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Phosphorus addition mitigates N_2O and CH_4 emissions in N-saturated subtropical forest, SW China

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Abstract

Chronically elevated nitrogen (N) deposition has led to severe nutrient imbalance in forest soils. Particularly in tropical and subtropical forest ecosystems, increasing N loading has aggravated phosphorus (P) limitation of biomass production, and has resulted in elevated emissions of nitrous oxide (N_2O) and reduced uptake of methane (CH_4), both of which are important greenhouse gases. Yet, the interactions of N and P and their effects on GHG emissions remain understudied. Here, we report N_2O and CH_4 emissions together with soil chemistry data for the a period of 18 months following P addition (79 kg P ha^{-1} yr^{-1}, applied as NaH_2PO_4 powder) to a N-saturated, Masson pine-dominated forest at TieShanPing (TSP), Chongqing, SW China. We observed a significant decline both in NO_3^- concentrations in soil water (at 5- and 20-cm depths) and in N_2O emissions, the latter by 3 kg N ha^{-1} yr^{-1}. We hypothesize that enhanced N uptake by plants and soil microbes in response to P addition, results in less available NO_3^- for denitrification. By contrast to most other forest ecosystems, TSP is a net source of CH_4. As for N_2O, P addition significantly decreased CH_4 emissions, turning the soil into a net sink. Based on our data and previous studies in South America and China, we believe that P addition relieves N-inhibition of CH_4 oxidation. Within the 1.5 years after P addition, no significant increase of forest growth was observed at TSP, but we cannot exclude that understory vegetation increased. Our study suggests that P fertilization of acid forest soils could mitigate GHG emissions in addition to alleviate nutrient imbalances and reduce losses of nitrogen through NO_3^- leaching and N_2O emission.

Key Word: N_2O and CH_4 emission, N saturation, Phosphate fertilization, soil CH_4 uptake, acid forest soil.

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1 Introduction

Anthropogenic activities have transformed the terrestrial biosphere into a net source of CH_4, N_2O and CO_2, leading to increased radiative forcing (Montzka et al., 2011; Tian et al., 2016). During the last decade, atmospheric concentrations of CO_2, CH_4, N_2O have increased at rates of 1.9 ppm yr^{-1}, 4.8 and 0.8 ppb yr^{-1}, respectively (Hartmann et al., 2013). In China, the exponential increase of reactive nitrogen (N) input into the biosphere since the 1970s has likely led to more carbon (C) being sequestered in the biosphere (Cui et al., 2013; Shi et al., 2015). However, enhanced emissions of N_2O and CH_4 due to chronic N pollution potentially offset the cooling effect by C sequestration (Liu and Greaver, 2009; Tian et al., 2011).

Microbial nitrification and denitrification in soils account for about 60% of N_2O emissions globally (Ciais et al., 2013; Hu et al., 2015). Although, microbial activity is often restricted in low pH soils of unproductive forests, surprisingly large N_2O emissions have been reported from acid, upland forest soils in South China (Zhu et al., 2013b). Reported average N_2O fluxes in humid, subtropical forests range from 2.0 to 5.4 kg ha^{-1} yr^{-1} (Fang et al., 2009; Tang et al., 2006; Zhu et al., 2013b), which by far exceeds global averages for temperate or tropical forest ecosystems (Werner et al., 2007; Zhuang et al., 2012). This has been attributed to frequently shifting aeration conditions during monsoonal summers, promoting both nitrification and denitrification (Zhu et al., 2013b) and to large soil NO_3^- concentrations due to efficient cycling of deposited N in acid subtropical soils (Yu et al., 2016).

Chronically elevated rates of N deposition (30-65 kg ha^{-1} yr^{-1}; Xu et al., 2015) have resulted in strong nutrient imbalances in southern Chinese forests, aggravating phosphorus (P) limitation (Du et al., 2016). Phosphorous deficiency in N-saturated forests restricts forest growth and thus

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55 constrains its capability to retain N (Huang et al., 2015; Li et al., 2016), resulting in ample
56 amounts of mineral N (NH_4^+ and NO_3^-) being present in the soil solution. Accordingly, Hall &
57 Matson (1999) observed larger N_2O emission in P-limited than in N-limited tropical forests after
58 1 year of repeated N addition. Likewise, previous N manipulation studies in forests of South
59 China reported pronounced stimulation of N_2O emissions by N addition (Chen et al., 2016;
60 Wang et al., 2014; Zheng et al., 2016), supporting the idea that P limitation causes forests to be
61 more susceptible to N saturation and N_2O-N loss. In an N-limited tropical montane forest in
62 southern Ecuador, P addition alone (10 kg P ha^{-1} yr^{-1}) had no effect on N_2O emissions during the
63 first two years. However, N_2O emission was smaller when P was added together with N (50 kg N
64 ha^{-1} yr^{-1}) than treatments with N addition alone (Martinson et al., 2013). After continued
65 fertilization for three years, also P addition alone reduced N_2O emissions at these sites (Müller et
66 al., 2015). In tropical China, with high N deposition (~ 36 kg ha^{-1} yr^{-1}; Mo et al., 2008), P
67 addition (150 kg P ha^{-1} yr^{-1}) to an old-growth forest revealed a similar pattern, with no initial
68 effect on N_2O emissions (0-2 years) but a significant longer term effect (3 to 5 years) (Chen et al.,
69 2016; Zheng et al., 2016). In a secondary tropical forests in South China, Wang et al. (2014)
70 found no effect on N_2O emissions of P alone (100 kg P ha^{-1} yr^{-1}), and in treatments combining P
71 with N (100 kg N ha^{-1} yr^{-1}), N_2O emissions even increased during the wet season. Meanwhile,
72 they observed a significant increase in soil microbial biomass after P addition, which is in line
73 with previous findings in tropical forest soils of South China (Liu et al., 2012). Thus, they
74 attributed the stimulating effect of P addition on N_2O emissions to the larger nitrification and
75 denitrification potential of the increased soil microbial biomass. This was also proposed by Mori
76 et al. (2014), based on results from a short-term incubation study with P addition, excluding plant
77 roots.

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As the sole biogenic sink for CH_4, upland soils play an important role in balancing terrestrial CH_4 emissions (Ciais et al., 2013; Dutaur and Verchot, 2007). Atmospheric CH_4 uptake in soil is mediated by the activity of methanotrophic bacteria, which oxidize CH_4 to CO_2 to gain energy for growth. Well-drained forest and grassland soils are dominated by yet uncultured, high-affinity methanotrophs residing in the upper soil layers (Le Mer and Roger, 2010). In addition to edaphic factors (pH and nutrients), other parameters affecting the diffusion of CH_4 into the soil (soil structure, moisture, temperature) are believed to be the major controllers for CH_4 uptake (Smith et al., 2003). A number of studies have shown that excess N affects CH_4 fluxes in forest soils (Liu and Greaver, 2009; Veldkamp et al., 2013; Zhang et al., 2008b). In general, N addition promotes CH_4 uptake in N-limited soils by enhancing growth and activity of methanotrophs, whereas excessive N input and N saturation inhibit CH_4 oxidation on an enzymatic level (Aronson and Helliker, 2010; Bodelier and Laanbroek, 2004). P addition experiments in N-enriched soils have shown positive effects on CH_4 uptake (Mori et al., 2013a; Zhang et al., 2011), but the underlying mechanisms, i.e. whether P addition affects the methanotrophic community in soils directly or alleviates the N-inhibition effect on CH_4 oxidation through enhanced N uptake (Mori et al., 2013b; Veraart et al., 2015), remain unresolved.

Subtropical forests in South China show strong signs of N saturation, with exceedingly high NO_3^- concentrations in soil water (Larssen et al., 2011; Zhu et al., 2013b). Little is known about how P addition affects N cycling and N_2O emission in these acidic, nutrient-poor soils. Likewise, the importance of increased mineral N concentrations for soil-atmosphere exchange of CH_4, and how this is affected by P fertilization remain to be elucidated for soils of the subtropics. Here, we assessed N_2O and CH_4 fluxes in an N-saturated subtropical forest in SW China under ambient N deposition and studied the effects of P addition on emission rates, nutrient availability and tree

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101 growth. The objectives were i) to quantify ambient N_2O and CH_4 emissions, ii) to test whether P
102 affects N cycling in a highly N-saturated forest and iii) to investigate the effect of P addition on
103 N_2O and CH_4 emission.

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2 Materials and Methods

2.1 Site description

The study site "TieShanPing" (TSP) is a 16.2 ha subtropical forest (29° 380 N, 106° 410 E; 450 m a.s.l.), about 25 km northeast of Chongqing, SW China. TSP is a naturally regenerated, secondary mixed coniferous-broadleaf forest, which developed after clear cutting in 1962 (Larssen et al., 2011). The forest stand is dominated by Masson pine (*Pinus massoniana*) and has a density of about 800 stems ha^{-1} (Huang et al., 2015). Having a monsoonal climate, TSP has a mean annual precipitation of 1028 mm, and a mean annual temperature of 18.2 °C (Chen and Mulder, 2007). Most of precipitation (> 70%) occurs during the summer period (April to September). The soil is a loamy yellow mountain soil, classified as Haplic Acrisol (WRB 2014), with a thin O horizon (< 2 cm). In the O/A horizon, soil pH is around 3.7, and the mean C/N and N/P ratios are 17 and 16, respectively. In the AB horizon, which has a slightly higher pH, mean C/N is well above 20. More details on soil properties are presented in Table 1.

