ORIGINAL ARTICLE

Effects of phosphorus addition on \( \text{N}_2\text{O} \) emissions from an \textit{Acacia mangium} soil in relatively aerobic condition

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ABSTRACT  Effects of phosphorus (P) addition on nitrous oxide (\( \text{N}_2\text{O} \)) emissions from an \textit{Acacia mangium} plantation soil was examined in relatively aerobic condition with carbon (C) and nitrogen (N) addition. We hypothesized that P addition reduced \( \text{N}_2\text{O} \) emissions through stimulated microbial N immobilization and subsequent decrease in inorganic N resources for producing \( \text{N}_2\text{O} \). We prepared the following four experimental sets; high C (glucose 2000 µg C g soil\(^{-1}\)) and water-filled pore space (WFPS) 40 % (H40), low C (glucose 100 µg C g soil\(^{-1}\)) and WFPS 40 % (L40), high C and WFPS 60 % (H60), and low C and WFPS 60 % (L60). Nitrogen (\( \text{NH}_4\text{NO}_3 \), 20 µg N g soil\(^{-1}\)) was also added to all soils. We prepared P-added soils (\( \text{Ca(H}_2\text{PO}_4 \)), 20 µg P g soil\(^{-1}\)) and non-added control to test the effects of P addition on \( \text{N}_2\text{O} \) emissions. Contrary to our hypothesis, P addition did not reduce \( \text{N}_2\text{O} \) emissions, although soil microbial N immobilization was stimulated by P addition in soils with low C addition. Stimulated total N cycling by P addition probably offset the decrease in soil inorganic N. Meanwhile P addition reduced soil microbial biomass N (MBN) content in H60, where \( \text{N}_2\text{O} \) emissions increased significantly by P addition. It was possible that the microbial growth reached its peak and started dying more quickly in P-added soils in H60 due to the favorable condition for microbes (higher C and water content). Thus we concluded that (i) P addition did not necessarily stimulate soil microbial N immobilization, and (ii) \( \text{N}_2\text{O} \) emissions might not decrease even if P addition stimulated soil microbial N immobilization.

Key words: microbial biomass, nitrogen immobilization, nitrous oxide, phosphorus, tropical tree plantation

INTRODUCTION

Increases in the atmospheric nitrous oxide (\( \text{N}_2\text{O} \)) emissions contribute to both global warming and the distraction of the stratospheric ozone layer (Ravishankara et al. 2009). Soils are major sources for the production of \( \text{N}_2\text{O} \), which are byproducts or intermediate products of microbial nitrification and denitrification, respectively (Wrage et al. 2001). Tropical soils are important sources of \( \text{N}_2\text{O} \). In recent years, fast-growing leguminous tree plantations has expanded in tropical Asia (FAO, 2006), and several studies have suggested that the plantations instead of indigenous tree species increase \( \text{N}_2\text{O} \) emissions from soils due to the high nitrogen (N)-fixing ability of the leguminous trees (Arai et al. 2008, Konda et al. 2008, 2010).

We recently reported that phosphorus (P) application reduced \( \text{N}_2\text{O} \) emissions from tropical leguminous tree plantation (Mori et al. 2013a), and attributed the result to stimulated plant N uptake in the P-limited tropical ecosystem (Cleveland et al. 2002, Elser et al. 2007). Our additional experiment verified the suggestion by showing that P addition tended to increase \( \text{N}_2\text{O} \) emissions from root-excluded plots, while clearly reduced from root-existing plots (Mori et al. 2014). Several other studies came to the same conclusion by demonstrating P application reduced \( \text{N}_2\text{O} \) emissions from acacia and eucalyptus plantation in tropical monsoon area (Zhang et al. 2014) or P-limited maize field (Baral et al. 2014).

