Dear Editor,

Thank you for considering the revision of our manuscript “Phosphorus addition mitigates $\text{N}_2\text{O}$ and $\text{CH}_4$ emissions in N-saturated subtropical forest, SW China” by Yu et al., for publication in Biogeosciences.

As suggested by the reviewers, we have reanalyzed our whole dataset with linear mixed effects models using R, and documented the details of statistical approaches in the last section of M&M. The discussion of treatment effects is now based on plot means instead of subplot measurement. Consequently, the presentation of our data in the Results and Figures has been changed. Also, we have made required revisions according to reviewers’ comments in the main text. More details regarding the revision (changes referred to the line numbers in the revised manuscript) can be found below. Also, the main manuscript marked with change (in red text) is supplemented in the end of this cover letter.

We thank you again for your effort and time spent on our manuscript.

Best regards,

Longfei Yu on behalf of all coauthors

longfei.yu@nmbu.no
Interactive comment on “Phosphorus addition mitigates N2O and CH4 emissions in N-saturated subtropical forest, SW China” by Longfei Yu et al.

Longfei Yu et al.
longfei.yu@nmbu.no
Received and published in BG Discussion on 03 Nov. 2016.

Referee #1

General comments:

Yu and co-authors present an interesting dataset of nitrous oxide (N2O) and methane (CH4) flux, nitrate (NO3-) concentration and other ancillary measurements from a forest ecosystem P-addition experiment conducted in a N-saturated (N-deposition is about 40 to 65 kg ha-1 yr-1) secondary Masson pine-dominated forest at TieShanPing (TSP), Chongqing, SW China (developed after a clear cut about 50 years ago) over a period of 18 months. After a single dose of P (applied as solid NaH2PO4*2H2O at a rate of 79.5 kg P ha-1) was added, Yu and co-authors found out that both N2O and CH4 emissions and NO3- concentrations in soil water decreased following P-addition during the 18-months period. They speculate that P-addition may have stimulated mineral N uptake by P-limited plants or microbes leading to decreased NO3- concentrations and N2O emissions. Concomitantly, decreasing mineral N concentrations may have relieved N inhibition of microbial CH4 oxidation leading to decreased CH4 emissions. Spatial and temporal dynamics of nutrient imbalances and their effects on biogeochemistry in forest ecosystems are very complex and difficult to decipher but important to understand in order to predict impacts of global change processes on trace gas fluxes in forest ecosystems. The results from Yu and co-authors are very interesting and valuable. Nutrient-addition experiments in whole forest ecosystems are very difficult to conduct but their results are often much more realistic than countless laboratory experiments trying to mimic whole ecosystem conditions.

R 1.0: We thank referee #1 for the appraisal and valuable comments intended to improve the presentation of the data. We have responded below to the comments.

However, I have major concerns and recommend a reanalysis of the dataset:

1) I would suggest to reanalyze the whole dataset using linear mixed effects models (Koehler et al. 2009 and Jones et al. 2016) to account for repeated measurements (monthly measurements over 1.5 years) and for within-group variance of a stratification (block-design of the study) which has not been done so far.

R 1.1: We agree with the referee that Linear Mixed Effects Models would be better to capture within-group variances, even though the overall result may not differ substantially. In the revision, we will document the details of our statistical tests for different parameters, and present our final outcomes with reanalyzed results.

2) Please take into account that all of your replicates in your plot are pseudo-replicates because they depend on one single block (which is your true replicate). In total you have three independent samples for the P-addition plots and three independent reference samples. Consequently, the dataset and figures should be reanalyzed and presented appropriately. If the blocks are so heterogenous you may show
patterns in different blocks or outliers but your whole discussion should focus on significant results of the reanalyzed dataset based on plot means and not subplots.

R 1.2: We agree with the referee’s suggestions and will change the manuscript accordingly.

3) I doubt that chronic N deposition alone has transformed TSP soils to a regional hotspot for N2O and CH4 emissions. You have not measured that. Changes in soil bulk density/soil compaction following a clear-cut about 50 years ago may be even more important. Water-filled pore space or gravimetric water content in soils are major controllers of N2O and CH4 production. These variables are almost always measured in soil trace gas flux studies and highly depend on soil bulk density which has also not been measured or is not presented in the manuscript. Since there was a clear-cut at the TSP site about 50 years ago and the soil type at this site is a Haplic Acrisol, where clay translocation processes in mineral horizons form clay-enriched horizons. Such a soil is prone to soil compaction. Especially in these clay-enriched horizons soil compaction may lead to increased bulk densities, may promote oxygen limitation and therefore increase rates of microbial denitrification and methanogenesis that eventually may lead to net CH4 and N2O emissions from this forest site. It would be great if authors could provide data about soil bulk densities, soil properties in general and soil water content variables.

R 1.3: N saturation, indicated by significant nitrate leaching from soils, has been previously confirmed at TSP, (Chen and Mulder, 2007; Huang et al., 2015; Zhu et al., 2013b). Accordingly, annual N2O fluxes previously observed at TSP were among the highest fluxes reported in literature (Zhu et al., 2013b). Chronic N deposition does not only increase soil N availability, but also contributes to soil acidification (Huang et al., 2016), which is believed to facilitate large N2O emission factors (N2O flux: total N input) (Liu et al., 2014). Therefore, based on the previous findings, our study mainly addresses the P-addition effect on GHG emissions at TSP.

We also agree with the referee that edaphic factors, specific to Haplic Acrisols, play a role in the N2O and CH4 emissions in TSP. Though WFPS was not directly monitored throughout our observation, the range of WFPS and its effect on N2O emissions in TSP have been well presented and studied by Zhu et al (2013b). The clear cut of the forests in the 1950s may affect the edaphic conditions in clay-rich horizon in soil, but not likely for the organic top horizon, where microbial activities such as denitrification were found to be strongest (Zhu et al., 2013a). In the revision, we will provide a better description of the soil properties for TSP referring to detailed data from previous studies (Sørbotten, 2011; Wang et al., 2007; Zhu et al., 2013b).

Minor comments:

Line 28 to 30: Nutrient imbalances in forest ecosystems and their effects on greenhouse gas emissions are very complex and shift in space and time. The present study analyzed effects of a single dose of P fertilizer on trace gas fluxes in an approximately 50-years old secondary forest over a period of 18 moths. It is simply too daring to extrapolate results from this special forest site to acid forest soils in general. Please do not speculate so much.