Annual N deposition at TSP measured in throughfall varies between 40 to 65 kg ha^{-1} and is dominated by NH_4^+ (Yu et al., 2016). According to regional data, annual P deposition via throughfall is < 0.40 kg ha^{-1} (Du et al., 2016). Strong soil acidification at TSP has resulted in severe decline in forest growth (Li et al., 2014; Wang et al., 2007), and in abundance and diversity of ground vegetation (Huang et al., 2015). Pronounced N saturation with strong NO_3^- leaching from the top soil has aggravated P deficiency (Huang et al., 2015). The total P content in the O/A horizon is ~ 300 mg kg^{-1}, while P_{Al} is smaller than 5 mg kg^{-1} (Table 1).

2.2 Experimental Design

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Three blocks, each having two 20 m * 20 m plots, were established near a hilltop on a gently sloping hillside. A 5-m buffer strip separated the two plots in each block. In each block, plots were assigned ad random to a reference (Ref) and a P treatment. On 4 May 2014, a single dose of P fertilizer was applied as solid $NaH_2PO_4{\cdot}2H_2O$, at a rate of 79.5 kg P ha^{-1}. The amount of P added was estimated from P adsorption isotherms (Supplementary Materials, Table S1 and Figure S1), to ensure significantly increased available P in TSP soil. To apply P fertilizer evenly, we divided each plot into a 5 m * 5 m grid and broadcasted the powdered fertilizer by hand in each grid cell. The P dose applied at TSP was intermediate as compared to the 10 kg P ha^{-1} yr^{-1} applied by Müller et al. (2015) to a mountain forest in Ecuador and the 150 kg P ha^{-1} yr^{-1} applied by Zheng et al. (2016) to a subtropical forest in South China.

Together with the addition of phosphate, the P-treated plots also received 59.0 kg ha^{-1} of sodium (Na). One month after the fertilizer application, Na^+ concentrations in soil water of the P treatments were about 5 mg L^{-1} at 5-cm depth and 3 mg L^{-1} at 20-cm depth (Table S2). Although somewhat larger than in the reference plots, the Na^+ concentration in soil water of the P treatments are unlikely to have exerted a strong negative impact on plant and microbial activities.

2.3 Sample collection and analyses

Within each plot, triplicates of ceramic lysimeters (P80; Staatliche Porzellanmanufaktur, Berlin) were installed at 5- and 20-cm soil depths in August 2013. To obtain water samples, 350-ml glass bottles with rubber stoppers were pre-evacuated, using a paddle pump, and connected to the lysimeters for overnight sampling. Between November 2013 and October 2015, we sampled soil pore water bi-monthly in the winter season and monthly during the growing season. All water samples were kept frozen during storage and transport. Concentrations of NH_4^+, NO_3^-, potassium

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147 (K^+), calcium (Ca^{2+}), and magnesium (Mg^{2+}) in soil water were measured at the Research Center
148 for Eco-Environmental Sciences (RCEES), Chinese Academy of Sciences, Beijing, using ion
149 chromatography (DX-120 for cations and DX-500 for anions).

150 In August 2013, soils from the O/A (0-3 cm), AB (3-8 cm) and B (8-20 cm) horizons were
151 sampled near the lysimeters for soil analysis. Total P and plant-available P contents were
152 monitored in samples collected from the O/A horizons every six months, starting two days
153 before P addition. Soil samples were kept cold (< 4 ºC) during transport and storage. Before
154 analysis, soil samples were air dried and sieved (2 mm). Soil pH was measured in soil
155 suspensions (10 g dry soil and 50 ml deionized water) using a pH meter (PHB-4, Leici, China).
156 Total soil C and N contents were determined on dried and milled samples, using a LECO
157 elemental analyzer (TruSpec@CHN, USA). To measure total P, 1 g dry soil was digested with 5
158 ml of 6 M H_2SO_4 (Singh et al., 2005) and measured as ortho-phosphate by the molybdenum blue
159 method (Murphy and Riley, 1962). Ammonium lactate (0.01 M)-extractable P and H_2O-
160 extractable P (P_{Al} and P_{H2O}, respectively) were measured as ortho-phosphate after extraction (1.5
161 g dry soil in 50 ml solution) (Singh et al., 2005). Ammonium oxalate (0.2 M)-extractable Fe, Al
162 and P were measured by inductive coupled plasma (7500; Agilent) after extraction (1.5 g dry soil
163 in 50 ml solution).

164 From August 2013 onwards, we measured N_2O and CH_4 emissions in triplicate in micro-plots
165 close to the lysimeters, using static chambers (Zhu et al., 2013b). To investigate the immediate
166 effect of P addition on N_2O emissions, we sampled the gas emissions once before (2 May) and
167 three times (7, 10 and 12 May) after the P application. Gas samples (20 ml) were taken 1, 5, 15
168 and 30 minutes after chamber deployment and injected into pre-evacuated glass vials (12 ml)
169 crimp-sealed with butyl septa (Chromacol, UK), maintaining overpressure to avoid

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contamination during sample transport. Mixing ratios of N_2O, CO_2 and CH_4 were analyzed using a gas chromatograph (Model 7890A, Agilent, US) at RCEES, equipped with an ECD for detection of N_2O (at 375 °C with 25 ml min^{-1} Ar/CH_4 as make up gas), a FID for CH_4 (250 °C; 20 ml min^{-1} N_2 as make-up gas) and a TCD for CO_2. Exchange rates between soil and atmosphere (emission/uptake) were calculated from measured concentration change in the chambers over time, applying linear or polynomial fits to the concentration data. Cumulative N_2O emissions over time were estimated by linear interpolation between measurement dates (Zhu et al., 2013b).

From October 2013 onwards, litterfall was collected during the first week of every month in five replicates per plot. Litterfall collectors were made of 1 m^2 nylon nets (1 mm mesh size), held in place by four wooden poles 0.8 m above the ground. Fresh litter was dried at 65°C. In early November 2013 and 2014 (at the end of the growing season), we collected current-year pine needles from several branches of three trees in each plot. The collected needles were dried at 65 °C and the dry weight of 500 needles was determined. A subsample was dried at 80 °C and finely milled prior to chemical analysis at the Chinese Academy of Forestry. Total C and N were measured using an elemental analyzer (FLASH 2000; Thermo Scientific; USA). The contents of K, Ca, Mg and P in the needles were determined by ICP-AES (IRIS Intrepid II; Thermo Scientific; USA) after digesting 0.25 g dry weight samples with 5 ml of ultra-pure nitric acid. In November 2013, and 2014, and in February of 2015, we measured the height and the diameter at breast height (DBH) of 6 to 10 Masson pines (only those with DBH > 5 cm) at each plot. These data were used to estimate the standing biomass of Masson pines based on standard allometric equations (Li et al., 2011; Zeng et al., 2008).

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192 Daily average air temperature and sum of precipitation were monitored by a weather station
193 (WeatherHawk 232, USA) placed on the roof at the local forest bureau, in about 1 km distance
194 from the sampling site (Yu et al., 2016).

195 **2.4 Statistical analyses**

196 Statistical analyses were performed with Minitab 16.2.2 (Minitab Inc., USA). All data were
197 tested for normality (Kolmogorov-Smirnov's test) and homoscedasticity (Levene's test) before
198 further analysis. If not normally distributed, the data were then normalized by logarithmic
199 transformation. Due to heterogeneity between blocks, data on gas fluxes and mineral N
200 concentrations are presented separately for each block. One-way ANOVA was used to evaluate
201 differences in gas fluxes, as well as nutrient concentrations in soil, soil water and plants between
202 treatments and blocks. Significance levels were set to $p < 0.05$, if not specified otherwise.

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3 Results

3.1 Nutrient concentrations in soil and soil water

Addition of P resulted in a significant increase in soil P content in the O/A horizon, both as P_{Al} and total P (Table 2). However, after 15 months, only P_{AL} indicated an enhanced P status, while total soil P did not differ significantly from background values at the reference sites. P addition had no significant effect on soil pH, or soil C and N content. The NO_3^- concentration in soil water collected at 5 cm depth varied seasonally, with significantly greater values (30-40 mg N L^{-1}) towards the start of the growing season in 2015 (April, Fig. S2), but not in 2014, likely due to dilution by abundant precipitation in February to March 2014. Addition of P resulted in significantly smaller NO_3^- concentrations in soil water at 5 and 20 cm depth in blocks 2 and 3 but not in block 1 (Fig. 1). In general, the concentration of NH_4^+ in soil water was small (< 0.6 mg L^{-1}) and not affected by P addition (Fig. S3). At both depths, mean soil water concentrations of Mg^{2+} and Ca^{2+} were significantly smaller in the P-treated than the reference plots, and the sum of charge of base cations declined significantly in response to P addition (Fig. S4).