On the other hand, direct effects of P addition on soil microbial activity has been rarely reported. Our previous reports (Mori et al. 2010, 2013b) showed that P addition with and without carbon (C) and/or N addition on \textit{Acacia mangium} plantation soils stimulated \( \text{N}_2\text{O} \) emission under...
relatively anaerobic condition, which is preferable for producing \( \text{N}_2\text{O} \) in our study site (Arai et al. 2008). The result was attributed to stimulated N cycling, activated denitrifying and/or nitrifying bacteria, and promoted anaerobic condition derived from stimulated heterotrophic microbial activities (Mori et al. 2013b). However the result might be different under (i) relatively aerobic condition in which nitrification is the main source of \( \text{N}_2\text{O} \), and (ii) condition with sufficient C, where strong microbial N immobilization restricts nitrification (Verhagen and Laanbroek 1991, 1992). Hall and Matson (1999) suggested that poor P availability in tropical soils cause higher \( \text{N}_2\text{O} \) emissions because P shortage limits microbial N immobilization and increase labile N that is usable for nitrification and/or denitrification. Their report implies that P addition may increase microbial N immobilization and reduce N resources for producing \( \text{N}_2\text{O} \), reducing the emissions under aerobic and C-rich condition where N immobilization is more dominant than nitrification (Verhagen and Laanbroek 1991). In the present study we demonstrated the effects of P addition on \( \text{N}_2\text{O} \) emissions under relatively aerobic condition of WFPS 40 % and 60 % with enough labile C (glucose was added), by a laboratory incubation experiment.

**MATERIALS AND METHODS**

**Soil sampling**

Soil sample (0–5 cm) was taken from a 0-year (immediately after harvesting) *Acacia mangium* plantation site in South Sumatra province, Indonesia (3° 42.05' S, 104° 00.13' E) in September 2007. The samples were mixed well and air-dried after sieving (2 mm) and kept dry until the incubation experiment. Annual precipitation and mean annual temperature of the study site is 2750 mm and 27.3°C, respectively (Hardjono et al. 2005). The period from April to September is relatively dry season and October to May is wet season, although there is no clear distinction. The study site is on the second generation of plantation activity. Although 85 g P of super phosphate (SP36, PT Pertrokimia, Gresik, East Java, Indonesia) and 35 g N of urea per tree was added to each planting hole before planting in 1999, no additional fertilizer was applied. Soils in the study area are classified as Acrisols derived from Tertiary sedimentary rock (Yamashita et al. 2008, Mori et al. 2013c). The soil had: total C, 36.4 mg C g\(^{-1}\); total N, 2.3 mg N g\(^{-1}\); available P, 3.2 mg P kg\(^{-1}\); particle density, 2.53; texture, clay. Total C and total N were analyzed with a CN coder (JM1000CN, J SCIENCE, Kyoto, Japan). The available P was determined by Bray-2 method (Nanjo 1997). The particle density was determined with pycnometer method.

**Incubation**

The following soil conditions were established; high C and water-filled pore space (WFPS) 40 % (H40), low C and WFPS 40 % (L40), high C and WFPS 60 % (H60), and low C and WFPS 60 % (L60). We prepared only the two WFPS conditions of 40 % and 60 % because it was difficult to prepare the homogeneous water condition if WFPS was lower than 40 %. Four sets of 500 g soil (air-dried) were prepared in plastic basins. Additional 5 g of fresh soil sample taken in November 2011 (0–5 cm surface soil from the same region) was shaken with 50 ml distilled water for 30 min, and 15 ml of the supernatants was added to each sample as microbial inoculant. Thirty minutes after the addition of inoculant and mixing the soils well, C and N were added as follows: twenty \( \mu \)g N g\(^{-1}\) as \( \text{NH}_4\text{NO}_3 \) and 100 \( \mu \)g C g\(^{-1}\) as glucose were added to L40 and L60 with 100 ml distilled water; to H40 and H60, we added 20 \( \mu \)g N g\(^{-1}\) as \( \text{NH}_4\text{NO}_3 \) and 2000 \( \mu \)g C g\(^{-1}\) as glucose with 100 ml distilled water. After the addition of C and N, the soils were mixed well again.

