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

Longfei Yu[1], Yihao Wang[2, 3], Xiaoshan Zhang[3], Peter Dörsch[1], Jan Mulder[1*]

[1]Faculty of Environmental Sciences and Natural Resource Management, Norwegian University of Life Sciences, Postbox 5003, N-1432 Aas, Norway.

[2]Chongqing Academy of Forestry, 400036, Chongqing, China.

[3]Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, 100085, Beijing, China

[*]Correspondence: Jan Mulder, tel. +47 67231852, E-mail jan.mulder@nmbu.no

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Abstract

12   Chronically elevated nitrogen (N) deposition has led to severe nutrient imbalance in forest soils.

13   Particularly in tropical and subtropical forest ecosystems, increasing N loading has aggravated

14   phosphorus (P) limitation of biomass production, and has resulted in elevated emissions of

15   nitrous oxide (N_2O) and reduced uptake of methane (CH_4), both of which are important

16   greenhouse gases. Yet, the interactions of N and P and their effects on GHG emissions remain

17   understudied. Here, we report N_2O and CH_4 emissions together with soil N and P data for a

18   period of 18 months following P addition (79 kg P ha^{-1} yr^{-1}, applied as NaH_2PO_4 powder) to an

19   N-saturated, Masson pine-dominated forest at TieShanPing (TSP), Chongqing, SW China. We

20   observed a significant decline both in NO_3^- concentrations in soil water (at 5- and 20-cm depths)

21   and in N_2O emissions, the latter by 3 kg N ha^{-1} yr^{-1}. We hypothesize that enhanced N uptake by

22   plants and soil microbes in response to P addition, results in less available NO_3^- for

23   denitrification. By contrast to most other forest ecosystems, TSP is a net source of CH_4. As for

24   N_2O, P addition significantly decreased CH_4 emissions, turning the soil into a net sink. Based on

25   our observation and previous studies in South America and China, we believe that P addition

26   relieves N-inhibition of CH_4 oxidation. Within the 1.5 years after P addition, no significant

27   increase of forest growth was observed at TSP, but we cannot exclude that understory vegetation

28   increased. Our study indicates that P fertilization of N-saturated, subtropical forest soils mitigates

29   N_2O and CH_4 emissions, in addition to alleviating nutrient imbalances and reducing losses of N

30   through NO_3^- leaching.

31   **Key Word:** N_2O and CH_4 emission, N saturation, Phosphate fertilization, soil CH_4 uptake, acid

32   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 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

constrains its capability to retain N (Huang et al., 2015; Li et al., 2016), resulting in ample amounts of mineral 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 after one year of repeated N addition. 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 (~ 36 kg 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 the major controllers for CH_4 uptake (Smith

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

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

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

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

89   (Aronson and Helliker, 2010; Bodelier and Laanbroek, 2004). P addition experiments in N-

90   enriched soils have shown positive effects on CH_4 uptake (Mori et al., 2013a; Zhang et al., 2011),

91   but the underlying mechanisms, i.e. whether P addition affects the methanotrophic community in

92   soils directly or alleviates the N-inhibition effect on CH_4 oxidation through enhanced N uptake

93   (Mori et al., 2013b; Veraart et al., 2015), remain unresolved.

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

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

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

97   the importance of increased mineral N concentrations for soil-atmosphere exchange of CH_4, and

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

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

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

101 growth. The objectives were i) to quantify N_2O and CH_4 emissions, ii) to investigate the effect of

102 P addition on N_2O and CH_4 emission.

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}. Soil water-filled pore space (10 cm) at TSP hilltop generally ranges from 50 to 70% (mean ~ 60%; Zhu et al., 2013b). More details on soil properties are given in Table 1.

Annual N deposition at TSP measured in throughfall varies between 40 and 65 kg ha^{-1} (dominated by NH_4^+; Yu et al., 2016), while the annual bulk N deposition is from 20 to 30 kg 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 at TSP has resulted in severe decline in forest growth (Li et al., 2014; Wang et al., 2007), and in decreased 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

125 horizon is ~ 300 mg kg^{-1}, while ammonium lactate-extractable P is smaller than 5 mg kg^{-1} (Table

126 1).

2.2 Experimental Design

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

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

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

131 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

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

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

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

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

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

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

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

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

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

141 somewhat larger than in the reference plots, the Na$^+$ concentration in soil water of the P

142 treatments are unlikely to have exerted a strong negative impact on plant and microbial activities.

