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The Biogeography of Ammonia-Oxidizing Bacterial Communities in Soil

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Abstract Although ammonia-oxidizing bacteria (AOB) are likely to play a key role in the soil nitrogen cycle, we have only a limited understanding of how the diversity and composition of soil AOB communities change across ecosystem types. We examined 23 soils collected from across North America and used sequence-based analyses to compare the AOB communities in each of the distinct soils. Using 97% 16S rRNA sequence similarity groups, we identified only 24 unique AOB phylotypes across all of the soils sampled. The majority of the sequences collected were in the *Nitrosospira* lineages (representing 80% of all the sequences collected), and AOB belonging to *Nitrosospira* cluster 3 were particularly common in our clone libraries and ubiquitous across the soil types. Community composition was highly variable across the collected soils, and similar ecosystem types did not always harbor similar AOB communities. We did not find any significant correlations between AOB community composition and measures of N availability. From the suite of environmental variables measured, we found the strongest correlation between temperature and AOB community composition; soils exposed to similar mean annual temperatures tended to have similar AOB communities. This finding is consistent with previous studies and suggests that temperature selects for specific AOB lineages. Given that distinct AOB taxa are likely to have unique functional attributes, the biogeographical patterns exhibited by soil AOB may be directly relevant to understanding soil nitrogen dynamics under changing environmental conditions.

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Introduction

The soil environment harbors an amazing diversity of microorganisms, and the composition of soil microbial communities can be highly variable across space. Despite considerable interest in understanding how microbial communities are structured across space [1–3], we still have a relatively limited understanding of the biotic and abiotic factors that may drive the observed spatial variability in community composition. In addition, because the vast majority of soil bacteria have not been cultivated, we can use studies that compare communities across a range of soil types to gain insight into the ecology, physiology, and life-history strategies of uncultivated microbial taxa.

A number of recent studies have examined the distribution of bacterial communities across a range of distinct soils. However, such studies are constrained by the high levels of soil bacterial diversity that (currently) make it

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difficult to survey whole communities at a high level of taxonomic resolution across a large number of individual samples. This limitation is less problematic when examining the distribution of individual bacterial taxa, which can be studied at reasonably fine levels of phylogenetic resolution. The ammonia-oxidizing bacteria, represented by the *Nitrosomonas* and *Nitrosospira* genera within the beta-subclass of the Proteobacteria [4], are particularly well suited for examining the distribution of soil microbes in space as the phylogenetic relationships within the ammonia-oxidizing bacteria (AOB) are reasonably well described [5]. In addition, unlike many other bacterial taxa found in soil, the group is represented by a relatively large number of cultured isolates. In addition, the AOB, along with the ammonia-oxidizing archaea [6, 7], perform a rate-limiting step of nitrification and play a key role in the regulation of soil nitrogen dynamics. For this reason, the study of AOB biogeography may have direct relevance to studies of soil biogeochemistry given that distinct AOB groups are likely to have different physiological and ecological attributes [8].

Despite the fact that AOB have been studied for decades, their biogeochemical importance, ubiquity, and the potential to serve as a "model taxon" for biogeographical studies, we know of no previous studies that have comprehensively examined the diversity and composition of AOB communities across a wide range of ecosystem types. We do know that distinct soils often harbor distinct AOB communities. A number of environmental factors, including vegetation type [9–11], soil nutrient levels [12–16], soil microclimate [13, 17, 18], and management practices [19–21], have been found to have an important influence on the spatial variability exhibited by AOB communities. However, because most studies have compared AOB communities across a rather limited number of samples, with one notable exception being the study by Avrahami and Conrad [18], it has been difficult to ascertain which soil biotic and abiotic characteristics are related to AOB community composition across larger spatial scales.

The present study was designed to examine the distribution of soil AOB across a range of ecosystem types and to determine if these distributional patterns are associated with specific soil and site characteristics. In addition, we wanted to estimate AOB diversity in the soil environment and assess how this diversity is apportioned across ecosystems. In particular, we address the following question: are differences in soil environmental characteristics associated with differences in AOB community composition and diversity? To address this question, we analyzed 23 soils collected from across North America and used a sequencing-based approach to survey and describe the AOB communities in each of these well-characterized soils.

