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Increased phosphorus availability mitigates the inhibition of nitrogen deposition on CH_4 uptake in an old-growth tropical forest, southern China

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Abstract. It is well established that tropical forest ecosystems are often limited by phosphorus (P) availability, and elevated atmospheric nitrogen (N) deposition may further enhance such P limitation. However, it is uncertain whether P availability would affect soil fluxes of greenhouse gases, such as methane (CH_4) uptake, and how P interacts with N deposition. We examine the effects of N and P additions on soil CH_4 uptake in an N saturated old-growth tropical forest in southern China to test the following hypotheses: (1) P addition would increase CH_4 uptake; (2) N addition would decrease CH_4 uptake; and (3) P addition would mitigate the inhibitive effect of N addition on soil CH_4 uptake. Four treatments were conducted at the following levels from February 2007 to October 2009: 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 $150\,kg\,N\,ha^{-1}\,yr^{-1}$ plus $150\,kg\,P\,ha^{-1}\,yr^{-1}$). Static chamber and gas chromatography techniques were used to quantify soil CH_4 uptake every month throughout the study period. Average CH_4 uptake rate was $31.2 \pm 1.1\,\mu g\,CH_4\text{-}C\,m^{-2}\,h^{-1}$ in the control plots. The mean CH_4 uptake rate in the N-addition plots was $23.6 \pm 0.9\,\mu g\,CH_4\text{-}C\,m^{-2}\,h^{-1}$, significantly lower than that in the controls. P-addition however, significantly increased CH_4 uptake by 24 % ($38.8 \pm 1.3\,\mu g\,CH_4\text{-}C\,m^{-2}\,h^{-1}$), whereas NP-addition ($33.6 \pm 1.0\,\mu g\,CH_4\text{-}C\,m^{-2}\,h^{-1}$) was not statistically different from the control. Our results suggest that increased P availability may enhance soil mathanotrophic activity and root growth, resulting in potentially mitigating the inhibitive effect of N deposition on CH_4 uptake in tropical forests.

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1 Introduction

Methane (CH_4) is considered the second most important greenhouse gas after carbon dioxide and with a global warming potential 25 times compare to carbon dioxide (CO_2) over a 100 year horizon (IPCC, 2007). The global atmospheric concentration of CH_4 has increased from a pre-industrial value of about 0.715 ppm to 1.732 ppm in the early 1990s, and 1.803 ppm in 2009 (IPCC, 2007; WMO, 2010), due primarily to the anthropogenic emissions from energy production, rice cultivation, ruminant animals, biomass burning and landfills (IPCC, 2007).

Major CH_4 sinks include tropospheric oxidation ($\sim 500\,Tg\,yr^{-1}$) and stratospheric loss ($\sim 40\,Tg\,yr^{-1}$) (Hein et al., 1997; Lelieveld et al., 1998). Upland oxic soil that are continuously emerged and exposed to atmospheric concentrations of CH_4 is another major sink of atmospheric CH_4, which consumes CH_4 through the activity of methanotrophs under aerobic conditions ($\sim 30\,Tg\,CH_4\,yr^{-1}$) (Lelieveld et al., 1998). Among upland soils, forest soils are probably the most efficient CH_4 sink (Le Mer and Roger, 2001).

Atmospheric methane originates mainly from a biological process. In forest soils, it is produced in anoxic layer by methanogenic bacteria during the anaerobic digesting of organic matter. Methane is also eliminated into forest soils by microbial oxidation (methanotrophy) in the aerobic zone and which oxidises atmospheric methane.Methanotrophs in forest soil use CH_4 as only a carbon (C) and energy source and oxygen availability is the main factor limiting their activity (Le Mer and Roger, 2001). Therefore, methanotrophy in forest soils is primarily dependent on physical factors controlling soil diffusion, e.g., water content and soil texture (Butterbach-Bahl and Papen, 2002; Templeton et al.,

2006). Teh et al. (2005) indicate that the effective diffusion at soil surface controls the overall rate of methane consumption. However, forest soils as biological sinks of CH_4 are also subjected to other abiotic and biotic controls. It is well known that methanotrophs have different temperature and pH optima. Effect soil temperature on methanotrophic community has been well studied (Mohanty et al., 2007). Soil pH seems to be a less important control for atmospheric methane oxidation (Kolb, 2009). Tree species would affect atmospheric CH_4 oxidation without altering community composition of soil methanotrophs (Menyailo et al., 2010), Jamali et al. (2011) found that termites offset 21 % of CH_4 consumed by soil in a tropical savanna woodland. Furthermore, it is believed that nitrogen (N) availability controls the activity and affects the community structure of methanotrophs in soil (Bodelier and Laanbroek, 2004).

