Links between Ammonia Oxidizer Community Structure, Abundance, and Nitrification Potential in Acidic Soils

Huaiying Yao, Yangmei Gao, Graeme W. Nicol, Colin D. Campbell, James I. Prosser, Limei Zhang, Wenyan Han and Brajesh K. Singh


Published Ahead of Print 13 May 2011.

Updated information and services can be found at:
http://aem.asm.org/content/77/13/4618

**SUPPLEMENTAL MATERIAL**

*These include:*

Supplemental material

**REFERENCES**

This article cites 54 articles, 11 of which can be accessed free at: http://aem.asm.org/content/77/13/4618#ref-list-1

**CONTENT ALERTS**

Receive: RSS Feeds, eTOCs, free email alerts (when new articles cite this article), more»
Links between Ammonia Oxidizer Community Structure, Abundance, and Nitrification Potential in Acidic Soils††

Huaiying Yao,1,* Yangmei Gao,1 Graeme W. Nicol,2 Colin D. Campbell,3 James I. Prosser,2 Limei Zhang,4 Wenyan Han,5 and Brajesh K. Singh 2,3,6,*

Key Laboratory of Environment Remediation and Ecological Health, Ministry of Education, Zhejiang University, Hangzhou 310029, China;1 Institute of Biological and Environmental Sciences, University of Aberdeen, Aberdeen AB24 3UU, United Kingdom;2 The James Hutton Institute, Craibstone, Aberdeen AB5 3QH, United Kingdom;3 Research Centre for Eco-environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China;4; Tea Research Institute, Chinese Academy of Agricultural Sciences, Hangzhou 310008, China;5; and Hawkesbury Institute for the Environment, University of Western Sydney, Penrith South, DC, NSW 1797, Australia6

Received 20 January 2011/Accepted 3 May 2011

Ammonia oxidation is the first and rate-limiting step of nitrification and is performed by both ammonia-oxidizing archaea (AOA) and bacteria (AOB). However, the environmental drivers controlling the abundance, composition, and activity of AOA and AOB communities are not well characterized, and the relative importance of these two groups in soil nitrification is still debated. Chinese tea orchard soils provide an excellent system for investigating the long-term effects of low pH and nitrogen fertilization strategies. AOA and AOB abundance and community composition were therefore investigated in tea soils and adjacent pine forest soils, using quantitative PCR (qPCR), terminal restriction fragment length polymorphism (T-RFLP) and sequence analysis of respective ammonia monoxygenase (amoA) genes. There was strong evidence that soil pH was an important factor controlling AOB but not AOA abundance, and the ratio of AOA to AOB amoA gene abundance increased with decreasing soil pH in the tea orchard soils. In contrast, T-RFLP analysis suggested that soil pH was a key explanatory variable for both AOA and AOB community structure, but a significant relationship between community abundance and nitrification potential was observed only for AOA. High potential nitrification rates indicated that nitrification was mainly driven by AOA in these acidic soils. Dominant AOA amoA sequences in the highly acidic tea soils were all placed within a specific clade, and one AOA genotype appears to be well adapted to growth in highly acidic soils. Specific AOA and AOB populations dominated in soils at particular pH values and N content, suggesting adaptation to specific niches.

Nitrification, the oxidation of ammonia to nitrate, is a critical step in the nitrogen cycle and has significant agricultural and environmental consequences for the availability of nitrogen as a plant nutrient, nitrate leaching to groundwater, and the release of greenhouse gases into the atmosphere. The rate-limiting step of nitrification, the conversion of ammonia to nitrite, can be performed by both ammonia-oxidizing archaea (AOA), within the proposed Thaumarchaeota (7, 38, 42), and ammonia-oxidizing bacteria (AOB). Both groups have been detected in a wide range of soil ecosystems (5, 10, 15, 29, 33). The potential for archaeal ammonia oxidation has been confirmed in laboratory enrichments and in isolates (13, 23, 28). In most soils, archaeal amoA genes are more abundant than those of bacteria, indicating that archaea could have a greater role in soil ammonia oxidation than AOB (10, 29, 36). Bacterial amoA genes are more abundant in some agricultural soils receiving additional nitrogen amendments (15, 26), whereas archaeal amoA genes are more abundant in soils where nitrification is fueled by mineralized organic nitrogen (34, 44).

