

REPORT

Diversity and composition of tropical soil nitrifiers across a plant diversity gradient and among land-use types

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Abstract

Ammonia-oxidizing bacteria (AOB) perform the rate-limiting step of nitrification, a key ecosystem process that in part determines the fate of nitrogen in ecosystems. However, little is known about the factors that determine soil AOB diversity or composition, especially in tropical systems. Using a set of study systems in Costa Rica, we examined whether plant diversity or land-use influenced AOB diversity or composition and whether AOB diversity or composition were associated with nitrification rates. We characterized the molecular diversity and composition of AOB via polymerase chain reaction amplification, cloning, and sequencing of 16S rDNA. We found that AOB diversity or composition did not change significantly across plant diversity treatments. In contrast, AOB differed among land-use types in some measures of diversity and in composition, and differences in AOB composition among land-use types were correlated with potential rates of nitrification. Our results suggest that anthropogenic changes of ecosystems can alter microbial communities in ways that may affect the processes they mediate.

Keywords

Ammonia-oxidizing bacteria, bacteria, biodiversity, community composition, La Selva Biological Station, land-use, microbes, nitrification, soil, tropics.

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INTRODUCTION

Human alteration of ecosystems continues to accelerate. There is strong evidence that the reduction of plant diversity, changes in disturbance regime, and modifications of the physical environment associated with these alterations have important implications for both ecosystem properties and processes. For example, some investigators have found that primary productivity is positively related to plant diversity, particularly at low levels of diversity (Loreau *et al.* 2001). Similarly, disturbance of tropical forests associated with agriculture or conversion to pasture often leads to increased nutrient fluxes during the first several years, but a reduction in nutrient turnover in the long term (Robertson 1984; Matson & Vitousek 1987; Matson *et al.* 1987; Vitousek *et al.* 1989; Keller *et al.* 1993; Reiners *et al.* 1994). Although it is clear that humans often alter ecosystem functioning through changes in land management, little is known about the role of soil microbial communities in these alterations.

Understanding the responses of microbial communities to anthropogenic change is important because microorganisms mediate many critical ecosystem processes, including organic matter decomposition, nutrient mineralization, and trace gas emission and consumption. The importance of microbial communities, in combination with our very limited knowledge about the basic patterns of microbial diversity and the processes driving those patterns (Horner-Devine *et al.* 2004), has led to a recent dramatic increase in attempts to understand the links between human management, plant communities, microorganisms, and ecosystem processes (van der Heijden *et al.* 1998; Cavigelli & Robertson 2000; Naeem *et al.* 2000; Agarwal 2003; Zak *et al.* 2003; Rudgers 2004).

One particularly important process that microorganisms mediate is the transformation of ammonium to nitrite, the rate-limiting step of nitrification (Hart *et al.* 1994). Nitrification can have important effects on the retention of nitrogen in ecosystems; for example, the transformation of ammonium to nitrate can increase the loss of nitrogen via

runoff and leaching to downstream ecosystems or through transfer of nitrogen to the atmosphere (Schlesinger 1997).

Ammonia oxidation in soil is primarily carried out by a specific group of autotrophic organisms within the β -*Proteobacteria*, the ammonia oxidizing bacteria (AOB; Prosser 1989). AOB are ubiquitous; they have been found in soil, freshwater, and marine environments (Kowalchuk & Stephen 2001). Because of their importance and ubiquity, they have been well studied over the past decade, and there is a growing body of knowledge regarding their genetics, physiology, and distribution. Terrestrial AOB are also monophyletic; this makes it possible to study them with recently developed molecular techniques that avoid the biases inherent in culture-based studies of microorganisms (Ward *et al.* 1990). All of these attributes suggest that studying AOB might be particularly fruitful in the effort to link land-use change, microbial communities, and ecosystem function.

Despite the interest in AOB noted above, very little is known about patterns of AOB diversity (i.e. the number of different types of ammonia oxidizers) and community composition (the identity of the ammonia oxidizers present) in the environment. Most past research has been based on laboratory isolates, which are known to represent a small proportion of environmental microorganisms (Ward *et al.* 1990; Smith *et al.* 2001). A few recent studies have utilized culture-independent molecular techniques to study AOB distributions, and these studies suggest that AOB can vary among different environments and can be sensitive to human management (Stephen *et al.* 1996; Bruns *et al.* 1999; McCaig *et al.* 1999; Phillips *et al.* 2000; Webster *et al.* 2002). However, very few of these studies have made statistical, quantitative comparisons of AOB diversity or composition across different environmental systems, and none have examined whether AOB vary with changes in plant species or plant species diversity while other variables remain constant. In addition, most of these studies have been conducted in temperate ecosystems; nothing is known regarding the response of AOB to environmental change in tropical soils.

