Community Structure of Ammonia Oxidizing Bacteria and Their Potential to Produce Nitrous Oxide and Carbon Dioxide in Acid Tea Soils

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Online Publication Date: 01 October 2008

To cite this Article

To link to this Article: DOI: 10.1080/01490450802402943
URL: http://dx.doi.org/10.1080/01490450802402943

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Community Structure of Ammonia Oxidizing Bacteria and Their Potential to Produce Nitrous Oxide and Carbon Dioxide in Acid Tea Soils

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The potentials of nitrous oxide (N2O) and carbon dioxide (CO2) production in acid tea soils from Indonesia and Japan were investigated in a laboratory incubation experiment, and the community structures of ammonia-oxidizing bacteria in these soils were characterized using PCR-DGGE approaches. The soils used were sampled from tea plantations in Shizuoka, Japan and in Bogor and Malino, Indonesia. All of the soils were acidic (pH 3.45 to 4.00). The N2O and CO2 production in Shizuoka was almost 5 times higher than in Bogor and Malino. All of the amoA gene sequences defined belong to the genus Nitrosospira sp. with cluster 2 and cluster 3a.

Keywords acid tea soils, Ammonia Oxidizing Bacteria (AOB), CO2, N2O

INTRODUCTION

Agriculture accounted for an estimated emission of 5.1 to 6.1 GtCO2-eq or about 10–12% of the total global anthropogenic emission of greenhouse gases (GHGs) in 2005. This trend is responsive to global change. Increases are expected as diets change and population growth increases food demand, and the future climate change may eventually release more soil carbon (IPPC 2007). Nitrous oxide (N2O) is produced in soils mainly by nitrification and denitrification processes. N2O production is affected by soil physical and chemical characteristics, such as water content, texture, associated O2 diffusion rate, temperature, availability of NO3−, NH4+, organic substrate, and pH (Davidson et al. 1993; Conrad 1996; Inubushi et al. 1996 and 2003; Bollmann and Conrad 1998; Tokuda and Hayatsu 2000; Jumadi et al. 2005). Nitrification is an aerobic process in which ammonium (NH4+) is oxidized to nitrate (NO3−). At sub-optimal oxygen concentrations, oxidation to NO3− is incomplete and some of the NH4+ is channeled into production of NO and N2O (Poth and Focht 1985). Therefore, nitrification can have important effects on retention of nitrogen in the ecosystem such as loss of nitrogen in the form of nitrate via runoff and leaching to downstream ecosystems or through transfer of N2O to the atmosphere.

Nitrification is a two-step process, consisting of conversion of ammonium to nitrite, which is subsequently converted to nitrate. These steps are carried out by two different group of organisms, the ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB). Most strains of AOB in soil belong to β-proteobacteria which includes the genera Nitrosomonas, and Nitrosospira which are mostly terrestrial. Some studies of AOB in acidic soil revealed the dominance of Nitrosospira-like sequences (Hasting et al. 2000; Nugroho et al. 2005; Schmidt et al.
In addition, Kowalchuk and Stephen (2001) reported that a molecular survey of AOB across a pH gradient has identified *Nitrosospira* cluster 2 as containing putative acidophilic AOB strain. Moreover, Laverman et al. (2001) and Backman et al. (2003) have detected a trend from domination by *Nitrosospira* cluster 3 in neutral pH agricultural plots toward *Nitrosospira* cluster 2 in acidic pH soil. However, cluster 4 and cluster 3 sequences have also been found recently in acidic soil (Backman et al. 2004; Jordan et al. 2005; Schmidt et al. 2007).

Nitrification can occur in a wide range of acid soils including agricultural field, tea plantation and forests, even in most acid soils (pH(H2O) ~3) (De Boer and Kowalchuk 2001). Jumadi et al. (2005) found that in the tropical acid soils in Indonesia, nitrification rate in tea soil was higher as compared to pine forest and potato soils, suggesting that N2O production in tea soil may also be higher than in pine and potato fields. However, number of AOB in tea soil was lower as compared to that in pine and potato soils.