The high affinity for total ammonium of Nitrosopumilus maritimus, the only cultivated AOA (31), suggests that this species may dominate ammonia oxidation in oligotrophic environments such as the open ocean. In soil ecosystems, nitrifiers and nitrification rates vary with vegetation type, location, and environmental conditions (16, 49). Soil pH is known to have a considerable effect on the activity and diversity of soil ammonia oxidizers (12, 33) and it has been suggested that the absence of nitrification activity in some highly acidic soils is the result of AOB sensitivity to low pH (11, 12), although growth and activity of some AOB at low pH may be possible through urease activity and aggregate formation (2, 8, 12). Specific AOA and AOB phylotypes have been found to be associated with soil pH across a pH gradient of 4.3 to 7.5, with an increase in the ratio of amoA transcript/gene abundance with decreasing and increasing pH for AOA and AOB communities, respectively (33). N fertilizer can also affect the activity and abundance of ammonia oxidizers (15, 19, 39, 49). However, the mechanisms controlling activity of a particular group of soil ammonia oxidizers or the potential for niche differentiation are not clear.
Soil samples for the study were collected from two sites. The first is located in the West Lake district of Hangzhou (30°11’N, 120°05’E), Zhejiang Province, China, where selected tea soils represent a wide range of orchard ages, fertilizers (urea), and lime applications. Since the N application rate is high in Hangzhou area, samples were also obtained from a second site, with two low-N-application tea orchard soils in Taihu county, Auhui Province, China (30°33’N, 116°20’E). Adjacent pine forest soils at each site were sampled to evaluate and compare the effect of vegetation on ammonia-oxidizing communities. The two sites are characterized by a subtropical wet monsoon climate with mean annual rainfall of 1,400 to 1,500 mm. All soils were Ultisols with kaolinite, chlorite, Fe, and Al oxides as the dominant clay minerals. The climate with mean annual rainfall of 1,400 to 1,500 mm. All soils were Ultisols with kaolinite, chlorite, Fe, and Al oxides as the dominant clay minerals.

The aim of this research was to determine how AOA and AOB community composition and abundance vary in response to soil pH and N input, to assess the relative contributions of AOA and AOB to soil nitrification, and to determine drivers of ammonia oxidizer community composition and activity. We hypothesized that AOA are responsible for most of the nitrification in acidic soils, that soil pH and N fertilizer input influence both ammonia oxidizer structure and nitrification potential, and that different AOA and AOB phylogenotypes occupy distinct pH ranges (i.e., niche differentiation). To achieve this, AOA and AOB community structure, abundance, and associated potential nitrification rates were determined in tea soils and in adjacent pine plantations.

### MATERIALS AND METHODS

**Study sites and soil sampling.** Soil samples for the study were collected from two sites. The first is located in the West Lake district of Hangzhou (30°11’N, 120°05’E), Zhejiang Province, China, where selected tea soils represent a wide range of orchard ages, fertilizers (urea), and lime applications. Since the N application rate is high in Hangzhou area, samples were also obtained from a second site, with two low-N-application tea orchard soils in Taihu county, Auhui Province, China (30°33’N, 116°20’E). Adjacent pine forest soils at each site were sampled to evaluate and compare the effect of vegetation on ammonia-oxidizing communities. The two sites are characterized by a subtropical wet monsoon climate with mean annual rainfall of 1,400 to 1,500 mm. All soils were Ultisols with kaolinite, chlorite, Fe, and Al oxides as the dominant clay minerals. The soils were developed on quaternary red earth. Triplicate samples were collected from three sampling plots randomly chosen within each tea orchard or pine forest. Six random soil cores (5-cm diameter by 15-cm length) were taken from each sample and mixed. Field moist soils were sieved to <2 mm, and visible pieces of plant material and soil animals were removed before use. Subsamples of each replicate were stored at ~80°C prior to DNA extraction.

The land use history and some physicochemical properties of the soils are presented in Table 1.

### Soil chemical analysis and potential nitrification rate.

Soil pH was measured using a glass electrode (soil/water, 1:2.5). Total organic C was determined by potassium dichromate oxidation, and total nitrogen was determined by Kjeldahl digestion and quantified using a continuous flow analyzer (Skalar, Delft, the Netherlands). Inorganic N (NH₄⁺-N and NO₃⁻-N) was extracted with 2 M KCl by shaking (1 h, 200 rpm) and filtering through a 0.45-μm-pore-size polysulfone membrane, before colorimetric determination using a continuous flow analyzer. Nitrification potential was determined according to the shaken-slurry method of Hart et al. (22). Fifteen grams of soil from each sample was preincubated at room temperature for 1 week and then mixed with 100 ml of 1.5 mM ammonium sulfate. After incubation for 2, 4, 22, and 24 h, 10-ml slurry samples were centrifuged and the supernatant was filtered through a 0.45-μm-pore-size membrane. NO₃⁻ content in the supernatant was immediately analyzed, as described above for KCl-extracted NO₃⁻-N. NO₃⁻ concentration increased linearly, and nitrification po-

### TABLE 1. Land use history, chemical properties, and abundance of AOA and AOB amoA genes g⁻¹ soil (dry weight) in tea and pine soils

