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 greenhouse gas emissions remain elusive. Here, we report N_2O and CH_4 emissions together with soil N and P data for a period of 18 months following a single P addition (79 kg P ha^{-1}, as NaH_2PO_4 powder) to an N-saturated, Masson pine-dominated forest soil at TieShanPing (TSP), Chongqing, SW China. We observed a significant decline in both NO_3^- concentrations in soil water (5- and 20-cm depths) and in soil N_2O emissions, following P application. We hypothesize that enhanced N uptake by plants in response to P addition, resulted in less available NO_3^- for denitrification. By contrast to most other forest ecosystems, TSP is a net source of CH_4. P addition significantly decreased CH_4 emissions and turned the soil from a net source into a net sink. Based on our observation 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 and P stimulation of forest N uptake by understory vegetation remains to be confirmed. Our study indicates that P fertilization of N-saturated, subtropical forest soils may mitigate N_2O and CH_4 emissions, in addition to alleviating nutrient imbalances and reducing losses of N through NO_3^- leaching.

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

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 N_2O-N 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 frequent shifts between aerobic and anaerobic conditions in soils during monsoonal summers, promoting alternating nitrification and denitrification (Zhu et al., 2013b) and causing 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 N 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

limits its capability to retain N (Huang et al., 2015; Li et al., 2016), resulting in ample amounts

of inorganic N (NH_4^+ and NO_3^-) being present in the soil solution. Accordingly, Hall & Matson

(1999) observed larger N_2O emission in P-limited than in N-limited tropical forests one year

after repeated addition of N. Likewise, previous N manipulation studies in forests of South China

reported pronounced stimulation of N_2O emissions by N addition (Chen et al., 2016; Wang et al.,

2014; Zheng et al., 2016), supporting the idea that P limitation causes forests to be more

susceptible to N saturation and N_2O-N loss. In an N-limited tropical montane forest in southern

Ecuador, P addition alone (10 kg P ha^{-1} yr^{-1}) had no effect on N_2O emissions during the first two

years. However, N_2O emission was smaller when P was added together with N (50 kg N ha^{-1} yr^{-1})

than in treatments with N addition alone (Martinson et al., 2013). After continued fertilization for

three years, also P addition alone reduced N_2O emissions at these sites (Müller et al., 2015). In

tropical China, with high N deposition ($\sim$ 36 kg N ha^{-1} yr^{-1}; Mo et al., 2008), P addition (150 kg

P ha^{-1} yr^{-1}) to an old-growth forest revealed a similar pattern, with no initial effect on N_2O

emissions (0-2 years) but a significant longer term effect (3 to 5 years) on N_2O emissions (Chen

et al., 2016; Zheng et al., 2016). In a secondary tropical forests in South China, Wang et al.

(2014) found no effect on N_2O emissions of P alone (100 kg P ha^{-1} yr^{-1}), and in treatments

combining P with N (100 kg N ha^{-1} yr^{-1}), N_2O emissions increased during the wet season.

Meanwhile, they observed a significant increase in soil microbial biomass after P addition, which

is in line with previous findings in tropical forest soils of South China (Liu et al., 2012). Thus,

they attributed the stimulating effect of P addition on N_2O emissions to the larger nitrification

and denitrification potential of the increased soil microbial biomass. This was also proposed by

Mori et al. (2014), based on results from a short-term incubation study with P addition, excluding

plant roots.

78   As the sole biogenic sink for CH_4, upland soils play an important role in balancing terrestrial

79   CH_4 emissions (Ciais et al., 2013; Dutaur and Verchot, 2007). Atmospheric CH_4 uptake in soil is

80   mediated by the activity of methanotrophic bacteria, which oxidize CH_4 to CO_2 to gain energy

81   for growth. Well-drained forest and grassland soils are dominated by yet uncultured, high-

82   affinity methanotrophs residing in the upper soil layers (Le Mer and Roger, 2010). In addition to

83   edaphic factors (pH and nutrients), parameters affecting the diffusion of CH_4 into the soil (soil

84   structure, moisture, temperature) are believed to be major controllers for CH_4 uptake (Smith et

85   al., 2003). A number of studies have shown that excess N affects CH_4 fluxes in forest soils (Liu

86   and Greaver, 2009; Veldkamp et al., 2013; Zhang et al., 2008b). In general, N addition promotes

87   CH_4 uptake in N-limited soils by enhancing growth and activity of methanotrophs, whereas

88   excessive N input and N saturation inhibit CH_4 oxidation on an enzymatic level by substrate

89   competition between CH_4 and NH_4^+ (Aronson and Helliker, 2010; Bodelier and Laanbroek,

90   2004). P addition experiments in N-enriched soils have shown positive effects on CH_4 uptake

91   (Mori et al., 2013a; Zhang et al., 2011), but the underlying mechanisms, i.e. whether P addition

92   affects the methanotrophic community directly or alleviates the N-inhibition effect on CH_4

93   oxidation through enhanced N uptake (Mori et al., 2013b; Veraart et al., 2015), remain

94   unresolved.

95   Subtropical forests in South China show strong signs of N saturation, with exceedingly high

96   NO_3^- concentrations in soil water (Larssen et al., 2011; Zhu et al., 2013b). Little is known about

97   how P addition affects N cycling and N_2O emission in these acidic, nutrient-poor soils. Likewise,

98   the importance of increased inorganic N concentrations for soil-atmosphere exchange of CH_4,

99   and how this is affected by P fertilization remain to be elucidated for soils of the subtropics. Here,

100   we assessed N_2O and CH_4 fluxes in an N-saturated subtropical forest in SW China under ambient

101   N deposition and studied the effects of P addition on emission rates, nutrient availability and tree

102   growth. We hypothesized that i) P addition stimulates forest growth; ii) stimulated forest growth

103   results in increased N uptake by trees and understory vegetation, and thus decreases the soil

104   inorganic N concentration; iii) P addition reduces soil N_2O emission and promotes CH_4 uptake.

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). TSP has a monsoonal climate, with mean annual precipitation of 1028 mm, and a mean annual temperature of 18.2 ^{o}C (Chen and Mulder, 2007a). Most of the precipitation (> 70%) occurs during summer periods (April to September). Soils are predominantly well-drained, 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. The soil bulk density of the O/A horizon (~ 5 cm) is about 0.75 g cm^{-3}. Generally, soil water-filled pore space (10 cm) on the hillslopes ranges from 50 to 70% (mean ~ 60%; Zhu et al., 2013b). More details on soil properties are given in Table 1.