Among the above factors, the effects of nitrogen deposition on CH_4 oxidation have received increasing attention (Steudler et al., 1989; Bodelier and Laanbroek, 2004; Tate et al., 2007; Zhang et al., 2008). Nitrogen addition alters the fluxes of greenhouse gases (GHGs, including CH_4) through regulating plant and microbial activities that are directly associated with GHGs production and consumption (Liu and Greaver, 2009). Steudler et al. (1989) first reported in a temperate forest that N fertilization reduced soil CH_4 uptake by 33 %. Extensive research has been conducted to investigate the relationship between CH_4 consumption and N input and it has been generally accepted that CH_4 uptake is inhibited by nitrogenous fertilization (Bodelier and Laanbroek, 2004; Chan et al., 2005). Laboratory studies indicate that oxidation of CH_4 by a variety of methanotrophs is competitively inhibited by N (Ferenci et al., 1975). In soils, the inhibition of CH_4 oxidation by ammonium is attributed to a competition at the level of the methane mono-oxygenase, a transfer of the methanotrophy activity towards nitrification (Castro et al., 1994) and the toxicity of NO_2 produced. Aluminium toxicity after extensive N input may also inhibit soil CH_4 uptake (Bradford et al., 2001a; Zhang et al., 2008). On the other hand, positive effects of increased N availability on CH_4 uptake rates were found in severely N-limited forests (Börjesson and Nohrstedt, 2000; Steinkamp et al., 2001). In addition, soil available phosphorus would also affect CH_4 flux by regulating changes in soil physico-chemical properties, plant root activities and soil microbial activities that are involved in CH_4 consumption. Increased P availability in forest soil would stimulate plant root growth and lead to higher water uptake, and then consequently lower soil water content which led to higher gas diffusion and, thus, higher CH_4 oxidation. Methanotrophs also needs phosphorus for community structure growth.

While N is often the primary limiting nutrient in temperate and boreal forests, phosphorus usually limits ecological processes in tropical and subtropical forests (Vitousek and Sanford, 1986; Cleveland et al., 2002; Vitousek et al., 2010). We studied the relationships between soil P availability and CH_4 flux in an old-growth tropical forest located in southern China where elevated N deposition has been well documented (Mo et al., 2006; Fang et al., 2008, 2011). Earlier studies in this old-growth forest showed that no N retention occurred, but rather a net loss of 8–16 kg N ha^{-1} yr^{-1} from the soil was estimated in the old-growth forest. In total, up to 60 kg N ha^{-1} yr^{-1} was leached from the old-growth forest, indicating that this forest was completely N saturated (Fang et al., 2008, 2011) from the chronic elevated N deposition in southern China. This interpretation is also supported by the results on litterfall production, which revealed no significant effects of N additions on total litterfall production in the old-growth forest (Mo et al., 2008). Studying forest CH_4 uptake and its relationship with soil available P and elevated N deposition is very important for evaluating the contribution of tropical forests to global climate change. Due to limited research in tropical forests, it is not clear how P availability would affect soil CH_4 uptake, and how P addition may interact with N deposition. This 33 months study experimentally tested the effects of P and N availabilities on soil CH_4 uptake. We hypothesized that: (1) N addition would inhibit soil CH_4 uptake as we found in a previous study in the same forest (Zhang et al., 2008); (2) P addition would increase soil CH_4 uptake due to that P addition world stimulate plant root growth and lead to higher water uptake, and then consequently lower WFPS which lead to higher gas diffusion and, thus, higher CH_4 uptake; (3) NP addition (interactive effect of N and P) would have less effect on soil CH_4 uptake comparing with N or P addition alone. This means that P addition would mitigate the inhibitive effect of N addition on soil CH_4 uptake.