3.2 N_2O and CH_4 fluxes: effects of P addition

During the experimental period, N_2O fluxes varied seasonally (Fig. 2), showing a significant relationship with daily precipitation (Fig. S5a), but not with daily mean temperature (Fig. S4b). In the reference plots, mean N_2O fluxes were generally below 50 µg N m^{-2} hr^{-1} in the dry, cool season, but reached up to 600 µg N m^{-2} hr^{-1} in the growing season (Fig. 2). Average and cumulative fluxes of N_2O differed greatly between the three blocks (Figs. 3 and 4, respectively), with the greatest annual emission observed in the reference plot (7.9 kg N ha^{-1}) of block 2. Mean N_2O fluxes during the 1.5 years after P addition were smaller in the P treatments than in the

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225 references, the differences being significant in blocks 2 and 3 (Fig. 3). Cumulative N_2O
226 emissions showed that P addition resulted in a decrease in N_2O emission by about 3 kg N ha^{-1} yr^{-1}
227 , which is a 57% reduction on average (Fig. 4). No immediate effects (within days) of P addition
228 on N_2O emission were observed (Fig. S6).

229 CH_4 fluxes varied greatly between blocks (Fig. 5). Net-emission of CH_4 was observed in
230 summer 2013 (~ 80 µg C m^{-2} hr^{-1}) in blocks 1 and 2, whereas block 3 showed CH_4 uptake. From
231 spring 2014 until October 2015, CH_4 fluxes were less variable in all blocks, with values
232 fluctuating around zero. A longer period of net-emission was observed in block 3 during the dry
233 season 2014. The fluxes did not correlate with either precipitation or air temperature (Fig.
234 S5c&d). In the 1.5 years following P addition, mean CH_4 fluxes indicated net CH_4 emission (~
235 +3.8 µg C m^{-2} hr^{-1}) in the reference plots (except for block 1), whereas net CH_4 uptake (~ -6.5 µg
236 C m^{-2} hr^{-1}) was observed in all P- treated plots (Fig. 6). The suppressing effect of P addition on
237 CH_4 emission was significant in blocks 2 and 3, similar to what was found for NO_3^-
238 concentrations and N_2O fluxes.

239 **3.3 The effect of P addition on tree growth**

240 Throughout the 2-year experimental period, we observed no change in tree biomass (138 t ha^{-1})
241 in response to P addition (Table S3). Likewise, there was no effect of P treatment on the 500-
242 needle weight (13 g on average). Between the two samplings in 2013 and 2014, we found
243 differences in chemical composition of the pine needles, but this effect was not linked to P
244 addition. Also, the C/N and N/P ratios of the needles (40 and 16, respectively) were hardly
245 affected by P addition. Monthly litterfall varied seasonally (Fig. S7), but no significant difference
246 was found between the reference and the P treated plots.

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4 Discussion

Background N_2O emission rates in the reference plots were relatively large, with mean values of 40 to 120 μg N m^{-2} hr^{-1} (Fig. 3). This is within the range previously reported for well-drained hillslope soils at TSP (Zhu et al., 2013b), but greater than the rates reported for other forests in South China. For instance, N_2O emission rates averaged 37 μg N m^{-2} hr^{-1} in unmanaged sites at Dinghushan (Fang et al., 2009; Tang et al., 2006) and up to 50 μg N m^{-2} hr^{-1} in N-fertilized sites (Zhang et al., 2008a). TSP reference plots emitted on average 5.3 kg N ha^{-1} yr^{-1} (Fig. 4), which is about 10% of the annual N deposition (50 kg ha^{-1} yr^{-1}) (Huang et al., 2015). These fluxes were well above average fluxes reported for tropical rainforests (Werner et al., 2007). High N_2O emissions at TSP are likely due to the large N deposition rates (Huang et al. 2015), as suggested by the similar trends indicated by data from a wide range of ecosystems (Liu et al., 2009). Also, warm-humid conditions during monsoonal summers may stimulate N_2O emissions (Ju et al., 2011), as monsoonal rainstorms triggered peak fluxes (Fig. S5a) (Pan et al., 2003). The positive correlation between precipitation and N_2O emission peaks may indicate the importance of denitrification as the dominant N_2O source. This is supported by recent ^{15}N tracing experiments at TSP (Zhu et al., 2013a; Yu et al., submitted).

Addition of P caused a significant decline in soil mineral N (predominantly NO_3^-) in two of three blocks (Fig. 2), particularly during summers, when NO_3^- concentrations were relatively high (Fig. S2). At the same time, annual N_2O emissions decreased by more than 50% (Figs. 3 and 4). These findings are consistent with a number of previous studies (Baral et al., 2014; Hall and Matson, 1999; Mori et al., 2014). The reduction of N_2O emissions in P treated soils was attributed to decreased mineral N content, most likely due to stimulated plant uptake and/or microbial

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assimilation. It is noteworthy, however, that there was no significant correlation between N_2O emission rates and soil water NO_3^- concentration in our study (Figs. 2 and S2), suggesting that the suppressing effect of P on N_2O emissions was indirect, probably by affecting the competition for mineral N between plant roots and microbes (Zhu et al., 2016). In contrast to our study, P-addition experiments in South Ecuador (Martinson et al., 2013) and South China (at Dinghushan Biosphere Reserve (DHSBR); Zheng et al., 2016) found no effect of a single P addition on N_2O emission during the first two years after application. However, significant reduction in N_2O emission was observed after three to five years with continuous P addition, both at the Ecuadorian and the Chinese site (Chen et al., 2016; Müller et al., 2015). For the montane forest site in Ecuador, the observed delay in N_2O emission response to P addition may be explained by the moderate amount of P added (10 kg P ha^{-1} yr^{-1}; Martinson et al., 2013). Moreover, the experiments were conducted in a forest with low ambient N deposition (~ 10 kg N ha^{-1} yr^{-1}) and N_2O fluxes (~ 0.36 kg N ha^{-1} yr^{-1} in the reference plot) (Martinson et al., 2013; Müller et al., 2015). By contrast, the DHSBR site in South China receives 36 kg of atmogenic N ha^{-1} yr^{-1}, which is only slightly smaller than the N deposition at our site (Huang et al., 2015), and showed larger N_2O emission rate than the Ecuadorian site (~ 0.88 kg N ha^{-1} yr^{-1} in the reference plot; Zheng et al., 2016). However, forests do not always display a straightforward relationship between N deposition and N_2O emissions. Manipulation experiments in the European NITREX project, for instance, revealed a much stronger correlation of N_2O emissions with soil NO_3^- leaching than with N deposition (Gundersen et al., 2012). Indeed, KCl-extractable mineral N at the DHSBR site (~ 40 mg kg^{-1}; Zheng et al., 2016) were several-fold smaller than at our site (> 100 mg kg^{-1}; Zhu et al., 2013b), indicating that DHSBR is less N-saturated than TSP. This suggests that the response of N_2O emission to P addition might depend on the N status of the soil.

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The fact that numerous studies found apparent suppression of N_2O emission in short-term experiments (< 2 years) in N + P treatments, but not in treatments with P alone, supports this idea (Müller et al., 2015; Zhang et al., 2014b; Zheng et al., 2016).

Other studies have observed increased N_2O emissions upon P addition (Mori et al., 2013c; Wang et al., 2014). In an *Acacia mangium* plantation, fertilized with P, Mori et al. (2013b&c) found that N_2O emissions were stimulated in the short-term but reduced in the long-term. While suppression of N_2O emission by P has been attributed to increased plant N uptake (Mori et al., 2014), increased N_2O emission are generally explained by enhanced microbial biomass (Liu et al., 2012) and denitrification activities (Ehlers et al., 2010; He and Dijkstra, 2015). N_2O emissions measured frequently after P addition at our site in May 2014 were not different from fluxes in untreated reference plots (Fig. S5). This may indicate that plant uptake at TSP is more important for the effect of P addition on N_2O emissions than changes in microbial activity, which are expected to occur more rapidly.

Two of three reference plots at TSP showed net CH_4 emission for extended periods of the year (Figs. 5 and 6). Also, long-term CH_4 fluxes sampled between 2012 and 2014 on TSP hillslopes near-by (Fig. S8; Zhu et al., unpublished data) showed net CH_4 emission. This is in contrast to the generally reported CH_4 sink function of forested upland soils (Ciais et al., 2013; Dutaur and Verchot, 2007). For example, CH_4 uptake rates reported for South Chinese forest soils range from 30 to 60 μg C m^{-2} hr^{-1} (Fang et al., 2009; Tang et al., 2006; Zhang et al., 2014a). As CH_4 fluxes at our sites were not correlated with climatic factors (Fig. S5c and d), CH_4 emissions cannot be explained by transiently wet conditions. One reason for the net-CH_4 emission observed at TSP could be inhibition of CH_4 oxidation activity by NH_4^+, as reported previously (Bodelier and Laanbroek, 2004; Zhang et al., 2014a). The concentration of NH_4^+ in the soil water was

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315 rather small (< 0.5 g L^{-1}; Fig. S3), which does not preclude, however, that NH_4^+ availability from
316 the soil exchangeable pool is high. Zhu et al. (2013b) found extraordinarily high KCL-
317 extractable NH_4^+ in TSP surface soils, likely reflecting the large atmogenic NH_4^+ input at our site
318 (Huang et al., 2015).