Annual inorganic N deposition at TSP measured in throughfall varies between 40 and 65 kg N ha^{-1} (dominated by NH_4^+; Yu et al., 2016), while the annual bulk N deposition is from 20 to 30 kg N ha^{-1} (Chen and Mulder, 2007b). According to regional data, annual P deposition via throughfall is < 0.40 kg ha^{-1} (Du et al., 2016). Strong soil acidification has been reported to cause severe decline in forest growth at TSP since 2001 (Li et al., 2014; Wang et al., 2007), and a decrease 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 at TSP

127 (Huang et al., 2015). The total P content in the O/A horizon is ~ 300 mg kg^{-1}, while ammonium

128 lactate-extractable P is smaller than 5 mg kg^{-1} (Table 1).

129 **2.2 Experimental Design**

130 Three blocks, each having two 20 m × 20 m plots, were established on well drained soils of a

131 gently sloping hillside. Adjacent plots were separated by at least 10-m buffer zone. In each block,

132 plots were randomly assigned to a Reference and a P treatment. On 4 May 2014, a single dose of

133 P fertilizer was applied as solid $NaH_2PO_4^{.}2H_2O$, at a rate of 79.5 kg P ha^{-1}. The amount of P

134 added was estimated from P adsorption isotherms (Supplementary Materials, Table S1 and

135 Figure S1), to ensure significantly increase in soil available P. To apply P fertilizer evenly, we

136 divided each plot into a 5 m * 5 m grid and broadcasted the powdered fertilizer by hand in each

137 grid cell. The P dose applied at TSP was intermediate as compared to the 10 kg P ha^{-1} yr^{-1}

138 applied by Müller et al. (2015) to a mountain forest in Ecuador and the 150 kg P ha^{-1} yr^{-1} applied

139 by Zheng et al. (2016) to a subtropical forest in South China.

140 The addition of $NaH_2PO_4^{.}2H_2O$ at the P-treated plots also resulted in an input of 59.0 kg ha^{-1} of

141 sodium (Na). One month after the fertilizer application, Na$^+$ concentrations in soil water of the P

142 treatments were about 5 mg L^{-1} at 5-cm depth and 3 mg L^{-1} at 20-cm depth (Table S2). Although

143 somewhat larger than in the Reference plots (0.52-1.31 mg L^{-1}), the Na$^+$ concentration in soil

144 water of the P treatments are unlikely to have exerted a significant negative impact on plant and

145 microbial activities.

146 **2.3 Sample collection and analyses**

147   Within each plot, three ceramic lysimeters (P80; Staatliche Porzellanmanufaktur, Berlin) were

148   installed at 5- and 20-cm soils near the plot centre in August 2013. To obtain water samples,

149   350-ml glass bottles with rubber stoppers were pre-evacuated, using a paddle pump, and

150   connected to the lysimeters for overnight sampling. Between November 2013 and October 2015,

151   we sampled soil pore water bi-monthly in the dry and dormant season and monthly during the

152   growing season. All water samples were kept frozen during storage and transport. Concentrations

153   of NH_4^+, NO_3^-, potassium (K^+), calcium (Ca^{2+}), and magnesium (Mg^{2+}) in soil water were

154   measured at the Research Center for Eco-Environmental Sciences (RCEES), Chinese Academy

155   of Sciences, Beijing, using ion chromatography (DX-120 for cations and DX-500 for anions).

156   In August 2013, soils from the O/A (0-3 cm), AB (3-8 cm) and B (8-20 cm) horizons were

157   sampled near the lysimeters for soil analysis. Total P and plant-available P contents were

158   monitored in samples collected from the O/A horizons every six months, starting two days

159   before P addition. Soil samples were kept cold (< 4 ^{o}C) during transport and storage. Before

160   analysis, soil samples were air dried and sieved (2 mm). Soil pH was measured in soil

161   suspensions (10 g dry soil and 50 ml deionized water) using a pH meter (PHB-4, Leici, China).

162   Total soil C and N contents were determined on dried and milled samples, using a LECO

163   elemental analyzer (TruSpec$^@$CHN, USA). To measure total P, 1 g dry soil was digested with 5

164   ml of 6 M H_2SO_4 (Singh et al., 2005) and measured as ortho-phosphate by the molybdenum blue

165   method (Murphy and Riley, 1962). Ammonium lactate (0.01 M)-extractable P and H_2O-

166   extractable P (P_{Al} and P_{H2O}, respectively) were measured as ortho-phosphate after extraction (1.5

167   g dry soil in 50 ml solution) (Singh et al., 2005). Ammonium oxalate (0.2 M)-extractable Fe, Al

168   and P were measured by inductively coupled plasma optical emission spectroscopy (ICP-OES,

169   Agilent, USA) after extraction (1.5 g dry soil in 50 ml solution).

170 From August 2013 onwards, we measured N_2O and CH_4 emissions in triplicate close to the

171 lysimeters, using static chambers (Zhu et al., 2013b). The measurements were conducted bi-

172 monthly in the dry and dormant season and monthly during the growing season, simultaneously

173 with the sampling of soil pore water. To investigate the immediate effect of P addition on N_2O

174 emissions, we also sampled the gas emissions once before (2 May) and three times (7, 10 and 12

175 May) after the P application. Gas samples were taken 1, 5, 15 and 30 minutes after chamber

176 deployment. 20 ml gas samples were injected into pre-evacuated glass vials (12 ml) crimp-sealed

177 with butyl septa (Chromacol, UK), maintaining overpressure to avoid contamination during

178 shipment. Mixing ratios of N_2O and CH_4 were analyzed using a gas chromatograph (Model

179 7890A, Agilent, USA) at RCEES, equipped with an ECD for detection of N_2O (at 375 ^{o}C with

180 25 ml min^{-1} Ar/CH_4 as make up gas), a FID for CH_4 (250 ^{o}C; 20 ml min^{-1} N_2 as make-up gas)

181 and a TCD for CO_2. Exchange rates between soil and atmosphere (emission/uptake) were

182 calculated from measured concentration change in the chambers over time, applying linear or

183 polynomial fits to the concentration data. Cumulative N_2O emissions over time were estimated

184 by linear interpolation between measurement dates (Zhu et al., 2013b).

