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Linkage of N$_2$O emissions to the abundance of soil ammonia oxidizers and denitrifiers in purple soil under long-term fertilization

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Abstract

Microbial nitrification and denitrification are responsible for the majority of soil nitrous (N$_2$O) emissions. In this study, N$_2$O emissions were measured and the abundance of ammonium oxidizers and denitrifiers were quantified in purple soil in a long-term fertilization experiment to explore their relationships. The average N$_2$O fluxes and abundance of the $amoA$ gene in ammonia-oxidizing bacteria during the observed dry season were highest when treated with mixed nitrogen, phosphorus and potassium fertilizer (NPK) and a single N treatment (N) using NH$_4$HCO$_3$ as the sole N source; lower values were obtained using organic manure with pig slurry (OM) and returning crop straw residue plus synthetic NH$_4$HCO$_3$ fertilizer at a ratio of 15%:85% (SRNPK). The lowest N$_2$O fluxes were observed in the treatment that used crop straw residue (SR) and in the control with no fertilizer (CK). Soil NH$_4^+$ provides the substrate for nitrification generating N$_2$O as a byproduct. The N$_2$O flux was significantly correlated with the abundance of the $amoA$ gene in ammonia-oxidizing bacteria ($r = 0.984$, $p < 0.001$), which was the main driver of nitrification. During the wet season, soil nitrate (NO$_3^-$) and soil organic matter (SOC) were found positively correlated with N$_2$O emissions ($r = 0.774$, $p = 0.041$ and $r = 0.827$, $p = 0.015$, respectively). The $nirS$ gene showed a similar trend with N$_2$O fluxes. These results show the relationship between the abundance of soil microbes and N$_2$O emissions and suggest that N$_2$O emissions during the dry season were due to nitrification, whereas in wet season, denitrification might dominate N$_2$O emission.

Key words: N$_2$O flux, abundance of functional gene, purple soil, wheat-maize cropping system.

INTRODUCTION

Nitrous oxide (N$_2$O) is an important greenhouse gas that also degrades stratospheric ozone (Denman et al. 2007). Agricultural soil is the primary source of N$_2$O (Helgason et al. 2005; Braker and Conrad 2011), accounting for approximately 60% of the total anthropogenic N$_2$O emissions (Syakila and Kroeze 2011). Microbial denitrification and nitrification in managed and natural soils contribute approximately 70% of the global N$_2$O emissions (Syakila and Kroeze 2011), and are responsible for the majority of N$_2$O emissions in agricultural soils (Braker and Conrad 2011). Ammonium oxidation, which is catalyzed by ammonia monoxygenase (AMO), is the first and rate-limiting step of nitrification when N$_2$O can be formed as a by-product (Firestone and Davidson 1989). Recent studies have shown that the AMO α subunit ($amoA$) genes are present not only in bacteria (AOB) but also in archaea (AOA) (Schleper et al. 2005). Denitrification is the stepwise reduction of nitrate (NO$_3^-$) to N$_2$ mediated by denitrifiers with intermediate gaseous products of nitric oxide (NO) and N$_2$O. Nitrite reductase (encoded by the $nirS$ or $nirK$ genes) and nitrous oxide reductase (encoded by nosZ) are the enzymes that reduce nitrite (NO$_2^-$) to NO and reduce N$_2$O to N$_2$ respectively. The abundance of nitrifiers and denitrifiers is thought to be important in regulating soil N$_2$O emissions (Cavigelli and Roberston 2010; Braker and Conrad...
A significant correlation was found between the abundance of the nirS, napA and narG denitrification genes and the N2O/(N2O+N2) ratio in grassland soils (Cuhel et al. 2010); however, limited studies have related the abundance of ammonia oxidizers to N2O production (Avrahami and Bohannan 2009). The community structure and activity of ammonia oxidizers and denitrifiers are affected by long-term nitrogen fertilization (Shen et al. 2008; Wallenstein et al. 2006; Hallin et al. 2009). Previous studies showed that the abundance and composition of AOB were more easily changed by long-term fertilization than those of AOA (Shen et al. 2006; Wang et al. 2009). Dambreville et al. (2006) reported that the application of organic or mineral fertilizer could affect both the composition and activity of the denitrifying community in a maize-cropped field. However, the response of both ammonium oxidizers and denitrifiers to fertilization and their effect on N2O emissions from agricultural soil is rarely reported.