319 P addition had a significant impact on CH_4 fluxes, changing the soil from a net source to a net
320 sink on an annual basis (Fig. 6). However, the uptake rates of CH_4 in the P treatments remained
321 smaller than those reported for forest soils in tropical China (Tang et al., 2006; Zhang et al.,
322 2008b). The stimulating effect of P addition on CH_4 uptake is consistent with previous studies
323 (Mori et al., 2013a, 2013b; Zhang et al., 2011), and has been attributed to lessening the NH_4^+
324 inhibition of methane oxidation. Unfortunately, we did not measure KCl-extractable NH_4^+ in our
325 study, but a decline of available NH_4^+, which is the substrate for nitrification, is likely as NO_3^-
326 concentrations in soil water were significantly smaller with in the P-treatments (Fig. 2). P
327 addition may also result in a change of the taxonomic composition of the methane oxidizing
328 community (Mori et al., 2013a; Veraart et al., 2015). Alternatively, CH_4 oxidation may be
329 stimulated by increased CH_4 diffusion into the soil, due to enhanced root growth and increased
330 transpiration in P-amended plots (Zhang et al., 2011). Given the high degree of N saturation of
331 TSP forest (Huang et al., 2015), it is likely that the reason for the observed reduction in CH_4
332 emissions in response to P fertilization was due to alleviating the NH_4^+ inhibition of the methane
333 monooxygenase enzyme (Veldkamp et al., 2013), rather than a direct P-stimulation of
334 methanotrophic activity (Veraart et al., 2015).

335 Shortly after fertilizer application, we observed a modest, albeit significant increase of Na^+
336 concentration in soil water (Table S2). Other studies have documented the toxicity of excess Na^+
337 in soil water to plant and microbial activities (Rengasamy et al., 2003; Wong et al., 2008).

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However, the occurrence of Na^+ toxicity at the treated plots, affecting N turnover processes, is unlikely, as Na^+ concentrations in soil water, within one month after application (Table S2), did not exceed 5 mg L^{-1}, far below the values commonly assumed to indicate the toxicity threshold (40 to 100 mg L^{-1}) (Bernstein 1975). The frequent precipitation in the humid forest of this study (Yu et al., 2016), both prior and following the addition of $NaH_2PO_4.2H_2O$ (Fig. 2), efficiently diluted and leached Na^+, thus minimizing toxic effects.

P application significantly increased plant-available P in the P-limited TSP soil (Table 2). Meanwhile, concentrations of leachable base cations (K^+, Mg^{2+}, Ca^{2+}) in soil water decreased (Fig. S4), as expected from the reduction of NO_3^- concentrations in the P-treatments (Mochoge and Beese, 1986). We observed no sign of stimulated forest growth or increased N uptake by plants within the relatively short period of our study (Table S3 and Fig. S7), which makes it difficult to link the observed reduction in mineral N in the soil solution to plant growth (Fig. 2). When interpreting the observed P effect on NO_3^- concentrations in soil water, several aspects need to be considered. Firstly, two years of observation may be too short to detect any significant NO_3^- uptake by plants, given the commonly large variabilities in tree biomass estimates (Alvarez-Clare et al., 2013; Huang et al., 2015). Secondly, a significant proportion of the added P, and of excess N, may have been assimilated by the understory biomass, which was not assessed in this study. Previously, understory vegetation has been reported to quickly respond to P addition (Fraterrigo et al., 2011). Thirdly, as long-term N saturation and acidification at TSP has reduced the forest health (Lu et al., 2010; Wang et al., 2007), we may not expect immediate response of forest growth to P addition. Large needle N/P ratios (17-22, Table S3) indicated that P limitation for tree growth was not relieved 1.5 years after P addition (Li et al., 2016). Therefore,

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360 enhanced N uptake by understory growth and/or soil microbial biomass may have been the main
361 mechanisms responsible for observed NO_3^- decline in the P-treated soil (Hall & Matson 1999).

362 Overall, our study demonstrates that chronically high N deposition has transformed TSP soils to
363 a regional hotspot for N_2O and CH_4 emission. Within the short experimental period of 1.5 years,
364 P fertilization was shown to significantly decrease NO_3^- concentrations in soil water and to
365 reduce both N_2O and CH_4 emissions. These findings provide a promising starting point for
366 improving forest management towards GHG abatement targets, taking into account the P and N
367 status of subtropical soils in the region.

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Biogeosciences Discuss., doi:10.5194/bg-2016-470, 2016
Manuscript under review for journal Biogeosciences
Published: 3 November 2016

Reference

Alvarez-Clare, S., Mack, M. C. and Brooks, M.: A direct test of nitrogen and phosphorus limitation to net primary productivity in a lowland tropical wet forest, Ecology, 94(7), 1540–1551, 2013.

Anon: World Reference Base for Soil Resources 2014, FAO, Rome., 2014.

Aronson, E. L. and Helliker, B. R.: Methane flux in non-wetland soils in response to nitrogen addition: A meta-analysis, Ecology, 91(11), 3242–3251, doi:10.1890/09-2185.1, 2010.

Bernstein, L.: Effects of salinity and sodicity on plant growth, Annual Review of Phytopathology, 13, 295-312, 1975.

Baral, B. R., Kuyper, T. W. and Van Groenigen, J. W.: Liebig's law of the minimum applied to a greenhouse gas: Alleviation of P-limitation reduces soil N_2O emission, Plant Soil, 374(1–2), 539–548, doi:10.1007/s11104-013-1913-8, 2014.

Bodelier, P. L. E. and Laanbroek, H. J.: Nitrogen as a regulatory factor of methane oxidation in soils and sediments, FEMS Microbiol. Ecol., 47(3), 265–277, doi:10.1016/S0168-6496(03)00304-0, 2004.

Chen, H., Gurmesa, G. A., Zhang, W., Zhu, X., Zheng, M., Mao, Q., Zhang, T. and Mo, J.: Nitrogen saturation in humid tropical forests after 6 years of nitrogen and phosphorus addition: Hypothesis testing, Funct. Ecol., 30(2), 305–313, doi:10.1111/1365-2435.12475, 2016.

Chen, X. and Mulder, J.: Indicators for nitrogen status and leaching in subtropical forest ecosystems, South China, Biogeochemistry, 82(2), 165–180, doi:10.1007/s10533-006-9061-3, 2007.

Ciais, P., Sabine, C., Bala, G., Bopp, L., Brovkin, V., Canadell, J., Chhabra, A., DeFries, R., Galloway, J., Heimann, M., Jones, C., Quéré, C. Le, Myneni, R. B., Piao, S. and Thornton, P.: Carbon and Other Biogeochemical Cycles. In: Climate Change 2013: The Physical Science Basis., edited by T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. B. and P. M. M. Stocker, Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA., 2013.

Cui, S., Shi, Y., Groffman, P. M., Schlesinger, W. H. and Zhu, Y.-G.: Centennial-scale analysis of the creation and fate of reactive nitrogen in China (1910-2010), Proc. Natl. Acad. Sci. U. S. A., 110(6), 2052–7, doi:10.1073/pnas.1221638110, 2013.

Du, E., de Vries, W., Han, W., Liu, X., Yan, Z. and Jiang, Y.: Imbalanced phosphorus and nitrogen deposition in China's forests, Atmos. Chem. Phys., (16), 8571–8579, doi:10.5194/acp-2015-984, 2016.

Dutaur, L. and Verchot, L. V.: A global inventory of the soil CH_4 sink, Global Biogeochem. Cycles, 21(4), 1–9, doi:10.1029/2006GB002734, 2007.

Ehlers, K., Bakken, L. R., Frostegård, Å., Frossard, E. and Bünemann, E. K.: Phosphorus limitation in a Ferralsol: Impact on microbial activity and cell internal P pools, Soil Biol.

412 Biochem., 42, 558–566, doi:10.1016/j.soilbio.2009.11.025, 2010.

413 Fang, Y., Gundersen, P., Zhang, W., Zhou, G., Christiansen, J. R., Mo, J., Dong, S. and Zhang,
414 T.: Soil–atmosphere exchange of N_2O, CO_2 and CH_4 along a slope of an evergreen broad-leaved
415 forest in southern China, Plant Soil, 319(1–2), 37–48, doi:10.1007/s11104-008-9847-2, 2009.

416 Fraterrigo, J. M., Strickland, M. S., Keiser, A. D. and Bradford, M. A.: Nitrogen uptake and
417 preference in a forest understory following invasion by an exotic grass, Oecologia, 167(3), 781–
418 791, doi:10.1007/s00442-011-2030-0, 2011.