185 From October 2013 onwards, litterfall was collected during the first week of every month in five

186 replicates per plot. Litterfall collectors were made of 1 m^2 nylon nets (1 mm mesh size), held in

187 place by four wooden poles 0.8 m above the ground. Fresh litter was dried at 65°C. In early

188 November 2013 and 2014 (at the end of the growing season), we collected current-year pine

189 needles from several branches of three trees in each plot. The collected needles were dried at

190 65 ^{o}C and the dry weight of 500 needles was determined. A subsample was dried at 80 ^{o}C and

191 finely milled prior to chemical analysis at the Chinese Academy of Forestry. Total C and N were

192 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 (marked in November 2013; DBH > 5 cm) at each plot. DBH was then used to estimate the standing biomass of Masson pines based on standard allometric equations (Li et al., 2011; Zeng et al., 2008).

Daily average air temperature and daily total precipitation were monitored from July 2013 to November 2015 by a weather station (WeatherHawk 232, USA) placed on the roof at the local forest bureau, in about 1 km distance from the sampling site (Yu et al., 2016).

2.4 Statistical analyses

Statistical analyses were performed using R version 3.3.1 (R Core Team, 2016). All data were tested for normality (Kolmogorov-Smirnov's test) and homoscedasticity (Levene's test) before further analysis. If not normally distributed, the data were normalized by logarithmic transformation. Considering heterogeneity among blocks, temporal variabilities of NO_3^- concentrations, N_2O and CH_4 fluxes were presented separately for each block. For time series data, we used linear mixed-effect (LME) models, to account for both repeated measurements and within-group variance of a stratification variable (block design). LME models were applied to test the effects of P addition on soil N_2O and CH_4 fluxes, NH_4^+, NO_3^-, K^+, Ca^{2+} and Mg^{2+} concentrations in soil water, as well as litterfall weight (Koehler et al., 2009; Müller et al., 2015). The analysis was based on data for plot means (the average of 3 subplot replicates) from three blocks. In LME models, treatments (Reference or P addition) were considered fixed effects, while sampling time and plots were treated as random effects. We then assessed the significance

215   of fixed effects through analysis of variance for LME models. One-way analysis of variance

216   (ANOVA) was conducted to examine the treatment effects on soil pH, nutrient contents in

217   organic matter, tree biomass, 500-needle weight and needle nutrient content for each sampling.

218   Significance levels were set to $p < 0.05$, if not specified otherwise.

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 both 5- and 20-cm depths (Fig. 1b). In general, the concentration of NH_4^+ in soil water was small (< 0.5 mg N L^{-1}) and not affected by P addition (Fig. 1a). 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 overall cationic charge declined significantly in response to P addition (Fig. S3).

3.2 N₂O and CH₄ fluxes: effects of P addition

In the Reference plots, N_2O fluxes varied seasonally (Fig. 2), showing a significant relationship with daily precipitation (Fig. S4a), but not with daily mean temperature (Fig. S4b). Mean N_2O fluxes were generally below 50 μg N_2O-N m^{-2} hr^{-1} in the dry, cool season, but reached values of up to 600 μg N_2O-N m^{-2} hr^{-1} in the growing season (Fig. 2). Cumulative N_2O emissions were estimated with seasonally averaged fluxes, and they differed greatly among the three blocks (Fig. 3), of which block 2 had the greatest annual N_2O emission (7.9 kg N ha^{-1}). CH_4 fluxes in the Reference plots also varied greatly among blocks (Fig. 5). Net emission of CH_4 was observed in

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241 summer 2013 (~ 80 μg CH_4-C m^{-2} hr^{-1}) in blocks 1 and 2, whereas block 3 showed net uptake.

242 From spring 2014 until October 2015, CH_4 fluxes were less variable in all blocks, with values

243 fluctuating around zero. A longer period of net emission was observed in block 3 during the dry

244 season 2014. The fluxes did not correlate with precipitation or air temperature (Figs. S5c&d).

245 Mean N_2O fluxes during the 1.5 years after P addition were significantly smaller in the P

246 treatment than in the Reference (Fig. 4). The P addition resulted in a 50% (3 kg N_2O-N ha^{-1} yr^{-1}

247 on average) reduction of cumulative N_2O emission (Fig. 3). No immediate effect (within days) of

248 P addition on N_2O emission was observed (Fig. S5).

249 In the 1.5 years following P addition, mean CH_4 fluxes indicated net CH_4 emission (~ 3.8 μg

250 CH_4-C m^{-2} hr^{-1}) in the Reference, whereas net CH_4 uptake (~ 6.5 μg CH_4-C m^{-2} hr^{-1}) was

251 observed in the P treatment (Fig. 6). The suppressing effect of P addition on CH_4 emission was

252 significant, in accordance with what was found for NO_3^- concentration and N_2O emission.

253 **3.3 The effect of P addition on tree growth**

254 Throughout the 2-year experimental period, we observed no significant change in tree biomass in

255 response to P addition (Table S3). Likewise, there was no effect of P treatment on the 500-needle

256 weight. Between the two samplings in 2013 and 2014, we found differences in chemical

257 composition of the pine needles, but the difference between the Reference and P treatment was

258 not significant. Also, the C/N and N/P ratios of the needles (40 and 16, respectively) were not

259 affected by P addition. Monthly litterfall varied seasonally in both Reference and P treatment

260 (Fig. S6), but no significant difference was found between the two treatments.

