 kg P ha^{-1} yr^{-1}),with a total of 20 plots (5 m × 5 m). Each plot was surrounded by a 5m wide buffer strip. Plot size and fertilizer level were referenced to the experiment in Costa Rica by Cleveland and Townsend (2006). All plots and treatments were assigned randomly. NH_4NO_3 and NaH_2PO_4 solutions were used as fertilizers and sprayed below the canopy using a backpack sprayer, bimonthly from February 2007 to October 2009. Fertilizer was weighed and mixed with 5L of water for each plot. Each control plot received 5L of water without fertilizer.

2.3 N_2O flux measurement

N_2O fluxes were measured from January 2007 before the first fertilizer application. Two static chambers were installed in each plot in November 2006, two months prior to the gas sampling. The chamber design and

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measurement method were adopted from Zhang et al. (2011). Gas fluxes were monitored monthly using a static chamber and a gas chromatograph (Agilent 4890D). Gas samples were collected from each chamber from 9:00−10:00 at local time, during which the greenhouse gas fluxes are closer to the daily means (Tang et al., 2006). Gas samples were taken with a 60-ml plastic syringe at 0, 15, and 30 min intervals after the chamber closure, and analyzed within 12 h in the gas chromatograph (Agilent 4890D) fitted with an electron capture detector (ECD) for N_2O. Calibration gases (N_2O at 321 ppbv, bottle's No. 070811) were obtained from the Institute of Atmospheric Physics, Chinese Academy of Sciences.

The calculation of N_2O fluxes followed the method of Holland et al. (1999), based on linear regression of chamber gas concentration across time. Atmospheric pressure was measured at the sampling sites using an air pressure gauge (Model THOMMEN 2000, Switzerland). Meanwhile, air temperature (enclosure), soil temperature (at 5 cm depth), and moisture (0−10 cm depth), were measured during each sampling. Soil moisture content was detected using a TDR-probe (Model Top TZS-I, China), and converted to water filled pore space (WFPS) according to the following formula:

WFPS = Vol / (1-SBD / 2.65)

SBD: soil bulk density (g cm^{-3}); Vol: volumetric water moisture (%); 2.65 is the density of soil particles (g cm^{-3}).

2.4 Soil sample analyses

Soil sampling was conducted in February 2007 (before the first fertilizer application) and August 2009 (during the study period). Five soil cores (2.5 cm inner diameter) were collected randomly from 0−10 cm soil depths and mixed by plot. Soil pH was measured in a soil/water (1:2.5) suspension. Soil organic carbon (C) was measured using dichromate oxidation and titration with ferrous ammonium sulfate (Liu, 1996). Soil microbial biomass C was measured using the chloroform fumigation-extraction method (Vance et al., 1987). Soil dissolved organic C was extracted with 0.5 M K_2SO_4 and analyzed using a total carbon analyzer (Shimadzu model TOC-500, Kyoto, Japan). Total N concentration was measured using semimicro-Kjeldahl digestion followed by detection of

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ammonium on a Wescan ammonia analyzer, and total P concentration was measured spectrophotometrically after acidified ammonium persulfate digestion (Anderson and Ingram, 1989). Soil available P was measured spectrophotometrically after extraction with acid-ammonium fluoride solution (Liu, 1996). Soil NH_4^+-N was measured spectrophotometrically by the indophenol blue method (Liu, 1996).

Soil nitrification rate was measured according to the *in situ* incubation method described by Raison et al. (1987). Briefly, 10 soil cores (2.5 cm inner diameter) were collected from each plot, 5 of which were brought to the laboratory for measurement of soil NO_3^-N using cadmium reduction followed by sulfanilamide-NAD reaction, and the remainders were returned to the plots for 1 month incubation. Nitrification rate was calculated from the difference between extractable NO_3^-N contents before and after incubation.

2.5 Statistical analyses

Repeated measures analysis of variance was used to examine the effect of fertilizer treatments on soil N_2O emission from February 2007 to October 2009. Two-way ANOVA was used to determine the treatment effects on soil N_2O emission. One-way ANOVA was used to determine the differences in soil properties among treatments. Linear regression analyses were used to determine the relationships between N_2O emission and soil WFPS / soil temperature in each forest. All analyses were conducted using the SPSS 16.0 for windows (SPSS Inc., Chicago, IL, USA). Statistically significant differences were recognized at $P < 0.05$, unless otherwise stated.

3 Results

3.1 Soil temperature

Soil temperature (at 5 cm depth) showed a similar pattern in all plots across the three forests, increasing from spring to summer and decreasing from fall to winter (Fig. 1). The mean soil temperature of the control plots during the study period was 21.79 ± 0.36, 22.60 ± 0.37, and 23.41 ± 0.39 °C in the old-growth, mixed, and pine

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forests, respectively. Repeated measures ANOVA highlighted significant differences ($P < 0.001$) in soil temperatures between each forest. In the mixed forest, soil temperature was significantly lower in P-addition plots ($P = 0.043$) compared to the control plots, while N- and NP-addition had no effect on soil temperature. No treatment effect was detected on soil temperature in the old-growth and pine forests, as determined by repeated measures ANOVA.

3.2 Soil WFPS

Soil WFPS (0−10 cm depth) increased in all forests from dry winter to wet spring, but decreased in summer (Fig. 2). Mean soil WFPS in control plots during the study period was 31.13 ± 1.06, 29.53 ± 1.15, and 28.31 ± 1.24 % in the old-growth, mixed, and pine forests, respectively. Repeated measures ANOVA showed no significant difference of soil WFPS in the control plots among three forests. N-, P-, and NP-addition had no significant effect on soil WFPS in any forest, as determined by repeated measures ANOVA.