419 Gundersen, P., Christiansen, J. R., Alberti, G., Brüggemann, N., Castaldi, S., Gasche, R., Kitzler,
420 B., Klemedtsson, L., Lobo-Do-Vale, R., Moldan, F., Rütting, T., Schleppi, P., Weslien, P. and
421 Zechmeister-Boltenstern, S.: The response of methane and nitrous oxide fluxes to forest change
422 in Europe, Biogeosciences, 9(10), 3999–4012, doi:10.5194/bg-9-3999-2012, 2012.

423 Hall, S. J. and Matson, P. A.: Nitrogen oxide emissions after nitrogen additions in tropical forests,
424 Nature, 400(July), 152, doi:10.1038/22094, 1999.

425 Hartmann, D. J., Klein Tank, A. M. G., Rusticucci, M., Alexander, L. V, Brönnimann, S.,
426 Charabi, Y. A.-R., Dentener, F. J., Dlugokencky, E. J., Easterling, D. R., Kaplan, A., Soden, B. J.,
427 Thorne, P. W., Wild, M. and Zhai, P.: Observations: Atmosphere and Surface, In: Climate
428 Change 2013: The Physical Science Basis., edited by T.F., D. Qin, G.-K. Plattner, M. Tignor,
429 S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. B. and P. M. M. Stocker, Cambridge University
430 Press, Cambridge, United Kingdom and New York, NY, USA., 2013.

431 He, M. and Dijkstra, F. A.: Phosphorus addition enhances loss of nitrogen in a phosphorus-poor
432 soil, Soil Biol. Biochem., 82, 99–106, doi:10.1016/j.soilbio.2014.12.015, 2015.

433 Hu, H. W., Chen, D. and He, J. Z.: Microbial regulation of terrestrial nitrous oxide formation:
434 Understanding the biological pathways for prediction of emission rates, FEMS Microbiol. Rev.,
435 39(5), 729–749, doi:10.1093/femsre/fuv021, 2015.

436 Huang, Y., Kang, R., Mulder, J., Zhang, T. and Duan, L.: Nitrogen saturation, soil acidification,
437 and ecological effects in a subtropical pine forest on acid soil in southwest China, J. Geophys.
438 Res. Biogeosciences, 120, 2457–2472, doi:10.1002/2015JG003048.Received, 2015.

439 Ju, X., Lu, X., Gao, Z., Chen, X., Su, F., Kogge, M., Römheld, V., Christie, P. and Zhang, F.:
440 Processes and factors controlling N_2O production in an intensively managed low carbon
441 calcareous soil under sub-humid monsoon conditions, Environ. Pollut., 159(4), 1007–1016,
442 doi:10.1016/j.envpol.2010.10.040, 2011.

443 Larssen, T., Duan, L. and Mulder, J.: Deposition and leaching of sulfur, nitrogen and calcium in
444 four forested catchments in China: implications for acidification, Environ. Sci. Technol., 45(4),
445 1192–8, doi:10.1021/es103426p, 2011.

446 Li, Y., Niu, S. and Yu, G.: Aggravated phosphorus limitation on biomass production under
447 increasing nitrogen loading: A meta-analysis, Glob. Chang. Biol., 22(2), 934–943,
448 doi:10.1111/gcb.13125, 2016.

449 Li, Z., Wang, Y., Liu, Y., Guo, H., Li, T., Li, Z. H. and Shi, G.: Long-term effects of liming on
450 health and growth of a Masson pine stand damaged by soil acidification in Chongqing, China,
451 PLoS One, 9(4), 1–9, doi:10.1371/journal.pone.0094230, 2014.

452 Li, Z., Yu, P., Y., W., Li, Z., Y., W. and Du, A.: Charcters of Litter-Fall in Damaged Pinus
453 massoniana Forests and Its Responses to Environmental Factors in the Acid Rain Region of
454 Chongqing, China, Sci. Silvae Sin., 47(8), 19–24, 2011.

455 Liu, L. and Greaver, T. L.: A review of nitrogen enrichment effects on three biogenic GHGs:
456 The CO_2 sink may be largely offset by stimulated N_2O and CH_4 emission, Ecol. Lett., 12(10),
457 1103–1117, doi:10.1111/j.1461-0248.2009.01351.x, 2009.

458 Liu, L., Gundersen, P., Zhang, T. and Mo, J.: Effects of phosphorus addition on soil microbial
459 biomass and community composition in three forest types in tropical China, Soil Biol. Biochem.,
460 44(1), 31–38, doi:10.1016/j.soilbio.2011.08.017, 2012.

461 Lu, X., Mo, J., Gilliam, F. S., Zhou, G. and Fang, Y.: Effects of experimental nitrogen additions
462 on plant diversity in an old-growth tropical forest, Glob. Chang. Biol., 16(10), 2688–2700,
463 doi:10.1111/j.1365-2486.2010.02174.x, 2010.

464 Martinson, G. O., Corre, M. D. and Veldkamp, E.: Responses of nitrous oxide fluxes and soil
465 nitrogen cycling to nutrient additions in montane forests along an elevation gradient in southern
466 Ecuador, Biogeochemistry, 112(1–3), 625–636, doi:10.1007/s10533-012-9753-9, 2013.

467 Le Mer, J. and Roger, P.: Production, oxidation, emission and consumption of methane by soils:
468 A review, Eur. J. Soil Biol., 37(2001), 2010.

469 Mo, J., Li, D. and Gundersen, P.: Seedling growth response of two tropical tree species to
470 nitrogen deposition in southern China, Eur. J. For. Res., 127(4), 275–283, doi:10.1007/s10342-
471 008-0203-0, 2008.

472 Mochoge, B. O. and Beese, F.: Leaching of plant nutrients from an acid forest soil after nitrogen
473 fertilizer application Audies on the leaching o f plant nutrients from soils after nitrogen, 91, 17–
474 29, 1986.

475 Montzka, S. A., Dlugokencky, E. J. and Butler, J. H.: Non-CO_2 greenhouse gases and climate
476 change, Nature, 476(7358), 43–50, doi:10.1038/nature10322, 2011.

477 Mori, T., Ohta, S., Ishizuka, S., Konda, R., Wicaksono, A. and Heriyanto, J.: Effects of
478 phosphorus application on CH_4 fluxes in an Acacia mangium plantation with and without root
479 exclusion, Tropics, 22(1), 13–17, 2013a.

480 Mori, T., Ohta, S., Ishizuka, S., Konda, R., Wicaksono, A. and Heriyanto, J.: Phosphorus
481 application reduces N_2O emissions from tropical leguminous plantation soil when phosphorus
482 uptake is occurring, Biol. Fertil. Soils, 50(1), 45–51, doi:10.1007/s00374-013-0824-4, 2014.

483 Mori, T., Ohta, S., Ishizuka, S., Konda, R., Wicaksono, A., Heriyanto, J., Hamotani, Y., Gobara,
484 Y., Kawabata, C., Kuwashima, K., Nakayama, Y. and Hardjono, A.: Soil greenhouse gas fluxes
485 and C stocks as affected by phosphorus addition in a newly established Acacia mangium
486 plantation in Indonesia, For. Ecol. Manage., 310, 643–651, doi:10.1016/j.foreco.2013.08.010,
487 2013b.

488 Mori, T., Ohta, S., Ishizuka, S., Konda, R., Wicaksono, A., Heriyanto, J. and Hardjono, A.:
489 Effects of phosphorus addition with and without ammonium, nitrate, or glucose on N_2O and NO
490 emissions from soil sampled under Acacia mangium plantation and incubated at 100 % of the
491 water-filled pore space, Biol. Fertil. Soils, 49(1), 13–21, doi:10.1007/s00374-012-0690-5, 2013c.

492 Müller, A. K., Matson, A. L., Corre, M. D. and Veldkamp, E.: Soil N_2O fluxes along an
493 elevation gradient of tropical montane forests under experimental nitrogen and phosphorus
494 addition, Front. Earth Sci., 3(October), 1–12, doi:10.3389/feart.2015.00066, 2015.

495 Murphy, J. and Riley, J. P.: A modified single method for the determination of phosphate in
496 natural waters, Anal. Chim. Acta, 27(27), 31–36, doi:10.1016/S0003-2670(00)88444-5, 1962.

497 Pan, F., Peters-Lidard, C. D. and Sale, M. J.: An analytical method for predicting surface soil
498 moisture from rainfall observations, Water Resour. Res., 39(11), 1314,
499 doi:10.1029/2003WR002142, 2003.

500 Rengasamy, P., Chittleborough D. and Helyar K.: Root-zone constraints and plant-based
501 solutions for dryland salinity, Plant and Soil, 257, 249-260, 2003.

502 Shi, Y., Cui, S., Ju, X., Cai, Z. and Zhu, Y.: Impacts of reactive nitrogen on climate change in
503 China, Sci. Rep., 5, 8118, doi:10.1038/srep08118, 2015.

504 Singh, B. R., Krogstad, T., Shivay, Y. S., Shivakumar, B. G. and Bakkegard, M.: Phosphorus
505 fractionation and sorption in P-enriched soils of Norway, Nutr. Cycl. Agroecosystems, 73(2–3),
506 245–256, doi:10.1007/s10705-005-2650-z, 2005.