2.3 Sample collection and analyses

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

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

146   350-ml glass bottles with rubber stoppers were pre-evacuated, using a paddle pump, and

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

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

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

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

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

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

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

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

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

156   before P addition. Soil samples were kept cold ($< 4\ ^oC$) during transport and storage. Before

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

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

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

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

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

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

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

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

165   and P were measured by inductive coupled plasma (7500; Agilent) after extraction (1.5 g dry soil

166   in 50 ml solution).

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

168   lysimeters, using static chambers (Zhu et al., 2013b). To investigate the immediate effect of P

169 addition on N_2O emissions, we sampled the gas emissions once before (2 May) and three times

170 (7, 10 and 12 May) after the P application. Gas samples (20 ml) were taken 1, 5, 15 and 30

171 minutes after chamber deployment and injected into pre-evacuated glass vials (12 ml) crimp-

172 sealed with butyl septa (Chromacol, UK), maintaining overpressure to avoid contamination

173 during sample transport. Mixing ratios of N_2O and CH_4 were analyzed using a gas

174 chromatograph (Model 7890A, Agilent, US) at RCEES, equipped with an ECD for detection of

175 N_2O (at 375 ^{o}C with 25 ml min^{-1} Ar/CH_4 as make up gas), a FID for CH_4 (250 ^{o}C; 20 ml min^{-1}

176 N_2 as make-up gas) and a TCD for CO_2. Exchange rates between soil and atmosphere

177 (emission/uptake) were calculated from measured concentration change in the chambers over

178 time, applying linear or polynomial fits to the concentration data. Cumulative N_2O emissions

179 over time were estimated by linear interpolation between measurement dates (Zhu et al., 2013b).

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

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

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

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

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

185 65 ^{o}C and the dry weight of 500 needles was determined. A subsample was dried at 80 ^{o}C and

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

187 measured using an elemental analyzer (FLASH 2000; Thermo Scientific; USA). The contents of

188 K, Ca, Mg and P in the needles were determined by ICP-AES (IRIS Intrepid II; Thermo

189 Scientific; USA) after digesting 0.25 g dry weight samples with 5 ml of ultra-pure nitric acid. In

190 November 2013, and 2014, and in February of 2015, we measured the height and the diameter at

191 breast height (DBH) of 6 to 10 Masson pines (only those with DBH > 5 cm) at each plot. These

192   data were used to estimate the standing biomass of Masson pines based on standard allometric

193   equations (Li et al., 2011; Zeng et al., 2008).

194   Daily average air temperature and sum of precipitation were monitored by a weather station

195   (WeatherHawk 232, USA) placed on the roof at the local forest bureau, in about 1 km distance

196   from the sampling site (Yu et al., 2016).

197   **2.4 Statistical analyses**

198   Statistical analyses were performed using R version 3.3.1 (R Core Team, 2016). All data were

199   tested for normality (Kolmogorov-Smirnov's test) and homoscedasticity (Levene's test) before

200   further analysis. If not normally distributed, the data were normalized by logarithmic

201   transformation. Considering heterogeneity among blocks, temporal variabilities of NO_3^-

202   concentrations, N_2O and CH_4 fluxes were presented separately for each block. For time series

203   data, we used linear mixed-effect (LME) models, to account for both repeated measurements and

204   within-group variance of a stratification variable (block design). LME models were applied to

205   test the effects of P addition on soil N_2O and CH_4 fluxes, NH_4^+, NO_3^-, K^+, Ca^{2+} and Mg^{2+}

206   concentrations in soil water, as well as litterfall (Koehler et al., 2009; Müller et al., 2015). The

207   analysis was based on data for plot means (the average of 3 subplot replicates) from three blocks.

208   In LME models, treatments (Reference or P addition) were considered fixed effects, while

209   sampling time and plots were treated as random effects. We then assessed the significance of

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

211   (ANOVA, Turkey post-hoc test) was conducted to examine the treatment effects on soil pH,

212   nutrient contents in organic matter, and data of tree growth. Significance levels were set to $p <$

213   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 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 sum of charge of dissolved base cations declined significantly in response to P addition (Fig. S3).

3.2 N$_2$O 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. S4a), 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 values of up to 600 μg 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). The greatest annual N_2O emission was 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

236 significantly smaller in the P treatment than in the Reference (Fig. 4). The P addition resulted in

237 a decrease in cumulative N_2O emission by about 3 kg N ha^{-1} yr^{-1} on average, which is a 50%

238 reduction (Fig. 3). No immediate effects (within days) of P addition on N_2O emission was

239 observed (Fig. S5).