In this report, we examine soil AOB diversity and composition along a plant diversity gradient and among land-use types in the tropical lowlands of Costa Rica. Changes in plant diversity, plant community composition, or land-use may drive differences in AOB communities through a number of mechanisms. For example, land-use can influence AOB via changes in ammonium availability due to fertilizer amendments or through changes in soil characteristics that influence rates of nitrogen cycling, including soil pH and soil moisture (Phillips *et al.* 2000; Kowalchuk & Stephen 2001). Differences in plant communities can affect soil AOB indirectly through variation in the type, complexity and amount of organic

matter input to soils (Angers & Caron 1998; Hooper *et al.* 2000). Although AOB are autotrophic and do not depend on organic matter inputs directly, differences in the magnitude or range of organic matter inputs may result in different or more variable rates of soil nitrogen mineralization (McLaugherty *et al.* 1985; Hobbie 1996), the main process that provides ammonium to soil AOB. Rooting depths and densities also vary among plant species, and increasing plant diversity may increase the variety of rooting allocation patterns, leading to higher soil physical heterogeneity; such heterogeneity has been observed to alter bacterial diversity and community composition (Horner-Devine *et al.* 2004). Plant diversity could also change soil moisture, temperature, or pH, factors that have all been observed to alter AOB diversity and/or community composition (Kowalchuk & Stephen 2001).

In our study system, we expected AOB diversity to increase with plant diversity due to potentially higher environmental heterogeneity. We also hypothesized that AOB composition would change with plant diversity due to the differences among plant species in root allocation, and potential effects of different plant species on environmental factors such as soil moisture, pH, and ammonium availability. Furthermore, we expected AOB diversity to be higher in forests than pastures because pastures are typically more highly disturbed, have more compacted soil, and have lower nitrogen mineralization rates than forests (Reiners *et al.* 1994); for these same reasons, we also expected AOB composition to vary among land-use types.

The potential importance of AOB diversity or composition to nitrification rates is also unknown. Past studies have noted coincident patterns between AOB composition and/or diversity and nitrification rates, but few have attempted to control for the environmental factors known to affect nitrification rates (e.g. soil moisture, O₂ or substrate availability, pH) (Bruns *et al.* 1999; McCaig *et al.* 1999; Kowalchuk *et al.* 2000; Phillips *et al.* 2000; Webster *et al.* 2002).

In this report, we ask whether AOB diversity or composition change with plant diversity or land-use type (forest, tree plantation, and pasture), which soil characteristics are most likely driving differences in AOB communities; and, finally, whether differences in AOB community composition are associated with differences in nitrification rates.

METHODS

Study sites

We utilized two study areas in the Atlantic tropical lowlands of Costa Rica at La Selva Biological Station. The first is an existing long-term experiment that directly manipulates plant community composition and diversity

while holding most other environmental variables constant. Although this experiment was established to study the influence of plant species composition, plant diversity, and disturbance regime on the productivity and sustainability of tree plantations (Haggard & Ewel 1995), we took advantage of its design to carry out a 2-year study on the effects of plant community composition and diversity on microbial diversity, composition, and function; this report focuses on our results for AOB. The experiment is located on an alluvial terrace that sustained cacao plantations for over 30 years prior to the experiment. In 1991, after all cacao vegetation was removed and the remnant vegetation burned, three different experiments were initiated (Haggard & Ewel 1995). We sampled from a subset of available plots from this experimental system, and chose plots that harboured three levels of plant diversity: one, three, and five species. At the one-species level, *Cordia alliodora* was grown in monoculture (three replicate plots: 40 × 30 m). Three-species combinations consisted of *Cordia* and two other understory species, an herb (*Heliconia imbricata*) and a palm (*Enterpe oleracea*; three replicate plots: 40 × 30 m). Five-species combinations consisted of *Cordia*, two other hardwoods (*Hyeronima alchorneoides* and *Cedrela odorata*), and two palm species (*Enterpe macrospadix* and *E. oleracea*; two replicate plots; 40 × 40 m). Each of the tree species in the plots is known to differ in phenology and rooting characteristics (Haggard & Ewel 1995); none are nitrogen-fixers.

Our second study consisted of three types of land-use (i.e. forest, pasture, and tree plantations). For the forest and pasture treatments, we used three replicate plots (40 × 30 m) that were located on the same soil type as the plant diversity experiment described above. All plots in these two treatment groups have been maintained with their current land cover for at least 3 decades. Typical canopy tree species of the forests include *Brosimum alicastrum*, *Bursera simaruba*, and *Cordia alliodora*; typical understory species include *Alchornea costaricensis*, *Bravaisia intergerrima*, *Casearia corymbosa*, and *Simira maxonii* (Hartshorn & Hammel 1994). The main species of grass in the pastures is *Ischaemum timorense* (Jack Ewel, pers. comm.). Tree plantations consisted of the pooled plots from the plant diversity experiment.