2 Materials and methods

2.1 Site description

This study was conducted in the 1200 ha Dinghushan Biosphere Reserve (DHSBR), which is located in the middle of Guangdong Province, southern China (112°10′ E, 23°10′ N). There is an old-growth evergreen broadleaf forest (mature forest) in this reserve. The old-growth forest has been well protected from human activity for more than 400 years (Wang et al., 1982; Mo et al., 2003; Tang et al., 2006). The average annual precipitation of 1927 mm in the reserve has a distinct seasonal pattern, with 75 % falling from March to August and only 6 % falling from December to February (Huang and Fan, 1982). The mean annual temperature is 21 °C with the January mean temperature of 12.6 °C and July mean temperature of 28.0 °C (Huang and Fan, 1982). Annual mean relative humidity is 80 % (Huang and Fan, 1982). The wet N deposition was 36–38 kg N ha^{-1} in the 1990s (Zhou and Yan, 2001). Precipitation N deposition in this region was 34.1 kg N ha^{-1} yr^{-1}, with roughly 1.5:1 NH_4^+ to NO_3^- ratio (Fang et al., 2011). Soil in the reserve is oxisols from

shale formation (Wu et al., 1982). The soil depth in the old-growth forest is more than 60 cm to the top of the C horizon (Mo et al., 2003). The forest in this experiment is situated on mountain slopes about 30°–35°.

2.2 Experimental treatment

Four treatments were established (each with five replicates) in 2007: 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 $150\,kg\,P\,ha^{-1}\,yr^{-1}$). A total of 20 plots of $5\,m \times 5\,m$ were established and each plot was surrounded by a 5-m-wide buffer strip. Plots size and fertilizer level were referenced to the experiment in Costa Rica by Cleveland and Townsend (2006). Field plots and treatments were laid out randomly. NH_4NO_3 and NaH_2PO_4 solutions were sprayed once every other month to the forest floor with a backpack sprayer starting from February 2007 and continued through October 2009. Fertilizer was weighed and mixed with 5 l of water for each plot. Each control plot received 5 l of water without fertilizer.

2.3 Field sampling and measurements

CH_4, CO_2 and nitrous oxide (N_2O) flux were measured from January 2007 before the first fertilizer application. Static collars were installed in each plot in November 2006, two months before the gas sampling. Gas fluxes were monitored once every month using the static chamber and a gas chromatograph (Agilent 4890D). The static chamber was a 25-cm-diameter by 16-cm-tall PVC pipe permanently anchored 8 cm into the soil. During gas collection, a 30-cm-tall removable cover chamber was attached tightly to the anchor ring with a rubber band. Gas samples were collected from each chamber from 09:00–10:00 LT. Diurnal studies in the adjacent forests found that greenhouse gas fluxes measured during the mid-morning (09:00–10:00 LT) were closer to the daily mean (Tang et al., 2006). The GHG concentrations remained linear for up to 100 min after the chamber was closed in our study. Gas samples were taken with a 60 ml plastic syringe at 0 and 30 min after the chamber closure. Before each sampling, syringes were flushed three times with chamber gas to mix the headspace. Laboratory tests showed that chambers and syringes were inert to N_2O, CO_2 and CH_4 (Steudler et al., 1989; Bowden et al., 1990). Gas samples were analysed within 12 h in a gas chromatograph (Agilent 4890D) equipped with a flame ionization detector (FID) for CH_4 and CO_2, and an electron capture detector (ECD) for N_2O. CO_2 was transformed into CH_4 via $(Ni)H_2$ before the FID analysis. Calibration gases (CH_4 at 1.87 ppm, CO_2 at 418 ppm, N_2O at 0.321 ppm, bottle's No. 070811) were obtained from the Institute of Atmospheric Physics, Chinese Academy of Sciences. In this paper, we report only data of soil CH_4 in the old-growth forest.

The calculation of GHG flux followed that described in Zhang et al. (2008), based on a linear regression of chamber gas concentration versus time (IAEA, 1992; Holland et al., 1999). Atmospheric pressure was measured at the sampling site using an air pressure gauge (Model THOMMEN 2000, Switzerland). 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 (Time Domain Reflectometry, Model Top TZS-I, China). Soil moisture (0–10 cm depth) values were converted to WFPS (Water Filled Pore Space) according to the following formula:

$$\mathrm{WFPS}\,(\%) = \mathrm{Vol}\,(\%)/(1 - \mathrm{SBD}(g\,cm^{-3})/2.65(g\,cm^{-3})) \quad (1)$$

where SBD is soil bulk density, Vol is volumetric water moisture and 2.65 is the density of quartz.