240 CH_4 fluxes varied greatly between blocks (Fig. 5). Net-emission of CH_4 was observed in summer

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

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

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

244 2014. The fluxes did not correlate with either precipitation or air temperature (Fig. S5c&d). In

245 the 1.5 years following P addition, mean CH_4 fluxes indicated net CH_4 emission (~ +3.8 µg C m^{-}

246 ^{2} hr^{-1}) in the Reference, whereas net CH_4 uptake (~ -6.5 µg C m^{-2} hr^{-1}) was observed in the P

247 treatment (Fig. 6). The suppressing effect of P addition on CH_4 emission was in accordance with

248 what was found for NO_3^- concentration and N_2O emission.

3.3 The effect of P addition on tree growth

250 Throughout the 2-year experimental period, we observed no change in tree biomass (138 t ha^{-1})

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

252 needle weight (13 g on average). Between the two samplings in 2013 and 2014, we found

253 differences in chemical composition of the pine needles, but this effect was not linked to P

254 addition. Also, the C/N and N/P ratios of the needles (40 and 16, respectively) were hardly

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

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

4 Discussion

Background N_2O emission rates in the Reference plots were relatively large (Fig. 2), with mean values close to 100 μg N m^{-2} hr^{-1} (Fig. 4). This is within the range of N_2O 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_2O emission rates averaged to 37 μg N m^{-2} hr^{-1} in unmanaged sites at Dinghushan (Fang et al., 2009; Tang et al., 2006) and 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. 3), which is about 10% of the annual N deposition (50 kg 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_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 (Pan et al., 2003). The positive correlation between precipitation and N_2O emission peaks (Fig. S4a) 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).

Addition of P caused a significant decline in soil mineral N (predominantly NO_3^-; 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), which attributed the reduction of N_2O emissions in P-treated soils decreased mineral N content, most likely as a consequence of stimulated plant uptake and/or microbial

279 assimilation. It is noteworthy that there was no significant correlation between N_2O emission

280 rates and soil water NO_3^- concentration in our study (Figs. 2 and S2), suggesting that the

281 suppressing effect of P on N_2O emissions was indirect, probably by affecting the competition for

282 mineral N between plant roots and microbes (Zhu et al., 2016). In contrast to our 1.5-year study,

283 P-addition experiments in South Ecuador (Martinson et al., 2013) and South China (at

284 Dinghushan Biosphere Reserve (Zheng et al., 2016) found no effect of a single P addition on

285 N_2O emission during the first two years after application. However, significant reduction in N_2O

286 emission was observed after three to five years with continuous P addition, both at the

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

288 site in Ecuador, the observed delay in N_2O emission response to P addition may be explained by

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

290 experiments were conducted in a forest with low ambient N deposition ($\sim$ 10 kg N ha^{-1} yr^{-1}) and

291 N_2O fluxes ($\sim$ 0.36 kg N ha^{-1} yr^{-1} in the Reference plots) (Martinson et al., 2013; Müller et al.,

292 2015). By contrast, the Dinghushan site in South China receives 28 kg N ha^{-1} yr^{-1} through wet

293 inorganic N deposition (Zheng et al., 2016), which is similar to the N deposition at our site (Chen

294 and Mulder, 2007b; Huang et al., 2015). They also observed larger N_2O emission rates ($\sim$ 0.88

295 kg N ha^{-1} yr^{-1} in the Reference plots) than in the Ecuadorian site. However, forests do not always

296 display a straightforward relationship between N deposition and N_2O emissions. Manipulation

297 experiments in the European NITREX project, for instance, revealed a much stronger correlation

298 of N_2O emissions with soil NO_3^- leaching than with N deposition (Gundersen et al., 2012).

299 Indeed, KCl-extractable mineral N at the Dinghushan site ($\sim$ 40 mg kg^{-1}; Zheng et al., 2016) is

300 several-fold smaller than at our site ($>$ 100 mg kg^{-1}; Zhu et al., 2013b), indicating that

301 Dinghushan is less N-rich than TSP. This suggests that the response of N_2O emission to P

302 addition might depend on the N status of the soil. The fact that numerous studies found apparent

303 suppression of N_2O emission in short-term experiments (< 2 years) in N + P treatments, but not

304 in treatments with P alone, supports this idea (Müller et al., 2015; Zhang et al., 2014b; Zheng et

305 al., 2016).

306 Other studies have observed increased N_2O emissions upon P addition (Mori et al., 2013c; Wang

307 et al., 2014). In an *Acacia mangium* plantation, fertilized with P, Mori et al. (2013b&c) found

308 that N_2O emissions were stimulated in the short-term but reduced in the long-term. While

309 suppression of N_2O emission by P has been attributed to increased plant N uptake (Mori et al.,

310 2014), increased N_2O emission are generally explained by enhanced microbial biomass (Liu et

311 al., 2012) and denitrification activity (Ehlers et al., 2010; He and Dijkstra, 2015). N_2O emissions

312 measured shortly after P addition at our site in May 2014 were not different from fluxes in

313 untreated reference plots (Fig. S5). This may indicate that plant uptake at TSP is more important

314 for the effect of P addition on N_2O emissions than changes in microbial activity, which are

315 expected to occur more rapidly.