Practically, tea plantations have received large amounts of nitrogen to maintain high-quality tea leaves, resulting in acidification below pH 5.0 and sometimes below pH 3.0 (Tokuda and Hayatsu 2000; Oh et al. 2006). Tokuda and Hayatsu (2001) also reported high rates of N2O production in 21 acidic tea soils in Japan. However, the numbers of abundant bacteria and actinomycetes in acid tea field soils are less than those in cultivated soils with neutral pH (Nioh et al. 1993).

Land use change can also alter the AOB in the soil. In Costa Rica, AOB differed among various land-use types in diversity and composition, which were correlated with potential rates of nitrification (Carney et al. 2004). Schmidt et al. (2007) showed a decrease in the relative abundance of AOB in an acidic spruce forest soil in Deepsyke, Southern Scotland from year 2002 to 2003. Therefore, the change of ecosystems can alter microbial communities in ways that may affect the processes they mediate (Carnet et al. 2004). Most of these studies have been conducted in temperate ecosystems and information on molecular characterization of AOB from acid tea soils of tropical ecosystems, as in Indonesia, is very limited if not unavailable.

The objective of this study was to assess the nitrification potential and N2O and CO2 production of some acid tea soils in Indonesia, relative to a Japanese acid tea soil with high nitrification potential, and characterize the community structures of AOB using the PCR-DGGE method based on 16S rDNA of amoA gene in these soils.

**MATERIALS AND METHODS**

**Soil Sampling and Site Description**

Two soils were sampled at the tea plantation (*Camellia sinensis*) in Malino district, South-Sulawesi province, Indonesia (05°15’S 119°54’E) at different elevations (1560 and 1500 m elevations with 250 m horizontal distance) Figure 1. The average total precipitation was 2050 mm year−1 and the average air temperature was 17.1°C (Source, Nittoh Tea Co.). The tea plantation in Malino started 17 years ago. The soils were classified as Typic Hapludalfs (U.S. Soil Taxonomy, 1998), described as a volcanic soil type (Center for Soil and Agroclimate Research, Indonesia, 2000). Another tea soil sample from Indonesia was taken in Puncak-Bogor district, West Java province (1200 m 06°43’S 106°58’E). The tea plants are up to 50 years old and the plantation was established in 1910. In the temperate region, tea soil was sampled in Shizuoka prefecture, Japan (240 m 34°50’N 138°06’E) with an annual precipitation of around 2200 mm and a mean temperature of about 15.0°C. Soil was classified as Typic Melanudand.

All soil samples were taken in triplicate at a depth of 0–10 cm, passed through a 2 mm mesh sieve, and stored at 4°C until use. Soil pH (Soil: H2O or KCl ratio 1:2.5, w/v) and EC (soil: H2O ratio 1:5, w/v) were measured using glass electrodes. Total carbon and nitrogen were measured using a C/N analyzer (MT 700 with an autosampler MTA 600, Yanaco, Japan). Soils were extracted with 0.5 M K2SO4 (1:5) solution by shaking for 30 min on reciprocal shaker and filtered with filter paper (Advantec No.6, Tokyo, Japan). The soluble organic carbon was analyzed by total organic carbon analyzer (TOC 5000, Shimadzu, Japan) and the amounts of ammonium (NH4+-N) and nitrate (NO3−-N) were determined by the nitroprusside (Anderson et al. 1989) and hydrazine reduction (Hayashi et al. 1997) methods, respectively. The chemical properties of all soil samples are summarized in Table 1.
Soil Incubation

Production of N₂O, CO₂ gases and nitrogen mineralization were determined in soil samples incubated in the laboratory. Composite samples of each soil was preincubated at 25°C for 7 days and then kept at 60% of water holding capacity (WHC) after treatment with a N source. The nitrogen source was urea applied at the rate of 150 mg N kg⁻¹ dry soil.