3.3 Soil properties

Soil pH did not change after addition of fertilizers in the old-growth and pine forests, but significantly decreased after NP-addition in the mixed forest (Table 2). Soil NH_4^+ concentrations were significantly increased after P- and NP-addition in the old-growth forest, while NP-addition significantly decreased soil NO_3^- and NH_4^+ concentrations in the old-growth and pine forests, respectively. N-addition significantly decreased soil total inorganic N ($NH_4^+ + NO_3^-$) concentrations in the pine forest. No treatment effect occurred on soil organic C in the old-growth and pine forests, while both P- and NP-addition significantly increased soil organic C in the mixed forest. Soil microbial biomass C was significantly increased by NP-addition in the old-growth forest and by N-, P- and NP-addition in the mixed forest. Although not always statistically significant, both P- and NP-addition increased soil available P concentrations in all the forests compared to the control plots.

3.4 Soil N_2O emission in control plots

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Soil N_2O emission was higher in all forests during spring and summer, and lower in fall and winter (Fig. 3). Mean soil N_2O emission was 13.98 ± 0.73, 9.92 ± 0.39, and 10.92 ± 0.53 μg N_2O-N m^{-2} h^{-1} in the old-growth, mixed, and pine forests, respectively (Fig. 4), with the significantly higher (P = 0.001) in the old-growth forest than in the mixed and pine forests. In the control plots, soil N_2O emission showed a significant positive linear relationship with soil temperature and WFPS across all forests (Fig. 5).

3.5 Soil N_2O emission after N and P addition

Effects of N- and P-addition on soil N_2O emission varied with forest type (Fig. 4). In the old-growth forest, mean N_2O emission during the study period was 24.66% higher in the N-addition plots (17.44 ± 1.09 μg N_2O-N m^{-2} h^{-1}), not significantly different in the P-addition plots (13.99 ± 0.81 μg N_2O-N m^{-2} h^{-1}), and 13.87% higher in the NP-addition plots (15.93 ± 0.86 μg N_2O-N m^{-2} h^{-1}), compared to the control plots (13.99 ± 0.73 μg N_2O-N m^{-2} h^{-1}). However, significant differences were confined to the N-addition treatment (P = 0.036). In the mixed forest, mean N_2O emission slightly increased by 0.71, 7.96 and 3.93% after N-, P-, and NP-addition, respectively. In the pine forest, N- and NP-addition slightly increased mean N_2O emission by 1.10 and 14.65%, respectively, while P-addition marginally decreased mean N_2O emission by 2.47%. In the mixed and pine forest, no significant differences among treatments were identified by repeated measures ANOVA.

Two-way ANOVA highlighted the significant positive effects of N-addition on N_2O emission in spring 2007, fall 2007, winter 2008 and fall 2008, and the marginal negative effects of P-addition in fall 2008 and summer 2009, in the old-growth forest (Table 3). In contrast, only a significant positive effect of N-addition occurred in winter 2008 in the mixed forest, and in spring 2007, fall 2008 in the pine forest. Interactive effects (P < 0.1) of combined N and P additions occurred in the old-growth (winter 2008), mixed (fall 2007, winter 2008 and winter 2009), and pine (summer 2007, winter 2009) forests.

3.6 Soil nitrification rate

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In the old-growth forest, N-addition significantly increased soil nitrification rate (P = 0.005), while P- and NP-addition had no significant effect (Fig. 6). In the mixed and pine forest, soil nitrification rate was not affected by N- or/and P-addition.

4 Discussion

4.1 N_2O emission in control plots

Soil N_2O emissions measured in the present study (9.9−13.9 μg N_2O-N m^{-2} h^{-1}) were comparable to previous reports from tropical forests (10.0−11.5 μg N_2O-N m^{-2} h^{-1}) (Kiese et al., 2008; Neto et al., 2011). However, our results were lower than those from adjacent forests (24.1−69.0 μg N_2O-N m^{-2} h^{-1}) (Tang et al., 2006; Zhang et al., 2008) and other tropical forests (16.3−77.1 μg N_2O-N m^{-2} h^{-1}) (Kiese et al., 2008; Davidson et al., 2008; Konda et al., 2010), and higher than those from many tropical/subtropical forests (1.0−8.7 μg N_2O-N m^{-2} h^{-1}) (Hall et al., 2004; Werner et al., 2006; Wang et al., 2010; Wieder et al., 2011). Taken together, these data suggest a high variation in N_2O emission among different study regions.

As expected, a generally higher seasonal N_2O emission and a significantly higher mean N_2O emission were identified in the old-growth forest than in the two younger forests (Fig. 3 and 4), suggesting that N_2O emission may vary depending on forest type. N_2O emission has been suggested to increases with succession (Verchot et al., 1999; Erickson et al., 2001), possibly due to the increase in soil N content (Erickson et al., 2002). For example, soil N enrichment due to the presence of N-fixing legume trees has been linked with higher N_2O emission (Erickson et al., 2002; Arai et al., 2008; Konda et al., 2010; Zhang et al., 2014). In addition, higher N_2O emission in N-rich soils has been reported by a study in adjacent forests with different soil N status (Zhang et al., 2008). These findings are consistent with our results in that the old-growth forest had higher inorganic N (NH_4^+ and NO_3^-) and total N content than the mixed and pine forests (Table 1). Given almost complete saturation of N in the old-growth forest (Fang et al., 2008), excess N in soils would be readily lost as dissolved organic and inorganic N (Fang et al., 2008, 2009), and N_2O gas (Zhang et al., 2008). Thus, our results further

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confirm that N-rich forests have a higher N_2O emission than N-limited forests.