<table>
<thead>
<tr>
<th>Soil no.</th>
<th>Land use</th>
<th>Site</th>
<th>NFI</th>
<th>Lime</th>
<th>pH</th>
<th>% Org C</th>
<th>% TN</th>
<th>PNR (mg NO₃⁻-N g⁻¹ h⁻¹)</th>
<th>amoA genes (10⁵ g⁻¹ soil)</th>
<th>AOB</th>
<th>AOA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tea, 3 yr</td>
<td>HZ</td>
<td>900</td>
<td>0</td>
<td>5.25</td>
<td>1.20</td>
<td>0.45</td>
<td>1.09</td>
<td>1.15</td>
<td>1.10</td>
<td>5.20</td>
</tr>
<tr>
<td>2</td>
<td>Tea, 7 yr</td>
<td>HZ</td>
<td>900</td>
<td>0</td>
<td>4.12</td>
<td>1.30</td>
<td>0.32</td>
<td>0.21</td>
<td>1.75</td>
<td>1.40</td>
<td>8.10</td>
</tr>
<tr>
<td>3</td>
<td>Tea, 8 yr</td>
<td>HZ</td>
<td>900</td>
<td>0</td>
<td>4.31</td>
<td>3.16</td>
<td>0.26</td>
<td>0.72</td>
<td>1.15</td>
<td>1.10</td>
<td>5.20</td>
</tr>
<tr>
<td>4</td>
<td>Tea, 10 yr</td>
<td>HZ</td>
<td>900</td>
<td>0</td>
<td>4.39</td>
<td>1.70</td>
<td>0.32</td>
<td>0.60</td>
<td>1.15</td>
<td>1.10</td>
<td>5.20</td>
</tr>
<tr>
<td>5</td>
<td>Tea, 16 yr</td>
<td>HZ</td>
<td>900</td>
<td>0</td>
<td>4.46</td>
<td>4.02</td>
<td>0.37</td>
<td>0.89</td>
<td>1.15</td>
<td>1.10</td>
<td>5.20</td>
</tr>
<tr>
<td>6</td>
<td>Tea, 20 yr</td>
<td>HZ</td>
<td>900</td>
<td>0</td>
<td>3.92</td>
<td>2.30</td>
<td>0.24</td>
<td>0.29</td>
<td>1.15</td>
<td>1.10</td>
<td>5.20</td>
</tr>
<tr>
<td>7</td>
<td>Tea, 40 yr</td>
<td>HZ</td>
<td>900</td>
<td>0</td>
<td>3.99</td>
<td>4.62</td>
<td>0.45</td>
<td>0.50</td>
<td>1.15</td>
<td>1.10</td>
<td>5.20</td>
</tr>
<tr>
<td>8</td>
<td>Tea, 45 yr</td>
<td>HZ</td>
<td>1,500</td>
<td>0</td>
<td>3.88</td>
<td>3.84</td>
<td>0.40</td>
<td>1.09</td>
<td>BDL</td>
<td>180</td>
<td>1.80</td>
</tr>
<tr>
<td>9</td>
<td>Tea, 50 yr</td>
<td>HZ</td>
<td>1,500</td>
<td>0</td>
<td>3.58</td>
<td>6.41</td>
<td>0.54</td>
<td>1.03</td>
<td>BDL</td>
<td>210</td>
<td>2.10</td>
</tr>
<tr>
<td>10</td>
<td>Tea, 60 yr</td>
<td>HZ</td>
<td>900</td>
<td>0</td>
<td>3.81</td>
<td>4.17</td>
<td>0.37</td>
<td>0.73</td>
<td>44.0</td>
<td>2.00</td>
<td>100.0</td>
</tr>
<tr>
<td>11</td>
<td>Tea, 90 yr</td>
<td>HZ</td>
<td>900</td>
<td>0</td>
<td>4.16</td>
<td>9.19</td>
<td>0.71</td>
<td>1.32</td>
<td>44.0</td>
<td>2.00</td>
<td>100.0</td>
</tr>
<tr>
<td>12</td>
<td>Tea, 4 yr</td>
<td>HZ</td>
<td>900</td>
<td>0</td>
<td>5.01</td>
<td>1.38</td>
<td>0.15</td>
<td>0.22</td>
<td>44.0</td>
<td>2.00</td>
<td>100.0</td>
</tr>
<tr>
<td>13</td>
<td>Tea, 4 yr</td>
<td>HZ</td>
<td>450</td>
<td>1,000</td>
<td>5.39</td>
<td>1.35</td>
<td>0.14</td>
<td>0.33</td>
<td>44.0</td>
<td>2.00</td>
<td>100.0</td>
</tr>
<tr>
<td>14</td>
<td>Tea, 4 yr</td>
<td>HZ</td>
<td>450</td>
<td>4,000</td>
<td>6.29</td>
<td>1.35</td>
<td>0.13</td>
<td>0.41</td>
<td>44.0</td>
<td>2.00</td>
<td>100.0</td>
</tr>
<tr>
<td>15</td>
<td>Tea, 5 yr</td>
<td>HZ</td>
<td>450</td>
<td>0</td>
<td>4.46</td>
<td>4.23</td>
<td>0.45</td>
<td>0.59</td>
<td>15.0</td>
<td>5.00</td>
<td>150.0</td>
</tr>
<tr>
<td>16</td>
<td>Tea, 5 yr</td>
<td>HZ</td>
<td>450</td>
<td>1,000</td>
<td>4.63</td>
<td>4.31</td>
<td>0.47</td>
<td>0.75</td>
<td>28.0</td>
<td>2.00</td>
<td>100.0</td>
</tr>
<tr>
<td>17</td>
<td>Tea, 5 yr</td>
<td>HZ</td>
<td>450</td>
<td>4,000</td>
<td>5.03</td>
<td>3.99</td>
<td>0.41</td>
<td>0.97</td>
<td>44.0</td>
<td>2.00</td>
<td>100.0</td>
</tr>
<tr>
<td>18</td>
<td>Pine, 50 yr</td>
<td>HZ</td>
<td>900</td>
<td>0</td>
<td>4.61</td>
<td>2.76</td>
<td>0.18</td>
<td>0.06</td>
<td>0.26</td>
<td>0.50</td>
<td>1.50</td>
</tr>
<tr>
<td>19</td>
<td>Tea, 10 yr</td>
<td>HZ</td>
<td>50</td>
<td>0</td>
<td>5.54</td>
<td>1.18</td>
<td>0.13</td>
<td>0.16</td>
<td>7.1</td>
<td>5.00</td>
<td>21.0</td>
</tr>
<tr>
<td>20</td>
<td>Tea, 39 yr</td>
<td>HZ</td>
<td>200</td>
<td>0</td>
<td>5.51</td>
<td>1.46</td>
<td>0.13</td>
<td>0.40</td>
<td>4.2</td>
<td>1.30</td>
<td>14.0</td>
</tr>
<tr>
<td>21</td>
<td>Pine, 30 yr</td>
<td>HZ</td>
<td>200</td>
<td>0</td>
<td>5.86</td>
<td>1.10</td>
<td>0.08</td>
<td>0.04</td>
<td>BDL</td>
<td>12.0</td>
<td>1.20</td>
</tr>
</tbody>
</table>