R 1.4: Our results have potential implications for other N-saturated subtropical forests, despite that further tests are needed. We will rephrase our conclusions and largely confine them to our site.

Line 127: change to: “In each block, plots were randomly assigned to a reference (Ref) and a P treatment.”
R 1.5: Agreed

Line 164-165: How often and when did you measure trace gas fluxes? Did you take water samples (NH4+ and NO3- concentrations) at the same time? A different sampling time may explain the lack of correlation between N2O emissions and NO3- concentrations.

R 1.6: Gas flux measurements were conducted monthly in the warm season and bi-monthly in the cold seasons. Soil water samples were retrieved on the same day the gas fluxes were measured and analyzed for NO3- and NH4+. A lack of correlation between N2O emissions and NO3- concentrations has been observed in other studies at TSP, e.g. by Zhu et al. (2013), who attributed this finding the non-limiting NO3- concentrations in the well-drained hillslope soils.

Line 251-254: Why do you use different units (μg N m-2 hr-1; kg N ha-1 yr-1) for the same variable?

We will revise the units, but stick to μg N2O-N m-2 h-1 for instantaneous fluxes (e.g. Figs. 2 and 5) and kg N2O-N ha-1 yr-1 for cumulative fluxes, as common in the GHG literature.

R 1.7: ok

We need to change to atmospheric

R 1.8: This has been discussed in R 1.3 and previously demonstrated by Zhu et al. (2013b).

Figures: Please provide information about sample size and if you use SD or SE in all of your figures.

R 1.9: ok


Referee #2

General comments The authors reported an experiment of P addition (79 kg P ha-1 yr-1, applied as NaH2PO4 powder) to an N saturated, Masson pine-dominated forest at TieShanPing (TSP), Chongqing, SW China for a period of 18 months. In the experiment, they measured soil fluxes of N2O and CH4, soil chemistry and plant growth. They found that P addition significantly decreased soil N2O emissions and turned the soil from CH4 emissions into a net sink. The experiment is appropriate. Data interpretation was logical and supported their conclusion. The study is for a type of ecosystem (subtropical, high ambient N deposition, N-saturated forest soil) for which such information is lacking. Their findings are interesting, can help us understand the interaction effect of N and P on greenhouse gas emission and also have implication for the forest management (such as P fertilization). In addition, the manuscript is also well organized and well written. I have only some minor concerns which I would like to discuss with the authors or maybe helpful for improving the manuscript, please see details below.
R 2.0: We thank referee #2 for the positive feedback. The comments are addressed point by point below.

Specific comments

1) P7, Line117. “annual N deposition at TSP measured in throughfall varies between 40-65 kg ha⁻¹”. What is the N deposition in precipitation there? Because N deposition in throughfall may be affected by difference in species, structure etc. of the forest, N deposition in precipitation is better data for comparing different forests.

R 2.1: The bulk deposition at TSP is in a range of 20 to 30 kg N ha⁻¹ yr⁻¹ (Chen and Mulder, 2007). We will add this to our site description.

2) P9, L165. Please give the locations (in the center of the plot?) where you measured the gas emission in the plots.

R 2.2: Chambers for flux measurements were deployed next to the lysimeters, which were randomly distributed near the center in each of the 20m * 20 m plots, at least 3 meters away from the border.

3) P9, L165-167. “to investigate the immediate effect of P addition on ...... (7, 10 and 12 May) after the P application.” Did you show these results in the result or discussion section? If not, please delete it.

R 2.3: Yes, the data were shown in Fig. S6 and result section (line 228). Other studies have reported stimulation of N₂O emission by P addition, presumably due to microbial response in soil (Mori et al., 2013; Wang et al., 2014). Hence, we included the short-term data for comparison.

4) P11, Line 195 Statistical analyses. In the Experimental Design, the author showed that three blocks were established and two plots in each block in the study forest. In each block, plots were assigned randomly to a reference (Ref) and a P treatment. Did you try One-way Repeated-Measures ANOVA to examine the treatment effect for the emission of CH₄ and N₂O, due to measuring the gas repeatedly.

R 2.4: We have used repeated measures ANOVA to compare the fluxes of CH₄ and N₂O as well as DIN concentrations among all our plots (Ref-1, Ref-2, Ref-3, P-1, P-2 and P-3). These six plots were compared as independent groups instead of three Ref plots as a group and three plots as the other. If we use N₂O fluxes as an example, our results showed that in blocks 2 and 3, ref-2 and ref-3 were significantly larger than P-2 and P-3, respectively. Indeed, we believe and appreciate the suggestion from referee #1, that Linear Mixed Effects Models are better for interpreting treatment effects in our study. In the revision, we will reanalyze our dataset and anchor our discussion within the outcome of a Mixed Effect Model. For comparing parameters for tree-growth and soil properties (single observations only), one-way ANOVA is used (as in the original manuscript).

5) P19, L362-363. “Overall, our study demonstrates that chronically high N deposition has transformed TSP soils to a regional hotspot for N₂O and CH₄ emission.” It is not clear for me. Could you explain it?

R 2.5: For details, please refer to R 1.3 and R1.8.

......” has been published in Biogeosciences, please replace Biogeosciences Discuss.

R 2.6: Thanks. We will change it in the new version of manuscript.

7) P27, L585, Table 1. How did you get n=6? Did you mixed samples in each plot?

R 2.7: Before P application, we sampled the soil (for background properties) three times within each plot. Since there was no treatment yet, we combined two plots in the same block for presentation of the data.

8) P28, L591, Table 2. The yearly variation for some data is big. For example, PAL in the ref plots was 5.4 in Aug. 2013, but was 13.4 in Aug. 2015. Do you have any explanation for it?

R 2.8: Every half year, we sampled soil in triplicates from each plot randomly. Significant spatial heterogeneity is common in surface soils in 20*20 m² plots, as the litterfall may directly affect surface soil composition.

Referee #3

This paper report the effects of P addition on leachate chemistry and gas exchange of N2O and CH4 in a high N deposition forest of the warm and humid part of China. The results are interesting and the paper easy to read. However, the statistical treatment is not ideal, since the repeated nature of the measurements seems not to have been considered in the model tests. I see this problem is well addressed by the other reviewers. This needs to be addressed although it will not change the outcome and major conclusions.

R 3.0: We thank referee #3 for the constructive and detailed suggestions. Regarding the statistical methods, we will reanalyze the dataset with Linear Mixed Effects Model as proposed by referee #1, so as to improve and justify the interpretation of our results. Please refer to our response to referee #1 for more details on statistical analyses.