In addition to soil N status, the availability of other nutrients may account for higher N_2O emission in the old-growth forest. Compared to the two younger forests, the old-growth forest had significantly higher soil dissolved organic C, total organic C, and microbial biomass C (Table 1), likely supporting a higher activity of nitrifying and denitrifying bacteria responsible for N_2O production (Zhang et al., 2008). N-rich and P-limiting conditions have previously been suggested to support higher N_2O emission (Zhang et al., 2008). In the present study, soil N:P ratios were significantly higher in the old-growth forest than in the mixed and pine forest (Table 1), suggesting that low availability of soil P may intensify N_2O emission under N-rich conditions (Zhang et al., 2014), thus indicating the potential interaction of N and P to control N_2O emission.

4.2 Effects of soil temperature and WFPS on N_2O emission

Soil temperature in all plots in the three forests showed a similar seasonal pattern, increasing from spring to summer and decreasing from fall to winter (Fig. 1). N_2O emission was positively correlated to soil temperature in all three forests (Fig. 5), which was consistent with previous studies in tropical forests (Butterbach-Bahl et al., 2004; Zhang et al., 2008; Zhu et al., 2013b; Zhang et al., 2014). However, mean soil temperature was highest in the pine forest, followed by the mixed and old-growth forests, which was inconsistent with the patterns of mean N_2O emission identified across forests (Fig. 4). This suggests a limited ability of soil temperature to explain the pattern in N_2O emission across forests with different soil N status.

In contrast to soil temperature, mean soil WFPS showed comparable dynamics to mean N_2O emission, with the highest in the old-growth forest and lowest in the pine forest (Fig. 2). In each forest, soil WFPS showed a positive relationship with N_2O emission (Fig. 5), as has previously been observed across forests with different soil N status (Zhang et al., 2008, 2014). Moreover, seasonal patterns in soil WFPS (Fig. 2) and N_2O emission were comparable in all forests (Fig. 3), suggesting that soil WFPS can predict the seasonal variance of N_2O

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emission, as follows. In spring, forest soil was enriched with inorganic N (accumulated during non-growing seasons) and had higher WFPS (increased in wet seasons); conditions that would increase microbial consumption of soil NH_4^+ and/or NO_3^- (Davidson et al., 2000), and thus greatly increase N_2O production (Davidson et al., 2000; Butterbach-Bahl et al., 2004; Werner et al., 2006). In summer, N_2O emission began to decrease given decreasing soil WPFS due to plant uptake and natural evaporation, (Fig. 3). In fall and winter, both the lower soil inorganic N (decreased after growing seasons) and WPFS (decreased in dry seasons) suppressed N_2O production. Accordingly, N_2O emission was highest in spring, declined in summer, and was lowest in fall and winter (Fig. 3). Thus, our findings suggest that soil WFPS may be a more appropriate predictor of N_2O emission in forests with different soil N status than soil temperature.

4.3 Effects of N addition on N_2O emission

As expected, N addition significantly increased mean N_2O emission in the old-growth forest, but not in the mixed and pine forests (Fig. 4), which was consistent with the results from adjacent forests (Zhang et al., 2008). In several N-rich forests, N_2O emission significantly increased after N addition (Hall and Matson, 1999; Venterea et al., 2003; Koehler et al., 2009; Zhang et al., 2014), whereas it was hardly impacted by N input in the N-limited forests (Davidson et al., 2000; Skiba et al., 2004), or only increased after chronic N addition (Magill et al., 2000; Hall and Matson, 2003). This indicates an important control of N_2O emission by soil N status (Zhang et al., 2008), as explained below.

As supported by our results, additional N inputs to N-rich forests exceed the ecosystems capacity for N retention, and thus less N is utilized (Aber et al., 1998). In the old-growth forest, we found no increase in soil organic C, microbial biomass C (Table 2), or litter decomposition rate (Mo et al., 2006) after N addition, whereas live fine root biomass was shown to decrease (Zhu et al., 2013a), suggesting that N addition no longer increases soil and plant C pools in this forest. Moreover, N fertilizer application rate was much larger than atmospheric N deposition rate, leading to excess soil N accumulating in the old-growth forest which would

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favor nitrifying and denitrifying bacteria (Zhang et al., 2008), and therefore significantly stimulated soil nitrification rate (Fig. 6), N_2O emission (Fig. 4) and NO_3^- leaching (Fang et al., 2009). As a result, no significant increase in soil inorganic N (NH_4^+ and NO_3^-) was observed after N addition in the old-growth forest (Table 2). Thus, in combination with previous findings, our results confirm that N addition will increase N_2O emission in N-rich forests.

In contrast, in N-limited forests, N is retained to support plant and microbial growth, and/or accumulation of soil organic matter (Aber et al., 1998; Harrington et al., 2001). In the N-limited mixed and pine forests, two N-limited ecosystems (Mo et al., 2006), despite no significant increase in soil inorganic N following N addition, a significant increase in soil microbial biomass C and a marginal increase in soil organic C was observed in the mixed forest (Table 2), as well as a significant increase in soil organic C after long-term N addition in the pine forest (Zheng et al., 2015). Both forests showed positive responses of litter decomposition rate to N addition (Mo et al., 2006), but no net N losses via NO_3^- leaching (Fang et al., 2008). In addition, nitrification rate showed no response to N addition in either forest (Fig. 6), and thus N_2O emission did not change (Fig. 4). Although rates of N addition in the present study were much higher than atmospheric N deposition, all above evidences suggest that N continue to be utilized and was not lost following N addition in our N-limited forests. This confirms our hypothesis that soil N_2O emission shows no response to N addition in N-limited forests (Zhang et al., 2008).