Rain-fed cropland in the Sichuan Basin (the largest agricultural region in south-western China) supplies 10% of the agricultural products of China by 7% of the national cropland in these areas. Regosol (locally known as purple soil) is widely distributed over an area greater than 160,000 km² in this region (Zhu et al. 2012), and the annual precipitation is concentrated in the period from July to October. In general, it is thought that nitrification contributes significantly to N2O formation during the dry season (approximately 85%) but less (approximately 30%) significantly during the wet season (Kiese et al. 2008), and denitrification is the dominant process for N2O production in wet, poorly drained soils (Wrage et al. 2001; Baily et al. 2012). To improve our knowledge of the relationships between the community abundance of nitrifiers and denitrifiers and N2O emissions from fertilized agricultural soils, we monitored N2O emissions from purple soil under various fertilization regimes using static chamber-gas chromatography and examined the abundances of ammonia oxidizers and the denitrifiers using real-time PCR with amoA, nirS and nosZ used as molecular indicators. The objective of this study was to determine (a) the effects of fertilization on the abundance of soil microbes and on N2O emissions from purple soil and (b) the relationships between the abundance of ammonium oxidizers and denitrifiers and N2O emissions during the dry and wet seasons.

MATERIALS AND METHODS

Field site
The study was conducted at the Yanting Agro-Ecological Experimental Station of Purple Soil (N31° 16', E105° 28'), the Chinese Academy of Sciences, which is located in the central Sichuan Basin of South-western China at an altitude of 400–600 m above sea level. The site is characterized by a moderate subtropical monsoon climate with an annual mean temperature of 17.3°C, a mean evaporation of 1305 mm, and a mean precipitation of 826 mm, with rainfall occurring mainly from July to October. Purple soil (Pup-Orthic Entisol according to the Chinese Soil Taxonomy, and Eutric Regosol according to the Food and Agriculture Organization (FAO) Soil Classification) is distributed widely in the region (Zhu et al. 2009). A typical purple soil with clay loam texture was selected as the experimental soil, which has a pH (H2O) of 8.28, a bulk density of 1.33 g cm⁻³ and contains 8.75 g kg⁻¹ organic carbon and 0.62 g kg⁻¹ total nitrogen.

Fertilization experiments
The fertilization experiments were set up in 2003 with six fertilization treatments as follows: single nitrogen fertilizer (N); mixed nitrogen, phosphorus and potassium fertilizer (NPK); organic manure with pig slurry (OM); organic manure with pig slurry with added NPK at a ratio of 40%:60% (total N applied (OMNPK)); crop straw residue (SR); and returning crop straw residue with added synthetic fertilizer at a ratio of 15%:85% (total N applied) (SRNPK). Ammonium bicarbonate, calcium superphosphate (90 kg P₂O₅ ha⁻¹), and potassium chloride (36 kg K₂O ha⁻¹) were used as the N, P, and K sources, respectively. The amount of N applied was maintained at 130 kg N ha⁻¹ during the dry season and 150 kg N ha⁻¹ during the wet season in all of the fertilization treatments except for the SR treatment which only accepts N from crop straws (equals to 15% of the N from other treatments). A treatment without the use of fertilizer (CK) served as the control. All of the treatments were replicated three times, and the experimental plots (4 × 6 m² each) were assigned using a completely randomized design under conventional rain-fed cultivation. The fertilizers were applied manually to the soil during sowing. Soils at depths of 0–15 cm were sampled on May 15 and September 27, 2012. Six subsamples were randomly collected within each sampling plot and bulked together as a sample. Fresh soil was sieved through a 2-mm mesh, and visible stones and vegetable matter were removed. One subsample was air-dried for chemical analysis, a second subsample was stored at 4°C before analysis, and the remaining subsample was immediately stored at −80°C for molecular analysis.