Ten grams of soil samples from each treatment were weighed in triplicate into 120 ml serum bottles that were sealed and incubated aerobically at 25°C for 35 days. Every 7 days the gases in the headspace of each bottle were taken to quantify the concentrations of N₂O and CO₂ using gas chromatographs (Shimadzu, GC 14B, Kyoto, Japan) equipped with an electron capture detector (ECD) and a thermal conductivity detector (TCD), respectively. Nitrogen mineralization potential was measured by the methods given.

The contributions of nitrification and denitrification to nitrous oxide production were distinguished by acetylene (C₂H₂) use as mentioned previously. N₂O production potentials were calculated from the increase in N₂O concentration over time (days). The N₂O produced in the presence of acetylene would be due to denitrification while the difference between N₂O produced with ambient air and that with acetylene would be due to nitrification.

Means and standard deviations of the data were calculated. Each mean was compared with others using the least significant differences (LSD=0.05) value by SPSS software (Ver.11.0 for windows, SPSS Inc., Chicago, USA).

DNA Extraction and PCR Amplification of amoA

Soil total DNA was extracted using a FastDNA SPIN Kit for soil (Bio 101, Inc. Vista, CA, USA) following the manufacturer’s instructions. In this procedure, cell lyses was performed by vigorous shaking in a mini-beadbeater (Biospec product, Wakenyaku, Co., Tokyo, Japan) at intensity speed of 4.8 for 30 seconds. DNA extracts were stored at −20°C before using as a template for subsequent PCR reaction.

One μl of DNA extract was used as template for PCR amplification with PCR-primer: AmoA-1F (forward) and AmoA-2R-TC (reverse), developed and tested by Rotthauwe et al. (1997) and Nicolaisen and Ramsing (2002). The primers system generated a specific PCR product 491 bp long and a nondegenerate amoA in reverse primer, a reliable screening tool to investigate AOB diversity in a wide range of environment (Nicolaisen and Ramsing 2002). These primers were used in our study.

PCR amplification was performed in a total volume of 50 μl reaction mixture containing 5 μl of 10x Ex taq buffer (20 mM Mg²⁺ concentration), 4 μl of dNTP mixture with final concentration of 2.5 mM of each, 0.5 μl of each primer (80 pmol μl⁻¹) and 0.3 μl of Ex Taq DNA polymerase (5 U μl⁻¹, a hot start version) and made to volume using a sterile distilled water (Otsuka Pharm., Japan). dNTP mixture, 10x Ex taq buffer, and Ex Taq DNA polymerase were purchased from Takara Bio Inc. Shiga, Japan.

The PCR reaction was run using a DNA thermocycler (Takara Bio Inc. model TP600, Japan). The thermal profile regime had a hot start at 94°C for 5 min (denaturation) and 35 cycles at 94°C for 50 seconds, followed by 60°C for 1 min, and then 72°C for 1 min. A final extension step was at 72°C for 6 min. Five μl of the PCR products were qualified on 2% agarose for 30 minutes at 100 V in 1 X TAE and visualized by UV transillumination (ATTO printgraph, model AE-6932)

### Table 1

<table>
<thead>
<tr>
<th>Tea Soil used</th>
<th>pH</th>
<th>EC (mS m⁻¹)</th>
<th>Total C (mg g⁻¹)</th>
<th>Total N (mg g⁻¹)</th>
<th>SOC (μg g⁻¹)</th>
<th>NH₄⁺ (μg g⁻¹)</th>
<th>NO₃⁻ (μg g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shizuoka</td>
<td>4.68</td>
<td>4.00</td>
<td>12.7</td>
<td>110</td>
<td>879</td>
<td>10.1</td>
<td>237</td>
</tr>
<tr>
<td>Bogor</td>
<td>4.44</td>
<td>3.82</td>
<td>7.4</td>
<td>37</td>
<td>659</td>
<td>5.1</td>
<td>37</td>
</tr>
<tr>
<td>Malino High</td>
<td>4.13</td>
<td>3.47</td>
<td>8.8</td>
<td>43</td>
<td>612</td>
<td>5.6</td>
<td>68</td>
</tr>
<tr>
<td>Malino Low</td>
<td>3.72</td>
<td>3.45</td>
<td>21.6</td>
<td>55</td>
<td>614</td>
<td>5.9</td>
<td>169</td>
</tr>
</tbody>
</table>