Below is my mainly minor comments listed by line number

70: ‘and’= ‘but’

R 3.1: Ok.

71: delete: ‘even’

R 3.2: Ok.

123: spell out what means PAL here at first appearance.

R 3.3: \( \text{PAL} \) means “ammonium lactate-extractable P, a common method to determine plant available P”. We will modify it in the revised manuscript.

126: 5-m buffer is a bit narrow, but cannot be changed
R 3.4: The designs of plot size and buffering strip were made with reference to Zheng et al. (2016) and Martinson et al. (2013). In line 126, the “5-m buffer strip separated the two plots in each block” actually means that 5-m buffer was included for each plot, thus resulting in 10-m distance between the borders of two neighboring plots. To avoid confusion, we will modify this sentence in the new version.

127: ‘ad’ = ‘at’ 130: ‘... in the TSP soil.’ 141: ‘Within each plot, three ceramic....’

R 3.5: We appreciate and accept the linguistic corrections from referee #3. Changes will be made in the revision.

145: ‘winter’ = ‘dormant’ or ‘dormant and dry’?

R 3.6: The “winter season” mainly refers to the “dormant and dry season”. We will rephrase it accordingly.

200: should be repeated ANOVA of some kind. It seems to me that the statistical analysis is not optimal.

R 3.7: Please refer to R2.4 for details.

206: check the subscript on P

R 3.8: We will check through and unify all the subscripts to “PAI”.

290: I am not in favor of discussing degrees of N-saturation; I would instead say ‘DHSRB is less N-rich with lower inorganic N availability than TSP’

R 3.9: We agree with it in general, but we will just use “less nitrate-leaching” to describe DHSRB in comparison to TSP.

303: ‘frequently’ = ‘shortly’

R 3.10: We assume that it refers to “frequently” in line 301 instead. We agree that “shortly” is more accurate.

306: delete ‘TSP’ here, implied in nearby

R 3.11: Thanks for the suggestion. We will delete “TSP”.

312-318: inhibition by NH4 cannot explain emission only lower uptake rates; so delete or reformulate

R 3.12: We are aware that the inhibition by ammonium affects gross methane uptake and not directly net emissions. However, the observed mean CH4 exchange rates (emission or uptake) at our TSP site was significantly smaller than reported in other subtropical forests from South China (Fang et al., 2009; Zhang et al., 2008) (Figs. 5 and 6). Therefore, it is reasonable to suggest that the inhibition of methane uptake by ammonium may have contributed to reverse net methane uptake to emission during “hotspots or hot moments” (Megonigal and Guenther, 2008) of methane production.
330: like line 290; reformulate

R 3.13: Changes will be made as presented in R3.9.

328-334: I would suggest that both reason (and others as well) may have contributed

R 3.14: As presented in the introduction section (line 91-93), “whether P addition affects the methanotrophic community in soils directly or alleviates the \( \text{NH}_4^+ \)-inhibition effect on \( \text{CH}_4 \) oxidation through enhanced N uptake” remains under debate (Veraart et al., 2015). In our case, we only have evidence for reduction in nitrate availability from soil water, supporting the “indirect” mechanism.

351-353: I do not understand this; what is the ‘tree biomass estimates’ doing here?

R 3.15: Other studies have documented that P limitation may restrict tree growth in Masson pine forests (Wang et al., 2007). Our hypothesis was that P addition may enhance tree growth and thus N uptake. As discussed in our manuscript, the tree biomass estimates show that no such effect occurred within two years after P addition

353-356: Why not, this should be simple and not much effort?

R 3.16: From the previous long-term study conducted at TSP forest (Huang et al., 2015), we have learned that the abundance of ground vegetation species is highly variable from year to year. This makes the evaluation of ground vegetation biomass really uncertain in a two-year scale (our study). In the long-term experiment, we have planned to include the measurements of ground vegetation.

436-38: something wrong in this ref 587: add ‘lactate’ 589, 594+595 add these lines to the table legend

R 3.17: We thank referee #3’s efforts on our manuscript. We will revise the manuscript according to the reviewer’s suggestions.
Details of revision:

Line 4: “Department of Environmental Sciences” changed to “Faculty of Environmental Sciences and Natural Resource Management”.

Line 17: “soil chemistry” to “soil N and P”

Line 28-30: “Our study suggests that P fertilization of N-saturated, subtropical forest soils could mitigate GHG emissions in addition to alleviate nutrient imbalances and reduce losses of nitrogen through NO₃⁻ leaching and N₂O emission.” changed to “Our study indicates that P fertilization of N-saturated, subtropical forest soils mitigates N₂O and CH₄ emissions, in addition to alleviating nutrient imbalances and reducing losses of N through NO₃⁻ leaching.”

Line 68-69: Add “on N₂O emissions” after “(3 to 5 years)”

Line 71: Delete “even”.

Line 83: Delete “other”

Line 101-102: Delete “ambient” and delete “ii) to test whether P affects N cycling in a highly N-saturated forest and”

Line 109-110: “Having a monsoonal climate, TSP has a mean annual precipitation” to “TSP has a monsoonal climate, with mean annual precipitation”

Line 112: “The soil is a loamy yellow mountain soil” changed to “Soils are predominantly well-drained, loamy yellow mountain soil”

Line 115-117: Add “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% (annual mean ~ 60%; Zhu et al., 2013b).”

Line 119-120: Add “while the annual bulk N deposition is from 20 to 30 kg ha⁻¹ (Chen and Mulder, 2007)”.

Line 122: Add “decreased” before “abundance”

Line 125: Change “PAl” to “ammonium lactate-extractable P”.

Line 128: Change “near a hilltop” to “well-drained soils of”

Line 129-130: Change “A 5-m buffer strip separated the two plots in each block. In each block, plots were assigned at random to a Reference and a P treatment” to “Adjacent plots were separated by at least 10-m buffer zone. In each block, plots were randomly assigned to a Reference and a P treatment”.
Line 138: “Together with the addition of phosphate, the P-treated plots also received 59.0 kg ha\(^{-1}\) of sodium (Na).” changed to “The addition of NaH\(_2\)PO\(_4\) \(2\)H\(_2\)O at the P-treated plots also resulted in an input of 59.0 kg ha\(^{-1}\) of sodium (Na).”

Line 144: Change “triplicates of” to “three”.