Measurements of N₂O emission
Soil N₂O emissions were sampled twice every week from 14 April to 15 May (during the dry season
with low water-filled pore space (WFPS)) and from 26 August to 26 September 2012 (during the wet season with high WFPS). Samples were collected from the headspace of static chambers from 9:00 to 11:00 am to obtain a daily average flux. The sampling methods were based on a method previously described by Chen et al. (2013) with minor modifications. The base frame was 0.5 m × 0.5 m × 0.1 m (length × width × height) and the top chamber was 0.5 m × 0.5 m × 0.5 m (length × width × height). The chambers were closed for 30 min, and gas samples (50 mL) were collected every 6 min using plastic syringes. The N₂O concentration was analyzed using a gas chromatograph (HP-5890 Series II, Hewlett Packard, Palo Alto, USA) equipped with an electron capture detector (ECD). The oven was operated at 55°C, and the ECD was operated at 330°C. N₂O fluxes were calculated from the initial slope of a non-linear regression of N₂O concentration within the chambers’ headspace against time (Kroon et al. 2008).

DNA extraction and quantitative real-time PCR
Total genomic DNA was extracted from the frozen soil (−80°C saved) samples using an Ultra Clean™ Soil DNA Isolation Kit (MoBio Laboratory, Carlsbad, USA) according to the manufacturer’s instructions. The abundance of functional genes related to nitrification (archaeal amoA and bacterial amoA) and denitrification (nirS and nosZ) were measured by Sybr Green-based quantitative real-time PCR (qPCR). Preliminary experiments targeting the nirK gene indicated that it was 6.3–16.6 times less abundant than the nirS gene (Fig. S1), and both the two gene types exhibited similar trends, however, nirS was found to be more sensitive to N and C amendment than nirK, which is consistent with other studies (Hallin et al. 2006; Yin et al. 2012). The nirS gene was therefore chosen as an indicator of nitrite reductase in subsequent work. The primer pair of Arch-amoAF and Arch-amoAR (Francis et al. 2005) was used to target archaeal amoA genes, whereas bacterial amoA genes were amplified using amoA-1F and amoA-2R (Rothhauwe et al. 1997). The nirS gene was amplified using primer scd3aF and R3cd (Michotey et al. 2000), and the nosZ genes of denitrifiers were amplified using primers NosZ2F and NosZ2R (Henry et al. 2006). The thermal-cycling conditions were as follows: 95°C for 2 min, followed by 40 cycles of 95°C for 10 s, 20 s at the specific annealing temperature and elongation at 72.5°C for 1 min. The annealing temperatures used as follows: 55°C for the primers amoA-1F and amoA-2R, 53°C for the primers Arch-amoAF and Arch-amoAR, 55°C for the primers cd3aF and R3cd, 64°C for the primers NosZ2F and NosZ2R. The reactions were conducted using an Applied Biosystems ABI 7500 Fast thermal cycler (Applied Biosystems, Streetsville, Canada) with a volume of 25 µL containing 1 µL of DNA template, 0.2 µM of each primer and 12.5 µL of SybrGreenqPCR Master Mix (Applied Biosystems, Foster City, CA, USA). Standard curves were obtained using serial dilutions (10⁴ to 10⁵ gene copies µl⁻¹) of plasmids, which were generated by amplifying the targeted genes (bacterial amoA, archaeal amoA, nirS and nosZ sequences) from the extracted DNA and cloning the products into the pMD18T-vector (Takara, Dalian, China). qPCR efficiency (E) was calculated according to the equation E = 10⁻¹/slope (Bustin 2000). Amplification efficiencies of 88% (archaeal amoA), 86% (bacterial amoA), 82% (nirS) and 83% (nosZ) were achieved, and the correlation coefficients (R²) were greater than 0.996 for all of the targeted genes.