* NFI, N fertilizer input (kg N h⁻¹ yr⁻¹); lime, lime input (kg CaCO₃ h⁻¹ yr⁻¹); Org C, soil organic carbon; TN, total N; PNR, potential nitrification rate (mg NO₃⁻-N g⁻¹ h⁻¹); HZ, Hangzhou, TH, Taihu; BDL, below detection limit. LSD₀.₀₅, least significant difference at the 0.05 level of probability. The values in this table are the averages of the measurements from triplicate soil samples.
tential \((\text{NO}_3^- - \text{N} \cdot \text{h}^{-1})\) was calculated from the rate of increase in \(\text{NO}_3^-\) concentration over time in the slurry using linear regression. The method of Hart et al. (22) involves adjustment of the slurry pH to 7.2 and potentially changes the activity of indigenous communities, selected in soils of different pHs. Potential nitrification rates were therefore also measured at natural soil pH, without adjustment of pH.

DNA extraction and qPCR. DNA was extracted from approximately 500 mg soil samples using the FastDNA SPIN kit for soil (Bio101, Vista, CA), per the manufacturer's instructions. Archaeal and bacterial amo\(\text{A}\) abundances were determined by quantitative PCR (qPCR) using an ABI 7500 thermocycler (Applied Biosystems, Foster City, CA) as described by Chen et al. (10). Bacterial amo\(\text{A}\) genes were quantified using primers amo\(\text{A}-1\)F and amo\(\text{A}-2\)R (37). For crenarchaeal amo\(\text{A}\), qPCR was performed with primers CrenamoA23f and CrenamoA616r (33). DNA concentration was determined by NanoDrop, and also analyzed using canonical variate analysis.

The second set of T-RFLP data for AOA was obtained by PCR amplification using the primer set amo111Ff and amo634Rr (6). The first set of primers in combination with the restriction enzyme (HpyCH4V) used has comparatively low discriminatory ability for some phylotypes, while the second set of primers provides better discrimination between some phylotypes, although primer amo111Ff does exhibit more than one mismatch to a large proportion of currently known AOA amo\(\text{A}\) sequences. For AOB analysis, the bacterial amo\(\text{A}\) partial gene fragment was PCR amplified using labeled primers amo\(\text{A}-1\)F and amo\(\text{A}-2\)R. The labeled PCR amplicons were cloned by agarose gel electrophoresis. A known copy number of linearized plasmid of an amo\(\text{A}\) gene clone was used as a standard for AOA or AOB qPCR (10). For all assays, amplification efficiency was 91 to 95% and \(r^2\) values were 0.97 to 0.99.