4 Discussion

N$_2$O emission rates in the Reference plots were relatively large (Fig. 2), with mean values close to 100 µg N$_2$O-N m^{-2} hr^{-1} (Fig. 4). This is within the range of N$_2$O emission rates 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$_2$O emission rates averaged to 37 µg N$_2$O-N m^{-2} hr^{-1} in unmanaged sites at Dinghushan (Fang et al., 2009; Tang et al., 2006) and 50 µg N$_2$O-N m^{-2} hr^{-1} in N-fertilized sites (Zhang et al., 2008a). TSP Reference plots emitted on average 5.3 kg N$_2$O-N ha^{-1} yr^{-1} (Fig. 3), which is about 10% of the annual N deposition (50 kg N ha^{-1} yr^{-1}) (Huang et al., 2015). These fluxes are well above average fluxes reported for tropical rainforests (Werner et al., 2007). Large N$_2$O emissions at TSP are likely due to the large N deposition rates (Huang et al., 2015). A similar trend of increasing N$_2$O emissions with increasing N deposition rates has been reported for a wide range of ecosystems (Liu et al., 2009). Also, warm-humid conditions during monsoonal summers may stimulate N$_2$O emissions (Ju et al., 2011), as monsoonal rainstorms trigger peak fluxes (Pan et al., 2003). The positive correlation between precipitation and N$_2$O emission peaks (Fig. S4a) may indicate the importance of denitrification as the dominant N$_2$O source. This is supported by recent ^{15}N tracing experiments at TSP (Yu et al., 2017; Zhu et al., 2013a).

Addition of P caused a significant decline in soil inorganic N in soil water (predominantly NO$_3^-$; Fig. 2), particularly during summers, when NO$_3^-$ concentrations were relatively large (Fig. S2). At the same time, annual N$_2$O 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), which attributed the reduction of N$_2$O emissions in P-treated soils to

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283   decreased NO_3^- availability and thus less denitrification. The attenuation of soil NO_3^- by P

284   addition at TSP may reflect stimulated N uptake by plants and/or soil microorganisms. In a

285   similarly N-rich, tropical forest in South China, Chen et al. (2016) reported a stimulation of net

286   N mineralization and nitrification after six years of bi-monthly P addition, despite reduced soil

287   NO_3^- concentration. Therefore, it is likely that plant uptake plays a more important role in P-

288   induced N retention than immobilization by soil microbes. However, during our study period of

289   two years, we did not find significant increase of N uptake based on tree biomass and foliar N

290   content measurements (Table S3). An alternative explanation could be that P addition stimulated

291   of N uptake by ground vegetation, which remains to be confirmed.

292   In contrast to our study, P-addition experiments in South Ecuador (Martinson et al., 2013) and

293   South China (at Dinghushan Biosphere Reserve (Zheng et al., 2016) found no effect of a single P

294   addition on N_2O emission during the first two years after application. However, significant

295   reduction in N_2O emission was observed after three to five years of continuous P addition, both

296   at the Ecuadorian and the Chinese site (Chen et al., 2016; Müller et al., 2015). For the montane

297   forest site in Ecuador, the observed delay in N_2O emission response to P addition may be

298   explained by the relatively low ambient N deposition ($\sim$ 10 kg N ha^{-1} yr^{-1}) and small N_2O fluxes

299   ($\sim$ 0.36 kg N ha^{-1} yr^{-1} in the Reference plots) (Martinson et al., 2013; Müller et al., 2015). In

300   addition, the moderate amount of P added (10 kg P ha^{-1} yr^{-1}; Martinson et al., 2013) could have

301   resulted in an insignificant P effect in the first two years. The Dinghushan site in South China

302   receives 36 kg inorganic N ha^{-1} yr^{-1} by throughfall (Chen et al., 2016; Fang et al., 2008), which is

303   similar to the inorganic N deposition at TSP (Chen and Mulder, 2007b; Huang et al., 2015).

304   However, soil KCl-extractable inorganic N ($\sim$ 40 mg N kg^{-1}; Zheng et al., 2016) and NO_3^-

305   leaching ($\sim$ 20 kg N ha^{-1} yr^{-1}; Fang et al., 2008) at the Dinghushan site are several-fold smaller

306 than at our site (~ 100 mg N kg^{-1} and ~ 50 kg N ha^{-1} yr^{-1}, respectively) (Huang et al., 2015; Zhu

307 et al., 2013b). Also, the mean N$_2$O emission rates in the reference plots (10 μg m^{-2} h^{-1}) at

308 Dinghushan were smaller than at TSP (> 50 μg m^{-2} h^{-1}; Fig. 4). These indicate that Dinghushan

309 forest has stronger N assimilation and is thus less N-rich than TSP forest. Therefore, we suggest

310 that the response of N$_2$O emission to P addition may depend on the N status of the soil. The fact

311 that numerous studies found apparent suppression of N$_2$O emission in short-term experiments (<

312 2 years) in N + P treatments, but not in treatments with P alone, supports this idea (Müller et al.,

313 2015; Zhang et al., 2014b; Zheng et al., 2016).

314 Another study in South China reported increased N$_2$O emissions during two years after P

315 addition, in a secondary mixed forest (Wang et al., 2014). While suppression of N$_2$O emission by

316 P has been attributed to increased plant N uptake (Mori et al., 2014), increased N$_2$O emission is

317 generally explained by enhanced microbial growth (Liu et al., 2012) and denitrification activity

318 (Ehlers et al., 2010; He and Dijkstra, 2015). P stimulation of N$_2$O emission by microbial

319 denitrification should be rather fast, as indicated by Mori et al. (2013c) in a short-term (one week)

320 incubation experiment with soils from an *Acacia mangium* plantation. Unlike Mori et al. (2013c),

321 we did not find increased N$_2$O emissions within a week after P addition at our site (Fig. S5). This

322 may suggest that the denitrifier community at TSP was not responsive to the P applied, probably

323 because TSP hillslope soils have large denitrification potentials (Zhu et al., 2013c).

324 The Reference plots at TSP showed net CH$_4$ emission for extended periods (Figs. 5 and 6). Also,

325 long-term CH$_4$ fluxes sampled between 2012 and 2014 on hillslope soils near-by (Fig. S7; Zhu et

326 al., unpublished data) showed net CH$_4$ emission. This is in contrast to the generally reported CH$_4$

327 sink function of forested upland soils (Ciais et al., 2013; Dutaur and Verchot, 2007). For

328 example, net CH$_4$ fluxes reported for well-drained, forest soils in South China range from -30 to

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329 -60 µg CH_4-C m^{-2} hr^{-1} (Fang et al., 2009; Tang et al., 2006; Zhang et al., 2014a). Since aerated

330 upland soils typically provide favourable conditions for microbial CH_4 uptake (Le Mer and

331 Roger, 2010), the net emission observed in our sites is unlikely to be due to enhanced CH_4

332 production, but rather due to supressed CH_4 consumption. One general explanation for the net