The sandy loam soils in both study areas are classified as mixed, isohyperthermic, andic, fluventic dysotropept. Earlier comparative research in this area indicated that these alluvial soils are rich in nitrogen and phosphorus relative to many areas in both tropical and temperate systems (Vitousek & Sanford 1986). Mean annual rainfall is 4 m and mean annual temperature is 24 °C. There is a short dry season between February and April, although mean monthly rainfall never falls below 0.1 m (Haggard & Ewel 1995).

Sampling

In November 2001, we used a 2.5 cm diameter soil auger to take 10 randomly located samples of soil from each plot to 10 cm depth. We composited and homogenized the 10 cores and hand-sieved the soil to remove large pieces of organic material (i.e. 2 mm or greater in size). Soils were stored on ice for approximately 3 days, after which soil nitrogen analyses were conducted and subsamples of soil for microbial community analysis were frozen at -80 °C for analysis at a later date. Subsamples of the remaining soil were air dried for soil pH and phosphorus analyses, dried at 60 °C for carbon analyses, or dried at 105 °C for soil moisture content (see Soil characteristics section).

Microbial community analyses

Extraction, amplification, cloning and sequencing of ammonia-oxidizer DNA

DNA was extracted from 0.5 g of soil using the Bio101 soil DNA extraction kit using the protocol recommended by the manufacturer (Qbiogene, Inc., Carlsbad, CA, USA). A region of the small subunit (16S) ribosomal genes present in the extracted DNA was amplified using a nested polymerase chain reaction (PCR) protocol and the ammonia oxidizer-specific primer sets β AOBf- β AOBr (McCaig *et al.* 1994) and CTO189f-CTO654r (Kowalchuk *et al.* 1997). PCR was carried out in 50 μ l reaction tubes, with a hot-start to reduce non-specific amplifications. The reaction mixture for the first PCR consisted of approximately 10 ng extracted soil DNA, 12.5 μ l of MasterAmp PCR premix F (Epicentre Technologies, Madison, WI, USA), 0.5 μ M of each β AOB primer, 2.5 U of *Taq* DNA polymerase LD (AmpliAmp, Applied Biosystems, Foster City, CA, USA) and 0.5 μ l bovine serum albumin (20 mg mL⁻¹). The first PCR conditions were as follows: initial denaturation at 94 °C for 3 min; 25 cycles of 94 °C (30 s), 55 °C (30 s), 72 °C (90 s); and a final extension at 72 °C for 10 min. The reactions of the second PCR consisted of 0.5 μ l of products from the first PCR, 12.5 μ l of MasterAmp PCR premix F, 0.5 μ M of each CTO primer, and 2.5 U of *Taq* DNA polymerase LD. We decreased the number of cycles in the second PCR to 20 to reduce PCR artifacts (Qiu *et al.* 2001), but kept all other PCR conditions the same. All PCR reactions were performed in triplicate and pooled prior to cloning.

We cloned PCR products with the TOPO TA Cloning Kit (Invitrogen Corp., San Diego, CA, USA) following the protocol of the manufacturer. Sequencing of a random subset of positive-screened clones was performed by the Genomics Technology Support Facility of Michigan State University (<http://genomics.msu.edu/>). We confirmed that the sequences were from AOBs by searching international

sequence databases using the Blast program (<http://www.ncbi.nlm.nih.gov/blast/>). These sequences have been deposited in GenBank under the accession numbers AY631475–AY631851.

Phylogenetic analysis of sequence data

We used the ARB software package (Ludwig & Strunk 1999) and the Ribosomal Database Project database (Maidak *et al.* 2001) to align the rDNA sequences in our clone library and to determine their phylogenetic affiliations. We screened for and excluded chimeric sequences using the program Chimera_Check (<http://rdp.cme.msu.edu/cgis/chimera.cgi?su=SSU>). To confirm that sequences flagged by this program were indeed chimeras, we used ARB to tree the part of the sequence that preceded and followed the breakpoint suggested by Chimera_Check. If the two parts showed affiliations with different reference sequences, they were considered chimeric and were excluded from further analyses.

We calculated similarity using 290 unambiguously aligned and informative positions, and built a neighbor-joining tree with ARB software. We assigned sequences into Operational Taxonomic Units (OTUs) based on 97% sequence similarity, the most commonly used OTU definition for bacteria (Stackebrandt & Rainey 1995; McCaig *et al.* 2001). When it was not possible to assign a sequence to an OTU unambiguously, we used complete-linkage clustering to resolve OTUs (Dunn & Everitt 1982).

Estimates of ammonia oxidizer diversity

We assessed ammonia oxidizer diversity with the software ESTIMATES (Colwell 1997) using three different methods, each of which reveals different properties regarding the presence, absence, and abundance of AOB: (1) observed richness, (2) non-parametric estimates of AOB richness (i.e. Chao1 and ACE), and (3) AOB diversity indices (i.e. Shannon's H' , Simpson's D), and Fisher's α (α). To account for differences in sampling effort (the number of sequenced clones from a given plot), we rarified our samples for all richness and diversity measures to compare treatment groups at the lowest common level of sampling effort (i.e. the lowest number of sequenced clones from any plot). For comparisons among plant diversity levels, we rarified to 28 observed sequences. For comparisons among land-use types, we rarified to 18 observed sequences.