Methods

Soil Collection and Characterization

Soils were collected from 23 individual sites that were selected to represent a range of ecosystem and soil types from throughout North America (Table 1). Many of these sites were located in established research areas, including a number of US Long-Term Ecological Research (LTER) sites (Table 1). All of the sites were minimally disturbed and none were used for intensive agriculture or are likely to have received large fertilizer inputs in recent history. Soils were collected near the height of the plant growing season at each site (spring and summer 2004). The upper 5 cm of mineral soil was collected from five to ten locations within a given plot of approximately 100 m^2 and composited into a single bulk sample. All soil samples were sieved to 4 mm, homogenized, and archived at −80°C. Additional details on the sample collection, site characterization, and the analysis of edaphic properties can be found in Fierer and Jackson [22] and the Table 1 caption.

Clone Library Construction

DNA was extracted from three replicate 0.5 g (wet weight) subsamples of each soil using the Bio101 Soil DNA extraction kit (Qbiogene, Carlsbad, CA, USA). The replicate DNA samples were composited and stored at −20°C prior to amplification. Small subunit ribosomal genes (16S rRNA genes) of bacterial ammonia oxidizers were amplified from each soil sample using the nested polymerase chain reaction (PCR) approach described in Carney et al. [10] with initial amplification using the βAOBf-βAOBr primer set [23] followed by amplification of the resulting amplicons with the CTO189f–CTO654r primer set [24]. This nested PCR approach captures a wide range of diversity of ammonia oxidizers within the beta-Proteobacteria but should also be specific for this group [24]. Previous work has shown that primer sets, such as the one used here, which target the16S rRNA gene yield AOB phylogenies that are topologically similar to those based on the amoA gene [5]. The amoA gene may provide more fine-scale phylogenetic resolution for detecting differences between AOB taxa, but the chosen 16S rRNA-targeting primer set makes it easier to detect nonspecific amplification and, given the broad breadth of our survey, very detailed resolution of individual AOB taxa was not essential for answering our research questions. PCRs were conducted in triplicate 25 μl reactions for each composite soil DNA sample. The initial PCR contained 1× PCR Buffer, 1.5 mmol l^{-1} $MgCl_2$, 5 μg bovine serum albumin, 200 μmol of each dNTP, 1 U of Taq polymerase (AmpliTaq, Applied Biosystems, Foster City, CA, USA.), 0.5 μM of each βAOB primer, and approximately 1.0 μl

Table 1 Site information and physiochemical properties of the 23 soils used in this study