Line 145: Add “near plot centre” after “at 5-cm and 20-cm soils”.

Line 148: Change “in the winter season” to “in the dry and dormant season”.

Line 167: Delete “in micro-plots”

Line 198-213: Linear mixed effect models have been applied to analyze time-series data, to account for both repeated measurements and within-group variance of a stratification variable (block design). The fixed effect (treatment by P or not) is then tested by analysis of variance. The whole dataset has been reanalyzed with more appropriate statistical methods using R. The most important details are added to the text.

Line 216: “P\(_{\text{AL}}\)” to “P\(_{\text{Ai}}\)”.

Results Section: The descriptions of differences in mean NO\(_3\)\(^-\) concentrations, N\(_2\)O and CH\(_4\) fluxes among three blocks are now removed (Line 223-224; Line 236-237; Line 246-248).

Line 232: Add “values of” before “up”

Line 259: Add “of N\(_2\)O emission rates” after “range”

Line 262: Delete “up to”

Line 266: Change “High” to “Large”

Line 277: Change “The” to “, which attributed the”, delete “was attributed to”

Line 278: Change “due to” to “as a consequence of”

Line 284: Delete “(DHSBR)”

Line 292: Change “DHSBR” to “Dinghushan”; same applied to Line 299 and 301

Line 293: “which is not strongly different from that” to “which is similar to the N deposition at our site”

Line 292-295: “By contrast, the DHSBR site in South China receives 36 kg of atomospheric 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\(_2\)O emission rate than the Ecuadorian site (~ 0.88 kg N ha\(^{-1}\) yr\(^{-1}\) in the reference plot; Zheng et al., 2016).” changed to “By contrast, the Dinghushan site in South China receives 28 kg N ha\(^{-1}\) yr\(^{-1}\) through wet inorganic N deposition (Zheng et al., 2016), which is similar to the
N deposition at our site (Chen and Mulder, 2007b; Huang et al., 2015). They also observed larger N$_2$O emission rates (~ 0.88 kg N ha$^{-1}$ yr$^{-1}$ in the Reference plots) than in the Ecuadorian site.”

**Line 301:** Change “less N-saturated” to “less N-rich”.

**Line 312:** Change “frequently” to “shortly”.

**Line 317:** “TSP” deleted.

**Line 320:** Change “for South Chinese forest soils” to “for well-drained, forest soils in South Chinese forest”

**Line 321-325:** Change “As CH$_4$ fluxes at our sites were not significantly correlated with climatic factors (Fig. S4c and d), CH$_4$ emissions cannot be explained by (transient) wet conditions.” to “Since aerated upland soils typically provide favourable conditions for microbial CH$_4$ uptake (Le Mer and Roger, 2010), the net emission observed in our sites is unlikely due to enhanced production, but rather by suppressed consumption.”

**Line 329:** Change “our” to “the TSP”

**Line 341:** Add “soil water loss due to”

**Line 341-342:** Change “the high degree of N saturation” to “the strong N enrichment”.

**Line 343:** Change “the” to “direct”

**Line 344:** Change “the methane monooxygenase enzyme (Veldkamp et al., 2013), rather than a direct P-stimulation” to “methane monooxygenase (Veldkamp et al., 2013), rather than to P-stimulation”

**Line 352:** “the toxicity threshold” to “toxicity”

**Line 352-354:** Change “The frequent precipitation in the humid forest of this study (Yu et al., 2016), both prior and following the addition of NaH$_2$PO$_4$.2H$_2$O (Fig. 2), efficiently diluted and leached Na”, thus minimizing toxic effects.” to “Frequent precipitation at TSP (Yu et al., 2016), both prior and following the addition of NaH$_2$PO$_4$.2H$_2$O (Fig. 2), apparently diluted and leached Na”, thus preventing toxic effects.”

**Line 357-358:** Add “which represent a major decline in mobile anions in the P-treated soils”

**Line 363-364:** Add “increase in tree growth, due to”

**Line 375-376:** “our study demonstrates that chronically high N deposition has transformed TSP soils to a regional hotspot for N$_2$O and CH$_4$ emission” changed to “our study suggests that N-saturated TSP soils act as a regional hotspot for N$_2$O and CH$_4$ emissions”.

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**Line 605:** Add “lactate”.

**Figs. 1, 4, 6, S3 and S6:** More appropriate statistics have been used to compare the data for the Reference and P treatments. Data used in the box whisker plots are now derived from the averages of subplot triplicates.

**Added references**


Phosphorus addition mitigates $\text{N}_2\text{O}$ and $\text{CH}_4$ 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

Article type: Research Article
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$_2$O) 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$_2$O and CH$_4$ emissions together with soil N and P data for a period of 18 months following P addition (79 kg P ha$^{-1}$ yr$^{-1}$, applied as NaH$_2$PO$_4$ powder) to an 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$_2$O 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$_2$O, P addition significantly decreased CH$_4$ emissions, turning the soil 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 at TSP, but we cannot exclude that understory vegetation increased. Our study indicates that P fertilization of N-saturated, subtropical forest soils mitigates N$_2$O and CH$_4$ emissions, in addition to alleviating nutrient imbalances and reducing losses of N through NO$_3^-$ leaching.

Key Word: N$_2$O 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 CH₄, N₂O and CO₂, leading to increased radiative forcing (Montzka et al., 2011; Tian et al., 2016). During the last decade, atmospheric concentrations of CO₂, CH₄, N₂O 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 N₂O and CH₄ 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 N₂O 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 N₂O emissions have been reported from acid, upland forest soils in South China (Zhu et al., 2013b). Reported average N₂O 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 NO₃⁻ 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$_2$O 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$_2$O 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$_2$O-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$_2$O emissions during the first two years. However, N$_2$O 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$_2$O 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$_2$O emissions (0-2 years) but a significant longer term effect (3 to 5 years) on N$_2$O 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$_2$O 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$_2$O emissions even 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$_2$O 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.
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$_2$O 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$_2$O 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
The objectives were i) to quantify ambient $N_2O$ and $CH_4$ emissions, ii) to test whether $P$ affects $N$ cycling in a highly $N$-saturated forest and iii) to investigate the effect of $P$ 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° 38’ N, 106° 41’ 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 °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...
horizon is ~ 300 mg kg$^{-1}$, while ammonium lactate-extractable P is smaller than 5 mg kg$^{-1}$ (Table 1).