We compared richness or diversity using a one-way analysis of variance (ANOVA) in two separate analyses. First, we determined whether there was a significant effect of plant diversity on AOB richness or diversity by comparing the results for the one, three, and five species plots. Second, we determined whether land-use type affected AOB richness or diversity by comparing forest, pasture, and

plantation (all the plant diversity plots combined) treatments.

Assessing differences in ammonia oxidizer community composition

We generated Bray–Curtis similarity matrices (Magurran 1988) of AOB data using normalized relative abundances of OTUs in each plot. With these similarity matrices, we performed a non-parametric two-way analysis of similarity (ANOSIM, Clarke & Warwick 2001) to test whether there were significant differences in microbial community composition among *a priori* defined treatment groups; we used separate ANOSIM analyses to test the effect of plant diversity or land-use type on AOB composition. We visualized shifts in community composition among treatment groups using non-metric multi-dimensional scaling (nMDS) of similarity matrices to produce two-dimensional ordination figures (Clarke 1993).

Identifying influential OTUs in discriminating between land uses

To determine which OTUs accounted for observed differences in AOB composition, we used the program SIMPER (Clarke & Warwick 2001). The program calculates the average dissimilarity between all pairs of intergroup samples (e.g. it compares each of the pasture plots to each of the forest plots) and tabulates the average percent contribution of each OTU to the dissimilarity observed between the groups. For this analysis, we compared the pooled forest and plantation plots to the pasture plots, as there was no significant difference in AOB composition between forest and plantation treatment groups (see Results).

Microbial biomass

We used phospholipid fatty acid composition (PLFA) to determine relative microbial biomass (Leckie *et al.* 2004). We extracted 4 g lyophilized soil using a modified Bligh and Dyer extraction and identified and quantified individual fatty acids using gas chromatography (White & Ringelberg 1998). Peaks were identified using bacterial fatty acid standards and MIDI peak identification software (MIDI, Inc., Newark, DE, USA). We estimated microbial biomass as the sum of the mass (in nmol) of each of the fatty acid groups present in a given soil.

Soil characteristics and their relation to changes in AOB composition

To determine environmental correlates of AOB composition, we examined the following soil characteristics in each plot of the study: extractable $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$, net nitrogen mineralization and nitrification, pH, moisture, Bray's phosphorus (P), Organic P, C : N and %C. For extractable nitrogen, 15 g subsamples of field moist soil

were extracted with 100 mL 2 N KCl by shaking for 1 h. The extract was passed through Whatmann #1 filters, and the filtrate was analyzed for $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ colorimetrically with an autoanalyzer (Alpkem Flow Solution IV). For net nitrogen mineralization, we incubated 15 g subsamples of field moist soil in 100 mL specimen cups with perforated caps for 21 days at constant field moisture at room temperature and determined extractable nitrogen concentrations as above. Net nitrogen mineralization was calculated as $\text{NH}_4\text{-N}$ plus $\text{NO}_3\text{-N}$ at the end of incubation minus initial $\text{NH}_4\text{-N}$ plus $\text{NO}_3\text{-N}$. Soil pH was measured on air-dried soil in 0.01 M CaCl_2 , using a 1 : 2 (w/v) soil : liquid ratio (Accumet Dual Channel pH/Ion/Conductivity Meter). Soil gravimetric moisture content was determined by oven drying 100 g subsamples of field moist soil at 105 °C for 48 h. We determined available P from air-dried soil using the Bray P1 method (Olsen & Sommers 1982) followed by colorimetric analysis using a Hitachi U-2001 spectrophotometer. We measured organic P using the Saunders and Williams ignition method (Olsen & Sommers 1982), again followed by colorimetric analysis. Percent C and C : N were determined using a Carlo Erba NA1500 Series II elemental analyzer using the thermal conductivity detector (TCD) with oven-dried soil (60 °C).

We tested whether variation in each of these soil properties (net nitrogen mineralization and nitrification, extractable NH_4 and NO_3 , pH, soil moisture, available P, Organic P, %C, and C : N) were related to differences in ammonia oxidizer community composition with the statistical software BVSTEP (Clarke & Warwick 2001). This program first generates a similarity matrix based on normalized Euclidean distances for each environmental variable, and selects the one giving the highest correlation coefficient (ρ) with the matrix of Bray–Curtis similarities. Each of the remaining abiotic variables is then added, and the one that improves the correlation most is retained in the model. This forward selection is continued until there is no improvement in the correlation coefficient beyond a threshold value (in our case 0.05). We emphasize that in this analysis, soil characteristics that are at other times presented as means across treatment groups (see Results) are converted to pairwise dissimilarities; thus, characteristics that do not significantly differ among treatment groups in an ANOVA may show a significant relationship to pairwise differences in AOB composition.