Terminal restriction fragment length polymorphism (T-RFLP). AOA T-RFLP profiles were obtained from two sets of FAM-labeled primers. For the first set of T-RFLP data, we used CrenamoA23f and CrenamoA616r (33) primers for AOA. The second set of T-RFLP data for AOA was obtained by PCR amplification using the primer set amo111Ff and amo634Rr (6). The first set of primers in combination with the restriction enzyme (HpyCH4V) used has comparatively low discriminatory ability for some phylotypes, while the second set of primers provides better discrimination between some phylotypes, although primer amo111Ff does exhibit more than one mismatch to a large proportion of currently known AOA amo\(\text{A}\) sequences. For AOB analysis, the bacterial amo\(\text{A}\) partial gene fragment was PCR amplified using labeled primers amo\(\text{A}-1\)F and amo\(\text{A}-2\)R. The labeled PCR amplicons were cloned by agarose gel electrophoresis and purified using an UltraClean DNA purification kit (MoBio Laboratories, CA). The first AOA samples were digested with restriction enzyme HpyCH4V, and the second AOA products were restricted using Rsal, as this enzyme has been reported to provide better T-RFLP profiles (10). AOB PCR products were restricted with MspI (47). After digestion, 2 μl of each sample was mixed with 0.3 μl of LIZ-labeled internal size standard and 12 μl of formamide. Fragment size analysis was carried out with an ABI PRISM 3000xl genetic analyzer (Applied Biosystems, Warrington, United Kingdom) (40). Fragment analysis of T-RFLP data was performed between 35 and 640 bp. All terminal restriction fragments (T-RFs) with fluorescence unit counts of <50 units and peaks with heights that were less than 2% of the total peak height were excluded from further analysis to avoid potential noise before calculating relative T-RF abundance (40).

Cloning and sequencing. DNA samples with different dominant T-RFs were used for cloning and sequencing analysis of AOA amo\(\text{A}\) sequences to assign phylogenetic affiliation to specific T-RFs. To investigate potential niche specialization over broader pH ranges, DNA extracted from each sample was pooled into three groups with soil pH in the ranges 3.5 to 4.4, 4.4 to 5.0, and 5.0 to 6.8. In total, three AOA amo\(\text{A}\) clone libraries and one AOB amo\(\text{A}\) clone library were constructed. Clones were generated using a TOPO TA cloning kit (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. A total of 120 AOA clones and 80 AOB clones were sequenced using vector-specific primers T3 and T7. AOA or AOB sequences (37 and 38 operational taxonomic units [OTUs] for AOA and AOB, respectively) were first used to construct a neighbor-joining tree using Mega software (39). One representative from each OTU (<2% nucleotide dissimilarity) was then used to construct the phylogenetic tree. Translated amo\(\text{A}\) gene sequences from this study were aligned with reference sequences using ClustalW implemented in BioEdit (21). Maximum likelihood and distance analyses were calculated using the Jones, Taylor, and Thornton (JTT) substitution model with site variation (invariable sites and four variable gamma rates) using PHYML (20) and PHYLIP (16), and a parsimony analysis was calculated using PHYLIP.

Statistical analysis. All analysis of variance (ANOVA), regression, and multivariate analyses were conducted using GenStat, 12th edition (VSN International, Oxford, United Kingdom). Mean values and least significant differences at the 95% level were calculated by a one-way ANOVA. The T-RFLP data were also analyzed using canonical variate analysis.

RESULTS

Soil pH and potential nitrification rate. Soil pH values ranged from 3.58 to 6.29 with highly significant differences between different soils (Table 1). The 50-year-old year tea orchards had the lowest pH, which may be due to a combination of high N fertilizer application (usually in the form of urea) and acidification by nitrification, plant growth, and acidic phytochemical inputs. Lime application caused a significant increase in soil pH, especially in the soils with low organic matter. Potential nitrification rate was positively correlated with total nitrogen (\(r^2 = 0.71, P < 0.001\)), organic C (\(r^2 = 0.67, P < 0.001\)), and N fertilizer application (\(r^2 = 0.27, P < 0.001\)) and negatively correlated with soil pH (\(r^2 = 0.28, P < 0.001\)). Measurement of potential nitrification without adjusting pH produced similar results and trends, but process rates were reduced to approximately 60 to 90% (see Table S1 in the supplemental material).