Thirty g soils (oven-dry base) were placed in 223 ml wide mouth jars for gas emission analysis, 5 g in 50 ml plastic bottles for inorganic N analysis, 5 g in 50 ml plastic bottles for dissolved organic C (DOC) and dissolved N (DN) analysis, and 5 g in a 50 ml glass bottles for soil microbial biomass analysis. For each analysis we took eight replications from each soil, four for P-added soils and four for non-added control. Two hours after the addition of inoculant, 20 \( \mu \)g P g\(^{-1}\) as \( \text{Ca}[\text{H}_2\text{PO}_4]\) were added to P-added soils, and water content was adjusted to WFPS 40 % or 60 %. Different chemical treatments did not cause the changes of the soil pH(\( \text{H}_2\text{O} \)) (values determined by 1:2.5 air-dried soil to distilled water ratio ± SE) (4.41 ± 0.01, 4.42 ± 0.01, 4.42 ± 0.00, and 4.41 ± 0.00 in low C, low C with P, high C, high C with P conditions, respectively; \( P = 0.47 \), one-way ANOVA, \( n = 3 \)). In the present experiment, the pre-incubation was not performed because we observed that the pre-incubated soils repelled water. Although the lack of the pre-incubation may cause the emissions of gases preserved in the soil pore spaces during the early period, we believe that air-dry soils would have not preserved much gases in their pore spaces. At the start of the incubation, all bottles were covered with polyethylene plastic wrap to prevent water evaporation except for wide mouth jars for gas sampling.
Gas samples were taken from the upper air of wide mouth jars at the start of the incubation, and the jars were immediately closed with butyl rubber stoppers equipped with sampling ports, and put into the incubator at 25°C under the dark. One day after the start of the incubation, gas samples were taken again and the jars were put back into the incubator again after taking butyl rubber stoppers off and covered with polyethylene plastic wrap. Gas samples at 2–3 days and 5–6 days were also taken in the same manner. All other soils for chemical and microbial analysis were also placed into the incubator at 25°C under the dark.

Gas and soil analysis

Nitrous oxide and CO₂ emissions were measured by calculating the changes of the gas concentrations during 0–1 days, 2–3 days, and 5–6 days. The N₂O concentration in the gas sample was analyzed using a gas chromatograph (GC-14B, SHIMADZU, Kyoto, Japan) equipped with an electron capture detector. The column, injector, and detector temperatures were kept at 60, 80 and 330°C, respectively. Argon containing 5% CH₄ was used as a carrier gas. The CO₂ concentration was analyzed with a gas chromatograph (GC-14B, SHIMADZU, Kyoto, Japan) equipped with a thermal conductivity detector, using He as a carrier gas. The column, injector and detector temperatures were kept at 60, 60 and 100°C, respectively.

One and 3 days after the start of the incubation experiment, soil analyses were conducted. We failed to conduct chemical and microbial analyses at 6 days. Both NH₄⁺ and NO₃⁻ were extracted by shaking the soils (5 g) incubated in the plastic bottles with 50 ml 2 M KCl for 1 h. Ammonium was determined by indophenol blue abroptiometry and NO₃⁻ by naphthyl ethylenediamine method using a flow injection analyzer (AQLA-700-NO, AQUA LAB, Japan). Dissolved organic C and DN were extracted by shaking soils (5 g) incubated in the plastic bottles with 25 ml 0.5 M K₂SO₄ for 30 min, and analyzed by a total organic carbon analyzer with a total organic nitrogen measurement unit (TOC-Vₐ/TNM-1, SHIMADZU, Kyoto, Japan). Soil microbial biomass C (MBC) and N (MBN) were determined by a chloroform fumigation extraction method (Vance et al. 1987). The incubated soils (5 g) in the glass bottles were exposed to CHCl₃ vapor for 24 h in a vacuum desiccator at 25°C. After residual CHCl₃ was removed, fumigated soils were shaken with 25 ml of 0.5 M K₂SO₄ extractant for 30 min and soluble C and N were extracted. Soil microbial biomass element contents were calculated from the differences of soluble C and N between the fumigated and unfumigated samples using conversion factors of 0.45 (Jenkinson et al., 2004). Soluble C and N were analyzed by a total organic carbon analyzer with a total organic nitrogen measurement unit (TOC-Vₐ/TNM-1, SHIMADZU, Kyoto, Japan).

Statistical analysis

Statistical analyses were performed by Excel 2013 with statistical add-in software (SSRI). The level of significance was examined by three-way (C amount, WFPS, and P addition) ANOVA followed by Tukey’s post hoc tests, assuming the normality of each data. Data were log-transformed if necessary. If interactions of the three factors were significant, we conducted simple main effect analysis. Since some data showed slightly lower gas flux than zero, we replaced the value with one-half of the minimum gas flux (0.031 ng N g⁻¹ soil⁻¹ day⁻¹) for further calculation (Gilbert 1987).