Nitrification potential and its link to AOB composition

We determined nitrification potential on soil from each plot using the soil slurry method (Hart *et al.* 1994). Briefly, 10 g of soil was shaken vigorously with 100 mL of 1 mM phosphate buffer solution (pH 7.2) and 1.5 mM $(\text{NH}_4)_2\text{SO}_4$ in 300 mL plastic specimen cups with perforated caps. We

took 10 mL subsamples of the slurry at 2, 4, 8, 10, and 12 h. We centrifuged the subsamples at $1500 \times g$ for 10 min, and froze 2 mL of the supernatant until NO_3^- concentration was determined using colorimetric analysis with an autoanalyzer (Alpkem Flow Solution IV). We analyzed the assay in triplicate for each soil. This approach controls all abiotic factors that are known to influence nitrification rate (i.e. ammonium and oxygen availability, diffusion limitation, temperature, pH). Although AOB abundance, which could influence this rate as well, was not estimated in this study, we did find that our estimate of overall microbial biomass was significantly and positively related to nitrification rates ($r^2 = 0.76$; $P < 0.0001$). We thus used overall biomass as a proxy for AOB abundance, and expressed nitrification rates per gram of overall microbial biomass.

We used a Mantel test (MANTEL 2.0) to test whether differences in AOB composition were related to nitrification potential rates. We transformed nitrification potential rates (both in terms of microbial biomass and without this transformation) into a similarity matrix based on normalized Euclidean distances for input into the MANTEL program. We used the Bray–Curtis similarity matrix as our measure of AOB composition.

RESULTS

We obtained a total of 376 AOB sequences that grouped into 56 OTUs, 15 of which were singletons or doubletons; see Appendix S1 in Supplementary Material for a complete list of the sequence accession numbers that correspond to each OTU. The sequences formed two distinct clusters within the β -Proteobacteria. One cluster was most closely related to *Nitrosomonas communis*, and contained 53 sequences and 19 OTUs (OTUs 1–19). The other cluster was most related to *Nitrospira multififormis* and contained 333 sequences and 37 OTUs (OTUs 20–56). These clusters correspond to clusters six and three of Stephen *et al.* (1996), respectively. The most abundant OTU (OTU #20) contained 94 individual sequences and was most closely affiliated with the genus *Nitrospira*.

Rarefaction curves revealed that although AOB communities in all plots were undersampled, the rates of OTU accumulation were beginning to decelerate (Fig. 1). Overall similarities among plots were relatively low; three-quarters of the pairwise similarities were between 5 and 40%, and there was a maximum similarity of 66% (data not shown).

Neither AOB diversity nor community composition changed significantly with plant diversity levels (Table 1; ANOSIM, $P > 0.05$). In contrast, AOB diversity changed with land-use by some measures (Table 1). Pasture plots harboured more diverse AOB than forest or plantation plots by some measures (observed richness, Simpson's diversity index, and Fisher's α ; Table 1). Pasture plots also harboured

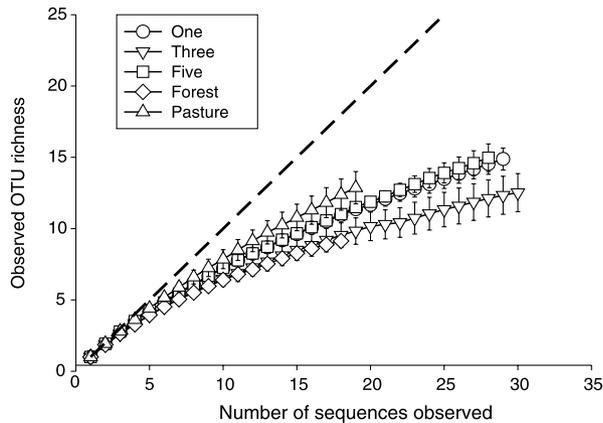


Figure 1 Rarefaction curves for monocultures, three-species combinations, five-species combinations, forest and pasture plots. Points represent means of replicate plots in each treatment and error bars are ± 1 SE. Because we take the mean across plots within a treatment group, the reported means are for the lowest sequence number in the treatment group. We show a dashed 1 : 1 line for reference.

AOB communities that were compositionally distinct from forest and plantation plots (ANOSIM, $P < 0.001$; Fig. 2). Since the only significant differences in composition were between pastures and forests/plantations, we focused our analyses of the drivers and consequences of AOB composition on these treatment groups.

SIMPER revealed that three OTUs (OTUs 20, 39, and 9) contributed most to the shift in composition between pasture and forest/plantation plots, together accounting for 27% of the difference between pasture and forest/planta-

tion plots (Table 2). Two were most closely associated with the genus *Nitrospira*, and one was most closely associated with the genus *Nitrosomonas*. We also found that pasture plots had a higher average relative abundance of *Nitrosomonas* vs. *Nitrospira* sequences (34.9%) than forest/plantation plots (10.2%; $F_{1,12} = 5.92$, $P < 0.05$). This analysis also highlights that the shift in AOB composition from forest/plantation to pasture is due to both the addition and loss of specific OTUs between these treatment groups (Table 2).