316 The Reference plots at TSP showed net CH_4 emission for extended periods of the year (Figs. 5

317 and 6). Also, long-term CH_4 fluxes sampled between 2012 and 2014 on hillslopes near-by (Fig.

318 S7; Zhu et al., unpublished data) showed net CH_4 emission. This is in contrast to the generally

319 reported CH_4 sink function of forested upland soils (Ciais et al., 2013; Dutaur and Verchot,

320 2007). For example, CH_4 uptake rates reported for well-drained, forest soils in South Chinese

321 forest range from 30 to 60 µg C m^{-2} hr^{-1} (Fang et al., 2009; Tang et al., 2006; Zhang et al., 2014a).

322 Since aerated upland soils typically provide favourable conditions for microbial CH_4 uptake (Le

323 Mer and Roger, 2010), the net emission observed in our sites is unlikely due to enhanced

324 production, but rather by supressed consumption. One reason for the net CH_4 emission at TSP

325   could be inhibition of CH_4 oxidation by NH_4^+, as reported previously (Bodelier and Laanbroek,

326   2004; Zhang et al., 2014a). The concentration of NH_4^+ in the soil water was rather small (< 0.5 g

327    $L^{-1;}$ Fig. 1), which does not preclude, however, that NH_4^+ availability from the soil exchangeable

328   pool is high. Zhu et al. (2013b) found extraordinarily high KCL-extractable NH_4^+ in TSP surface

329   soils, likely reflecting the large atmogenic NH_4^+ input at the TSP site (Huang et al., 2015).

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

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

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

333   2008b). The stimulating effect of P addition on CH_4 uptake is consistent with previous studies

334   (Mori et al., 2013a, 2013b; Zhang et al., 2011), and has been attributed to lessening the NH_4^+

335   inhibition of methane oxidation. Unfortunately, we did not measure KCl-extractable NH_4^+ in our

336   study, but a decline of available NH_4^+, which is the substrate for nitrification, is likely as NO_3^-

337   concentrations in soil water were significantly smaller with in the P-treatments (Fig. 1). P

338   addition may also result in a change of the taxonomic composition of the methane oxidizing

339   community (Mori et al., 2013a; Veraart et al., 2015). Alternatively, CH_4 oxidation may be

340   stimulated by increased CH_4 diffusion into the soil, due to enhanced root growth and increased

341   soil water loss due to transpiration in P-amended plots (Zhang et al., 2011). Given the strong N

342   enrichment of TSP forest (Huang et al., 2015), it is likely that the reason for the observed

343   reduction in CH_4 emissions in response to P fertilization is due to alleviating direct NH_4^+

344   inhibition of methane monooxygenase (Veldkamp et al., 2013), rather than to P-stimulation of

345   methanotrophic activity (Veraart et al., 2015).

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

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

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

349 2008). However, the occurrence of Na$^+$ toxicity at the treated plots, affecting N turnover

350 processes, is unlikely, as Na$^+$ concentrations in soil water, within one month after application

351 (Table S2), did not exceed 5 mg L^{-1}, far smaller than the values commonly assumed to indicate

352 toxicity (40 to 100 mg L^{-1}) (Bernstein 1975). Frequent precipitation at TSP (Yu et al., 2016),

353 both prior and following the addition of NaH$_2$PO$_4$.2H$_2$O (Fig. 2), apparently diluted and leached

354 Na$^+$, thus preventing toxic effects.

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

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

357 (Fig. S3), as expected from the reduction of NO$_3^-$ concentrations in the P-treatments, which

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

359 observed no sign of stimulated forest growth or increased N uptake by plants within the

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

361 observed reduction in mineral N in the soil solution (Fig. 1) to plant growth. When interpreting

362 the observed P effect on NO$_3^-$ concentrations in soil water, several aspects need to be considered.

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

364 growth, due to NO$_3^-$ uptake by plants, given the commonly large variabilities in tree biomass

365 estimates (Alvarez-Clare et al., 2013; Huang et al., 2015). Secondly, a significant proportion of

366 the added P, and of excess N, may have been assimilated by the understory biomass, which was

367 not assessed in this study. Previously, understory vegetation has been reported to quickly

368 respond to P addition (Fraterrigo et al., 2011). Thirdly, as long-term N saturation and

369 acidification at TSP have reduced forest health (Lu et al., 2010; Wang et al., 2007), we may not

370 expect immediate response of forest growth to P addition. Large needle N/P ratios (17-22, Table

371 S3) indicated that P limitation for tree growth was not relieved 1.5 years after P addition (Li et al.,

372 2016). Therefore, enhanced N uptake by understory growth and/or soil microbial biomass may

373 have been the main mechanisms responsible for observed NO_3^- decline in the P-treated soil (Hall

374 & Matson 1999).