### 2.2 Experimental Design

Three blocks, each having two 20 m * 20 m plots, were established on well drained soils of a gently sloping hillside. Adjacent plots were separated by at least 10-m buffer zone. In each block, plots were randomly assigned to a Reference and a P treatment. On 4 May 2014, a single dose of P fertilizer was applied as solid NaH$_2$PO$_4$.2H$_2$O, 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 increase in soil available P. 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.

The addition of NaH$_2$PO$_4$.2H$_2$O at the P-treated plots also resulted in an input of 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, three ceramic lysimeters (P80; Staatliche Porzellanmanufaktur, Berlin) were installed at 5- and 20-cm soils near the plot centre 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 dry and dormant season and monthly during the growing season. All water samples were kept frozen during storage and transport. Concentrations of NH$_4^+$, NO$_3^-$, potassium (K$^+$), calcium (Ca$^{2+}$), and magnesium (Mg$^{2+}$) in soil water were measured at the Research Center for Eco-Environmental Sciences (RCEES), Chinese Academy of Sciences, Beijing, using ion chromatography (DX-120 for cations and DX-500 for anions).

In August 2013, soils from the O/A (0-3 cm), AB (3-8 cm) and B (8-20 cm) horizons were sampled near the lysimeters for soil analysis. Total P and plant-available P contents were monitored in samples collected from the O/A horizons every six months, starting two days before P addition. Soil samples were kept cold (< 4 °C) during transport and storage. Before analysis, soil samples were air dried and sieved (2 mm). Soil pH was measured in soil suspensions (10 g dry soil and 50 ml deionized water) using a pH meter (PHB-4, Leici, China).

Total soil C and N contents were determined on dried and milled samples, using a LECO elemental analyzer (TruSpec®CHN, USA). To measure total P, 1 g dry soil was digested with 5 ml of 6 M H$_2$SO$_4$ (Singh et al., 2005) and measured as ortho-phosphate by the molybdenum blue method (Murphy and Riley, 1962). Ammonium lactate (0.01 M)-extractable P and H$_2$O-extractable P (P$_{Al}$ and P$_{H2O}$, respectively) were measured as ortho-phosphate after extraction (1.5 g dry soil in 50 ml solution) (Singh et al., 2005). Ammonium oxalate (0.2 M)-extractable Fe, Al and P were measured by inductive coupled plasma (7500; Agilent) after extraction (1.5 g dry soil in 50 ml solution).

From August 2013 onwards, we measured N$_2$O and CH$_4$ emissions in triplicate in micro-plots close to the lysimeters, using static chambers (Zhu et al., 2013b). To investigate the immediate
effect of P addition on N₂O emissions, we sampled the gas emissions once before (2 May) and three times (7, 10 and 12 May) after the P application. Gas samples (20 ml) were taken 1, 5, 15 and 30 minutes after chamber deployment and injected into pre-evacuated glass vials (12 ml) crimp-sealed with butyl septa (Chromacol, UK), maintaining overpressure to avoid contamination during sample transport. Mixing ratios of N₂O and CH₄ were analyzed using a gas chromatograph (Model 7890A, Agilent, US) at RCEES, equipped with an ECD for detection of N₂O (at 375 °C with 25 ml min⁻¹ Ar/CH₄ as make up gas), a FID for CH₄ (250 °C; 20 ml min⁻¹ N₂ as make-up gas) and a TCD for CO₂. 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 N₂O 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 m² 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).

Daily average air temperature and sum of precipitation were monitored 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$_2$O 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$_2$O and CH$_4$ fluxes, NH$_4^+$, NO$_3^-$, K$^+$, Ca$^{2+}$ and Mg$^{2+}$ concentrations in soil water, as well as litterfall (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 of fixed effects through analysis of variance for LME models. One-way analysis of variance (ANOVA, Turkey post-hoc test) was conducted to examine the treatment effects on soil pH, nutrient contents in organic matter, and data of tree growth. 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 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$_2$O 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$_2$O fluxes were generally below 50 $\mu$g N m$^{-2}$ hr$^{-1}$ in the dry, cool season, but reached values of up to 600 $\mu$g N m$^{-2}$ hr$^{-1}$ in the growing season (Fig. 2). Cumulative N$_2$O emissions were estimated with seasonally averaged fluxes, and they differed greatly among the three blocks (Fig. 3). The greatest annual N$_2$O emission was observed in the Reference plot (7.9 kg N ha$^{-1}$) of block 2. Mean N$_2$O fluxes during the 1.5 years after P addition were
significantly smaller in the P treatment than in the Reference (Fig. 4). The P addition resulted in a decrease in cumulative N\textsubscript{2}O emission by about 3 kg N ha\textsuperscript{-1} yr\textsuperscript{-1} on average, which is a 50% reduction (Fig. 3). No immediate effects (within days) of P addition on N\textsubscript{2}O emission was observed (Fig. S5).

CH\textsubscript{4} fluxes varied greatly between blocks (Fig. 5). Net-emission of CH\textsubscript{4} was observed in summer 2013 (~ 80 \mu g C m\textsuperscript{-2} hr\textsuperscript{-1}) in blocks 1 and 2, whereas block 3 showed CH\textsubscript{4} uptake. From spring 2014 until October 2015, CH\textsubscript{4} fluxes were less variable in all blocks, with values fluctuating around zero. A longer period of net-emission was observed in block 3 during the dry season 2014. The fluxes did not correlate with either precipitation or air temperature (Fig. S5c&d). In the 1.5 years following P addition, mean CH\textsubscript{4} fluxes indicated net CH\textsubscript{4} emission (~ +3.8 \mu g C m\textsuperscript{-2} hr\textsuperscript{-1}) in the Reference, whereas net CH\textsubscript{4} uptake (~ -6.5 \mu g C m\textsuperscript{-2} hr\textsuperscript{-1}) was observed in the P treatment (Fig. 6). The suppressing effect of P addition on CH\textsubscript{4} emission was in accordance with what was found for NO\textsubscript{3}\textsuperscript{-} concentration and N\textsubscript{2}O emission.