Soil processes and characteristics varied among land-use types (Table 3). Net nitrogen mineralization steadily and significantly decreased from forests to plantations to pastures ($P < 0.05$), as did net nitrification; the latter result was marginally significant ($P = 0.052$). Pasture plots tended to have higher $\text{NH}_4\text{-N}$ than either forest or plantation plots at the time of sampling, but this trend again was not significant. Conversely, forests had significantly higher $\text{NO}_3\text{-N}$ than either plantations or pastures ($P < 0.01$). Data from four sampling dates over 2 years show similar trends in the relative sizes of these nitrogen pools among land-use types (Carney 2003). Soil pH was significantly lower in the pastures ($P < 0.01$), and forests had higher microbial biomass than either other land-use ($P < 0.001$). Although not significant, there was a trend in soil moisture from the highest values in forests to the lowest values in plantations. Organic P and %C were significantly higher in the forests than plantations ($P < 0.05$). The BVSTEP model that was most related to differences in AOB composition among land-use types included net nitrogen mineralization, extractable NH_4 , pH, and soil moisture ($\rho = 0.452$). Of these four, the single variable that was most highly correlated to differences in AOB composition was soil moisture ($\rho = 0.253$).

Table 1 Rarefied estimates and indices of ammonia oxidizer diversity among plant diversity levels and land-use types. Values are means (standard error). Diversity was estimated using Operational Taxonomic Units defined as groups with $\geq 97\%$ sequence similarity.

Diversity metric	Plant diversity gradient				Land-use types			
	One	Three	Five	Significance	Plantation	Forest	Pasture	Significance
S_{obs}	14.53 (0.72)	12.12 (1.30)	14.97 (0.97)	ns	10.44 ^a (0.41)	9.13 ^a (0.29)	12.39 ^b (1.10)	*
S_{Chao1}	35.33 (7.15)	22.29 (2.80)	43.50 (20.36)	ns	27.62 (3.29)	15.16 (1.55)	27.91 (1.26)	ns
S_{ACE}	46.30 (14.80)	25.61 (4.62)	54.46 (24.57)	ns	32.60 (4.88)	19.77 (1.48)	35.28 (3.30)	ns
D	10.83 (1.05)	7.89 (0.83)	11.87 (0.79)	ns	10.91 ^a (1.12)	7.27 ^a (0.93)	21.64 ^b (7.53)	*
H'	2.36 (0.057)	2.14 (0.18)	2.38 (0.025)	ns	2.10 (0.062)	1.94 (0.062)	2.34 (0.18)	ns
α	12.51 (1.59)	8.94 (1.89)	13.38 (2.24)	ns	11.49 ^a (1.05)	7.48 ^a (0.57)	19.87 ^b (5.00)	*
Sample size	3	3	2		8	3	3	
Sequence each plot	(29, 33, 37)	(32, 35, 36)	(26, 33)		†	(19,19,18)	(19,19,20)	

S_{obs} , observed richness; S_{Chao1} , Chao1 estimated richness; S_{ACE} , ACE estimated richness; D , Simpson's diversity index; H' , Shannon's diversity index; α , Fisher's α diversity index; Sample size, number of field replicates; Sequence each plot, the number of sequences in each replicate plot.

Values are significant at * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ in a one-way ANOVA; ns, not significant. Letters denote differences in pairwise means.

†See plant diversity plots for number of sequences in each plot.

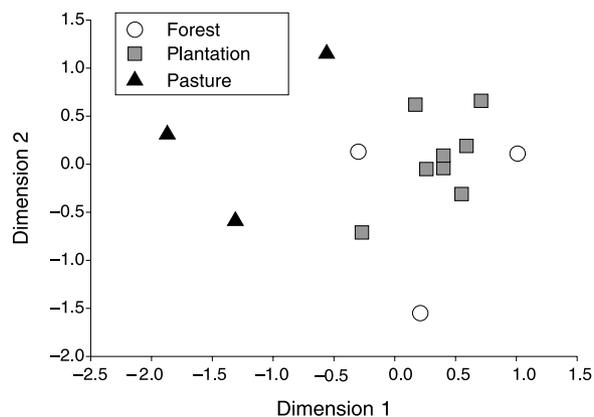


Figure 2 Bray–Curtis similarity values of AOB composition among land-use types as represented by non-metric multi-dimensional scaling.

Nitrification potential also varied among land-use types, tending to decrease from forest to plantation to pasture plots (Table 3). AOB composition was significantly related to nitrification potential rates ($g = 5.978$; $P < 0.01$); this result was still significant when differences in overall microbial abundance were controlled for (see Methods; $g = 6.112$; $P < 0.01$).