Soil samples were collected in February 2007 (before the first fertilizer application) and February 2009 (after two years of fertilization) for the physical and chemical properties. Five soil cores (2.5 cm inner diameter) were collected randomly from each of the 20 plots at 0–10 cm soil depths and combined to one composite sample. The litter layer was carefully removed before the soil sampling. The pH of the soil sample was measured in a 1:2.5 soil/water suspension. Total N concentration was determined by the micro-Kjeldahl digestion followed by the analysis of ammonium on a Wescan ammonia analyser, while total P concentration was analysed colorimetrically after acidified ammonium persulfate digestion (Anderson and Ingram, 1989). Available P was extracted with 0.03 *M* ammonium fluoride and 0.025 *M* hydrochloric acid and analysed colorimetrically (Anderson and Ingram, 1989).

2.4 Statistical analysis

Repeated measures of Analysis of Variance (ANOVA, PROC MIXED with AR(1) from SAS – SAS Institute Inc., Cary NC, USA) was used to examine the effect of fertilizer treatments on soil GHG fluxes from February 2007 to October 2009. Two-way ANOVA (PROC GLM from SAS) was used to examine the effects of N and P addition. One-way ANOVA was used to examine the difference in soil pH, NH_4^+, NO_3^- and available P among treatments. Linear regression analysis was performed by Origin 8.0 (OriginLab Corporation, Northampton, MA USA) to examine the relationship between CH_4 fluxes and soil WFPS contents and soil temperature. Out of 680 observations, three were identified as outliers, which were probably caused by chamber leaks, abnormally high WFPS, and other unknown factors, and were removed from the data analyses. Statistical significant differences were set at p values <0.05 unless otherwise stated. Mean values $\pm$ 1 standard error are given in the text.

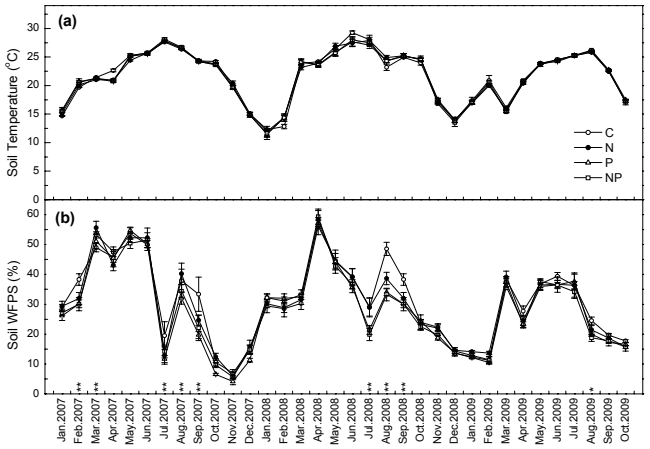

Fig. 1. Soil temperature at 5cm depth **(a)** and soil WFPS **(b)** during the study period. Error bars represent standard error of means (N = 5). Asterisk (*) and double asterisk (**) indicates significant differences between control and at least one of the experiment treatments at $p < 0.1$ and $p < 0.05$, respectively.

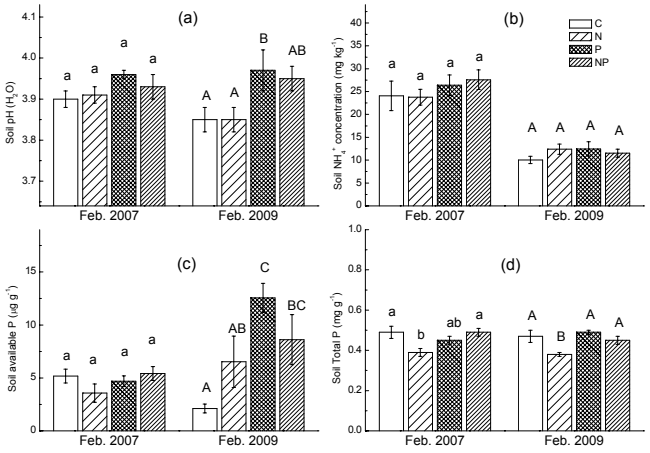

Fig. 2. Soil pH **(a)** , ammonia nitrogen **(b)** , available P **(c)** and total P **(d)** at 5 cm depth in February 2007 and February 2009. Error bars represent standard error of means (N = 5). Different letters denote significant difference ($p < 0.05$) between treatments by the Repeated Measurement ANOVA.