Soil analysis
Soil NO₃-N and NH₄-N were extracted with 2 M KCl and assayed using a continuous-flow analyzer (model AA3, Bran + Luebbe, Norderstedt, Germany). The total nitrogen (TN, mg g⁻¹) was determined using semi-micro Kjeldahl digestion using Se, CuSO₄, and K₂SO₄ catalysts. The soil organic carbon (SOC, mg g⁻¹) was determined based on dichromate oxidation and titration with (NH₄)₂Fe(SO₄)₂·6 H₂O. The soil volumetric water content was measured at depths of 0–5 cm using EC-5 dielectric soil moisture sensors (Decagon Devices, Pullman, WA, USA). The WFPS was calculated based on the volumetric water content and soil bulk density (Ding et al. 2007). The soil temperature at a depth of 5 cm was monitored using ECT temperature sensors (Decagon Devices). The daily precipitation and air temperature data were obtained from the meteorological station at the Yanting Agro-Ecological Experimental Station, which is located 50 m from the experimental site.

Statistical analysis
Statistical analyses were performed using the software SPSS Statistics Client 19.0 (SPSS, Inc., Chicago, USA) and Origin 8.0 (Origin Lab Ltd., Northampton, USA). A One-way analysis of variance (ANOVA) combined with Duncan’s Multiple Range test was performed to test the differences among treatments. Pearson’s test was used to determine (i) whether N₂O emissions were significantly correlated with soil ammonium, nitrate, or SOC and (ii) whether microbial abundances were significantly correlated with soil ammonium, nitrate, SOC or N₂O emissions.
RESULTS

Soil conditions
The air temperature was greater than 18.3°C during the observation period, and the average temperature and total precipitation during the observed dry season were 21.6°C, 27 mm, respectively, whereas during the wet season, the values were 21.2°C, 209 mm, respectively. The soil WFPS was generally lower than 60% during the dry season and higher than 60% during the wet season. The CK and N treatments exhibited the highest average soil temperature and WFPS values, with values of 22.9°C and 61.3% and 22.0°C and 58.8%, respectively. Lower average soil temperatures and WFPS values were observed for the SRNPK, OM and OMNPK treatments (Fig.1). The application of pig slurry and crop straw for 9 years resulted in greater soil TN and SOC contents than did the chemical fertilizer treatments. NH$_4^+$ was released gradually from straw residues and was highest for the SR and SRNPK treatments during the late periods of crop growth (p < 0.05). The single nitrogen fertilizer treatment resulted in the highest NO$_3^-$ during the dry season and lowest NO$_3^-$ during the wet season (p < 0.05) (Table 1).

Average N$_2$O fluxes during the dry and wet seasons
The average N$_2$O fluxes during the dry and wet seasons differed under different fertilization regimes (Fig.2). During the dry season, the average N$_2$O emissions from the NPK and N plots were highest, reaching 0.05 and 0.04 mgN$_2$O m$^{-2}$ h$^{-1}$, respectively, followed by OM, OMNPK and SRNPK. The N$_2$O fluxes in SR and CK plots were significantly lower than in the other plots (p < 0.01). During the wet season, the OM treatment exhibited the highest average N$_2$O flux (0.05 mgN$_2$O m$^{-2}$ h$^{-1}$, p < 0.01), followed by the SR, NPK, SRNPK and OMNPK treatments. The lowest average N$_2$O fluxes were found in the plots receiving no fertilizer or low input single N treatments.