333 CH_4 emission at TSP could be inhibition of CH_4 oxidation by NH_4^+, which competes with CH_4

334 for the active site at the methane monooxygenase enzyme (Bodelier and Laanbroek, 2004; Zhang

335 et al., 2014a). The concentration of NH_4^+ in the soil water was rather small (< 0.5 g L^{-1}; Fig. 1),

336 which does not preclude, however, that NH_4^+ availability from the soil exchangeable pool may

337 have been high. Zhu et al. (2013b) found extraordinarily high KCL-extractable NH_4^+ in TSP

338 surface soils, likely reflecting the large atmogenic NH_4^+ input at the TSP site (Huang et al.,

339 2015). On the other hand, Reay and Nedwell (2004) found that NO_3^- inhibits methanotrophic

340 activity in acidic soils, where NH_3 is scarce. Possible mechanisms are the toxicity of

341 denitrification intermediates (e.g. NO_2^-; Wang and Ineson, 2003) and the osmotic effect of high

342 NO_3^- concentration (Hütsch et al., 1996). This deduction can be supported by the high NO_3^-

343 concentration in the acidic soils at TSP (Figs. 1 and S2).

344 P addition had a significant impact on CH_4 fluxes, changing the soil from a net source to a net

345 sink on an annual basis (Fig. 6). However, the uptake rates of CH_4 in the P treatments remained

346 smaller than those reported for forest soils in tropical China (Tang et al., 2006; Zhang et al.,

347 2008b). The stimulating effect of P addition on CH_4 uptake is consistent with previous studies

348 (Mori et al., 2013a, 2013b; Zhang et al., 2011), and has been attributed to alleivating N inhibition

349 of methane oxidation. P addition may also result in a change of the taxonomic composition of the

350 methane oxidizing community (Mori et al., 2013a; Veraart et al., 2015). Alternatively, CH_4

351 oxidation may be stimulated by increased CH_4 diffusion into the soil, due to enhanced root

352   growth and increased soil water loss due to transpiration in P-amended plots (Zhang et al., 2011).

353   Given the strong N enrichment of TSP forest (Huang et al., 2015), it is likely that the reason for

354   the observed reduction in CH_4 emissions in response to P fertilization is due to alleviating direct

355   NH_4^+ inhibition of methane monooxygenase (Veldkamp et al., 2013), rather than due to P-

356   stimulation of methanotrophic activity (Veraart et al., 2015).

357   Shortly after fertilizer application, we observed a modest, albeit significant increase of Na^+

358   concentration in soil water (Table S2). Other studies have documented the potential toxicity of

359   excess Na^+ in soil water to plant and microbial activities (Rengasamy et al., 2003; Wong et al.,

360   2008). However, Na^+ toxicity to a degree affecting N turnover processes in our plots is unlikely,

361   as Na^+ concentrations in soil water, within one month after application (Table S2), did not

362   exceed 5 mg L^{-1}, which is far smaller than the values commonly assumed to cause toxicity (40 to

363   100 mg L^{-1}) (Bernstein 1975). Frequent precipitation at TSP (Yu et al., 2016), both prior and

364   following the addition of $NaH_2PO_4.2H_2O$ (Fig. 2), may have diluted and leached Na^+, thus

365   preventing toxic effects.

366   P application significantly increased plant-available P in the P-limited TSP soil (Table 2).

367   Meanwhile, concentrations of leachable base cations (K^+, Mg^{2+}, Ca^{2+}) in soil water decreased

368   (Fig. S3), as expected from the reduction of NO_3^- concentrations in the P-treatments, which

369   represent a major decline in mobile anions in the P-treated soils (Mochoge and Beese, 1986). We

370   observed no sign of stimulated forest growth or increased N uptake by trees within the relatively

371   short period of our study (Table S3 and Fig. S6), making it difficult to link the observed

372   reduction in inorganic N in the soil solution (Fig. 1) to plant growth. When interpreting the

373   observed P effect on NO_3^- concentrations in soil water, several aspects need to be considered.

374   Firstly, two years of observation may be too short to detect any significant increase in tree

375 growth, due to NO_3^- uptake, given the commonly large variabilities in tree biomass estimates

376 (Alvarez-Clare et al., 2013; Huang et al., 2015). Secondly, a significant proportion of the added

377 P, and of excess N, may have been assimilated by the understory vegetation, which was not

378 assessed in this study. Previously, understory biomass has been reported to quickly respond to P

379 addition (Fraterrigo et al., 2011). Thirdly, as long-term N saturation and acidification at TSP

380 have reduced forest health (Lu et al., 2010; Wang et al., 2007), we may not expect immediate

381 response of forest growth to P addition. Large needle N/P ratios (17-22, Table S3) indicated that

382 P limitation for tree growth was not relieved 1.5 years after P addition (Li et al., 2016). Therefore,

383 enhanced N uptake by understory growth may have been the main mechanisms responsible for

384 observed NO_3^- decline in the P-treated soil (Hall & Matson 1999).

385 Our study suggests that N-saturated TSP soils act as a regional hotspot for N_2O (Zhu et al.,

386 2013b) and CH_4 emissions. Within the short experimental period of 1.5 years, P fertilization was

387 shown to significantly decrease NO_3^- concentrations in soil water and to overall reduce N_2O and

388 CH_4 emissions. These findings provide a promising starting point for improving forest

389 management towards GHG abatement targets, taking into account the P and N status of

390 subtropical soils in the region.

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622 **Table 1** Ambient soil properties of the experimental plots at Tieshanping (TSP). Values are
623 means and standard deviations in parenthesis (n = 6)$^\phi$. Soils were sampled in August 2013.