3 Results

3.1 Soil temperature and WFPS

Soil temperature (at 5 cm depth) followed the air temperature in all plots, with temperatures increased from spring to summer and decreased from fall to winter (Fig. 1a). There was no treatment effect on soil temperature during the study period. Soil WFPS (0–10 cm depth) rose following the increased precipitation from dry winters to wet springs but decreased in summer, possibly due to plant uptake and higher evaporation, despite the high amount of precipitation in summer (Fig. 1b). Repeated measurement ANOVA showed that soil WFPS was significantly lower in the P-addition and NP-addition plots ($p = 0.011$ and $p = 0.018$, respectively) than in the control plots. However, there was no treatment effect in the N-addition plots ($p = 0.165$).

3.2 Other soil properties

Nitrogen and P treatments changed soil nutrient conditions (Fig. 2). Soil pH increased significantly in the P-addition plots ($p = 0.021$) and was a little higher in the NP-addition plots ($p = 0.062$), while no change in the N-addition plots ($p = 0.933$) (Fig. 2a) compared with the control plots. After 24 months of the treatment, a six-fold of soil available P was observed in P-addition plots ($p = 0.0003$) and four-fold in NP-addition plots ($p = 0.012$), compared to the controls (Fig. 2c). Furthermore, total P was significantly lower in N-addition plots compared to control plots ($p = 0.001$). This pattern is the same as pre-treatment (Fig. 2d). Soil NH_4^+ concentrations did not show any significant change in any treatment plots (N-addition, P-addition and NP-addition) compared to the controls ($p = 0.163$, 0.152 and 0.368, respectively) (Fig. 2b).

3.3 Soil CH_4 fluxes

Pre-treatment gas measurement (January 2007) showed no difference in different treatment plots compared to control plots ($p = 0.807$, 0.559 and 0.961, respectively, in N, P, NP treatment plots). After fertilization treatment, repeated measurement ANOVA showed that P addition significantly increased soil CH_4 uptake while N addition significantly decreased CH_4 uptake (Fig. 3). The mean soil CH_4 uptake rate was 31.2 ± 1.1 μg CH_4-C m^{-2} h^{-1} in the control plots during the 33 months study period. Soil CH_4 uptake in the P-addition plots was significantly higher (mean CH_4 uptake rate was 38.8 ± 1.3 μg CH_4-C m^{-2} h^{-1}, $p = 0.0068$). On the contrary, N-addition significantly inhibited soil CH_4 uptake (mean CH_4 uptake rate was 23.6 ± 0.9 μg CH_4-C m^{-2} h^{-1}, $p = 0.007$). No significant difference was observed between NP-addition plots (33.6 ± 1.0,μg CH_4-C m^{-2} h^{-1}) and the controls ($p = 0.177$).

CH_4 uptake was higher in summer and fall (when the soil was low in water content) and was lower in spring (when the soil was wet) and winter (when the soil was cold). Two-way ANOVA showed a significant positive P effect on CH_4 uptake in the summers of 2007, 2008 and 2009, fall 2007, and the springs of 2008 and 2009 (Fig. 4), and a negative N effect in the springs of 2008 and 2009, and summer 2009 (Fig. 4). In summer 2007, CH_4 uptake in the P-addition and NP-addition plots was 26.6 % and 27.3 % higher than in the control plots ($p = 0.049$ and 0.051, respectively). CH_4 uptake in the N-addition plots was reduced by 30.3 % in fall