![Figure 1](image-url) Precipitation, temperature and WFPS during observed period.
The values presented are means; the standard errors of temperature and moisture data are not shown for figure clarity. Soil temperature was measured at a depth of 5 cm. WFPS represents water-filled pore space and is a measure of soil moisture. N-single nitrogen fertilizer, NPK-mixed nitrogen, phosphorus and potassium fertilizer, OM-organic manure with pig slurry, OMNPK-organic manure with pig slurry with added NPK at a ratio of 40%:60% (total N applied), SR-crop straw residue, SRNPK-returning crop straw residue with added synthetic fertilizer at a ratio of 15%:85% (total N applied), CK-no fertilizer.
The abundance of archaeal amoA genes was 6–135 times higher than that of bacterial amoA genes (ANOVA with Duncan’s Multiple Range test, p < 0.01, n = 3) during the dry season. The abundance of amoA genes from bacteria (AOB) was highest in the chemical fertilizer treatments (NPK and N), followed by the treatments with added organic matter (OMNPK, OM and SRNPK); the SR treatment, which only received 15% of the N used in the other treatments, and CK, which received no fertilizer ranked lowest (ANOVA with Duncan’s Multiple Range test, p < 0.01, n = 3) (Fig.3). During the dry season, the abundance of archaeal amoA genes was significantly higher in the OM and SR treatments i.e., fertilization with organic matter (p < 0.01, n = 3), and the treatments exhibiting the next most abundant archaeal amoA genes were SRNPK, NPK and OMNPK. The treatments without organic matter (N and CK) exhibited the lowest (p < 0.05, n = 3). The nirS and nosZ genes were quantified to assess the abundance of denitrifiers, and the soil nosZ gene abundance under the tested fertilization regimes exhibited the same trend with archaeal amoA genes. The nirS gene also exhibited a similar trend, with the organic matter fertilization resulting in the greatest abundance, followed by the organic and inorganic fertilization and then NPK, N, and CK.

Abundance of ammonia-oxidizing bacteria, ammonia-oxidizing archaea and denitrifiers and the effects of fertilizer management

The abundance of archaeal amoA genes was 6–135 times higher than that of bacterial amoA genes (ANOVA with Duncan’s Multiple Range test, p < 0.01, n = 3) during the dry season. The abundance of amoA genes from bacteria (AOB) was highest in the chemical fertilizer treatments (NPK and N), followed by the treatments with added organic matter (OMNPK, OM and SRNPK); the SR treatment, which only received 15% of the N used in the other treatments, and CK, which received no fertilizer ranked lowest (ANOVA with Duncan’s Multiple Range test, p < 0.01, n = 3) (Fig.3).

During the dry season, the abundance of archaeal amoA genes was significantly higher in the OM and SR treatments i.e., fertilization with organic matter (p < 0.01, n = 3), and the treatments exhibiting the next most abundant archaeal amoA genes were SRNPK, NPK and OMNPK. The treatments without organic matter (N and CK) exhibited the lowest (p < 0.05, n = 3). The nirS and nosZ genes were quantified to assess the abundance of denitrifiers, and the soil nosZ gene abundance under the tested fertilization regimes exhibited the same trend with archaeal amoA genes. The nirS gene also exhibited a similar trend, with the organic matter fertilization resulting in the greatest abundance, followed by the organic and inorganic fertilization and then NPK, N, and CK.

The abundance of the functional genes during the wet season was generally lower than that observed during the dry season, however, most of the tested genes