375 Overall, our study suggests that N-saturated TSP soils act as a regional hotspot for N_2O and CH_4

376 emissions. Within the short experimental period of 1.5 years, P fertilization was shown to

377 significantly decrease NO_3^- concentrations in soil water and to reduce both N_2O and CH_4

378 emissions. These findings provide a promising starting point for improving forest management

379 towards GHG abatement targets, taking into account the P and N status of subtropical soils in the

380 region.

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603 **Table 1** Background soil properties of the experimental plots at Tieshanping (TSP). Values are

604 means and standard deviations in parenthesis (n = 6). Soils were sampled in August 2013.

	Soil Layer	pH	Total C g kg^{-1}	Total N g kg^{-1}	Total P 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_{H2O} mg kg^{-1}	P_{Al} mg kg^{-1}	Al_{ox} mg kg^{-1}	Fe_{ox} mg kg^{-1}	P_{ox} mg kg^{-1}	P_{ox} / (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)

605 P_{H2O} = Water-extractable P, P_{Al} = Ammonium lactate-extractable P,

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

607 * Data not available

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

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

610   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)^{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}

611   † Different letters indicate significant differences (p < 0.05).

612

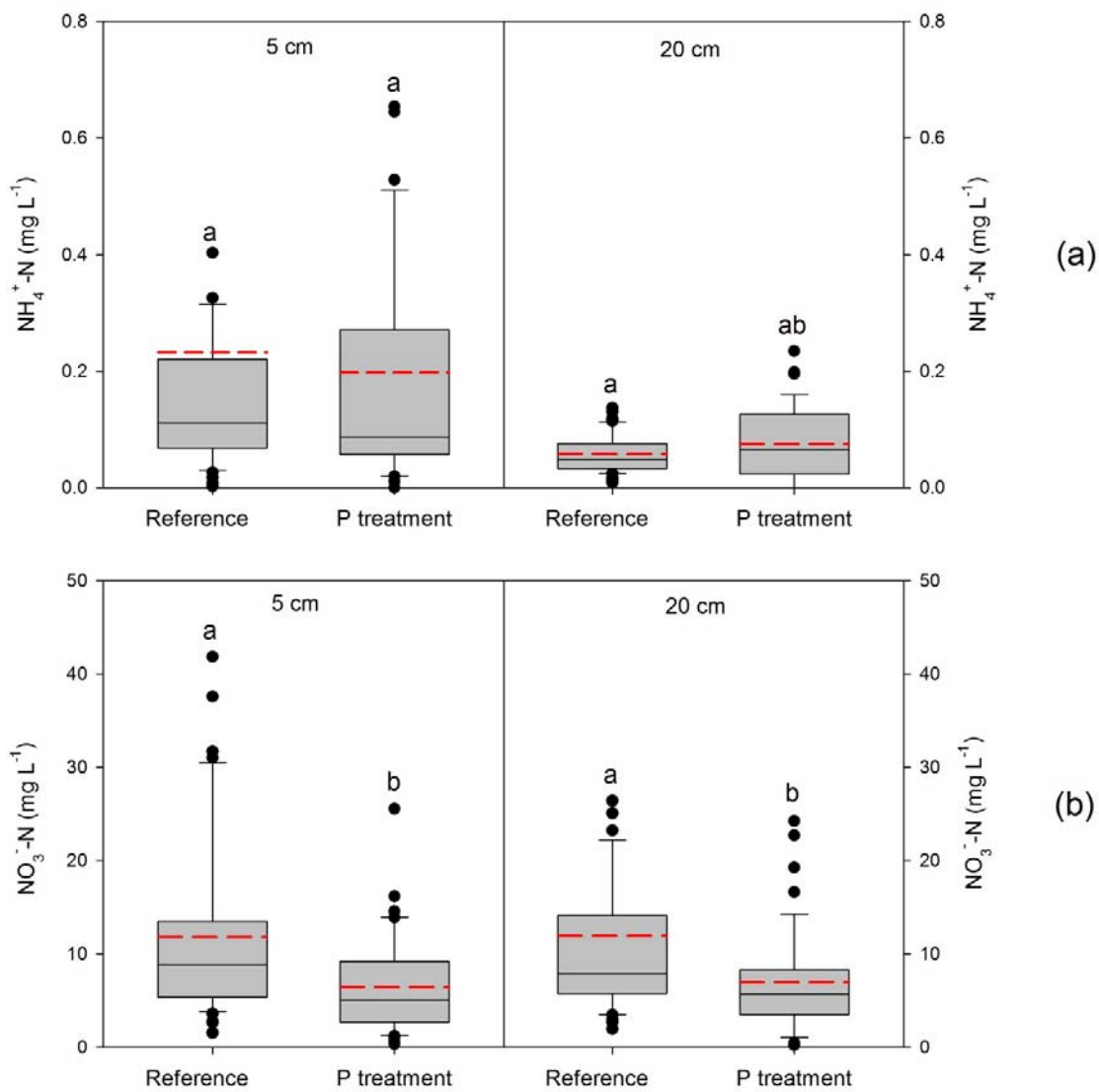



Fig. 1 Box whisker plots of NH_4^+ (a) and NO_3^- (b) concentration in soil water at 5- and 20-cm

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 ($p < 0.05$).