### 3.3 The effect of P addition on tree growth

Throughout the 2-year experimental period, we observed no change in tree biomass (138 t ha\textsuperscript{-1}) in response to P addition (Table S3). Likewise, there was no effect of P treatment on the 500-needle weight (13 g on average). Between the two samplings in 2013 and 2014, we found differences in chemical composition of the pine needles, but this effect was not linked to P addition. Also, the C/N and N/P ratios of the needles (40 and 16, respectively) were hardly affected by P addition. Monthly litterfall varied seasonally in both Reference and P treatment (Fig. S6), but no significant difference was found between the two treatments.
4 Discussion

Background \( \text{N}_2\text{O} \) emission rates in the Reference plots were relatively large (Fig. 2), with mean values close to 100 \( \mu \text{g N m}^{-2} \text{ hr}^{-1} \) (Fig. 4). This is within the range of \( \text{N}_2\text{O} \) 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, \( \text{N}_2\text{O} \) emission rates averaged to 37 \( \mu \text{g N m}^{-2} \text{ hr}^{-1} \) in unmanaged sites at Dinghushan (Fang et al., 2009; Tang et al., 2006) and up to 50 \( \mu \text{g N m}^{-2} \text{ hr}^{-1} \) 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 \( \text{N}_2\text{O} \) emissions at TSP are likely due to the high 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 \( \text{N}_2\text{O} \) emissions (Ju et al., 2011), as monsoonal rainstorms triggered peak fluxes (Pan et al., 2003). The positive correlation between precipitation and \( \text{N}_2\text{O} \) emission peaks (Fig. S4a) may indicate the importance of denitrification as the dominant \( \text{N}_2\text{O} \) source. This is supported by recent \( ^{15}\text{N} \) tracing experiments at TSP (Zhu et al., 2013a).

Addition of P caused a significant decline in soil mineral N (predominantly \( \text{NO}_3^- \); Fig. 2), particularly during summers, when \( \text{NO}_3^- \) concentrations were relatively high (Fig. S2). At the same time, annual \( \text{N}_2\text{O} \) 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 \( \text{N}_2\text{O} \) emissions in P-treated soils decreased mineral N content, most likely due to as a consequence of stimulated plant uptake and/or
microbial assimilation. It is noteworthy that there was no significant correlation between N₂O emission rates and soil water NO₃⁻ concentration in our study (Figs. 2 and S2), suggesting that the suppressing effect of P on N₂O emissions was indirect, probably by affecting the competition for mineral N between plant roots and microbes (Zhu et al., 2016). In contrast to our 1.5-year 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 N₂O emission during the first two years after application. However, significant reduction in N₂O 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 N₂O 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 N₂O fluxes (~ 0.36 kg N ha⁻¹ yr⁻¹ in the Reference plots) (Martinson et al., 2013; Müller et al., 2015). By contrast, the (DHSBR)-Dinghushan site in South China receives 28 kg N ha⁻¹ yr⁻¹ through wet inorganic N deposition (Zheng et al., 2016), which is not strongly different from that similar to the N deposition at our site (Chen and Mulder, 2007b; Huang et al., 2015). They also observed larger N₂O emission rates (~ 0.88 kg N ha⁻¹ yr⁻¹ in the Reference plots) than in the Ecuadorian site. However, forests do not always display a straightforward relationship between N deposition and N₂O emissions. Manipulation experiments in the European NITREX project, for instance, revealed a much stronger correlation of N₂O emissions with soil NO₃⁻ leaching than with N deposition (Gundersen et al., 2012). Indeed, KCl-extractable mineral N at the (DHSBR)-Dinghushan site (~ 40 mg kg⁻¹; Zheng et al., 2016) is several-fold smaller than at our site (> 100 mg kg⁻¹; Zhu et al., 2013b), indicating that (DHSBR)-Dinghushan is less N-
saturated-N-rich than TSP. This suggests that the response of \( \text{N}_2\text{O} \) emission to P addition might depend on the N status of the soil. The fact that numerous studies found apparent suppression of \( \text{N}_2\text{O} \) 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 \( \text{N}_2\text{O} \) emissions upon P addition (Mori et al., 2013c; Wang et al., 2014). In an \textit{Acacia mangium} plantation, fertilized with P, Mori et al. (2013b&c) found that \( \text{N}_2\text{O} \) emissions were stimulated in the short-term but reduced in the long-term. While suppression of \( \text{N}_2\text{O} \) emission by P has been attributed to increased plant N uptake (Mori et al., 2014), increased \( \text{N}_2\text{O} \) emission are generally explained by enhanced microbial biomass (Liu et al., 2012) and denitrification activity (Ehlers et al., 2010; He and Dijkstra, 2015). \( \text{N}_2\text{O} \) emissions measured frequently shortly 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 \( \text{N}_2\text{O} \) emissions than changes in microbial activity, which are expected to occur more rapidly.

The Reference plots at TSP showed net \( \text{CH}_4 \) emission for extended periods of the year (Figs. 5 and 6). Also, long-term \( \text{CH}_4 \) fluxes sampled between 2012 and 2014 on TSP-hillslopes near-by (Fig. S7; Zhu et al., unpublished data) showed net \( \text{CH}_4 \) emission. This is in contrast to the generally reported \( \text{CH}_4 \) sink function of forested upland soils (Ciais et al., 2013; Dutaur and Verchot, 2007). For example, \( \text{CH}_4 \) uptake rates reported for well-drained, forest soils in South Chinese forest range from 30 to 60 \( \mu \text{g C m}^{-2} \text{ hr}^{-1} \) (Fang et al., 2009; Tang et al., 2006; Zhang et al., 2014a). Since aerated upland soils typically provide favourable conditions for microbial \( \text{CH}_4 \) uptake (Le Mer and Roger, 2010), the net emission observed in our sites is unlikely due to enhanced production, but rather by suppressed consumption. One reason for the net \( \text{CH}_4 \) emission
at TSP could be inhibition of CH$_4$ oxidation by NH$_4^+$, as reported previously (Bodelier and Laanbroek, 2004; Zhang et al., 2014a). The concentration of NH$_4^+$ in the soil water was rather small (< 0.5 g L$^{-1}$; Fig. 1), which does not preclude, however, that NH$_4^+$ availability from the soil exchangeable pool is high. Zhu et al. (2013b) found extraordinarily high KCl-extractable NH$_4^+$ in TSP surface soils, likely reflecting the large atmogenic NH$_4^+$ input at the TSP site (Huang et al., 2015).