DISCUSSION

Our study is the first, to our knowledge, to examine AOB diversity along a plant diversity gradient. As with other studies attempting to relate species diversity to ecosystem function (e.g. Tilman & Downing 1994), the design of our study system potentially confounded the effects of plant diversity and plant community composition as plant diversity increased in this study, so did the likelihood that

OTU #*	Closest relative	Mean relative abundance (%) in forest and plantation	Mean relative abundance (%) in pasture	Contrib.%	Cum.%
20	<i>Nitrospira multififormis</i>	28.9	6.9	13.09	13.09
39	<i>N. multififormis</i>	0	15.1	8.99	22.08
9	<i>Nitrosomonas communis</i>	0.3	8.8	5.17	27.26
47	<i>N. multififormis</i>	1.6	0	4.91	32.17
7	<i>N. communis</i>	1.5	5.3	3.47	35.64

Contrib.% is the percent of difference between pasture and forest/plantation plots for which this OTU accounts; Cum.% is the cumulative percent of the difference accounted for by each additional OTU. The overall average dissimilarity between pasture and forest/plantation plots is 83.91%.

*See Appendix S1 in Supplementary Material for a list of sequence accession numbers belonging to each OTU.

Table 2 The five most important Operational Taxonomic Units (OTUs) in discriminating AOB composition between land-use types

Table 3 Soil and microbial characteristics among land-use types. Values are means (standard error)

Soil property	Forest	Plantation	Pasture	Significance
Net mineralization ($\text{mg kg}^{-1} 21 \text{ days}^{-1}$)	14.42 ^a (4.19)	6.92 ^{ab} (1.16)	2.84 ^b (2.64)	*
Net nitrification ($\text{mg kg}^{-1} 21 \text{ days}^{-1}$)	18.48 (4.11)	11.87 (1.61)	8.99 (0.34)	ns ($P = 0.0524$)
Nitrification Potential ($\text{mg kg}^{-1} \text{ h}^{-1}$)	1.36 ^a (0.20)	0.37 ^b (0.029)	0.19 ^b (0.049)	***
Extractable $\text{NH}_4\text{-N}$ (mg kg^{-1})	5.37 (0.27)	5.37 (0.87)	7.40 (3.27)	ns
Extractable $\text{NO}_3\text{-N}$ (mg kg^{-1})	8.71 ^a (0.38)	4.02 ^b (0.49)	4.21 ^b (1.41)	**
pH (in CaCl_2)	5.82 ^a (0.12)	5.61 ^a (0.074)	5.18 ^b (0.068)	**
% Moisture	44.78 (1.73)	39.88 (0.93)	42.22 (3.34)	ns
Bray's P (mg kg^{-1})	17.10 (4.05)	21.12 (8.69)	7.75 (1.87)	ns
Organic P (mg kg^{-1})	1032.75 ^a (54.08)	864.75 ^b (26.49)	914.08 ^{ab} (58.87)	*
C : N	10.58 (0.15)	11.19 (0.36)	10.86 (0.067)	ns
%C	4.59 ^a (0.66)	3.12 ^b (0.15)	3.65 ^{ab} (0.40)	*
Microbial biomass (nmol PLFA g^{-1})	78.84 ^a (4.57)	39.37 ^b (3.01)	49.03 ^b (1.16)	***

Values are significant at * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ in a one-way ANOVA; ns, not significant. Letters denote differences in pairwise means.

a study site contained a species that had a strong effect on microbial community composition (i.e. the sampling effect; Wardle 1999). However, contrary to our expectations, we found no significant differences in AOB diversity or composition among plant diversity levels. This is likely due to the lack of differences among plant diversity levels in soil moisture ($F_{2,5} = 2.95$; $P = 0.142$), pH ($F_{2,5} = 1.64$; $P = 0.283$), or net nitrogen mineralization ($F_{2,5} = 2.02$; $P = 0.230$) despite differences in canopy cover, rooting depth, litter production, and litter chemistry (Haggar & Ewel 1995). Soil $\text{NH}_4\text{-N}$ changed with plant diversity at the time of sampling ($F_{2,5} = 6.19$; $P = 0.0443$), but this difference was either transient or was not sufficient to affect the relative abundance of the most common AOB types. We might expect to find the greatest differences in AOB composition or diversity among systems that vary substantially in factors known to influence rates of nitrogen cycling (e.g. soil moisture and/or porosity, pH, plant litter quality or quantity, soil organic matter quality or quantity, or fertilizer additions). It is also possible, however, that differences in AOB composition exist in these sites that are driven primarily by relatively rare AOB types; such differences might not have been detected because it was not possible to exhaustively sample the AOB community.