616

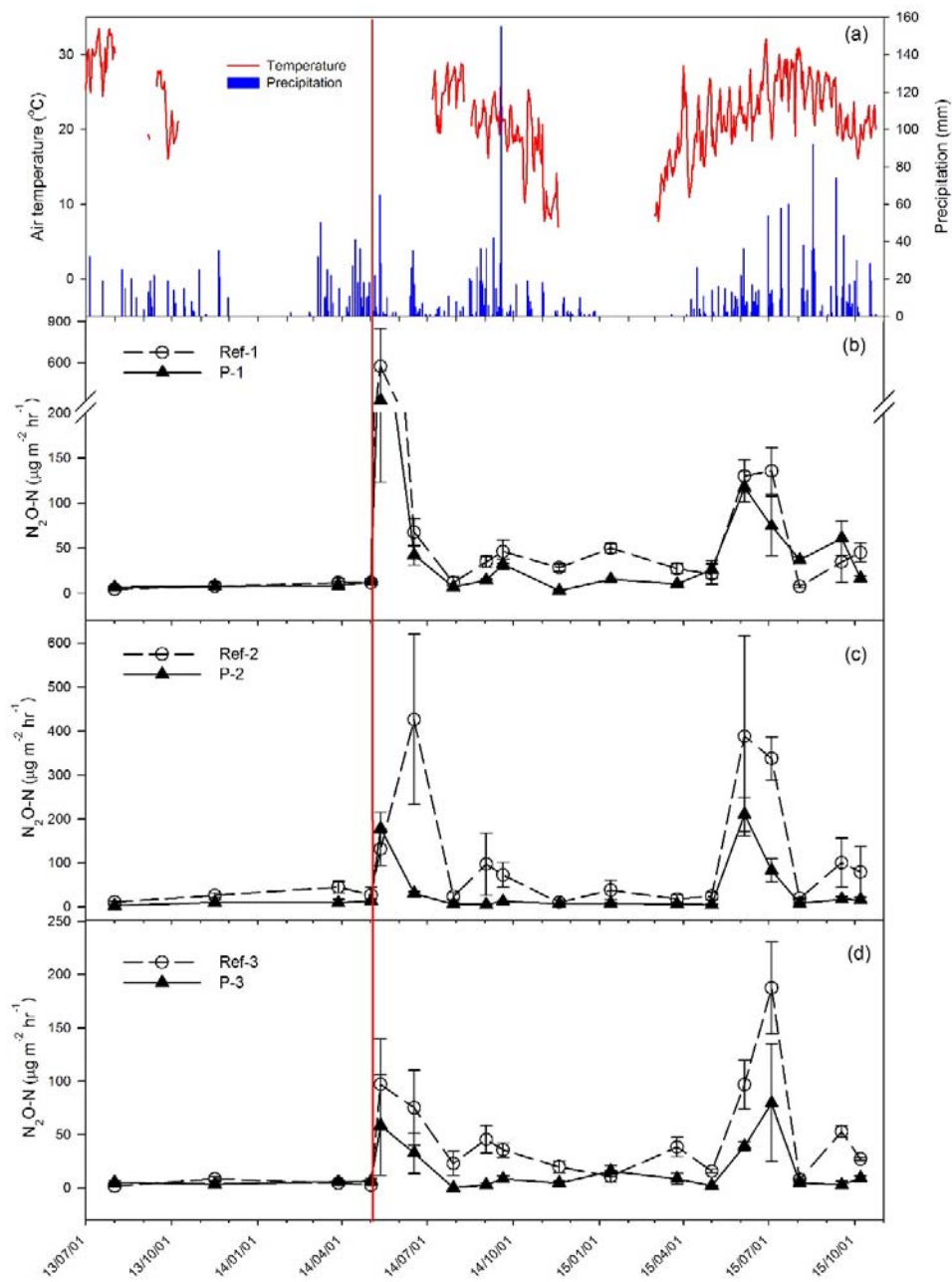
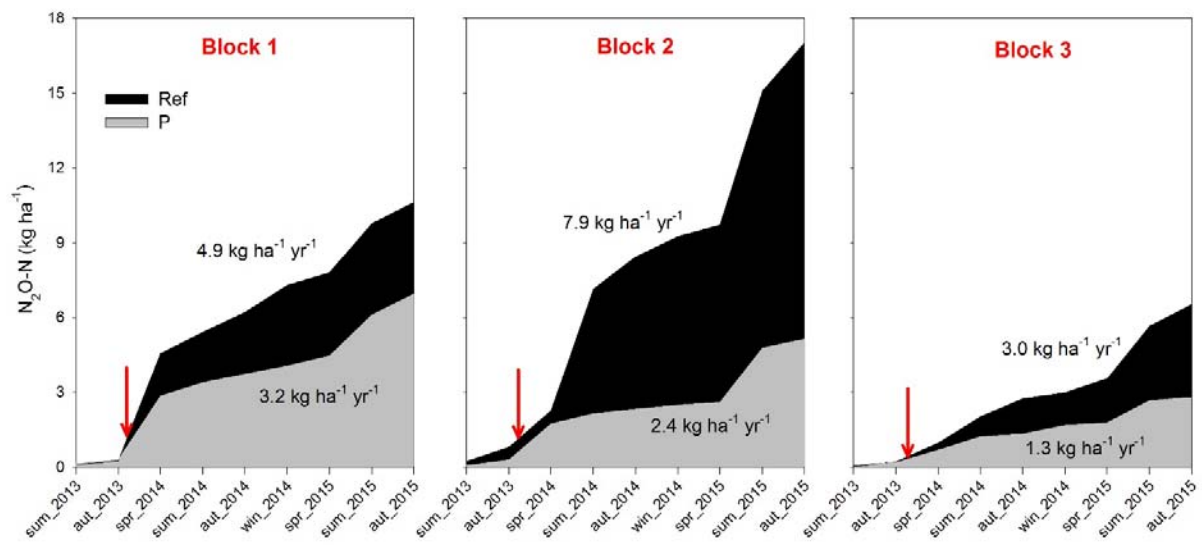
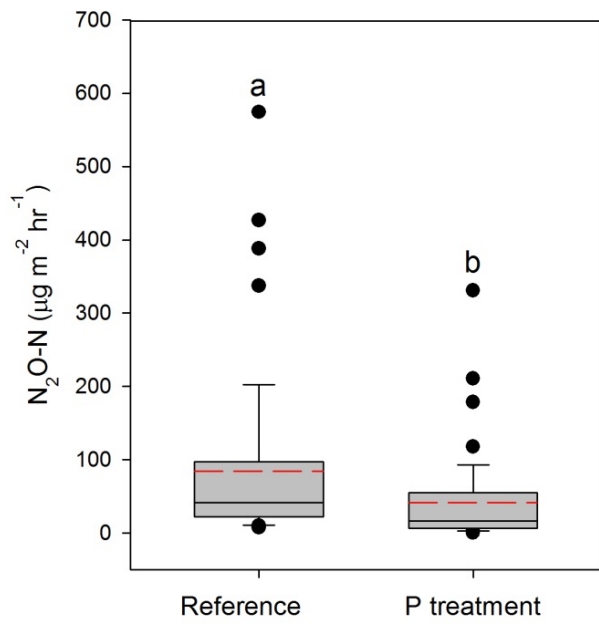



Fig. 2 Daily mean air temperature and precipitation (a), and monthly mean N₂O fluxes (±SE) in
the References (Ref) and P treatments in each of the three blocks (b-d); the red vertical line gives
the date of P addition (4 May, 2014).

620

621 **Fig. 3** Cumulative N$_2$O emissions for three blocks in the References (Ref) and P treatments from

622 summer 2013 to autumn 2015; the red arrows refer to the date of P addition (4 May, 2014).

623

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

625   after the P addition; red dashed lines indicate mean values; different letters indicate significant

626   difference ($p < 0.05$).