507 Smith, K. a., Ball, T., Conen, F., Dobbie, K. E., Massheder, J. and Rey, A.: Exchange of
508 greenhousegases between soil and atmosphere: interactions of soil physical factors and
509 biological processes, Eur. J. Soil Sci., 54(December), 779–791, doi:10.1046/j.1365-
510 2389.2003.00567.x, 2003.

511 Tang, X., Liu, S., Zhou, G., Zhang, D. and Zhou, C.: Soil-atmospheric exchange of CO_2, CH_4,
512 and N_2O in three subtropical forest ecosystems in southern China, Glob. Chang. Biol., 12(3),
513 546–560, doi:10.1111/j.1365-2486.2006.01109.x, 2006.

514 Tian, H., Lu, C., Ciais, P., Michalak, A. M., Canadell, J. G., Saikawa, E., Huntzinger, D. N.,
515 Gurney, K. R., Sitch, S., Zhang, B., Yang, J., Bousquet, P., Bruhwiler, L., Chen, G.,
516 Dlugokencky, E., Friedlingstein, P., Melillo, J., Pan, S., Poulter, B., Prinn, R., Saunois, M.,
517 Schwalm, C. R. and Wofsy, S. C.: The terrestrial biosphere as a net source of greenhouse gases
518 to the atmosphere, Nature, 531(7593), 225–228, doi:10.1038/nature16946, 2016.

519 Tian, H., Xu, X., Lu, C., Liu, M., Ren, W., Chen, G., Melillo, J. and Liu, J.: Net exchanges of
520 CO_2, CH_4, and N_2O between China's terrestrial ecosystems and the atmosphere and their
521 contributions to global climate warming, J. Geophys. Res. Biogeosciences, 116(2), 1–13,
522 doi:10.1029/2010JG001393, 2011.

523 Veldkamp, E., Koehler, B. and Corre, M. D.: Indications of nitrogen-limited methane uptake in
524 tropical forest soils, Biogeosciences, 10(8), 5367–5379, doi:10.5194/bg-10-5367-2013, 2013.

525 Veraart, A. J., Steenbergh, A. K., Ho, A., Kim, S. Y. and Bodelier, P. L. E.: Beyond nitrogen:
526 The importance of phosphorus for CH_4 oxidation in soils and sediments, Geoderma, 259–260,
527 337–346, doi:10.1016/j.geoderma.2015.03.025, 2015.

528 Wang, F., Li, J., Wang, X., Zhang, W., Zou, B., Neher, D. a and Li, Z.: Nitrogen and phosphorus
529 addition impact soil N_2O emission in a secondary tropical forest of South China., Sci. Rep., 4,
530 5615, doi:10.1038/srep05615, 2014.

531 Wang, Y., Solberg, S., Yu, P., Myking, T., Vogt, R. D. and Du, S.: Assessments of tree crown
532 condition of two Masson pine forests in the acid rain region in south China, For. Ecol. Manage.,
533 242(2–3), 530–540, doi:10.1016/j.foreco.2007.01.065, 2007.

534 Werner, C., Butterbach-Bahl, K., Haas, E., Hickler, T. and Kiese, R.: A global inventory of N_2O
535 emissions from tropical rainforest soils using a detailed biogeochemical model, Global
536 Biogeochem. Cycles, 21(3), doi:10.1029/2006GB002909, 2007.

537 Wong, V.N.L., Dalal, R.C., Greene, R.S.B.: Salinity and sodicity effects on respiration and
538 microbial biomass of soil, Biology and Fertility of Soils, 44, 943-953, 2008.

539 Xu, W., Luo, X. S., Pan, Y. P., Zhang, L., Tang, A. H., Shen, J. L., Zhang, Y., Li, K. H., Wu, Q.
540 H., Yang, D. W., Zhang, Y. Y., Xue, J., Li, W. Q., Li, Q. Q., Tang, L., Lu, S. H., Liang, T., Tong,
541 Y. A., Liu, P., Zhang, Q., Xiong, Z. Q., Shi, X. J., Wu, L. H., Shi, W. Q., Tian, K., Zhong, X. H.,
542 Shi, K., Tang, Q. Y., Zhang, L. J., Huang, J. L., He, C. E., Kuang, F. H., Zhu, B., Liu, H., Jin, X.,
543 Xin, Y. J., Shi, X. K., Du, E. Z., Dore, A. J., Tang, S., Collett, J. L., Goulding, K., Sun, Y. X.,
544 Ren, J., Zhang, F. S. and Liu, X. J.: Quantifying atmospheric nitrogen deposition through a
545 nationwide monitoring network across China, Atmos. Chem. Phys., 15(21), 12345–12360,
546 doi:10.5194/acp-15-12345-2015, 2015.

547 Yu, L., Zhu, J., Mulder, J. and Dörsch, P.: Multiyear dual nitrate isotope signatures suggest that
548 N-saturated subtropical forested catchments can act as robust N sinks, Glob. Chang. Biol., In
549 Press, doi:10.1111/gcb.13333, 2016.

550 Zeng, L., Wang, P., Xiao, W., Wan, R., Huang, Z. and Pan, L.: Allocation of Biomass and
551 Productivity of Main Vegetations in Three Gorges Reservoir Region, Sci. Silvae Sin., 44(8), 16–
552 22, 2008.

553 Zhang, T., Zhu, W., Mo, J., Liu, L. and Dong, S.: Increased phosphorus availability mitigates the
554 inhibition of nitrogen deposition on CH 4 uptake in an old-growth tropical forest, southern China,
555 Biogeosciences, 8(9), 2805–2813, doi:10.5194/bg-8-2805-2011, 2011.

556 Zhang, W., Mo, J., Yu, G., Fang, Y., Li, D., Lu, X. and Wang, H.: Emissions of nitrous oxide
557 from three tropical forests in Southern China in response to simulated nitrogen deposition, Plant
558 Soil, 306(1–2), 221–236, doi:10.1007/s11104-008-9575-7, 2008a.

559 Zhang, W., Mo, J., Zhou, G., Gundersen, P., Fang, Y., Lu, X., Zhang, T. and Dong, S.: Methane
560 uptake responses to nitrogen deposition in three tropical forests in southern China, J. Geophys.
561 Res. Atmos., 113(11), 1–10, doi:10.1029/2007JD009195, 2008b.

562 Zhang, W., Wang, K., Luo, Y., Fang, Y., Yan, J., Zhang, T., Zhu, X., Chen, H., Wang, W. and
563 Mo, J.: Methane uptake in forest soils along an urban-to-rural gradient in Pearl River Delta,
564 South China., Sci. Rep., 4, 5120, doi:10.1038/srep05120, 2014a.

565 Zhang, W., Zhu, X., Luo, Y., Rafique, R., Chen, H., Huang, J. and Mo, J.: Responses of nitrous
566 oxide emissions to nitrogen and phosphorus additions in two tropical plantations with N-fixing
567 vs. non-N-fixing tree species, Biogeosciences Discuss., 11(1), 1413–1442, doi:10.5194/bgd-11-
568 1413-2014, 2014b.

569 Zheng, M., Zhang, T., Liu, L., Zhu, W., Zhang, W. and Mo, J.: Effects of nitrogen and
570 phosphorus additions on nitrous oxide emission in a nitrogen-rich and two nitrogen-limited

571 tropical forests, Biogeosciences, 13, 3503–3517, doi:10.5194/bg-2015-552, 2016.

572 Zhu, Q., Riley, W.J., Tang, J., Koven, C.D.: Multiple soil nutrient competition between plants,
573 microbes, and mineral surfaces: model development, parameterization, and example applications
574 in several tropical forests, Biogeosciences 13, 341-36, doi:10.5194/bg-13-341-2016, 2016.

575 Zhu, J., Mulder, J., Bakken, L. and Dörsch, P.: The importance of denitrification for N_2O
576 emissions from an N-saturated forest in SW China: results from in situ ^{15}N labeling experiments,
577 Biogeochemistry, 116(1–3), 103–117, doi:10.1007/s10533-013-9883-8, 2013a.

578 Zhu, J., Mulder, J., Wu, L. P., Meng, X. X., Wang, Y. H. and Dörsch, P.: Spatial and temporal
579 variability of N_2O emissions in a subtropical forest catchment in China, Biogeosciences, 10(3),
580 1309–1321, doi:10.5194/bg-10-1309-2013, 2013b.

581 Zhuang, Q., Lu, Y. and Chen, M.: An inventory of global N_2O emissions from the soils of
582 natural terrestrial ecosystems, Atmos. Environ., 47, 66–75, doi:10.1016/j.atmosenv.2011.11.036,
583 2012.