RESULTS

Gas emissions

Interactive effects of two factors (C amount*WFPS, WFPS*P addition) on N₂O emissions were significant at 0–1 day (Table 1, Fig. 1ab). Simple main effect analysis suggested that N₂O emissions were higher in WFPS 60% than 40% at both lower and higher C conditions, and higher in higher C conditions than lower C conditions at WFPS 60% but not at 40%. It was also suggested that at both control soils and P-added soils N₂O emissions were higher in WFPS 60% than 40%, and P-added soils showed higher emissions at WFPS 60% but not at 40%. Three factors influenced N₂O emissions at 2–3 day with significant interaction (Table 1, Fig. 1ab). N₂O emissions were higher in higher C conditions and higher WFPS. P addition reduced N₂O emissions except at WFPS 40%. At 5–6 day, only WFPS influenced N₂O emissions, with higher emissions at WFPS 60% than at 40%. CO₂ emissions were influenced by all three factors (C amount, WFPS, and P addition) with significant interaction at all of the 0–1 day, 2–3 day, and 5–6 day (Table 1, Fig. 1cd). CO₂ emissions were generally higher in higher C conditions and at higher WFPS. P addition stimulated CO₂ emissions except at lower C condition at 2–3 day and 5–6 day, and at WFPS 40% at 5–6 day.
### Soil microbial C and N

Three factors (C amount, WFPS, and P addition) influenced MBC contents at 1 day with significant interaction (Table 2). At WFPS 40%, MBC contents were not different among different C conditions and treatment. At WFPS 60%, higher C conditions caused higher MBC, and the difference was higher in soils without P addition. Regardless of C condition, MBC contents were higher in WFPS 60% than in WFPS 40%. P addition reduced MBC contents at higher C condition. At 3 day, MBC contents were higher in higher C conditions, but not influenced by other factors (Table 2). Interactive effects of two factors (C amount*WFPS, C amount*P addition, and WFPS*P addition) on MBN contents were significant at 1 day. The results of simple main effect analyses were as follows: MBN contents were higher at WFPS 60% than 40% at both C conditions; MBN contents were higher at lower C conditions at WFPS 40%, but not at 60%; P addition increased MBN contents in lower C conditions, but reduced in higher C conditions; at WFPS 40%, P addition increased MBN contents, but reduced at WFPS 60%. At 3 day, only C conditions affected MBN contents with higher contents in higher C conditions (Table 2).

### Soil chemical properties

Carbon amount and WFPS had interactive effects on DOC contents at 1 day (Table 3). At higher C conditions, DOC contents were higher at WFPS 40% than 60%, but at lower C conditions there were no differences between WFPS 40% and 60%. At both WFPS 40% and 60%, higher C conditions showed higher DOC contents. All three factors (C amount, WFPS, and treatment addition) influenced DOC contents at 3 day with significant interaction (Table 3). At WFPS 40%, higher C conditions had higher DOC contents and P addition reduced DOC contents. At WFPS 60%, higher C conditions caused higher DOC contents with larger differences in soils without P addition. P addition reduced DOC contents in higher C conditions but not in lower C condition. DN contents were not influenced by any factors at 1 day, but at 3 day, C amount and treatment had significantly influenced interactively (Table 3). At both control and P-added soils, DN contents were higher at lower C conditions. P addition reduced DN contents at higher C conditions, but not in lower C conditions.

No factors influenced NH₄⁺ contents at 1 day, but at 3 day lower C condition caused higher NH₄⁺ contents (Table 4). At 1 day, no factors influenced NO₃⁻ contents (Table 4).
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Three of C amount, WFPS, and P addition influenced NO$_3^-$ contents at 3 day with significant interaction (Table 4). At both WFPS 40% and 60%, higher C conditions caused lower contents of NO$_3^-$. NO$_3^-$ contents were higher at WFPS 60% than at WFPS 40% in higher C condition, but no significant differences were observed in lower C condition. P addition reduced NO$_3^-$ contents at higher C condition. At WFPS 40%, only H40+P showed lower contents than others.

**DISCUSSION**

Higher $N_2O$ emissions in higher WFPS in the present study were in accordance with most of the other study (for example, Davidson et al. 2000, Yanai et al. 2007). Higher labile C addition also caused the higher $N_2O$ emissions as previously reported (Nobre et al. 2001), indicating that denitrification was activated in the present study, because denitrifiers need organic C as energy source but nitrifier does not. Although we tried to set the soil condition aerobic, it is possible that there were some microsites in the soil with heterogeneity and anaerobic condition developed in the microsites.