Table 1 Soil properties under different long-term fertilizer regimes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NH₄⁺ (mg kg⁻¹)</th>
<th>NO₃⁻ (mg kg⁻¹)</th>
<th>TN (g kg⁻¹)</th>
<th>SOC (g kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry season</td>
<td>11.759 ± 0.339 b</td>
<td>5.001 ± 0.669 b</td>
<td>0.774 ± 0.048 c</td>
<td>7.73 ± 0.46e</td>
</tr>
<tr>
<td>Wet season</td>
<td>0.443 ± 0.264 b</td>
<td>2.278 ± 0.352 c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>12.165 ± 0.348ab</td>
<td>22.828 ± 6.832a</td>
<td>0.925 ± 0.069bc</td>
<td>9.76 ± 0.12de</td>
</tr>
<tr>
<td>SR</td>
<td>12.926 ± 0.302a</td>
<td>9.629 ± 0.538 b</td>
<td>1.175 ± 0.152 ab</td>
<td>14.04 ± 0.76ab</td>
</tr>
<tr>
<td>OM</td>
<td>12.050 ± 0.225ab</td>
<td>9.172 ± 0.531 b</td>
<td>1.094 ± 0.064ab</td>
<td>14.63 ± 0.50ab</td>
</tr>
<tr>
<td>NPK</td>
<td>12.518 ± 0.206ab</td>
<td>7.042 ± 1.349 b</td>
<td>0.986 ± 0.033abc</td>
<td>10.90 ± 0.52 cd</td>
</tr>
<tr>
<td>OMNPK</td>
<td>11.538 ± 0.136c</td>
<td>5.951 ± 0.402 b</td>
<td>1.043 ± 0.066ab</td>
<td>12.29 ± 0.76bc</td>
</tr>
<tr>
<td>SRNPK</td>
<td>12.017 ± 0.283ab</td>
<td>8.669 ± 1.250 b</td>
<td>1.217 ± 0.084a</td>
<td>13.75 ± 1.16ab</td>
</tr>
</tbody>
</table>

Note: Different letters in the same row represent significant differences (P < 0.05) between treatments within the same season, according to Duncan test. Abbreviations of fertilizer regime as described in “Material and methods” section.

Figure 2 Average N₂O fluxes in observed dry and wet season. Bars represent one standard error.
showed similar trends to those observed during the dry season under the tested fertilization regimes, especially the bacterial amoA and nirS genes. For AOA, the archaeal amoA gene abundance ranked in the following order with no significant differences: OM > SR > OMNPK > CK > NPK > SRNPK > N. The abundance of the nosZ genes also did not exhibit any significant differences during the wet season, and the following trend was observed (from high to low): fertilization with organic matter (OM and SR treatments), fertilization with organic matter and synthetic fertilizer (SRNPK and OMNPK) and the remaining regimes (CK, NPK and N) (Fig.3).

DISCUSSION

During the dry season, N₂O emissions increased with rising exogenous NH₄⁺ content, and NH₄⁺ was the substrate for nitrification, indicating that nitrification might contribute to N₂O production. The significant correlation between the average N₂O fluxes and abundance of bacterial amoA ($r = 0.984$, $p < 0.001$, Table 2) further confirmed that nitrification might be the dominant N₂O production process. Mao et al. (2011) reported that AOB might be more active than AOA in soils amended with ammonia. No significant correlation was observed between the AOA and the average N₂O flux during the dry season (Table 2), which implies that AOB, rather than AOA, contributes to nitrification in this study. Di et al. (2010) also published findings showing that the total N₂O emissions from grazed grasslands with high ammonium concentrations were significantly related to the amoA gene copy numbers from AOB rather than those from the AOA community.

During the wet season, the OM and SR treatments resulted in higher N₂O fluxes because manure and crop straw provided a greater amount of organic carbon for denitrification (Lampe et al. 2006; Senbayram et al. 2012). The SOC was closely related to N₂O fluxes during the wet season (Table 2), and organic carbon might trigger denitrification by providing energy for denitrifiers and enhancing respiration through the consumption of oxygen-creating anoxic micro-sites (Firestone 1982; Attard et al. 2011; Senbayram et al. 2012). The N₂O fluxes were lower in the OMNPK and SRNPK treatments than in the OM and SR treatments, which indicated that exogenous carbon became the main factor for N₂O production and suggested that denitrification might play a more important role than nitrification during the wet season. We found that the correlation coefficient between the abundance of nirS from denitrifiers and