Quantitative PCR determination of amo\(\text{A}\) gene abundance. Abundances of putative AOA and AOB were assessed by quantifying their respective amo\(\text{A}\) genes (Table 1). Archaeal amo\(\text{A}\) gene abundance ranged from 5.5 × 10^8 to 2.4 × 10^9 g^-1 soil and was lower in the pine soils than the tea orchard soils. Soil potential nitrification rate increased with AOA amo\(\text{A}\) gene abundance in all samples (\(P < 0.001\)) and in the tea orchard soils only (\(P = 0.014\)), but there was no significant correlation between AOA amo\(\text{A}\) abundance and soil pH (\(P = 0.071\)) (see Table S2 in the supplemental material). Bacterial amo\(\text{A}\) abundance was lower than that of archaea in all soils. In one pine soil (Taihu) and two highly acidic tea orchard soils, bacterial amo\(\text{A}\) abundance was below the detection limit (2.0 × 10^4 g^-1 soil). AOB abundance decreased significantly with decreasing pH (\(P < 0.001\)) but did not correlate with nitrification potential (\(P = 0.255\)). Interestingly, there was a sharp increase in the ratio of archaeal/bacterial amo\(\text{A}\) abundance below pH 4 (Fig. 1).

T-RFLP analysis of AOA and AOB communities. T-RFLP data using CrenamoA23f and CrenamoA616r in combination with HpyCH4V enzyme produced a total of 14 T-RFs in all samples and were used for all multivariate statistical analysis. PCA analysis of the T-RFLP data showed that the scores of the
first component were significantly ($P = 0.001$) correlated with soil pH. Canonical variate analysis, using all 14 T-RFs, showed a significant difference between results of different lime treatments but no significant difference between results of N fertilizer applications (Fig. 2). AOA T-RF166 had highest relative abundance (on average 49%) in the tested soils and may include several sequence types, as indicated by cloning and sequencing data (see below). AOA T-RF79 and T-RF205 had similar levels of dominance (10 to 15%) in all soil samples within the pH range 5.4 to 5.8, irrespective of site and land use type. T-RFLP profiles obtained from primer set amo111f and amo643r in combination with the RsaI enzyme produced 20 T-RFs in all the samples. T-RF 101 was the major T-RF and had the highest relative abundance (>50%) in the highly acidic tea soils with pHs lower than 4.4. Because the second set of primers has low specificity (occasionally amplifying non-AOA sequences), they were used only for confirmation and identification of T-RFs obtained using the first primer set and T-RFs originating from this set of primers were here identified by the restriction enzyme used (RsaI) followed by the T-RF size in base pairs.

A total of nine AOB T-RFs were obtained from soils, and AOB T-RF60, T-RF156, and T-RF256 had high relative abundances of 43%, 33% and 13%, respectively. PCA of the T-RFLP data showed that the scores of the first component were significantly ($P = 0.003$) correlated with soil pH. Canonical variate analysis showed a significant difference between limed and unlimed soils, but no significant difference between the low and high lime treatments (Fig. 3). There was also a significant difference between the highly fertilized soils (900 kg N ha$^{-1}$ yr$^{-1}$) and other less-fertilized samples (Fig. 3).

**Analysis of AOA and AOB amoA sequences.** All major AOA and AOB amoA T-RFs were characterized by cloning and sequencing. Finally, 14 representative AOA sequences and 11 AOB sequences were selected for detailed analysis and the construction of phylogenetic trees. AOA were also classified using two sets of T-RFLP data, and exactly the same 14 representative AOA sequences were obtained (Table 2). The size of T-RF in Table 2 was determined by computation analysis and was close to that determined experimentally.

Clones of AOA T-RF166(Rsa473) were quite different from the other T-RF166 clones (Rsa101, Rsa187, Rsa203, Rsa293, Rsa554) in the phylogenetic tree (Fig. 4). All sequences belonging to AOA T-RF49 and T-RF79 fell within the soil/sed-
Nitrosospira ble 2). According to the nomenclature of Avrahami and T-RF217(Rsa244) and fell within the soil/sediment cluster (Ta-

timent cluster. However, the clones of AOA T-RF217 and T-RF205 were distributed into two and three subclusters, respectively. Most AOA T-RF217 clones belonged to T-RF205 were distributed into two and three subclusters, 

mance cluster. However, the clones of AOA T-RF217 and T-RF217(Rsa244) TRF217 TRF244 FN869058 5.0–6.8 17