4.4 Effects of P addition on N_2O emission

No significant change in mean N_2O emission was observed following P addition in any of the study forests (Fig. 4), allowing us to reject the hypothesis that P addition causes great decrease in N_2O emission in the old-growth forest than in two younger forests. This finding was inconsistent with many previous studies conducted *in situ* (Mori et al., 2014; Zhang et al., 2014) or in laboratories (Sundareshwar et al., 2003; Mori et al., 2010, 2013; Baral et al., 2014). For example, Mori et al. (2014) and Zhang et al. (2014) reported that P addition significantly

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decreased N_2O emission in a leguminous and non-leguminous plantation, respectively. Under laboratory conditions, Sundareshwar et al. (2003) found a negative response of sediment N_2O emission to nitrate addition. Based on a pot experiment with maize, Baral et al. (2014) also suggested that alleviation of P limitation would decrease N_2O emission. The major mechanism of this P-driven decrease in N_2O emission is the increased plant uptake of soil N due to higher P availability, which therefore reduces N availability for nitrifying and denitrifying bacteria (Mori et al., 2010). However, several incubation experiments found a positive response of N_2O emission to P addition (Mori et al., 2010, 2013), with authors suggesting that P addition might stimulate soil N cycles for nitrification and denitrification and/or might alleviate soil P limitation of nitrifying and denitrifying bacteria. In contrast, a lack of response of N_2O emission to P addition has rarely been reported, especially for natural forests (Wang et al., 2014), and the mechanism remains poorly understood.

Based on the present study, we propose that a lack of response of N_2O emission to P addition may be attributed to failure of soil N immobilization, or N uptake stimulated by short-term P addition. P fertilization has been suggested to decrease soil N substrates (or increase soil N immobilization), and thus suppress N_2O production (Sundareshwar et al., 2003; Mori et al., 2010, 2014; Zhang et al., 2014). However, we found no significant change in soil total inorganic N (NH_4^+ plus NO_3^-) after approximately 2 years of P addition in all forests, despite a significant increase in NH_4^+ in the old-growth forest (Table 2). Moreover, soil nitrification rate remained stable after P addition in all forests (Fig. 6), suggesting that P addition did not affect N_2O production in the present study. Yet, in a recent study, significant decreases in soil inorganic N and N_2O emission occurred after 6 years of P addition in an old-growth forest (Chen et al., 2015), indicating that N_2O emission may remain stable following short-term P addition, but decrease after long-term addition in N-rich forests. We further suggest studies to identify whether long-term P addition will also decrease N_2O emission in N-limited forests.

4.5 Effects of combined N and P additions on N_2O emission

Consistent with our hypothesis, mean N_2O emission showed no response to combined N and P additions in all

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forests (Fig. 4), suggesting that P alleviated the stimulating effect of N addition on N_2O emission in the old-growth forest; as has been reported by several previous studies. For example, Hall and Matson (2003) reported that N addition significantly increased soil N_2O emission but N and P addition had no effect in a P-limited forest. Using a pot experiment, Baral et al. (2014) found that N_2O emission was highest under N fertilization treatment, but reduced after P fertilization in a P-limited soil/sand mixture. Zhang et al. (2014) also reported that N_2O emission significantly increased with N addition but not with NP addition in a leguminous plantation. However, our results were inconsistent with those of Mori et al. (2013) and Wang et al. (2014), who suggested that both N- and NP-addition significantly increased N_2O emission.

Currently, two mechanisms of the P alleviation of N_2O emission are plausible. First, P addition may alleviate P limitation of plants, and thus increase plant uptake of N (Hall and Matson, 1999; Baral et al., 2014; Sundareshwar et al., 2003). Second, P addition may alleviate P limitation of soil microbes and therefore increase microbial N immobilization (Sundareshwar et al., 2003). Both pathways will reduce soil N substrates available for N_2O production. Although plant and microbial N contents were not measured in this study, our recent studies in the old-growth forest found no effect of 5 years of P- and NP-addition on fine root N contents (Zhu et al., 2013a), while 4 years of P- and NP-addition tended to increase soil microbial biomass N (Liu et al., 2013). This suggests that P alleviation of the N stimulation on N_2O emission in our old-growth forest was likely attributed to an increase in microbial N immobilization rather than plant N uptake. Accordingly, NP addition did not significantly affect soil total inorganic N (NH_4^+ plus NO_3^-) (Table 2), and thus soil nitrification rate (Fig. 6), which in turn did not affect N_2O emission. Therefore, our findings suggest that P addition will alleviate the stimulating effects of N on N_2O emission in the N-rich forest, potentially resulting from an increase in microbial N immobilization.

5 Conclusions

To our knowledge, this is the first study to examine how N and P interact to control soil N_2O emission in

tropical forests with different soil N status. Our results confirm that N-rich forests have higher N_2O emission than N-limited forests, and N addition will merely increase N_2O emission in N-rich forests, as less N is utilized in N-rich soils. However, neither P- nor NP-addition affects N_2O emission in both N-rich and N-limited forests, which suggests that P addition potentially alleviates N stimulation of N_2O emission in N-rich forests; the underlying mechanism potentially being microbial N immobilization. Therefore, P fertilization can be used to reduce soil N_2O emission in N-rich forests under atmospheric N deposition, but we suggest more investigations to definitively assess this management strategy and the importance of P in regulating N cycles from regional to global scales.

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Table 1. General characteristics of the 0−10cm mineral soils in the three study forests.

Forest type	Old-growth forest	Mixed forest	Pine forest
pH value (H_2O)	3.90(0.02) b	4.02(0.03) a	3.99(0.04) ab
NH_4^+ (mg kg^{-1})	2.41(0.32) a	1.36(0.08) b	2.43(0.25) a
NO_3^- (mg kg^{-1})	4.26(0.25) a	1.33(0.18) c	3.27(0.46) b
Dissolve organic C (mg kg^{-1})	709.22(33.65) a	552.32(13.91) b	573.16(25.15) b
Soil organic C (%)	4.05(0.15) a	2.77(0.22) b	2.91(0.29) b
Microbial biomass C (mg kg^{-1})	551.89(38.50) a	75.92(7.04) c	165.64(10.27) b
Available P (mg kg^{-1})	2.14(0.36) a	0.93(0.10) b	1.10(0.18) b
Total N (g kg^{-1})	1.58(0.11) a	1.14(0.15) b	1.10(0.14) b
Total P (mg g^{-1})	0.49(0.03)	0.51(0.02)	0.49(0.03)
N:P ratios	3.24(0.19) a	2.23(0.22) b	2.25(0.28) b

Notes: Soil samples were collected in February 2007. Values are means with standard error in parentheses (n = 5). Different lowercase letters indicate significant differences among forests, as determined by one-way ANOVA ($P < 0.05$).