Biogeosciences Discuss., doi:10.5194/bg-2016-470, 2016
Manuscript under review for journal Biogeosciences
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584 **Table 1** Background soil properties of the experimental plots at Tieshanping (TSP). Values are

	Soil Layer	pH	Total C	Total N	Total P	C/N	N/P
			g kg^{-1}	g kg^{-1}	mg kg^{-1}		
	O/A (0-3 cm)	3.7 (0.1)	80.7 (32.3)	4.8 (1.7)	308 (57)	17.0 (2.5)	15.5 (5.7)
Block 1	AB (3-8 cm)	3.8 (0.0)	23.9 (9.3)	1.3 (0.6)	-*	20.0 (3.0)	-
	B (8-20 cm)	3.9 (0.2)	8.6 (1.2)	< 0.05	-	-	-
	O/A (0-3 cm)	3.6 (0.1)	77.6 (13.4)	4.7 (0.8)	297 (44)	16.7 (1.3)	15.7 (2.8)
Block 2	AB (3-8 cm)	3.7 (0.1)	20.2 (5.3)	1.0 (0.3)	-	21.4 (3.3)	-
	B (8-20 cm)	3.9 (0.1)	7.1 (1.6)	< 0.05	-	-	-
	O/A (0-3 cm)	3.6 (0.1)	67.0 (15.5)	3.8 (0.8)	223 (45)	17.4 (0.6)	17.2 (3.7)
Block 3	AB (3-8 cm)	3.6 (0.1)	21.0 (7.9)	1.1 (0.5)	-	24.5 (4.6)	-
	B (8-20 cm)	3.8 (0.1)	7.2 (1.5)	< 0.05	-	-	-
	Soil Layer	P_{H2O}	P_{Al}	Al_{ox}	Fe_{ox}	P_{ox}	P_{ox} /
		mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	(Al_{ox} + Fe_{ox})
	O/A (0-3 cm)	< 5.0	5.8 (1.4)	1700 (513)	1933 (350)	85.8 (22.6)	0.025 (0.008)
Block 1	AB (3-8 cm)	< 5.0	2.1 (0.6)	1217 (243)	1692 (493)	47.1 (22.0)	0.016 (0.007)
	B (8-20 cm)	< 5.0	< 1.0	1083 (90)	1158 (249)	29.3 (28.6)	0.012 (0.011)
	O/A (0-3 cm)	< 5.0	5.9 (1.0)	1500 (238)	1792 (215)	79.2 (21.5)	0.024 (0.007)
Block 2	AB (3-8 cm)	< 5.0	1.6 (0.4)	925 (149)	1517 (320)	37.2 (10.7)	0.016 (0.006)
	B (8-20 cm)	< 5.0	< 1.0	892 (209)	1033 (413)	16.1 (10.5)	0.009 (0.007)
	O/A (0-3 cm)	< 5.0	4.1 (0.9)	1367 (180)	1667 (168)	50.7 (10.9)	0.017 (0.003)
Block 3	AB (3-8 cm)	< 5.0	4.4 (4.0)	1075 (128)	1350 (150)	24.8 (8.3)	0.010 (0.002)
	B (8-20 cm)	< 5.0	< 1.0	992 (130)	875 (138)	8.0 (2.0)	0.004 (0.001)

585 means and standard deviations in parenthesis (n = 6)†.

586

587 P_{H2O} = Water extractable P, P_{Al} = Ammonium extractable P,

588 Al_{ox} = Oxalate extractable Al, Fe_{ox} = Oxalate extractable Fe, P_{ox} = Oxalate extractable P.

589 † Soils were sampled in August 2013.

590 * Data not available

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591 **Table 2** Soil pH, C, N and P contents in the O/A horizon (0-3 cm) in Reference and phosphate (P)

		pH	Total C	Total N	C/N	P_{Al}	Total P
			g kg^{-1}	g kg^{-1}		mg kg^{-1}	mg kg^{-1}
13/08/02	Ref	3.7 (0.1)^{bc†}	8.3 (2.3)^{ab}	0.5 (0.1)^{bcd}	16.9 (1.1)^{bcd}	5.4 (1.4)^{c}	292 (46)^{bc}
	P	3.6 (0.1)^{c}	6.7 (2.0)^{b}	0.4 (0.1)^{bd}	17.1 (2.1)^{bc}	5.1 (1.3)^{c}	260 (70)^{c}
14/05/02	Ref	3.7 (0.1)^{abc}	12.2 (4.2)^{a}	0.9 (0.3)^{a}	13.7 (1.5)^{e}	19.0 (8.0)^{c}	336 (65)^{bc}
	P	3.8 (0.2)^{abc}	9.0 (3.5)^{ab}	0.7 (0.2)^{abc}	14.2 (2.8)^{de}	13.7 (5.2)^{c}	270 (72)^{bc}
14/05/10	Ref	3.8 (0.1)^{abc}	9.9 (2.1)^{ab}	0.7 (0.2)^{ab}	14.0 (0.7)^{e}	15.4 (7.0)^{c}	304 (49)^{bc}
	P	3.9 (0.3)^{ab}	8.0 (1.9)^{ab}	0.6 (0.1)^{bcd}	14.3 (1.3)^{cde}	174 (114)^{a}	572 (242)^{a}
14/12/02	Ref	3.8 (0.1)^{abc}	10.5 (3.6)^{ab}	0.7 (0.3)^{ab}	14.5 (1.3)^{cde}	14.2 (7.4)^{c}	328 (102)^{bc}
	P	3.9 (0.2)^{abc}	9.5 (2.1)^{ab}	0.7 (0.1)^{abc}	14.0 (0.8)^{e}	66 (24)^{ab}	442 (106)^{ab}
15/08/02	Ref	3.9 (0.2)^{ab}	8.3 (2.2)^{ab}	0.4 (0.1)^{cd}	20.5 (2.5)^{a}	13.4 (6.2)^{c}	291 (61)^{bc}
	P	4.0 (0.2)^{a}	6.5 (1.9)^{b}	0.3 (0.1)^{d}	19.7 (2.2)^{ab}	57 (36)^{ab}	383 (136)^{bc}

592 treatments. Values are means and standard deviations in parenthesis (n = 9).

593

594 $^{\theta}$ P addition was conducted on 14/05/04, after the first two sampling dates.

595 † Different letters indicate significance in difference.

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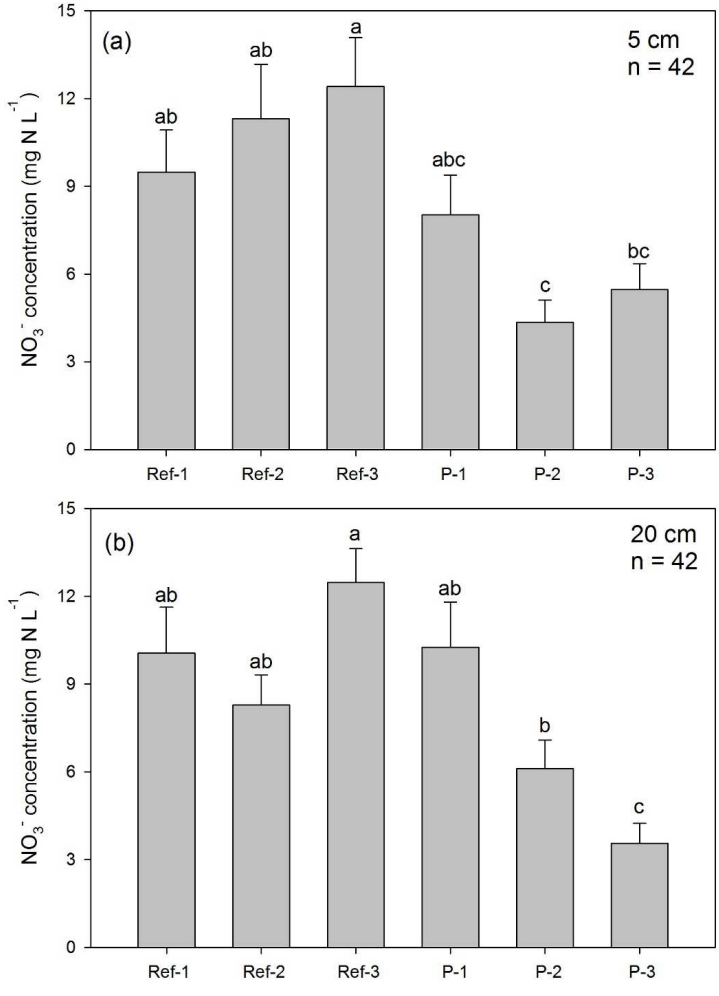

596

597 **Fig. 1** Mean NO_3^- concentrations in soil water at 5 (a) and 20 (b) cm depths from three blocks
598 with Reference and P treatment, 1.5 years after P addition; different letters indicate significant
599 differences between the treatments and blocks