**Chemical properties of all soil samples used**

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after staining with ethyldium bromide for 30 minutes and washing with distilled water. The total DNA of soils were extracted and reamplified in duplicate.

**Denaturing Gradient Gel Electrophoresis (DGGE), Sequencing and Phylogenetic Analysis**

DGGE analysis was performed on the D-Code system (Bio-Rad, Hercules, Calif.). PCR products were applied directly into 7% (wt/vol) polyacrylamide gels in 1X TAE (40 mM Tris base (pH 7.2), 20 mM acetic acid, 0.5 M EDTA 1 mM) with 35–60% denaturing gradient (urea and formamide). One hundred percent denaturing acrylamide was defined as 7 M urea and 40% formamide. A top gel without denaturant was cast above the 60% denaturing gradient (urea and formamide). One hundred µl of PCR products were loaded into each lane and run at 60 V for 15 hours at constant temperature of 60°C and visualized after staining with ethyldium bromide for 30 minutes and washed twice with distilled water.

Prominent DGGE bands were excised from the gel with a sterile razor blade, placed in 1.5 ml plastic tube with 50 µl of sterile distilled water, and vortexed gently for 30 seconds before overnight incubation at 4°C. After centrifugation at 2000 rpm for 20 seconds, 1 µl of liquid was used as template for PCR reactions performed under the conditions described above and then separated on DGGE again after only a single band appeared. The single band was excised as described, and then subjected as template for PCR reactions performed under the conditions described before without GC clamp on forward primer. The quantity of DNA recovered from gel acrylamide after PCR reactions were determined on agarose gel electrophoresis as explained above.

The remaining PCR products were purified using Suprex® (Takara Bio. Inc. Shiga, Japan) according to manufacturer’s instructions. One µl of purified PCR product was used as a template for cycle sequencing reaction performed with a DNA sequencing kit- BigDye® Terminator v3.0 (Applied Biosystem, Foster City, CA, USA) with forward and reserve primers run separately. The sequencing reaction was performed for 30 cycles at 95°C for 30 sec (denaturation), at 60°C for 30 sec. (annealing) and at 75°C for 95 sec (extension). Prior to DNA sequence analyzes with an ABI 3100 genetic analyzer (Applied Biosystems, USA) the reaction products were purified with Centri-Sep Columns (Princeton Separation, NJ) following manufacturer’s instructions.

Phylogenetic relationships of the aligned sequences were inferred using neighbor-joining. A bootstrap analysis with 1000 replicates was made for all database sets to evaluate the stability of phylogeny. Phylogenetic analyses were conducted in MEGA 4 (Tamura et al. 2007). The nucleotide sequences in this study have been deposited in the DNA databank of Japan (DDBJ. URL: http://www.ddbj.nig.ac.jp/) under Accession no. AB370215 through AB370227.

**RESULTS AND DISCUSSION**

**Soil Properties, N₂O and CO₂ Productions and Nitrogen Mineralization Potential**

Table 1 shows some chemical properties of the tea plantation soils in Shizuoka, Japan and Indonesia (Bogor and Malino sites). Soil pH was categorized as acid ranging from 3.72 to 4.68 for soils in Malino sites). EC (mS m⁻¹) values were in the range of 7.4 to 21.6. The Shizuoka tea soil was characterized by greater soluble organic carbon (SOC) content. Total carbon content was almost 3 times higher compared with Bogor or Malino soils. Shizuoka tea soil also contained much more mineral nitrogen (NH₄⁺ and NO₃⁻) than other tea soils.