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Table 2. Effects of N and P addition on soil properties in the three study forests.

Treatment	pH	NH_4^+ (mg kg^{-1})	NO_3^- (mg kg^{-1})	$NH_4^+ + NO_3^-$ (mg kg^{-1})	Soil organic C (%)	Microbial biomass C (mg kg^{-1})	Available P (mg kg^{-1})
				Old-growth forest			
C	3.83(0.02)	5.52(0.20) b	5.48(0.54) a	11.00(0.37)	4.02(0.48)	732.90(80.93) b	1.84(0.10) b
N	3.78(0.04)	6.42(0.36) ab	5.24(0.58) a	11.67(0.68)	5.08(0.58)	682.07(25.47) b	3.44(0.53) b
P	3.85(0.04)	7.04(0.61) a	3.75(0.69) ab	10.79(0.29)	5.27(0.14)	756.29(55.70) ab	14.11(4.03) a
NP	3.88(0.03)	7.01(0.62) a	3.24(0.35) b	10.25(0.59)	4.70(0.50)	975.28(109.52) a	6.94(1.86) ab
				Mixed forest			
C	4.05(0.03) a	6.28(0.35)	2.53(0.14)	8.81(0.27)	1.71(0.16) b	305.00(23.45) b	1.38(0.15) b
N	4.05(0.02) a	7.12(0.69)	2.45(0.30)	9.58(0.99)	2.45(0.30) ab	405.92(41.89) a	4.79(1.30) ab
P	4.06(0.04) a	6.38(0.42)	1.90(0.14)	8.28(0.48)	3.02(0.31) a	404.73(32.54) a	5.50(1.27) a
NP	3.96(0.03) b	7.00(0.62)	2.80(0.54)	9.80(0.83)	3.20(0.41) a	432.06(29.18) a	2.78(0.51) ab
				Pine forest			
C	3.98(0.05)	6.99(0.65) a	3.39(0.17)	10.38(0.71) a	3.24(0.39)	523.61(41.81)	0.51(0.15) b
N	3.98(0.04)	5.74(0.31) ab	3.27(0.23)	9.01(0.26) b	2.90(0.10)	507.60(44.06)	0.29(0.07) b
P	3.95(0.04)	6.23(0.34) ab	3.41(0.29)	9.64(0.41) ab	3.37(0.25)	468.84(43.11)	2.58(0.70) a
NP	3.93(0.04)	5.61(0.34) b	3.46(0.54)	9.07(0.27) ab	2.96(0.23)	488.85(22.60)	2.68(0.97) a

Notes: Soil samples were collected in August 2009. Values are means with standard error in parentheses (n = 5). Different lowercase letters represent significant difference among treatments in each forest, as determined by one-way ANOVA ($P<0.05$).

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Table 3. *P* value of two-way repeated measures ANOVA of seasonal N_2O fluxes in the three study forests.

Seasons	Spring 2007	Summer 2007	Fall 2007	Winter 2008	Spring 2008	Summer 2008	Fall 2008	Winter 2009	Spring 2009	Summer 2009
					Old-growth forest					
N	**0.001**	0.470	**0.048**	**0.021**	0.631	0.761	**0.029**	0.253	0.567	0.775
P	0.328	0.519	0.552	0.265	0.383	0.931	**0.090**	0.356	0.524	**0.052**
N×P	0.531	0.748	0.556	**0.034**	0.751	0.519	0.782	0.565	0.202	0.172
					Mixed forest					
N	0.881	0.667	0.253	**0.017**	0.304	0.866	0.609	0.446	0.989	0.349
P	0.601	0.948	0.462	0.128	0.522	0.649	0.570	0.958	0.277	0.102
N×P	0.721	0.487	**0.084**	**0.043**	0.814	0.440	0.470	**0.089**	0.509	0.711
					Pine forest					
N	**0.027**	0.101	0.934	0.255	0.612	0.793	**0.045**	0.907	0.762	0.651
P	0.559	0.117	0.152	0.600	0.743	0.875	0.898	0.234	0.912	0.410
N×P	0.491	**0.024**	0.163	0.431	0.685	0.194	0.400	**0.097**	0.834	0.434

Notes: Spring from April to June, summer from July to September, fall from October to December and winter from January to March. *P* values that are less than 0.1 are marked by bold type.