627

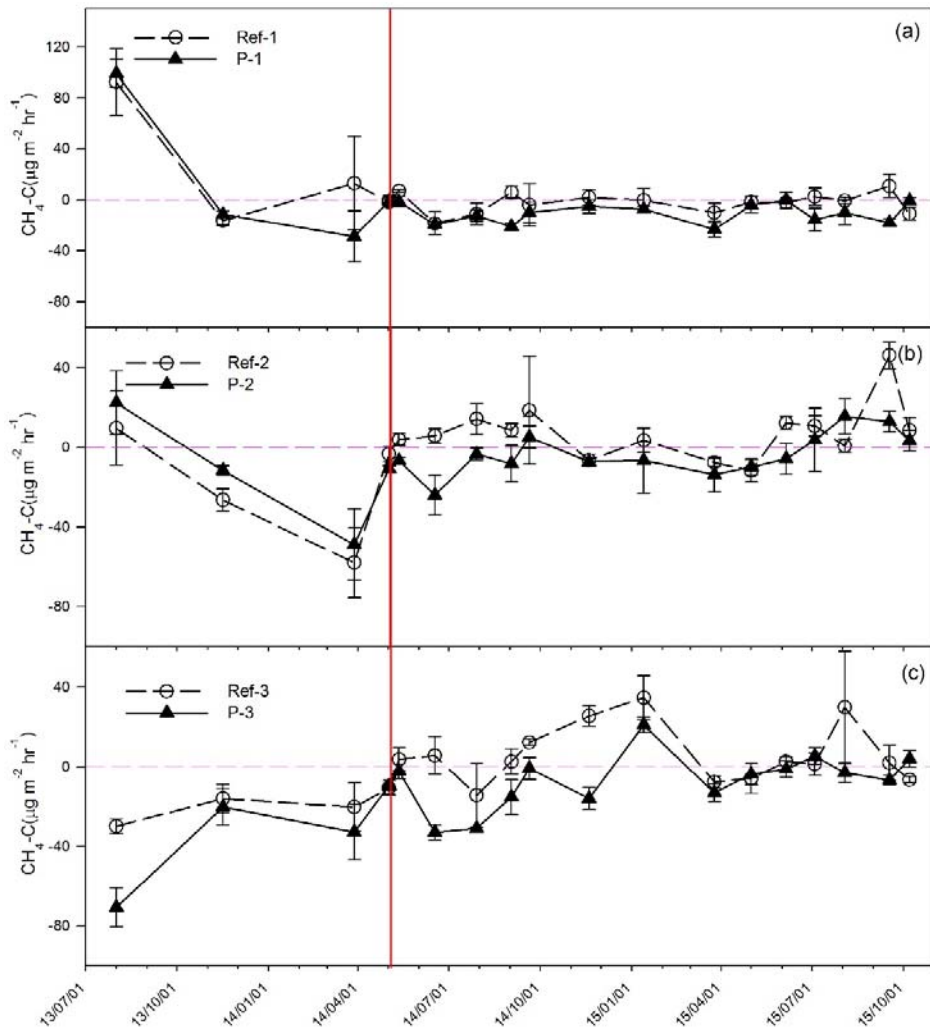
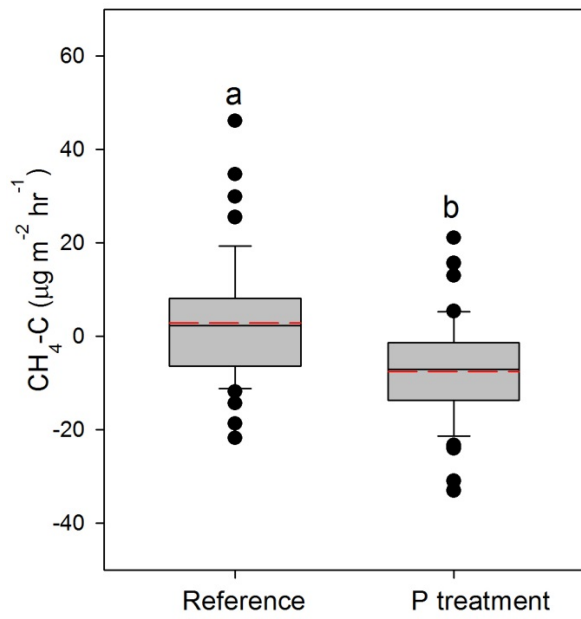



Fig. 5 Monthly mean CH_4 fluxes (±SE) in the References (Ref) and P treatments for three blocks (a-c); the horizontal broken line indicates zero flux the red vertical line refers to the date of P addition (4 May, 2014).

631

632   **Fig. 6** Box whisker plots of CH_4 fluxes in the Reference and P treatment throughout 1.5 years

633   after the P addition; red dash lines indicate mean values; the different letters indicate significant

634   difference ($p < 0.05$).