We demonstrated P addition increased the microbial N immobilization when added with 100 µg C (lower C conditions) at 0–1 day, in harmony with our hypothesis (Table 2). Sundareshwar et al. (2003) demonstrated that P addition stimulated microbial N immobilization and reduced N which is available for $N_2O$ producer, leading to lower $N_2O$ emissions. Hall and Matson (1999, 2003) suggested that tropical soils with limited P availability restricted soil microbial N immobilization, providing more available N for nitrification and/or denitrification process. Their suggestion implies that P addition stimulates microbial N immobilization and reduces $N_2O$ emissions. Nevertheless, the increase in microbial N immobilization observed in our experiment at 1 day (Table 2) was not
associated with reduced N\textsubscript{2}O emissions at 0–1 day at lower C condition (P addition rather increased the N\textsubscript{2}O emissions at WFPS 60 %, Table 1). Stimulated total N cycling by P addition (Mori et al. 2010), including N mineralization, nitrification, denitrification, and microbial N immobilization, probably offset the decrease in soil inorganic N. This is supported by data showing no differences in soil inorganic N contents and DN contents between P-added soils and soils without P addition at 1 day (Table 3 and 4). Higher MBN contents in P-added soils in higher C condition at 1 day (see also that H60 and WFPS 60 %). H60, high C and WFPS 60 %. H60, high C and WFPS 60 %. SE, standard error. MBC, microbial biomass carbon. MBN, microbial biomass nitrogen.

Table 2. Microbial biomass C and N contents.

<table>
<thead>
<tr>
<th>treatment</th>
<th>MBC (µg C g soil(^{-1}))</th>
<th>MBN (µg N g soil(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 day</td>
<td>3 day</td>
</tr>
<tr>
<td></td>
<td>Aver.</td>
<td>SE</td>
</tr>
<tr>
<td>Averages and standard errors</td>
<td>L40–P</td>
<td>68.5</td>
</tr>
<tr>
<td></td>
<td>L40+P</td>
<td>139.3</td>
</tr>
<tr>
<td></td>
<td>H40–P</td>
<td>81.2</td>
</tr>
<tr>
<td></td>
<td>H40+P</td>
<td>107.4</td>
</tr>
<tr>
<td></td>
<td>L60–P</td>
<td>306.3</td>
</tr>
<tr>
<td></td>
<td>L60+P</td>
<td>370.7</td>
</tr>
<tr>
<td></td>
<td>H60–P</td>
<td>1778.3</td>
</tr>
<tr>
<td></td>
<td>H60+P</td>
<td>1393</td>
</tr>
</tbody>
</table>

Notes: \(^{1}\)Three-way ANOVA was performed after values were log-transformed. L40, low C (100 µg glucose) and water-filled pore space (WFPS) 40 %. H40, high C (2000 µg glucose) and WFPS 40 %. L60, low C and WFPS 60 %. H60, high C and WFPS 60 %. SE, standard error. MBC, microbial biomass carbon. MBN, microbial biomass nitrogen.

Contrary to our hypothesis, P addition did not increase microbial N immobilization in higher C conditions (H60 and H40, 2000 µg C added), rather reduced at higher C condition at 1 day (especially at H60, Table 2). The favorable nutrient condition provided by P addition in combination with high C addition possibly accelerated the growth rate of fast-growing microbial copiotrophs, although PLFA analysis or 16 S rRNA analysis will be needed to confirm this idea. It is possible that the microbial growth reached its peak and microbes started dying more quickly in P-added soils, which caused lower MBN contents in P-added soils in higher C condition at 1 day (see also that MBC contents in H60 were lower in 3 day than in 1 day, Table 2). Possible reasons for the stimulated N\textsubscript{2}O emissions by P addition at WFPS 60 % at 0–1 day are stimulated N cycling (Mori et al. 2010), promoted anaerobic condition (Mori et al. 2013b), and re-mineralization of dead microbes (Wu and Brooks 2005) provided by quick microbial growth and death.