	Soil Layer	pH	Total C $\mathrm{g\ kg^{-1}}$	Total N $\mathrm{g\ kg^{-1}}$	Total P $\mathrm{mg\ kg^{-1}}$	C/N	N/P
	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_{Al} $\mathrm{mg\ kg^{-1}}$	Al_{ox} $\mathrm{mg\ kg^{-1}}$	Fe_{ox} $\mathrm{mg\ kg^{-1}}$	P_{ox} $\mathrm{mg\ kg^{-1}}$	P_{ox} / ($Al_{ox} + Fe_{ox}$)
	O/A (0-3 cm)	5.8 (1.4)	1700 (513)	1933 (350)	85.8 (22.6)	0.025 (0.008)
Block 1	AB (3-8 cm)	2.1 (0.6)	1217 (243)	1692 (493)	47.1 (22.0)	0.016 (0.007)
	B (8-20 cm)	< 1.0	1083 (90)	1158 (249)	29.3 (28.6)	0.012 (0.011)
	O/A (0-3 cm)	5.9 (1.0)	1500 (238)	1792 (215)	79.2 (21.5)	0.024 (0.007)
Block 2	AB (3-8 cm)	1.6 (0.4)	925 (149)	1517 (320)	37.2 (10.7)	0.016 (0.006)
	B (8-20 cm)	< 1.0	892 (209)	1033 (413)	16.1 (10.5)	0.009 (0.007)
	O/A (0-3 cm)	4.1 (0.9)	1367 (180)	1667 (168)	50.7 (10.9)	0.017 (0.003)
Block 3	AB (3-8 cm)	4.4 (4.0)	1075 (128)	1350 (150)	24.8 (8.3)	0.010 (0.002)
	B (8-20 cm)	< 1.0	992 (130)	875 (138)	8.0 (2.0)	0.004 (0.001)

624 P_{Al} = Ammonium lactate-extractable P,

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

626 $^\phi$ Water-extractable P was below a detection limit of 5 mg kg^{-1}, thus not presented in table,

627 [*] Data not available

628   **Table 2** Soil pH, C, N and P contents in the O/A horizon (0-3 cm) in the References (Ref) and P

629   treatments. Values are means and standard deviations in parenthesis (n = 9). P addition was

630   conducted on 14/05/04, after the first two sampling dates.

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

631 † Different letters indicate significant differences between References and P treatments (p < 0.05).

632
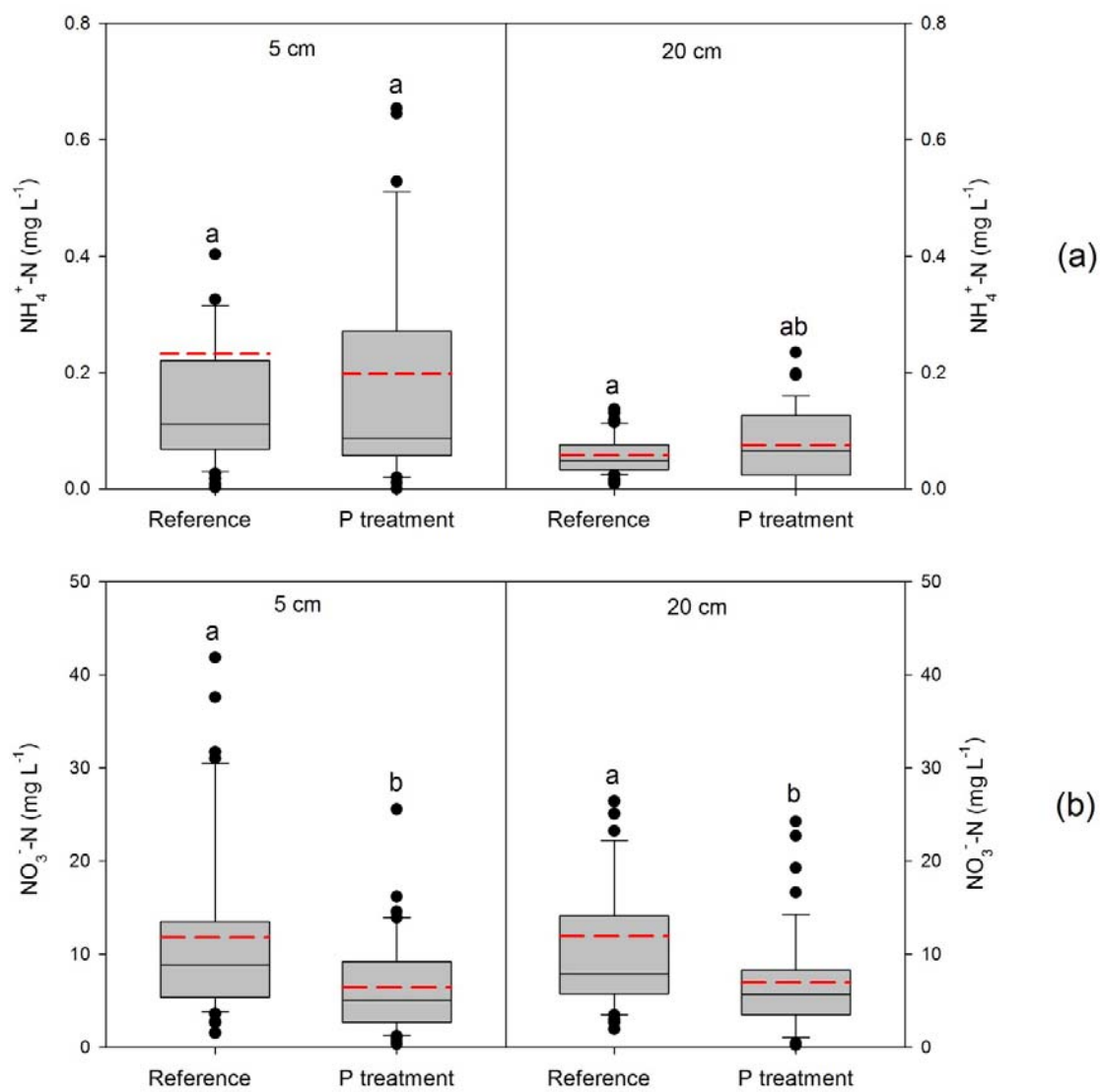
Fig. 1 Box whisker plots of NH_4^+ (a) and NO_3^- (b) concentration in soil water at 5- and 20-cm

depths in the References and P treatments, throughout 1.5 years after the P addition; red dashed

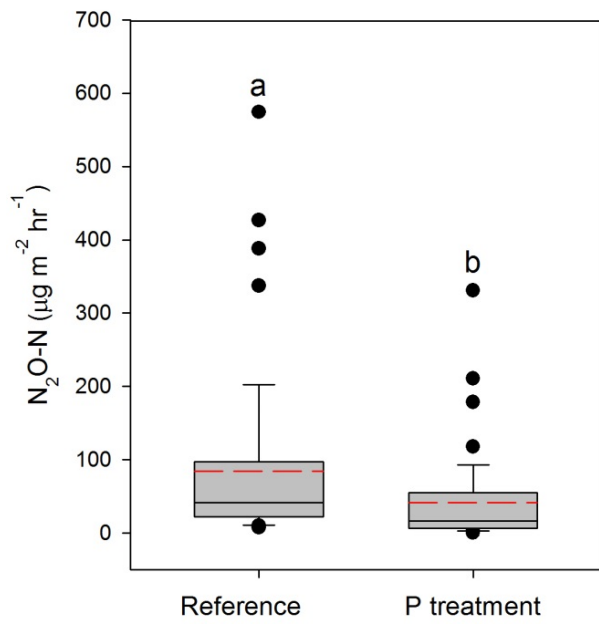lines indicate mean values; different letters indicate significant differences ($p < 0.05$).