The EC values were higher in Malino low soil than in Malino high soil probably because of downward movement of soluble salts from the upper to lower field. The lower pH among soils might be caused by excess nitrogen input (Bowman et al. 1995). This phenomenon could also be attributed to decomposition of fallen tea leaves, which induced an increase of Al³⁺ in the soil, therefore leading to soil acidification (Wang et al. 1997; Oh et al. 2006).

The higher amounts of soluble organic carbon and nitrogen in Shizuoka tea soils may be due to the intensive application of organic matter (chicken and cow manures amendment) and nitrogen fertilizers that were applied annually at rate of 800 kg ha⁻¹ y⁻¹. Meanwhile, in Indonesian tea soils, chemical fertilizer was applied at 450 kg ha⁻¹ y⁻¹ combined with cow manure 150 kg ha⁻¹ y⁻¹ particularly at the Malino tea plantation site (Jumadi et al. 2005). Nitrate content at the lower elevation was almost 3 times higher as compared to that at the higher elevation, most probably due to the run-off or leaching of NO₃⁻ from the upper site.

The N₂O and CO₂ production from tea soil of Shizuoka was remarkably higher than other soil samples. In Shizuoka soil, N₂O peaked at 1 week incubation then decreased towards the end of incubation (Figure 2a). The same pattern was observed in Bogor soil, but the concentration of N₂O produced was 5 times lower than Shizuoka soil. Malino soils produced more N₂O during the second week than decreased until 5 weeks. Malino low soil showed a higher N₂O potential capacity than Malino high soil (p < 0.05).

Figure 2b shows the cumulative production of CO₂ within 5 weeks incubation. CO₂ production in Shizuoka soil was also 5 times higher compared to other soils. Bogor soil produced less CO₂ than Malino soils. No difference was found in CO₂ production in Malino soils sampled at different elevations. The reasons for differences in production of N₂O and CO₂ from soil samples were possibly primarily related to organic carbon and nitrogen contents of the soils. Tea soils usually receive high levels of nitrogen fertilizer to maintain good quality of leaves. Thus, tea soils are also expected to emit large amounts of N₂O and CO₂ to the atmosphere.

Organic matter application in the soil is expected to affect carbon pools, soil microbial activity, and rates of soil nitrogen
Hayatsu (2000) was also 5 times higher than that in this study. Moreover, the amount of nitrogen applied by Tokuda and Hayatsu (2000) was added to the soil, N2O was produced constantly at a rate 3800 ng N2O-N g−1 d−1 throughout the 14-day incubation period. This rate was higher as compared with the N2O rate measured in this study in Shizouka soil (2700 ng N2O-N g−1) after 7 days of incubation. The difference might be due to the nitrogen amendment. Tokuda and Hayatsu (2000) applied NH4+ and NO3− together, which could have enhanced the synergisms of nitrification and denitrification such that both processes occurred continuously. Meanwhile, nitrogen was amended only as urea in this study. Moreover, the amount of nitrogen applied by Tokuda and Hayatsu (2000) was also 5 times higher than that in this study.

Nitrification and denitrification are the main processes leading to N2O production in soil. It is generally known that nitrification is the predominant N2O producing process under moderately moist (aerobic) condition and that denitrification is the predominant process under wet (anaerobic) condition when NH4+ plus NO3− are available (Bouwman 1996; Inubushi et al. 1996; Conrad 1998). In more detail, at 60% WHC the NO and N2O released by denitrification increase with decreasing O2 partial pressure and reach a maximum under anoxic condition (Bollmann and Conrad 1998). Field scale studies in the tropical region of Indonesia showed that N2O production by nitrification occurred even in moist soil (50–70% of soil moisture) and could be repressed up to 55.8–80% by using a nitrification inhibitor (Hadi et al. 2008; Jumadi et al. 2008).