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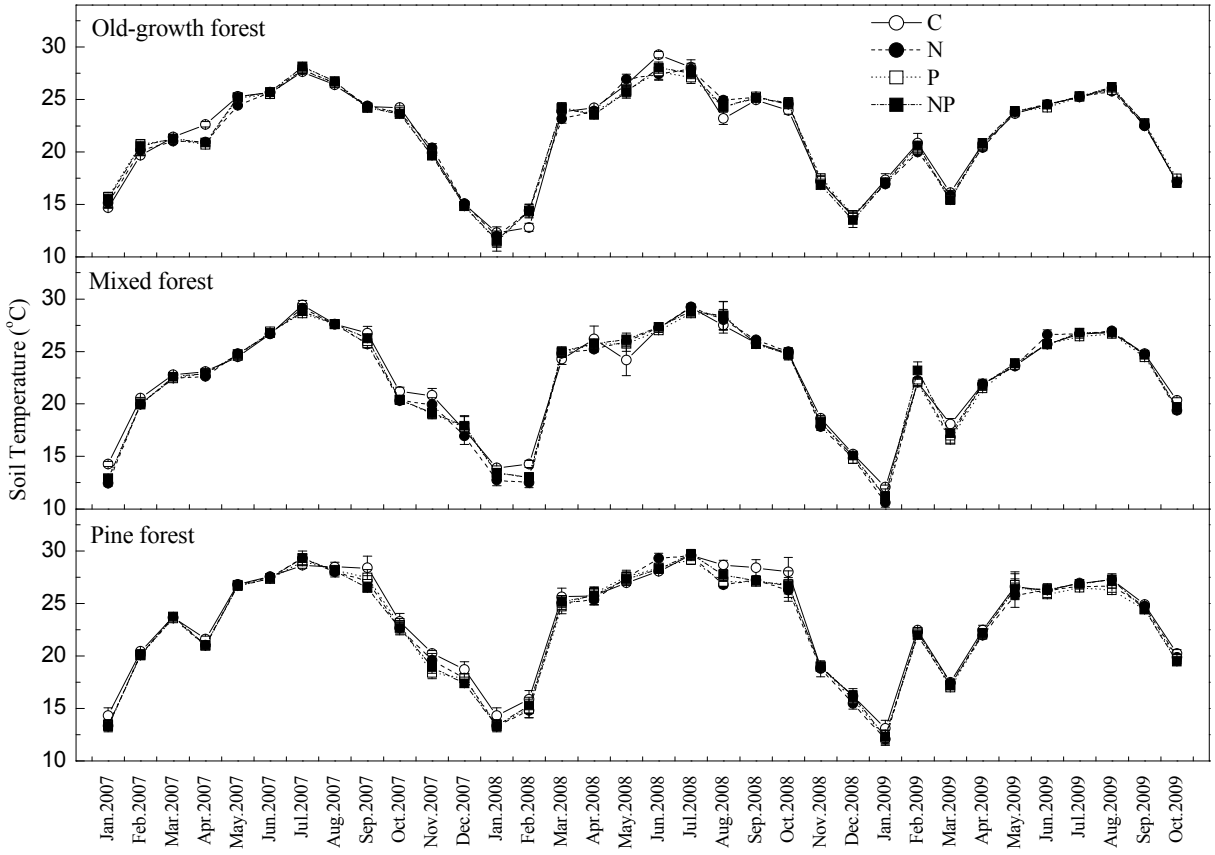

Fig. 1 Monthly soil temperature in the three study forests of Dinghushan Biosphere Reserve (DHSBR) from January 2007 to October 2009.

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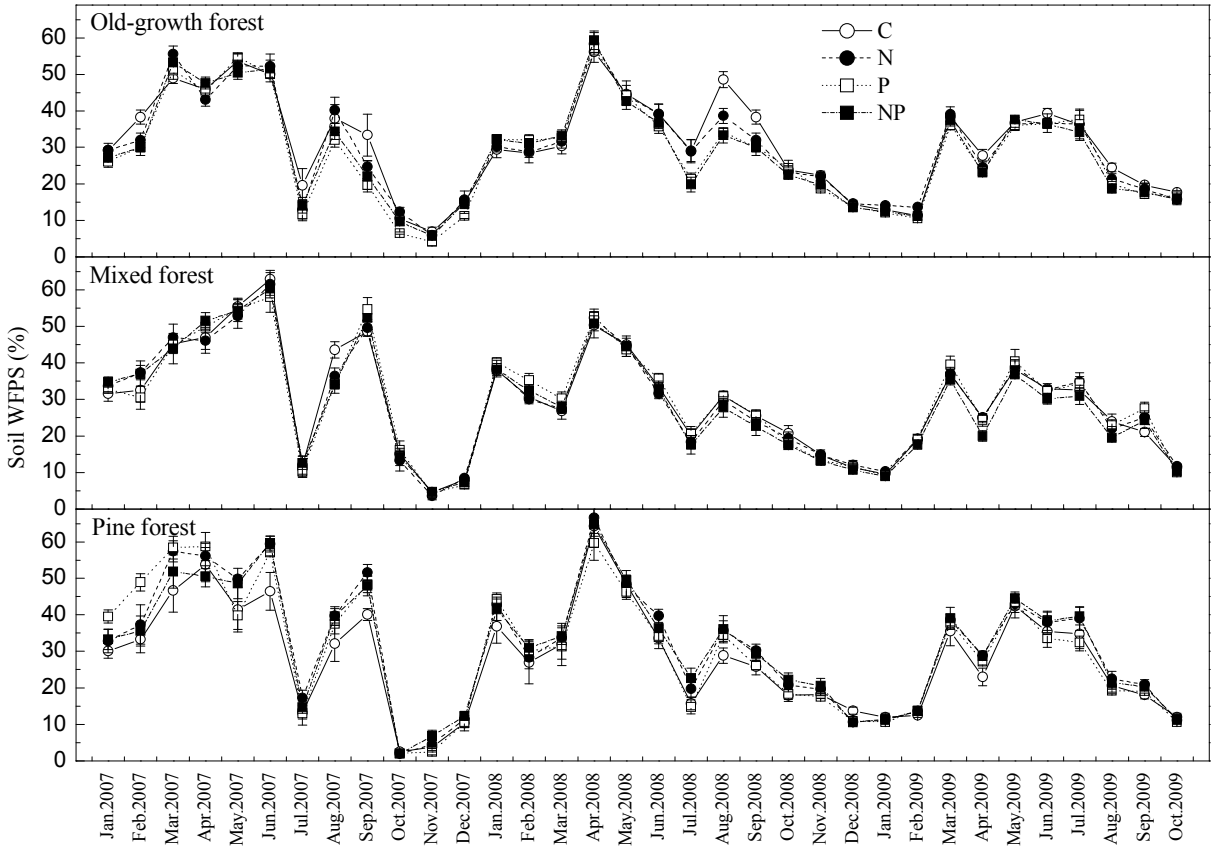

Fig. 2 Monthly soil WFPS in the three study forests of Dinghushan Biosphere Reserve (DHSBR) from January 2007 to October 2009.