![Figure 3](image-url)
average N$_2$O fluxes was 0.611 (p = 0.145) in the wet season. Hai et al. (2009) reported that the use of organic fertilizers (manure and straw) increased the abundance of nirS genes in a tropical agro-ecosystem. In our study, although nosZ gene copies were positively correlated with N$_2$O emissions during the wet season, nosZ gene copies showed no significant differences (p > 0.05) among the fertilizer regimes, and nirS genes were significantly more abundant after the SR and OM treatments than after the other treatments, with the exception of the NPK treatment. Moreover, the OM and SR treatments resulted in the highest N$_2$O fluxes, and the nirS exhibited a similar trend with N$_2$O fluxes (Fig. 2, 3c and 3d). To sum up, this study suggests that nirS was more sensitive than nosZ to the effect of fertilizer regimes on N$_2$O fluxes during the wet season. However, the presence of a gene does not mean that the encoded protein is expressed, and the abundance of genes cannot be directly linked to functional processes (e.g., N$_2$O production) (Truu et al. 2009). Both mRNA and proteins should be further studied to better explain the mechanisms of N$_2$O emissions. In addition, our study found a significant correlation between archaeal amoA and N$_2$O emissions during the wet season (Table 2), indicating that N$_2$O emissions might occur as a by product of nitrification or an intermediate product of nitrifier denitrification (Ma et al. 2008). Previous research has shown that positive correlation occurred between N$_2$O emissions and the nirS, nosZ and archaeal amoA gene abundances (Majeed et al. 2013), and N$_2$O production by AOA was also reported (Santoro et al. 2011). Therefore, the role of AOA should be explored further.

Moreover, favorable WFPS levels for nitrification and denitrification have been reported to be 30–70% and 70–90%, respectively (Davidson 1993; McTaggart et al. 1997; Zhang et al. 2012). The finding that WFPS was generally lower than 60% during the dry season and higher than 60% during the wet season supports our conclusions. Similar conclusions were also obtained for a lowland tropical rainforest ecosystem in which: nitrification contributed significantly to N$_2$O formation during the dry season (approximately 85%) but less significantly (approximately 30%) during the wet season (Kiese et al. 2008).

CONCLUSIONS

Using real-time PCR and static chamber- gas chromatography, we found that the abundance of the amoA gene of ammonia-oxidizing bacteria was significantly correlated with N$_2$O emissions during the dry season, whereas nirS showed similar trend N$_2$O emissions during the wet season. Our findings suggest that nitrification might dominate N$_2$O emissions during the dry season, and nirS was more sensitive than nosZ to the effect of fertilizer regimes on N$_2$O fluxes during the wet season.

ACKNOWLEDGMENTS

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SUPPLEMENTARY MATERIAL

The supplementary material for this article is available online from: http://dx.doi.org/10.1080/00380768.2015.1049930.

REFERENCES


Table 2 Correlation coefficients between average N$_2$O fluxes, abundance of the functional genes and soil properties

<table>
<thead>
<tr>
<th>Average N$_2$O fluxes (mgN$_2$O Om$^{-1}$h$^{-1}$)</th>
<th>archaeal amoA (gene copies kg DW soil$^{-1}$)</th>
<th>bacterial amoA (gene copies kg DW soil$^{-1}$)</th>
<th>nirS</th>
<th>nosZ</th>
<th>NO$_3^{-}$ (mg kg$^{-1}$)</th>
<th>SOC (g kg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry season</td>
<td>-0.072 *</td>
<td>0.984**</td>
<td>0.056</td>
<td>-0.223</td>
<td>0.308</td>
<td>-0.126</td>
</tr>
<tr>
<td>Wet season</td>
<td>0.852*</td>
<td>-0.206</td>
<td>0.611</td>
<td>0.892**</td>
<td>0.774*</td>
<td>0.827*</td>
</tr>
</tbody>
</table>

Note: TN, AP, NH$_4$+ and WFPS had no significant correlations with average N$_2$O fluxes, which were not shown in the table. * means <0.05, ** means <0.01.

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