Acetylene concentration of 10 Pa has been used to block nitrofication in soils without affecting denitrification (Robertson and Tiedje 1987). The production of N2O from Malino low and Bogor soils in the absence and presence of acetylene in closed bottles showed that N2O production was associated with nitrification and denitrification in different proportions. The N2O production potential in the absence of acetylene (air ambient) was 165 and 16 µg-N g−1 dry soils in the Malino low and Bogor soils, respectively. The N2O production from nitrification in Malino low soil was 71 µg-N g−1 dry soil or 43%, while it was 6 µg-N g−1 dry soil or 37% in Bogor soil based on the difference in N2O production with and without acetylene. In the presence of acetylene, N2O production from denitrification was estimated at 57% in Malino low soil and 63% in Bogor soil.

The experiments indicated that the contribution of nitrification to N2O production was less than that of denitrification particularly in Bogor soil. However, these proportions were even lower compared to another study using Shizouka tea soil where the production of N2O from denitrification was 76% even under aerobic conditions (Tokuda and Hayatsu 2000). These results show the importance of denitrification in the formation of N2O in acid tea soils.

Kester et al. (1997) also reported that denitrification was the main sources of N2O in acid forest and grassland soils. In addition, the previous study showed that the number of denitrifiers by MPN (most probable number) method in Malino tea soil was significantly higher than in pine forest and potato soils, but that of ammonium oxidizers was lower in the tea soil. Moreover, Malino tea soil produced much more N2O compared to other soils (Jumadi et al. 2005). However, the tea soils examined were acidic, contained a larger amount of organic matter and were incubated aerobically, so that the production of N2O from heterotrophic nitrification may have also occurred. Inubushi et al. (1996) suggested that N2O was mostly produced from autotrophic nitrification in soil that has a low soil pH value and high organic matter content like Andosols in Japan.

The nitrogen mineralization potential of Shizouka tea soil was not significantly different than that of Malino Low soil (p < 0.05), but was significantly higher than that of Malino high soil and Bogor soil (Figure 3). The lowest mineralization potential was found in Bogor soil (4.8 µg-N g−1 dry soil day−1), which was 2 times lower than Shizouka soils. However, no correlation was found between nitrogen mineralization and N2O production among soils. Therefore, the N2O productions in the soil samples were mostly from denitrification, although production of N2O

**FIG. 2.** N2O and CO2 production during incubation. Vertical bars indicated standard deviations.
FIG. 3. Nitrogen mineralization potential. Vertical bars indicated standard deviations. At each sampling time for each soil, means followed by the same letter are not significantly different (LSD \( p < 0.05 \)).

by heterotrophic nitrification and autotrophic nitrification also occurred.

Ammonia-Oxidizing Bacteria Community Structure

Diversity of AOB community structure in acid tea soils in Shizuoka, Japan and Indonesia (Bogor and Malino sites) were investigated with DGGE method approaches with a nondegenerate primer set (AmoA-1F/ AmoA-2R-TC) as tested by Nicolaisen and Ramsing (2002). Results from duplicate soil samples indicated fine reproducibility of the DGGE banding patterns. One replicate of DGGE pattern is shown in Figure 4.

Shizuoka tea soil had more number of bands in DGGE pattern compared to other tea soils in Indonesia. However, several bands found in Bogor (e.g., bands 10 and 11) and Malino tea soils (e.g., bands 12 and 13) could not be detected in Shizuoka soil, indicating different diversity of AOB community structure among tea soil samples. The sequences of amoA were used to reconstruct a phylogenetic tree including other environment amoA clones from database of DNA bank.