TRF217(Rsa203) TRF217 TRF203 FN869065 4.4–6.8 6

TRF205(Rsa187) TRF205 TRF187 FN869067 4.4–6.8 3

TRF205(Rsa79) TRF205 TRF79 GU396238 3.5–6.8 8

TRF79(Rsa108) TRF79 TRF108 GU396238 3.5–6.8 8

TRF79(Rsa291) TRF79 TRF291 GU396249 3.5–6.8 7

TRF166(Rsa101) TRF166 TRF101 GU396241 3.5–4.4 56

TRF166(Rsa187) TRF166 TRF187 FN869065 3.5–5.0 6

TRF166(Rsa203) TRF166 TRF203 GU396244 4.4–6.8 4

TRF166(Rsa293) TRF166 TRF293 GU396253 4.4–6.8 7

TRF166(Rsa473) TRF166 TRF473 FN869072 5.0–6.8 8

TRF166(Rsa554) TRF166 TRF554 FN869068 4.4–6.8 3

TRF205(Rsa79) TRF205 TRF293 GU396257 4.4–6.8 2

TRF205(Rsa101) TRF205 TRF101 GU396248 4.4–6.8 8

TRF205(Rsa187) TRF205 TRF187 FN869061 4.4–6.8 5

TRF217(Rsa203) TRF217 TRF203 FN869059 4.4–6.8 1

TRF217(Rsa244) TRF217 TRF244 FN869058 5.0–6.8 17

TRF49(Rsa203) TRF49 TRF203 FN869067 4.4–6.8 6

TRF205(Rsa187) TRF205 TRF187 FN869065 3.5–5.0 6

TRF205(Rsa79) TRF205 TRF79 GU396238 3.5–6.8 8

TRF166(Rsa293) TRF166 TRF293 GU396253 4.4–6.8 7

TRF166(Rsa473) TRF166 TRF473 FN869072 5.0–6.8 8

TRF166(Rsa554) TRF166 TRF554 FN869068 4.4–6.8 3

TRF205(Rsa79) TRF205 TRF293 GU396257 4.4–6.8 2

TRF205(Rsa101) TRF205 TRF101 GU396248 4.4–6.8 8

TRF205(Rsa187) TRF205 TRF187 FN869061 4.4–6.8 5

TRF217(Rsa203) TRF217 TRF203 FN869059 4.4–6.8 1

TRF217(Rsa244) TRF217 TRF244 FN869058 5.0–6.8 17

abundance was negatively correlated with soil pH (see Fig. S3 in the supplemental material). The average AOB T-RF256 relative abundance was less than that for T-RF156. The peak had a wide pH range (pH > 3.8), and its relative abundance was negatively correlated with soil pH (see Fig. S3 in the supplemental material).

The relative abundances of several T-RFs were significantly (P < 0.05) correlated with N fertilizer application. For example, the relative abundances of AOA T-RF49 decreased and AOA T-RF166 increased, respectively, with increasing fertilizer input (see Fig. S4 in the supplemental material). AOB T-RF256 was not detected in soil with low fertilizer input, and the relative abundance of AOB T-RF156 was negatively correlated with fertilizer input (see Fig. S5 in the supplemental material).

**DISCUSSION**

**Abundance and activity of ammonia oxidizers.** Archael and bacterial amoA abundances were much higher in the tea orchards than in adjacent pine soils and correlated with potential nitrification rates. This may be explained by the fact that the tea orchards have been fertilized over long periods whereas the pine forest soils have never received any fertilizer treatment. Nitrogen input may therefore influence abundance, but only two pine sites were investigated. Previous studies (15) showed that N fertilizer application stimulates soil nitrification and ammonia oxidizer abundance. Moreover, the differences in litter quality and quantity may account for differences in the nitrification rate and ammonia oxidizer abundance, since vegetation type was considered an important factor in determining the activity and abundance of nitrifiers (39, 50, 52).

AOB abundance decreased significantly with decreasing pH, indicating that pH was an important factor controlling AOB abundance in the soil and consistency with other reports of higher AOB abundance in neutral or slightly alkaline conditions (33, 39). However, no significant correlation was observed between AOA abundance and pH in this study. AOA were present over a wider pH range with some populations adapted to highly acidic soils. AOA were generally more abundant than AOB, and the ratio of AOA/AOB amoA gene abundance increased with decreasing soil pH. This suggests that AOA were the dominant ammonia oxidizers in acid soils (33).

The trends in specific gene abundance may reflect different preferences of archael and bacterial ammonia oxidizers for the available ammonia concentration or other differences in physiology and metabolism. The available ammonia concentration decreases with decreasing pH due to ionization of ammonium, and this is believed to the major reason for reduced activity of ammonia oxidation at low pH (12). Although effects of pH and ammonia concentration were not distinguished in this study, pH-associated differences may have resulted from other differences in physiology and metabolism. Measurement of potential nitrification with natural pH produced similar trends but lower rates compared to results with the neutral pH.
The results suggest that AOA may perform nitrification at low pH as well as at neutral pH.