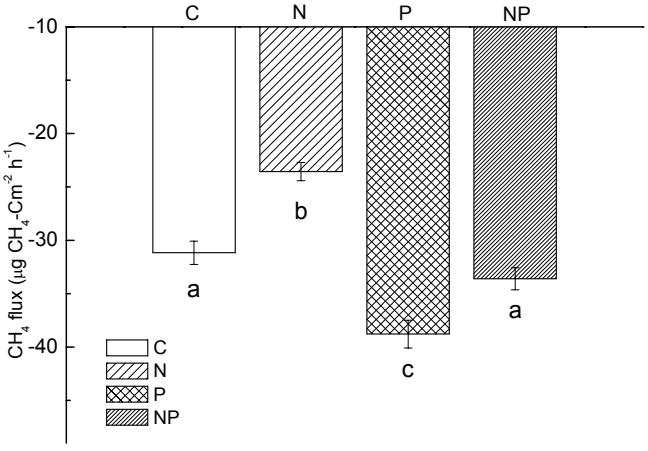

Fig. 3. Comparisons of mean soil CH_4 fluxes between treatments from 2007 to 2009 after N and P additions. Bars indicate ±1 SE, N = 5. Different letters denote significant difference ($p < 0.05$) between treatments by the Repeated Measurement ANOVA.

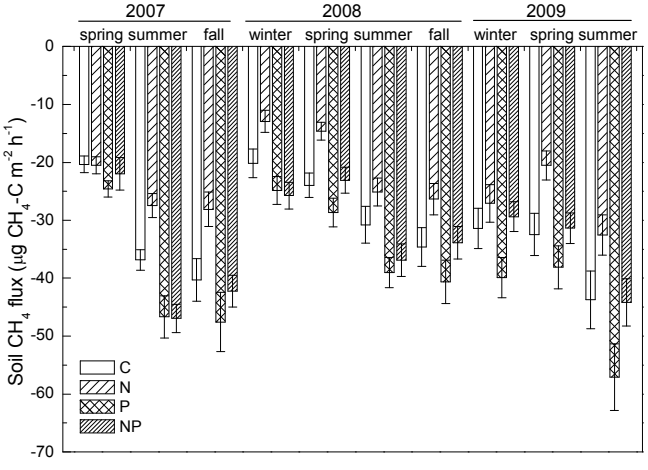

Fig. 4. Seasonal pattern of soil CH_4 uptake (April 2007 to September 2009). Bars indicate ±1 SE, N = 5. Spring from April to June, summer from July to September, fall from October to December and winter from January to March.

2007 ($p = 0.052$), 39.1 % in spring 2008 ($p = 0.034$) and 36.7 % in spring 2009 ($p = 0.027$). In addition, CH_4 uptake was increased by 30.6 % in the P-addition plots in summer 2009 ($p = 0.038$).

CH_4 fluxes and soil WFPS were positively correlated in the control plots and N and P addition plots (Fig. 5). Under similar WFPS conditions, CH_4 uptake was the highest in P-addition plots and lowest in N-addition plots. CH_4 flux was not correlated to soil temperature.

4 Discussion

4.1 Compare with other tropical forests

The annual CH_4 uptake rates in our old-growth tropical forest site in southern China ranged from 2.3 to 3.4 kg CH_4-C ha^{-1} yr^{-1} (11 months in 2007, 12 months in 2008, 10 months in 2009), and were higher in the dry year (2007) than in the wet year (2008). The CH_4 fluxes, quantified in this study, are similar to the previous reported CH_4 fluxes measured at an adjacent forest (Tang et al., 2006; Zhang et al., 2008) and other parts of tropical Southwest China (Werner et al., 2006; Yan et al., 2008; Wang et al., 2010). The rates of CH_4 uptake in other tropical forests are also similar, ranging from 0.8 to 4.73 kg CH_4-C ha^{-1} yr^{-1} (Steudler et al., 1991; Kiese et al., 2003; Davidson and Nepstad, 2004; Davidson et al., 2008).

4.2 Effects of soil temperature and pH

Temperature variation has a minor impact on soil CH_4 uptake in our studied forest, which is consistent with previous results from the adjacent forest (Tang et al., 2006). In temperate or boreal regions, in contrast, soil CH_4 oxidation was generally positively correlated with soil temperature (Crill, 1991; King, 1997). Castro et al. (1995) observed that methanotrophy was affected by soil temperature between −5 and 10 °C, but in our site, almost all of soil temperatures were between 10 and 30 °C (Fig. 1a). The higher pH after P addition might contribute to the increase in CH_4 uptake rate in this forest. Most of the soils oxidised CH_4 over a pH range of $3 \sim 7.5$ supporting in situ observations (Born et al., 1990). Bacteria extracted from boreal acidic forest soils had their highest CH_4 oxidation activity at pH 5.8 (Amaral et al., 1998). Soil pH in this acidic old-growth forest almost below 4 or even lower. Bradford et al. (2001b) observed that the drop in pH in a beech forest in the UK contributed to a decrease in soil CH_4 uptake. However, in some acidic peat soils, the rates of methane uptake were only slightly influenced by pH at values between 4.0 and 6.0 and decreased sharply at values below and above this range, although oxidation still occurred below pH 4.0 (Hanson and Hanson, 1996). Therefore, P addition increased soil pH in our study which may lead to increased methane oxidation.