We assumed the decrease in N\textsubscript{2}O emissions by P addition observed at 2–3 day was because the emissions peak shifted to the earlier, due to the quicker microbial
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Table 3. Dissolved organic C and dissolved N contents.

<table>
<thead>
<tr>
<th>treatment</th>
<th>DOC (μg C g soil⁻¹)</th>
<th>DN (μg N g soil⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 day</td>
<td>3 day</td>
</tr>
<tr>
<td>L40−P</td>
<td>941.6</td>
<td>15.9</td>
</tr>
<tr>
<td>L40+P</td>
<td>929.2</td>
<td>22.1</td>
</tr>
<tr>
<td>H40−P</td>
<td>2575</td>
<td>14.4</td>
</tr>
<tr>
<td>H40+P</td>
<td>2535.4</td>
<td>5.7</td>
</tr>
<tr>
<td>L60−P</td>
<td>1004.6</td>
<td>39.6</td>
</tr>
<tr>
<td>L60+P</td>
<td>985.9</td>
<td>50.7</td>
</tr>
<tr>
<td>H60−P</td>
<td>1971.2</td>
<td>133.5</td>
</tr>
<tr>
<td>H60+P</td>
<td>2139.9</td>
<td>81.2</td>
</tr>
</tbody>
</table>

P values of three-way ANOVA

- C amount: P < 0.001<br>
- WFPS: P < 0.001<br>
- P addition: P = 0.67<br>
- C amount*WFPS: P < 0.001<br>
- C amount*P addition: P = 0.30<br>
- WFPS*P addition: P = 0.40<br>
- C amountWFPS*P addition: P = 0.35

Notes: † Three-way ANOVA was performed after values were log-transformed. L40, low C (100 μg glucose) and water-filled pore space (WFPS) 40%. H40, high C (2000 μg glucose) and WFPS 40%. L60, low C and WFPS 60%. H60, high C and WFPS 60%. SE, standard error. DOC and DN shows dissolved organic carbon and dissolved nitrogen extracted by 0.5 M K₂SO₄, respectively.

Table 4. Inorganic N contents.

<table>
<thead>
<tr>
<th>treatment</th>
<th>NH₄⁺ (μg N g soil⁻¹)</th>
<th>NO₃⁻ (μg N g soil⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 day</td>
<td>3 day</td>
</tr>
<tr>
<td>L40−P</td>
<td>25.6</td>
<td>1.9</td>
</tr>
<tr>
<td>L40+P</td>
<td>28.4</td>
<td>1.9</td>
</tr>
<tr>
<td>H40−P</td>
<td>28.2</td>
<td>1.1</td>
</tr>
<tr>
<td>H40+P</td>
<td>24.1</td>
<td>2.4</td>
</tr>
<tr>
<td>L60−P</td>
<td>26.6</td>
<td>1.7</td>
</tr>
<tr>
<td>L60+P</td>
<td>24.7</td>
<td>2.8</td>
</tr>
<tr>
<td>H60−P</td>
<td>23.3</td>
<td>2.7</td>
</tr>
<tr>
<td>H60+P</td>
<td>22.1</td>
<td>2.3</td>
</tr>
</tbody>
</table>

P values of three-way ANOVA

- C amount: P = 0.23<br>
- WFPS: P = 0.12<br>
- P addition: P = 0.48<br>
- C amount*WFPS: P = 0.50<br>
- C amount*P addition: P = 0.32<br>
- WFPS*P addition: P = 0.77<br>
- C amountWFPS*P addition: P = 0.23

Notes: † Three-way ANOVA was performed after values were log-transformed. L40, low C (100 μg glucose) and water-filled pore space (WFPS) 40%. H40, high C (2000 μg glucose) and WFPS 40%. L60, low C and WFPS 60%. H60, high C and WFPS 60%. SE, standard error.
growth and death (for example, see the lower DN in H60+P than H60–P, Table 3). However, it is also possible that increased microbial N immobilization restricted nitrification and denitrification as we hypothesized. Microbial N pool may have rapidly changed into insoluble dead-microbial N fraction, which caused no difference (or rather decrease) in MBN contents. We have no evidence to support the idea. Although the whole processes were not understood yet, we demonstrated that (i) P addition was not necessarily associated with increase in soil microbial N pool, and (ii) N, N emissions might not decrease even if P addition stimulated soil microbial N immobilization.

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