Land-use treatments in our study incorporated plots that varied substantially in plant communities, soil characteristics, and disturbance history. In these plots, we found that some measures of AOB diversity changed with land-use (Table 1). Observed richness and two diversity indices indicated that pasture plots harboured more types of AOB than either plantation or forest plots. However, when diversity was extrapolated using estimation techniques, the difference between land-use types disappears, suggesting that more exhaustive sampling might remove the trends in richness we observed. We therefore cannot conclude that pasture plots are necessarily more diverse than either forest or plantation plots; however, it is unlikely that pasture plots are less diverse in AOB than native forest soils.

These observations are in contrast to our expectations, as well as results from a previous study that showed that native soils had higher AOB phylogenetic diversity than human-managed soils (Bruns *et al.* 1999). In that study, Bruns *et al.* (1999) found that AOB sequences from native soils were distributed among more of seven distinct phylogenetic clusters than managed soils. In contrast, our sequences grouped into two of seven proposed AOB clusters (i.e. clusters three and six from Stephen *et al.* 1996), and each land-use type harboured AOB from both of these clusters. The difference between the two studies may be due to the low-intensity management of the pastures in our study. Bruns *et al.* (1999) examined an intensively managed system with high nutrient inputs and intense physical disturbance, whereas the pastures in our study area were subjected to

cattle grazing with little fertilizer input or tillage. However, it is important to note that even in the intensively managed system utilized by Bruns *et al.* (1999), changes in qualitative phylogenetic diversity between native and cultivated soils are not always evident (Phillips *et al.* 2000). Other studies suggest that AOB may be sensitive to the type of fertilizer application (Oved *et al.* 2001), soil pH (Stephen *et al.* 1998; Kowalchuk *et al.* 2000), soil temperature (Avrahami *et al.* 2003), and the heterogeneity of soil characteristics (Webster *et al.* 2002), all of which can change differentially depending on management.

Although we found no compelling differences in the diversity of AOB among land uses, we found that pasture soils harboured AOB that were compositionally distinct from those in both other land-use types (Fig. 2), a difference that may be related to rates of nitrogen cycling among these systems. Others have observed that rates and dynamics of nitrogen mineralization and nitrification are higher in undisturbed tropical forests than in older secondary succession forests (our plantation plots are in an equivalent state of development) or older pastures (Keller & Reiners 1994). Our data support these general trends, showing a steady decline in net nitrogen mineralization across forest, plantation, and old pasture plots (Table 3). This decline in net nitrogen mineralization is likely to affect AOB in soil, as they depend on ammonium for energy. In fact, we observed that net nitrogen mineralization was one of four interrelated factors that were related to differences in AOB composition (along with soil moisture, pH, and $\text{NH}_4\text{-N}$).

In exploring which OTUs were responsible for the observed differences in composition, we found that most OTUs played a small role in discriminating between treatment groups (Table 2). The most discriminating OTU was also the most abundant among all plots and was most closely related to *Nitrosospira multiformis*. The majority of our sequences were most closely related to this species, whereas other studies of environmental AOB using the same primers have found that environmentally retrieved sequences cluster with many different *Nitrosospira* species (McCaig *et al.* 1999; Phillips *et al.* 2000; Webster *et al.* 2002). While our soils were dominated by *Nitrosospira*, which is in agreement with many other studies of soil AOB (Kowalchuk & Stephen 2001), we also found that the relative proportion of *Nitrosomonas* species was higher in pasture than forest. It is thought that *Nitrosomonas* may become more dominant in nutrient rich environments or where soils are amended with fertilizer (Hastings *et al.* 1997). All soils in our study area are relatively fertile alluvial soils, perhaps accounting for the presence of members of this genus in the forest and plantation plots, and pasture plots have likely been fertilized periodically since their inception. This shift in composition among both *Nitrosospira*-like and *Nitrosomonas*-like organisms may have implications for ecosystem function, given the differences in

activity observed even among different AOB strains (Jiang & Bakken 1999a,b).

We found a significant relationship between AOB composition and nitrification rates using an approach that controls for factors other than AOB on this process rate; this relationship remained significant even when we accounted for differences in overall microbial abundance. Although these results are novel and suggestive, they need to be interpreted with caution. We used overall microbial biomass as a proxy for relative AOB abundance because it was highly significantly related to nitrification rates, but there may be differences in AOB abundance that we did not account for in this study. Future work will address this issue using newly developed culture-independent methods for directly estimating AOB abundance.

Our study is the first to quantitatively compare AOB diversity and composition across plant diversity and land-use gradients in a tropical ecosystem. We observed that soil AOB diversity and composition were insensitive to changes in plant diversity or composition, but changed significantly with land use. Furthermore, we observed that changes in AOB composition due to land-use change are significantly associated with changes in the potential rates of nitrification. These results suggest that anthropogenic changes of ecosystems can alter microbial communities in ways that may affect the processes they mediate.

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SUPPLEMENTARY MATERIAL

The following material is available from <http://www.blackwellpublishing.com/products/journals/suppmat/ele/ele628/ele628sm.htm>

Appendix S1

Comprehensive list of sequence accession numbers and the OTUs into which they were binned.

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