4.3 Effects of soil WFPS

Soil CH_4 uptake was negatively correlated with the soil WFPS (a better measurement of soil moisture) in this study (Fig. 5), which was consistent with previous publications (Born et al., 1990; Castro et al., 1995; Kiese et al., 2003). Steinkamp et al. (2001) reported similarly that soil moisture was the dominant factor controlling CH_4 uptake when soil temperature was >10 °C. Methanotrophs use CH_4 as only a C and energy source and oxygen availability is the main factor limiting their activity (Le Mer and Roger, 2001). Lower

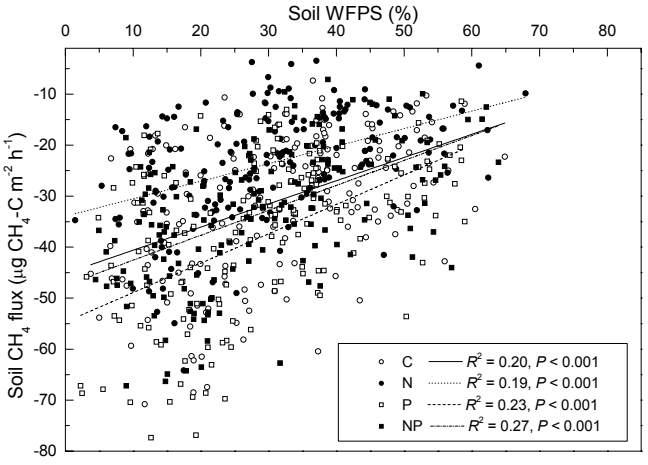

Fig. 5. Correlations between CH_4 flux and soil WFPS under different fertilization treatments. Under similar soil WFPS conditions, CH_4 uptake was higher in P-addition plots and lower in N-addition plots.

WFPS after P addition in our study might lead to higher soil aeration and more available oxygen and atmospheric methane to methanotrophs.

4.4 Effects of P and N fertilization

We found P addition significantly increased CH_4 uptake, N addition decreased CH_4 uptake, and P addition mitigated the negative N effect in this 33 months field experiment, as hypothesized. We believe this is the first experimental testing of N and P limitation on soil CH_4 flux in tropical and subtropical forests. The treatment effects were particularly strong in the summers and falls, when soil uptakes of CH_4 were the highest (Fig. 4). Phosphorus fertilization was often conducted in agricultural ecosystems. On planted rice soils, phosphorus addition significantly decreased CH_4 emission (Lu et al., 1999) probably by increasing methanotrophic potential (Joulian et al., 1998). We infer that this response of CH_4 uptake to the experimental P additions could be resulted from increased soil diffusion due to plant root growth. In our study, we found that soil WFPS was decreased after phosphorus addition, particularly in the summers (growing season) with a higher methane oxidation. This decreasing of soil WFPS was likely due to increased plant water consumption after P addition. Since phosphorus is a common limiting nutrient in tropical forests (Herbert et al., 2003; Wardle et al., 2004), low phosphorus availability due to strong sorption is often the main constraint on plant growth on highly weathered tropical soils (Vitousek, 1984). Ostertag (2001) also observed P-fertilizer leads to a greater root turnover in 4.1 million year-old forests in Hawaii. In these forests, two years fertilization of 150 kg ha^{-1} yr^{-1} did not increase total soil phosphorus probably due to serious plant uptake under high N deposition. Phosphorous deficiency in plant growth in this old-growth forest is also partially supported by the results on litterfall study, which showed that annual total litterfall fluxes were significantly increased after P addition (Liu et al., 2011). Thus, P addition might stimulate plant roots sorption of phosphorus and water uptake which lead to lower soil WFPS in this forest. Higher water uptake by plant roots would subsequently result in higher soil diffusion. Therefore, higher diffusion for oxygen and atmospheric methane would increase methanotrophy activities. In addition, since phosphorus is a common limiting nutrient in tropical forests (Herbert et al., 2003; Wardle et al., 2004), microbial activity could be strongly P limited (Cleveland et al., 2002). Increased soil CH_4 uptake in this study may be due to stimulated methanotrophy activities after increased P availability in soil. In a companion study, we found that microbial biomass increased significantly by P addition (Liu et al., 2011). However, this could be a minor contribution to the increased CH_4 uptake due to methane oxidizing bacteria representing only a very small portion of the total soil microbial biomass. On the other hand, phosphorus fertilizer may increase the mineralization of organic P (Ofori-Frimpong and Rowell, 1999) and reduce Al^{3+} toxicity. Increased soil P levels might stabilize soil aluminum and iron through geochemical reaction of adsorption (Frossard et al., 1995). This coupling effect decreased aluminum toxicity to methanotrophs (Nanba and King, 2000). However, in our experiment, we found no treatment effect on soil exchangeable Al^{3+} (data was not shown here).