Soil Code	Location	Latitude	Longitude	Elevation (m.a.s.l)	Dominant plant species	MAT (°C)	MAP (mm)	Soil texture	% Organic C	C/N ratio	soil pH	DIN	Net N min
BF1	Bousson Experimental Forest, PA	41.58	80.05	390	*Acer saccharum, Fagus grandifolia, Prunus serotina*	7.8	1000	Loam	6.44	13.7	4.05	53.7	0.01
BZ2	Bonanza Creek LTER, AK	64.8	148.25	300	*Picea glauca*	−2.9	260	Silt loam	3.03	20.5	5.16	18.2	0.29
BZ3	Bonanza Creek LTER, AK	64.8	148.25	300	*Picea glauca*	−2.9	260	Silt loam	3.73	20.8	5.36	6.1	0.19
CF1	Catskills, NY	42.12	74.1	800	*Tsuga canadensis*	5.3	1300	Silt loam	4.33	17	3.56	12.8	1.74
CL2	Calhoun Experimental Forest, SC	34.62	81.67	150	*Bromus* spp.	15.9	1250	Sandy loam	2.27	12.8	5.57	8.8	0.29
CL3	Calhoun Experimental Forest, SC	34.62	81.67	150	*Pinus taeda*	15.9	1250	Loamy sand	1.21	24.3	4.89	0.8	0.15
CL4	Calhoun Experimental Forest, SC	34.62	81.67	150	*Hordeum* sp.	15.9	1250	Sandy loam	1.71	13.7	5.03	29.1	0.28
CO1	Fort Collins, CO	40.4	105.7	2400	*Geum rossii, Silene acaulis, Poa* spp.	−3	600	Sand	1.59	19.1	6.13	9.6	0.47
CO3	Shortgrass Steppe LTER, CO	40.8	104.83	1500	*Bouteloua gracilis, Buchloe dactyloides*	9.3	322	Sandy loam	0.82	11.7	6.02	9.9	0.29
DF1	Duke Forest, NC	35.97	79.08	163	*Pinus taeda, Liquidambar styraciflua, Liriodendron tulipifera*	14.6	1100	Sandy loam	2.78	37.7	5.37	2.0	0.06
DF2	Duke Forest, NC	35.97	79.08	163	*Liriodendron tulipifera, Liquidambar styraciflua, Carya* sp., *Ostrya virginiana*	14.6	1100	Loam	5.45	18.3	6.84	21.5	0.87
DF3	Duke Forest, NC	35.97	79.08	150	*Quercus alba*	14.6	1100	Loamy sand	1.7	25.8	5.05	0.1	0.31
HI2	Kohala Peninsula, HI	20.08	155.7	1000	*Pennisetum clandestinum*	22.8	750	Sandy loam	15.88	11	6.32	36.7	0.56
HI3	Kohala Peninsula, HI	20.28	154	1500	*Pennisetum clandestinum*	22.8	1000	Loamy sand	18.24	11.2	6.53	0.9	0.02
KP2	Konza Prarie LTER, KS	39.1	96.6	100	*Andropogon gerardii, Sorghastrum nutans, Schizachyrium scoparium*	12.5	835	Silt loam	4.62	13.4	6.5	10.9	0.15
KP3	Konza Prarie LTER, KS	39.1	96.6	100	*Juniperus virginiana*	12.5	835	Silt loam	6.89	17.7	7.92	6.3	0.12
LQ3	Luquillo LTER, Puerto Rico	18.3	65.83	700	*Cyrilla racemiflora*	20.5	4500	Sandy loam	6.41	22.6	4.67	35.4	0.70
MD2	Mojave Desert, CA	34.9	115.6	967	*Larrea tridentata, Ambrosia dumosa, Yucca shidigera*	21	150	Loamy sand	0.42	8.8	7.65	12.8	0.05
MD3	Mojave Desert, CA	35.2	115.65	776	*Opuntia echinocarpa, Echinocactus polycephalus*	21	150	Sandy loam	0.12	6.8	7.9	8.8	0.27
MD5	Mojave Desert, CA	35.2	115.87	776	*Larrea tridentata, Ambrosia dumosa, Yucca schidigera*	21	150	Loamy sand	0.57	9.5	8.07	7.5	0.08
SR1	Sedgwick Reserve, CA	34.7	120.05	300	*Quercus douglasii, Bromus* spp.	17.2	500	Loam	4.59	11.1	6.84	13.6	0.37
SR2	Sedgwick Reserve, CA	34.68	120.03	300	*Artemisia californica, Salvia leucophylla*	17.2	500	Sandy loam	1.46	11	8	18.9	0.55
SR3	Sedgwick Reserve, CA	34.68	120.05	300	*Bromus* spp., *Hordeum murinum*	17.2	500	Loam	3.3	11	6.95	22.8	0.28

MAT mean annual temperature, *MAP* mean annual precipitation, *DIN* sum of K_2SO_4-extractable NH_4^+ and NO_3^- concentrations (μg N soil^{-1}), *net N min* potential net nitrogen mineralization rate measured over a 50-day incubation period under controlled conditions (μg N soil^{-1} day^{-1})

All longitudes are west, all latitudes are north, and all sites are within the USA. The dominant plant species at each site were determined in a qualitative manner at the time of sample collection. Data on soil and site characteristics were collected as described in Fierer and Jackson [22].