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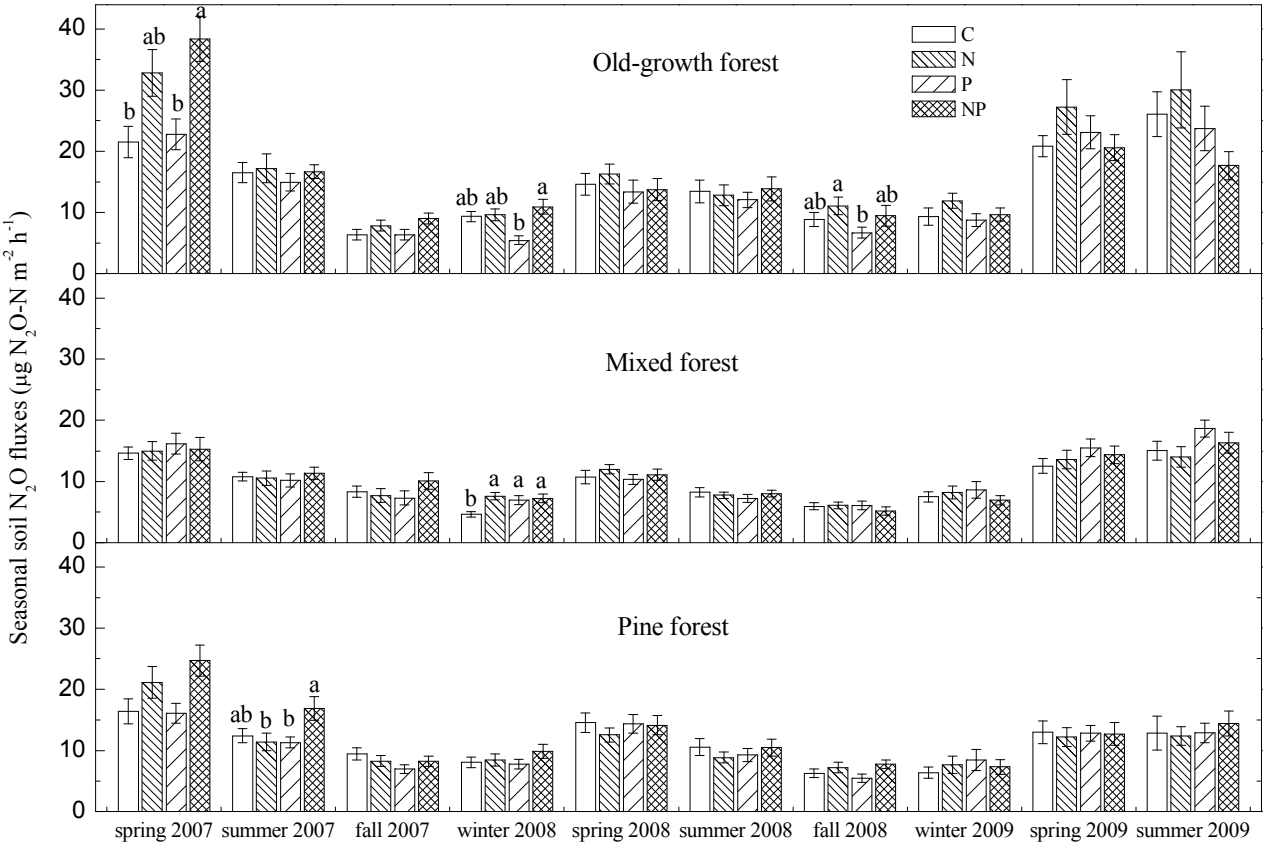

Fig. 3 Seasonal variation of N_2O fluxes in the three study forests during the sampling periods. Each error bar represents standard error of mean N_2O fluxes from 5 plots (n = 5), and the data of N_2O fluxes in each plot has been averaged by season (3 months) before analyses. Different lowercase letters within each season represent significant differences among treatments, as determined by repeated measures ANOVA ($P < 0.05$).

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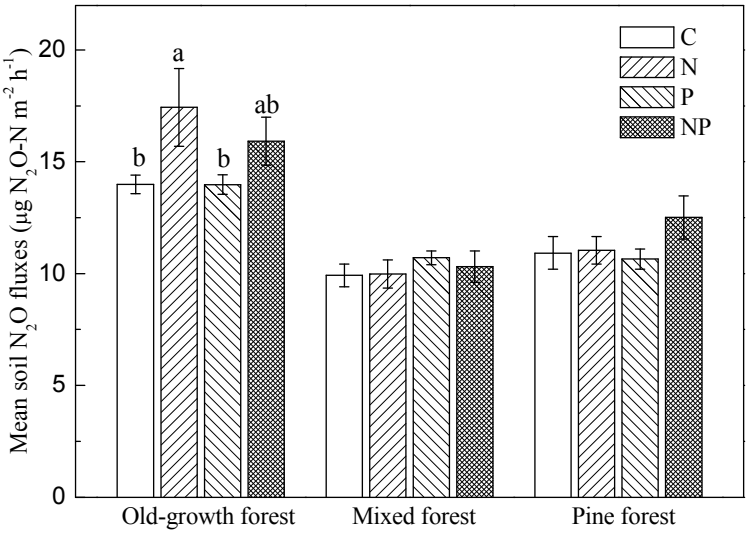

Fig. 4 Effects of N- and P-addition on mean soil N_2O fluxes from February 2007 to October 2009. Each error bar represents standard error of mean N_2O fluxes from 5 plots (n = 5), and the data of N_2O fluxes in each plot has been averaged from the whole sampling period (33 months) before analyses. Different lowercase letters within each forest represent significant differences among treatments, as determined by repeated measures ANOVA ($P < 0.05$).