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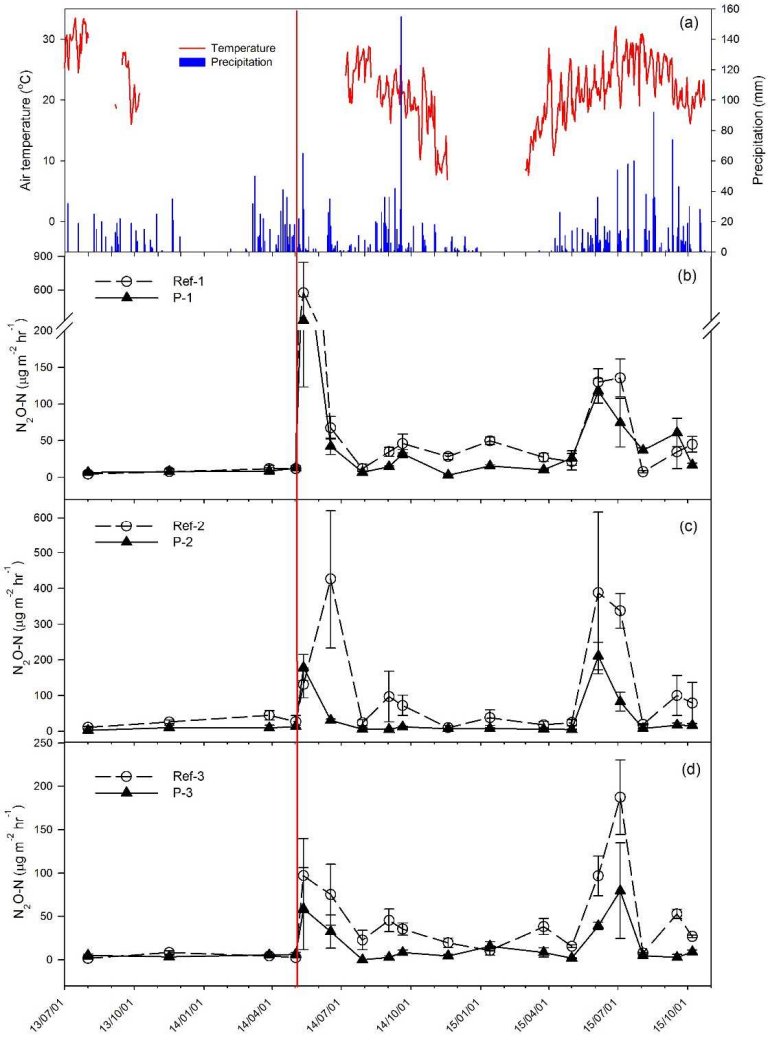

600

601 **Fig. 2** Daily mean air temperature and precipitation (a), and monthly mean N_2O fluxes in
602 Reference and P treatments in each of the three blocks (b-d); the red line gives the date of P
603 addition.

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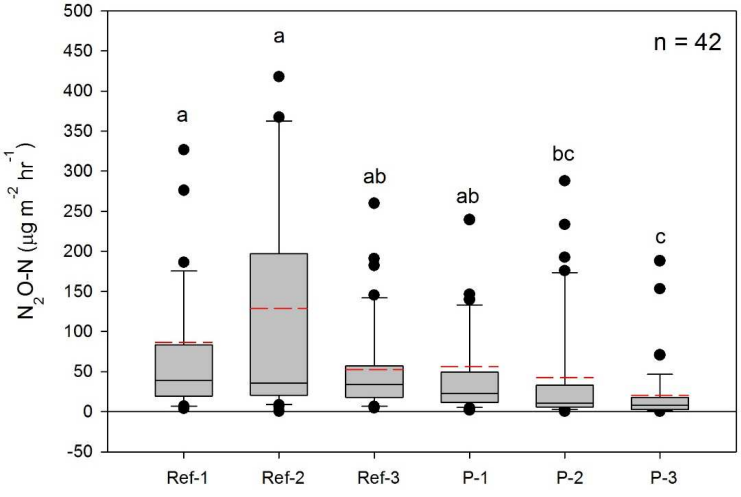

Fig. 3 Box whisker plots for N_2O fluxes in three blocks with Reference and P treatments throughout 1.5 years after the P addition; red dash lines indicate mean values; different letters indicate significant differences between treatments and blocks.

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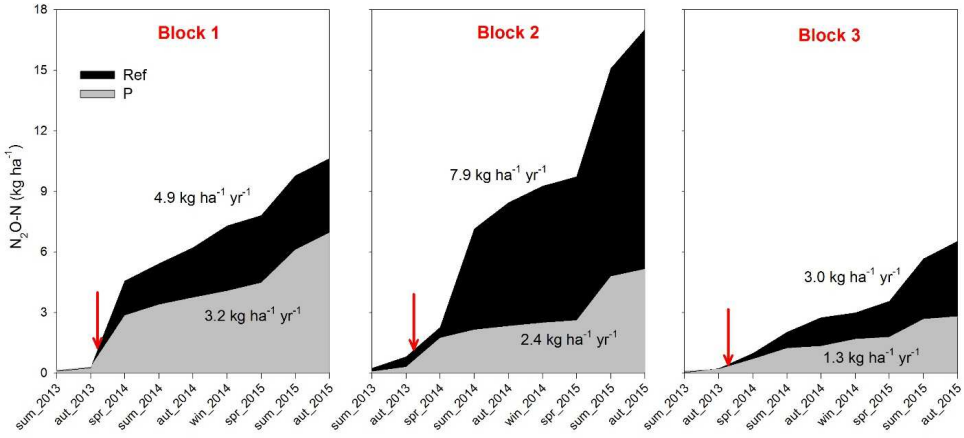

608

609 **Fig. 4** Cumulative N_2O emissions for three blocks with Reference and P treatments during two
610 years; the red arrows refer to the date when P addition was conducted.

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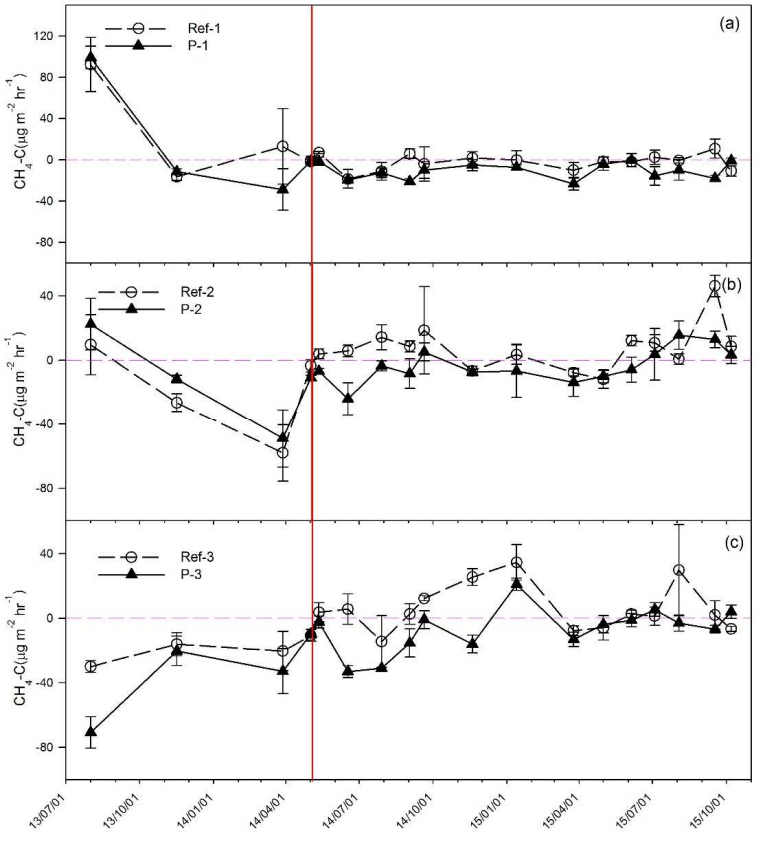

611

612 **Fig. 5** Monthly mean CH_4 fluxes for three blocks (a-c) with Reference and P treatments during
613 two years; the horizontal broken line indicates zero flux the red line refers to the date of P
614 addition.

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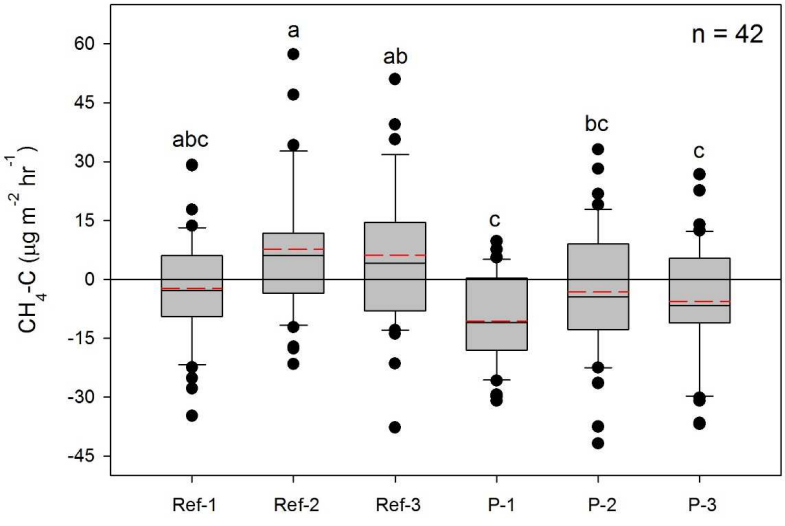

615

616 **Fig. 6** Box whisker plots of CH_4 fluxes for three blocks with Reference and P treatments 1.5
617 years after the P addition; red dash lines indicate mean values; the small letters indicate the
618 significance levels among the treatments and blocks.