**AOA and AOB community structure.** Ammonia-oxidizing archaeal and bacterial community composition determined by T-RFLP analysis varied with liming and correlated with soil pH, suggesting that soil pH is a key factor controlling the community composition. Nicol et al. (33) also found effects of soil pH on AOA and AOB communities. Nitrogen fertilizer is also believed to influence AOA and AOB community composition (14, 24, 30, 47, 48). In this study, AOB community composition was influenced by high input of N fertilizer (900 kg N ha⁻¹ yr⁻¹). AOB amoA genes were not detected in the highest-N application treatments (1,500 kg N ha⁻¹ yr⁻¹) but fertilizer treatment did not affect AOA T-RFLP patterns. Consequently, data suggest that the effect of N fertilizer on ammonia oxidizer community composition was smaller than the soil pH effect. Moreover, the changes in AOB community composition caused by N fertilizer input may also be partly due to a decrease in soil pH through continued nitrification.

**Relative importance of AOA and AOB in highly acidic soils.** Regression analysis showed a significant positive relationship between nitrification potential and archaeal, but not bacterial, amoA abundance, suggesting that nitrification is driven by AOA in acidic soils. AOB amoA gene abundance was below...
detection limits in the heavily N-fertilized (1,500 kg N ha\(^{-1}\) yr\(^{-1}\)) and low-pH tea soils, but nitrification potential and AOA abundance were high. The relative role of AOA and AOB has been debated since Leininger et al. (29) first reported the dominance of AOA in soil (36). Furthermore, Chen et al. (10) suggested that AOA are dominant in the rhizosphere in paddy soils and were influenced more by exudation from rice roots. However, AOA abundance and activity did not increase with N fertilizer input and AOB and not AOA contributed to nitrification in grassland soils receiving high nitrogen inputs (15). Distinctions may be due to different land use and environmental conditions. Evolutionary considerations suggest that archaea can grow under conditions of extreme salinity, temperature, and pH and low ammonia availability (18, 31), which do not support growth of bacteria and eukaryotes (46). Therefore, it is possible that AOA lack the competitive advantage in some grassland soils but can adapt and flourish in highly acidic soils. Low pH may explain the higher relative abundance of AOA compared to AOB in tea orchards.

**Niche differentiation and potential activity of ammonia oxidizer phylotypes.** Soil pH is clearly a major factor influencing niche separation of AOA and AOB. The relative abundances of several AOA and AOB T-RFs [e.g., AOA T-RF49 and T-RF217(Rsa244) and AOB T-RF256] correlated with soil pH and were dominant in specific pH ranges. N fertilizer input also influenced relative abundance, but this may be a secondary effect due to reduction in soil pH due to increased levels of nitrification. For example, the relative abundances of AOB T-RF 60 and T-RF256 and of AOB T-RF156 increased and decreased, respectively, with increasing N fertilizer input. Soil pH has previously been shown to be a major driver of AOB (43) and AOA (33) community structure and that of bacteria (17) and contrasts with the suggestion (25) that amoA genes may be too conserved to reflect ecological differences.

T-RFLP and sequence analyses suggest that T-RF166(Rsa101), represented by the amoA gene sequence (GU396241), dominates highly acidic tea orchard soils with pHs of <4.4. These soils had very high AOA/AOB amoA gene ratios (>15) and high nitrification potential. Consequently, the dominant representative AOA phylotype in these highly acidic soils may have high activity and play an important role in nitrification. The negative relationship between the abundance of AOA T-RF205 and nitrification potential also suggests that this phylotype may have low activity in Chinese soils.

The relative contributions of AOA and AOB to soil nitrification remain a topic of debate (36). Here we have provided evidence that AOA dominate the ammonia oxidizer community in acidic soils, and, along with potential nitrification data, these results suggest that AOA may be responsible for most of the nitrification in these highly acidic environments. The study demonstrates that different ammonia oxidizer phylotypes occupy distinct pH niches, which in turn provides new insight into the relative dominances of AOA and AOB and niche differentiation of individual phylotypes in natural environments.

**ACKNOWLEDGMENTS**

This work was financially supported by the Royal Society of Edinburgh and the National Science Foundation of China (grant no. 30871600 and 41090283). C.D.C. and B.K.S. are funded by the Scottish Government, Rural and Environment Research and Analysis Directorate. G.W.N. is funded by a NERC Advanced Fellowship (grant NE/D010195/1).

Nadine Thomas, Lucinda Robinson, Duncan White, Clare Cameron, and Cecile Gubry-Rangin are gratefully acknowledged for technical assistance and advice.

**REFERENCES**

AMMONIA OXIDIZER IN ACIDIC SOILS


Downloaded from http://aem.asm.org/ on September 26, 2012 by Research Center of Eco-Environmental Sciences, CAS