636
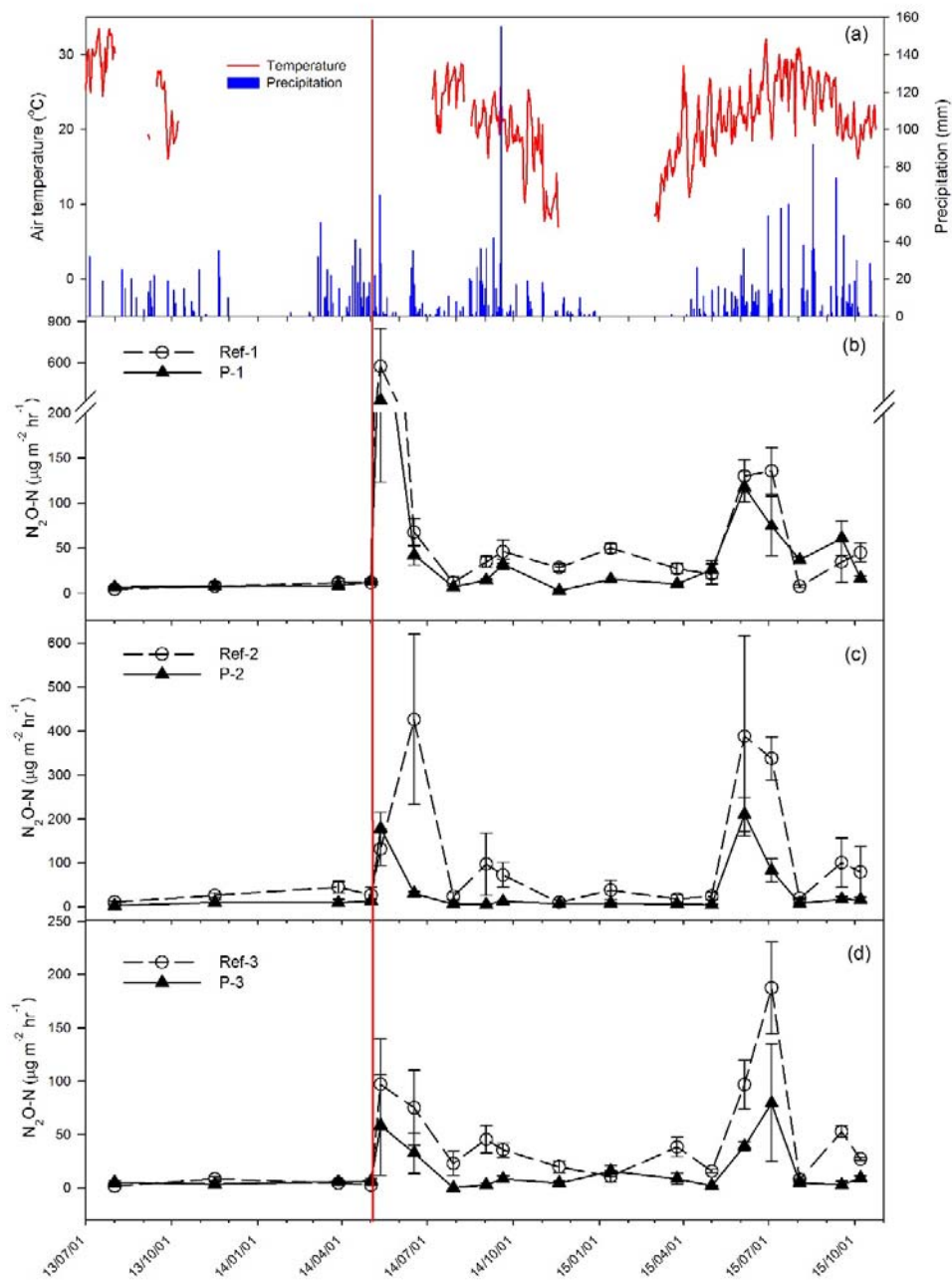
Fig. 2 Daily mean air temperature and precipitation (a), and monthly mean N₂O fluxes (±SE) in
the References (Ref) and P treatments in each of the three blocks (b-d); the red vertical line gives
the date of P addition (4 May, 2014).
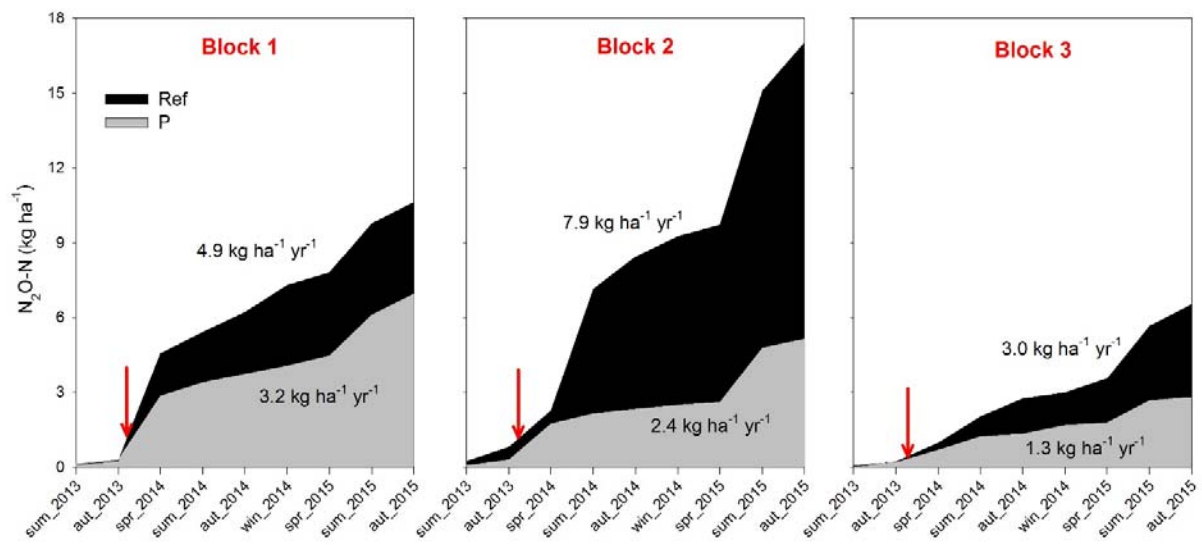
**Fig. 3** Cumulative N$_2$O emissions for three blocks in the References (Ref) and P treatments from summer 2013 to autumn 2015; the red arrows refer to the date of P addition (4 May, 2014).