P addition had a significant impact on CH$_4$ fluxes, changing the soil from a net source to a net sink on an annual basis (Fig. 6). However, the uptake rates of CH$_4$ in the P treatments remained smaller than those reported for forest soils in tropical China (Tang et al., 2006; Zhang et al., 2008b). The stimulating effect of P addition on CH$_4$ uptake is consistent with previous studies (Mori et al., 2013a, 2013b; Zhang et al., 2011), and has been attributed to lessening the NH$_4^+$ inhibition of methane oxidation. Unfortunately, we did not measure KCl-extractable NH$_4^+$ in our study, but a decline of available NH$_4^+$, which is the substrate for nitrification, is likely as NO$_3^-$ concentrations in soil water were significantly smaller with in the P-treatments (Fig. 1). P addition may also result in a change of the taxonomic composition of the methane oxidizing community (Mori et al., 2013a; Veraart et al., 2015). Alternatively, CH$_4$ oxidation may be stimulated by increased CH$_4$ diffusion into the soil, due to enhanced root growth and increased soil water loss due to transpiration in P-amended plots (Zhang et al., 2011). Given the high degree of N saturation strong N enrichment of TSP forest (Huang et al., 2015), it is likely that the reason for the observed reduction in CH$_4$ emissions in response to P fertilization is due to alleviating the direct NH$_4^+$ inhibition of methane monooxygenase (Veldkamp et al., 2013), rather than to P-stimulation of methanotrophic activity (Veraart et al., 2015).
Shortly after fertilizer application, we observed a modest, albeit significant increase of Na\(^+\) concentration in soil water (Table S2). Other studies have documented the potential toxicity of excess Na\(^+\) in soil water to plant and microbial activities (Rengasamy et al., 2003; Wong et al., 2008). 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 smaller than the values commonly assumed to indicate toxicity (40 to 100 mg L\(^{-1}\)) (Bernstein 1975). Frequent precipitation at TSP (Yu et al., 2016), both prior and following the addition of NaH\(_2\)PO\(_4\).2H\(_2\)O (Fig. 2), apparently diluted and leached Na\(^+\), thus preventing 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. S3), as expected from the reduction of NO\(_3^+\) concentrations in the P-treatments, which represent a major decline in mobile anions in the P-treated soils (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. S6), making it difficult to link the observed reduction in mineral N in the soil solution (Fig. 1) to plant growth. 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 increase in tree growth, due to 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 have reduced 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, enhanced N uptake by understory growth and/or soil microbial biomass may have been the main mechanisms responsible for observed NO$_3^-$ decline in the P-treated soil (Hall & Matson 1999).

Overall, our study suggests that N-saturated TSP soils act as a regional hotspot for N$_2$O and CH$_4$ emissions. Within the short experimental period of 1.5 years, P fertilization was shown to significantly decrease NO$_3^-$ concentrations in soil water and to reduce both N$_2$O and CH$_4$ emissions. These findings provide a promising starting point for improving forest management towards GHG abatement targets, taking into account the P and N status of subtropical soils in the region.
5 Acknowledgement

Longfei Yu thanks the China Scholarship Council (CSC) for supporting his PhD study. Support from the Norwegian Research Council to project 209696/E10 ‘Forest in South China: an important sink for reactive nitrogen and a regional hotspot for N₂O?’ is gratefully acknowledged. We thank Prof. Wang Yanhui, Prof. Duan Lei, Dr. Wang Zhangwei, Zhang Yi, Zhang Ting, Zou Mingquan for their help during sample collection and data analysis. Dr. Zhu Jing is gratefully acknowledged for unpublished data on long-term CH₄ fluxes in the TSP catchment.
Reference


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Table 1 Background soil properties of the experimental plots at Tieshanping (TSP). Values are means and standard deviations in parenthesis ($n = 6$). Soils were sampled in August 2013.

<table>
<thead>
<tr>
<th>Soil Layer</th>
<th>pH</th>
<th>Total C g kg$^{-1}$</th>
<th>Total N g kg$^{-1}$</th>
<th>Total P mg kg$^{-1}$</th>
<th>C/N</th>
<th>N/P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block 1 O/A (0-3 cm)</td>
<td>3.7 (0.1)</td>
<td>80.7 (32.3)</td>
<td>4.8 (1.7)</td>
<td>308 (57)</td>
<td>17.0 (2.5)</td>
<td>15.5 (5.7)</td>
</tr>
<tr>
<td>AB (3-8 cm)</td>
<td>3.8 (0.0)</td>
<td>23.9 (9.3)</td>
<td>1.3 (0.6)</td>
<td>-</td>
<td>* 20.0 (3.0)</td>
<td>-</td>
</tr>
<tr>
<td>B (8-20 cm)</td>
<td>3.9 (0.2)</td>
<td>8.6 (1.2)</td>
<td>&lt; 0.05</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>O/A (0-3 cm)</td>
<td>3.6 (0.1)</td>
<td>77.6 (13.4)</td>
<td>4.7 (0.8)</td>
<td>297 (44)</td>
<td>16.7 (1.3)</td>
<td>15.7 (2.8)</td>
</tr>
<tr>
<td>Block 2 AB (3-8 cm)</td>
<td>3.7 (0.1)</td>
<td>20.2 (5.3)</td>
<td>1.0 (0.3)</td>
<td>-</td>
<td>21.4 (3.3)</td>
<td>-</td>
</tr>
<tr>
<td>B (8-20 cm)</td>
<td>3.9 (0.1)</td>
<td>7.1 (1.6)</td>
<td>&lt; 0.05</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>O/A (0-3 cm)</td>
<td>3.6 (0.1)</td>
<td>67.0 (15.5)</td>
<td>3.8 (0.8)</td>
<td>223 (45)</td>
<td>17.4 (0.6)</td>
<td>17.2 (3.7)</td>
</tr>
<tr>
<td>Block 3 AB (3-8 cm)</td>
<td>3.6 (0.1)</td>
<td>21.0 (7.9)</td>
<td>1.1 (0.5)</td>
<td>-</td>
<td>24.5 (4.6)</td>
<td>-</td>
</tr>
<tr>
<td>B (8-20 cm)</td>
<td>3.8 (0.1)</td>
<td>7.2 (1.5)</td>
<td>&lt; 0.05</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Soil Layer</th>
<th>$P_{H2O}$ mg kg$^{-1}$</th>
<th>$P_{Al}$ mg kg$^{-1}$</th>
<th>$Al_{ox}$ mg kg$^{-1}$</th>
<th>$Fe_{ox}$ mg kg$^{-1}$</th>
<th>$P_{ox}$ mg kg$^{-1}$</th>
<th>$P_{ox}/(Al_{ox} + Fe_{ox})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block 1 O/A (0-3 cm)</td>
<td>&lt; 5.0</td>
<td>5.8 (1.4)</td>
<td>1700 (513)</td>
<td>1933 (350)</td>
<td>85.8 (22.6)</td>
<td>0.025 (0.008)</td>
</tr>
<tr>
<td>AB (3-8 cm)</td>
<td>&lt; 5.0</td>
<td>2.1 (0.6)</td>
<td>1217 (243)</td>
<td>1692 (493)</td>
<td>47.1 (22.0)</td>
<td>0.016 (0.007)</td>
</tr>
<tr>
<td>B (8-20 cm)</td>
<td>&lt; 5.0</td>
<td>&lt; 1.0</td>
<td>1083 (90)</td>
<td>1158 (249)</td>
<td>29.3 (28.6)</td>
<td>0.012 (0.011)</td>
</tr>
<tr>
<td>O/A (0-3 cm)</td>
<td>&lt; 5.0</td>
<td>5.9 (1.0)</td>
<td>1500 (238)</td>
<td>1792 (215)</td>
<td>79.2 (21.5)</td>
<td>0.024 (0.007)</td>
</tr>
<tr>
<td>Block 2 AB (3-8 cm)</td>
<td>&lt; 5.0</td>
<td>1.6 (0.4)</td>
<td>925 (149)</td>
<td>1517 (320)</td>
<td>37.2 (10.7)</td>
<td>0.016 (0.006)</td>
</tr>
<tr>
<td>B (8-20 cm)</td>
<td>&lt; 5.0</td>
<td>&lt; 1.0</td>
<td>892 (209)</td>
<td>1033 (413)</td>
<td>16.1 (10.5)</td>
<td>0.009 (0.007)</td>
</tr>
<tr>
<td>O/A (0-3 cm)</td>
<td>&lt; 5.0</td>
<td>4.1 (0.9)</td>
<td>1367 (180)</td>
<td>1667 (168)</td>
<td>50.7 (10.9)</td>
<td>0.017 (0.003)</td>
</tr>
<tr>
<td>Block 3 AB (3-8 cm)</td>
<td>&lt; 5.0</td>
<td>4.4 (4.0)</td>
<td>1075 (128)</td>
<td>1350 (150)</td>
<td>24.8 (8.3)</td>
<td>0.010 (0.002)</td>
</tr>
<tr>
<td>B (8-20 cm)</td>
<td>&lt; 5.0</td>
<td>&lt; 1.0</td>
<td>992 (130)</td>
<td>875 (138)</td>
<td>8.0 (2.0)</td>
<td>0.004 (0.001)</td>
</tr>
</tbody>
</table>