The inhibitive effect of N input on CH_4 uptake has been reported extensively. Zhang et al. (2008) suggested that the response of forest soil CH_4 uptake on N fertilization possibly depends on soil N status. They compared the CH_4 uptake under various N additions in three tropical forests with very different N conditions and found that N addition in N-saturated old-growth forest significantly decreased soil CH_4 uptake while in N-limited young forests N addition had a limited effect. Singh et al. (1997) observed in a natural gradient of tropical forests, sites with higher soil mineral nitrogen concentrations had lower CH_4 uptake. In a Puerto Rican wet forest, Steudler et al. (1991) found that maximum reduction in CH_4 uptake coincided with the highest soil NH_4^+, suggesting that nitrogen-CH_4 linkage observed in temperate forests (Steudler et al., 1989) may also function in tropical forests. NH_4^+ can reduce the growth rate of many methanotrophs by inhibiting CH_4 oxidation (Whittenbury et al., 1970). Laboratory studies indicate that oxidation of CH_4 by a variety of methanotrophs is competitively inhibited by nitrogen (Ferenci et al., 1975). In soils, the inhibition of CH_4 oxidation by ammonium is attributed to a competition at the level of the methane mono-oxygenase, a transfer of the methanotrophy activity towards nitrification (Castro et al., 1994) and the toxicity of NO_2 produced. Schnell and King (1994) observed that nitrite, the end product of methanotrophic ammonia oxidation, was a more effective inhibitor of CH_4 oxidation than ammonium. This inhibition becomes

irreversible and can only be released at CH_4 concentrations higher than 100 ppm (King and Schnell, 1994). In this old-growth forest, we observed that atmospheric CH_4 concentrations was around 2 ppm and even in the soil can not come up to 100 ppm. We suggest that the inhibition of CH_4 uptake by ammonium has occurred and persisted. However, we did not observe soil NH_4^+ concentration change after treatments in this study, which might be due to the rapid volatile and leaching of the fertilization before our soil sampling.

5 Conclusions

Nitrogen deposition would weaken the function of upland forest soils as CH_4 sinks by its inhibitive effect on soil CH_4 uptake and by decreasing availability of phosphorus due to continuous nitrogen deposition and phosphorus limitation in the tropical forests of southern China and other parts of the world. Galloway et al. (1994) forecasted a large increase in nitrogen deposition from fossil-fuel, growing population and agricultural activity for year 2020 in those regions. However, P addition could reverse such trends by stimulating forest soil CH_4 uptake. In this 33 months field experiment, we showed that increased P availability might mitigate the inhibitive effect of N addition on soil CH_4 uptake in this N-saturated old-growth tropical forest. While average annual consumption of CH_4 was enhanced after P addition and decreased after N addition, there was no difference between the NP-addition plots and the controls (i.e., the negative effect of N addition on CH_4 uptake was reduced by P addition). As far as we know, our study is among the first field study experimentally testing the responses of soil CH_4 uptake to N and P additions in tropical forests. Results from our study suggest that phosphorus fertilization in tropical forests could be one of the future choices for mitigating the inhibitive effect of N deposition on soil CH_4 uptake.

Supplementary material related to this article is available online at: http://www.biogeosciences.net/8/2805/2011/bg-8-2805-2011-supplement.pdf.

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