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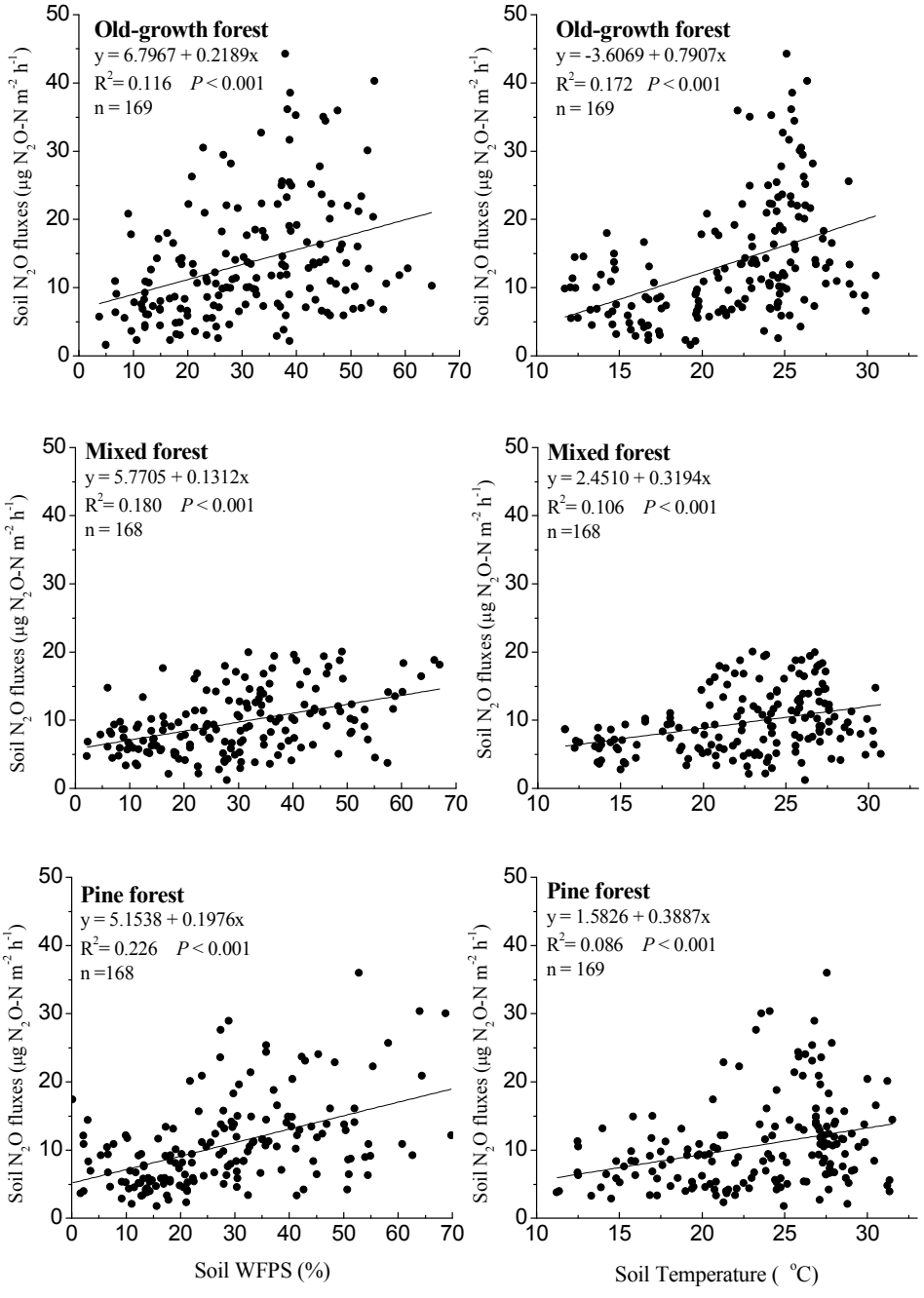

Fig. 5 Relationships between N_2O fluxes and soil WFPS (and temperature) in the three study forests, as determined by linear regression analyses.

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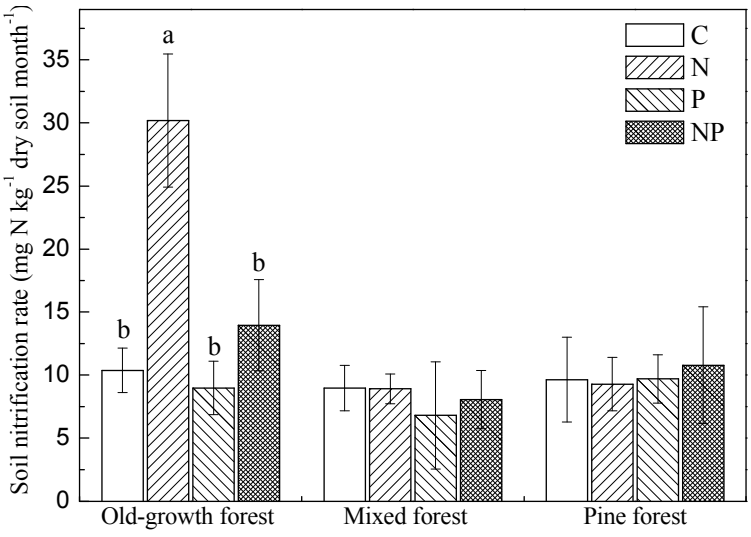

Fig. 6 Effects of N- and P-addition on soil nitrification rate in the three study forests in August 2008. Error bars represent standard errors (n = 5). Different lowercase letters within each forest represent significant differences among treatments, as determined by one-way ANOVA ($P < 0.05$).