All the band sequences belong to the genus *Nitrosospira* and not to genus *Nitrosomonas*-like species, as confirmed by sequence determination. The cluster of *Nitrosospira* amoA used in this study was defined by Avrahami et al. (2002, 2003). Genus *Nitrosospira* was frequently observed by other studies in soil (Stephen et al. 1998; Hasting et al. 2000; Avrahami et al. 2003; Jordan et al. 2005; Chu et al. 2007). All of amoA sequences found in this study were defined within 2 clusters, namely Cluster 2 and Cluster 3a (Figure 5). Sequences of Shizuoka tea soil resulted in both clusters (Cluster 2 and Cluster 3a). Meanwhile, sequences from Bogor and Malino tea soils appeared only in 1 cluster, Clusters 3a and 2, respectively. Therefore, amoA sequences from 2 sites of tea soil plantation in Indonesia were defined in different *Nitrosospira* communities.

The amoA sequences were grouped in 2 clusters (Clusters 2 and 3a), indicating that the community structure of AOB in tea soil samples are low in diversity. *Nitrosospira* cluster 2 is a common cluster detected in acid soils (Stephen et al. 1998; Kowalchuck et al. 2000; Avrahami et al. 2003; Jordan et al. 2005). In acid soils, AOB can maintain activity in microsite with high pH, through simultaneous hydrolyzes of urea and through attachment to the surface (Allison and Prosser 1991, 1993). Shizuoka and Malino tea soil samples have low pH ranging from 3.45 to 4.00 \( (\text{KCl}) \), which might also be a possible reason that the amoA sequences in this study belong to Cluster 2. But, even in low pH in Bogor soil (3.82 \( \text{KCl} \)), the *Nitrosospira* Cluster 2 was not found suggesting that there is variability of AOB with low pH.

In Shizuoka and Bogor soils, amoA sequences were also related in cluster 3a. Based on 16S rRNA amoA, Cluster 3 is composed mainly of sequences from neutral pH soil library (Stephen et al. 1996), found dominant in early succession of soils having relatively high ammonium concentration (Kowalchuk et al. 2000). However, the *Nitrosospira* cluster 3a were also found in the acidic conifer forest soil in San Bernardino, California located along the well-documented gradient of nitrogen and ozone exposure (Jordan et al. 2005). Avrahami et al. (2003) suggested that the cluster 3a and 3b were not necessarily dominant at high ammonium concentration, but, they were found also at most temperatures. Therefore, it is suggested that cluster 3a and 3b showed different trends with respect to temperature and ammonium, reflecting the high versatility of ammonia oxidizer within these clusters.

Tea soil samples from Shizuoka and Bogor sites have a long history of cultivation (30 and 50 years, respectively), and intensive application of nitrogen fertilizer. Therefore, the *Nitrosospira* cluster 3a is supposed to be dominant in soils.
under long-term tea cultivation, because the cluster 3a was not found in Malino tea soil where plantation is relatively new (17 years).

The results of this study confirmed the hypothesis that acid-tolerant AOB (\textit{Nitrosospira} Cluster 2) or those generally found in high ammonium content soils (\textit{Nitrosospira} Cluster 3) would preferably occupy the highly nitrogen impact (Jordan et al. 2005). Moreover, Chu et al. (2007) reported that \textit{Nitrosospira} cluster 3 became dominant after long-term nitrogen fertilizer in a sandy loam texture under rotation crops of winter wheat and summer maize. Therefore, we suggest that \textit{Nitrosospira} cluster 3a may also be abundant in acid soils cultivated over a long-term with nitrogen and organic fertilizer applications.

The common distribution of amoA sequences Clusters 2 and 3a from Shizouka tea soils to other tea soils, indicate that different biogeographical and physicochemical properties such as pH, levels of soluble organic carbon and soil nitrate, may not greatly influence the composition of the community.

This study concludes that fertilization and organic amendments could affect the physicochemical properties of tea soils in Shizouka, Japan and Indonesia (Bogor and Malino sites), which in turn may influence the potential of N2O and CO2 production and nitrogen mineralization. Furthermore, this study is expanded knowledge of the biogeographical range and niches for \textit{Nitrosospira} gene Clusters 2 and 3a to acidic soil of tea plantation.
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