643
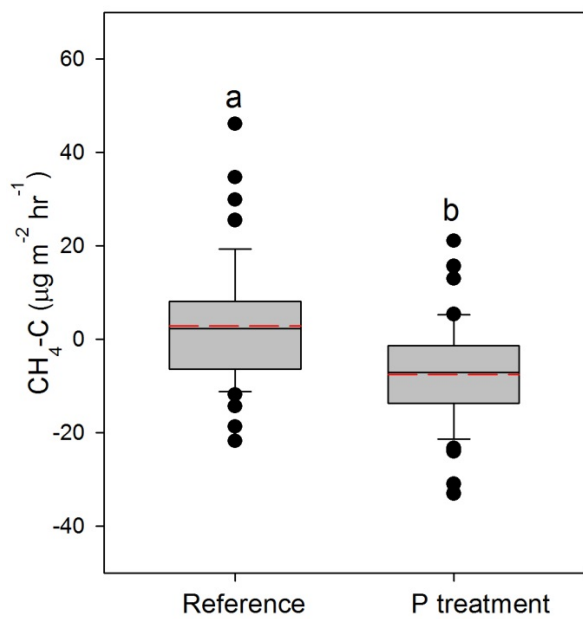
Fig. 4 Box whisker plots for N_2O fluxes in the Reference and P treatment throughout 1.5 years

after the P addition; red dashed lines indicate mean values; linear mixed-effect models were used

to test the P treatment effect; different letters indicate significant difference ($p < 0.05$).
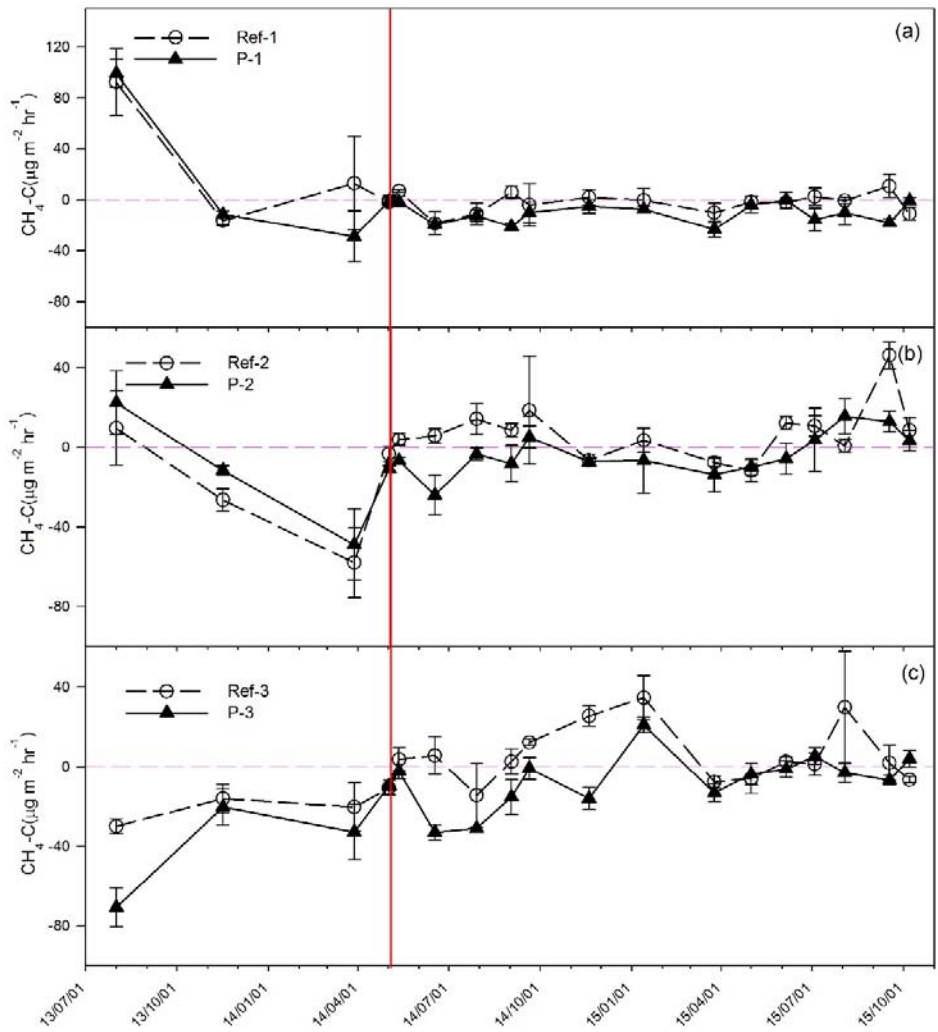
Fig. 5 Monthly mean CH₄ fluxes (±SE) in the References (Ref) and P treatments for three blocks (a-c); the horizontal broken line indicates zero flux the red vertical line refers to the date of P addition (4 May, 2014).

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Fig. 6 Box whisker plots of CH_4 fluxes in the Reference and P treatment throughout 1.5 years
after the P addition; red dash lines indicate mean values; linear mixed-effect models were used to
test the P treatment effect; the different letters indicate significant difference ($p < 0.05$).