$P_{H2O}$ = Water-extractable P, $P_{Al}$ = Ammonium lactate-extractable P, $Al_{ox}$ = Oxalate extractable Al, $Fe_{ox}$ = Oxalate extractable Fe, $P_{ox}$ = Oxalate extractable P.

* Data not available
Table 2 Soil pH, C, N and P contents in the O/A horizon (0-3 cm) in the References (Ref) and P treatments. Values are means and standard deviations in parenthesis (n = 9). P addition was conducted on 14/05/04, after the first two sampling dates.

<table>
<thead>
<tr>
<th>Date</th>
<th>Treatment</th>
<th>pH</th>
<th>Total C (g kg⁻¹)</th>
<th>Total N (g kg⁻¹)</th>
<th>C/N</th>
<th>P₂O₅ (mg kg⁻¹)</th>
<th>Total P (mg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13/08/02</td>
<td>Ref</td>
<td>3.7 (0.1)</td>
<td>8.3 (2.3)</td>
<td>0.5 (0.1)</td>
<td>16.9 (1.1)</td>
<td>5.4 (1.4)</td>
<td>292 (46)</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>3.6 (0.1)</td>
<td>6.7 (2.0)</td>
<td>0.4 (0.1)</td>
<td>17.1 (2.1)</td>
<td>5.1 (1.3)</td>
<td>260 (70)</td>
</tr>
<tr>
<td>14/05/02</td>
<td>Ref</td>
<td>3.7 (0.1)</td>
<td>12.2 (4.2)</td>
<td>0.9 (0.3)</td>
<td>13.7 (1.5)</td>
<td>19.0 (8.0)</td>
<td>336 (65)</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>3.8 (0.2)</td>
<td>9.0 (3.5)</td>
<td>0.7 (0.2)</td>
<td>14.2 (2.8)</td>
<td>13.7 (5.2)</td>
<td>270 (72)</td>
</tr>
<tr>
<td>14/05/10</td>
<td>Ref</td>
<td>3.8 (0.1)</td>
<td>9.9 (2.1)</td>
<td>0.7 (0.2)</td>
<td>14.0 (0.7)</td>
<td>15.4 (7.0)</td>
<td>304 (49)</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>3.9 (0.3)</td>
<td>8.0 (1.9)</td>
<td>0.6 (0.1)</td>
<td>14.3 (1.3)</td>
<td>174 (114)</td>
<td>572 (242)</td>
</tr>
<tr>
<td>14/12/02</td>
<td>Ref</td>
<td>3.8 (0.1)</td>
<td>10.5 (3.6)</td>
<td>0.7 (0.3)</td>
<td>14.5 (1.3)</td>
<td>14.2 (7.4)</td>
<td>328 (102)</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>3.9 (0.2)</td>
<td>9.5 (2.1)</td>
<td>0.7 (0.1)</td>
<td>14.0 (0.8)</td>
<td>66 (24)</td>
<td>442 (106)</td>
</tr>
<tr>
<td>15/08/02</td>
<td>Ref</td>
<td>3.9 (0.2)</td>
<td>8.3 (2.2)</td>
<td>0.4 (0.1)</td>
<td>20.5 (2.5)</td>
<td>13.4 (6.2)</td>
<td>291 (61)</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>4.0 (0.2)</td>
<td>6.5 (1.9)</td>
<td>0.3 (0.1)</td>
<td>19.7 (2.2)</td>
<td>57 (36)</td>
<td>383 (136)</td>
</tr>
</tbody>
</table>

† Different letters indicate significant differences (p < 0.05).
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).
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 N₂O 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).
Fig. 4 Box whisker plots for N$_2$O fluxes in the Reference and P treatment throughout 1.5 years after the P addition; red dashed lines indicate mean values; different letters indicate significant difference (p < 0.05).
**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.
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; the different letters indicate significant difference (p < 0.05).