template DNA. Amplification was accomplished by initial denaturation at 94°C for 3 min followed by 25 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 90 s with a final extension at 72°C for 10 min. The second PCR reaction was conducted with 0.5 μl of amplicons from the first reaction, 1× PCR Buffer, 1.5 mmol l^{-1} $MgCl_2$, 200 μmol of each dNTP, 1 U of Taq polymerase (AmpliTaq, Applied Biosystems, Foster City, CA, USA.), 0.5 μM of each CTO primer, and approximately 1.0 μl template DNA. PCR conditions were identical to those described for the first PCR reaction except that the cycle number was reduced to 20 to reduce PCR artifacts. After this final amplification, the triplicate PCR reactions were pooled together and run on a 1% agarose gel to check amplicon lengths. Amplicons were purified using Qiagen Quickgel extraction kits (Qiagen, Valencia, CA, USA) and cloned using the TOPO TA cloning kit (Invitrogen, Carlsbad, CA, USA) following the manufacturer's directions. Sequencing of randomly selected clones was conducted at the Laboratories of Analytical Biology of the Smithsonian Institution, Suitland, MD, USA.

Sequence Analyses

After removing sequences of insufficient length or quality, the remaining sequences (886 in total) were aligned using the NAST alignment utility [25]. After alignment, we used the GreenGenes "classify" utility (http://greengenes.lbl.gov/) to identify sequences that were not likely to belong to bacterial ammonia oxidizers. Out of the 886 sequences, 170 were classified as Burkholderiales (mainly Comamonadaceae), 14 as Methylophilales, 15 as Rhodocyclales. We ended up with a total of 645 sequences after removing sequences that do not belong to the target group and sequences that were a poor match (<75% sequence identity over 300 bp) to anything in the database and/or chimeric.

To further refine the alignment and confirm that the sequences were all closely related to known bacterial ammonia oxidizers, we used RaxML [26] to construct a tree with the remaining 645 sequences plus 30 sequences from previously described ammonia oxidizers (namely the *Nitrosomonas* and *Nitrosospira* genera) that were aligned together using the NAST alignment utility. A careful examination of the resulting tree revealed that 40 of the remaining sequences were not likely to be closely related to the target groups and these sequences were thus removed from the analyses. After this screening process, 602 quality sequences remained with 23–46 individual sequences per soil sample. The nonredundant sequences from this study have been submitted to the GenBank database.

To estimate AOB diversity across all of the collected soils, we estimated the numbers of phylotypes at various phylogenetic levels using Fastgroup II [27], with sequences dereplicated using percent sequence similarity with gaps at the 99%, 97%, and 95% similarity levels. Rarefaction analyses on the phylotype estimates were conducted using EstimateS [28]. Diversity in each community was estimated using Faith's index of phylogenetic diversity [29] as implemented in Phylocom [30]. Faith's index of phylogenetic diversity (PD) denotes the proportion of branch lengths represented by one community relative to the total of all branch lengths across all communities, providing a relative estimate of the overall phylogenetic (tree) breadth within a given community. In this case, we used Faith's PD to identify the relative proportions of phylogenetic diversity harbored by the AOB communities in each of the individual soils. This approach has distinct advantages over phylotype-based (i.e., operational taxonomic unit-based) approaches in that it assesses the overall phylogenetic diversity of a given community not just diversity at a single level of taxonomic resolution that is often defined arbitrarily.

The pairwise phylogenetic distance between AOB communities from individual soil samples was determined using two distinct approaches: the UniFrac algorithm [31, 32] and a phylotype-based approach. The UniFrac algorithm measures the overall degree of phylogenetic divergence between sets of communities by quantifying the fraction of branch lengths from one phylogenetic tree that are unique to a given community. The UniFrac metric, therefore, allows us to compare community phylogenies in a more integrated manner than the phylotype-based approach which assesses community differences at a single level of taxonomic resolution by defining phylotypes at a sequence similarity level that is chosen somewhat arbitrarily [32]. For the UniFrac analyses, we used a maximum likelihood tree generated with MEGA [33] rooted with a *Pseudomonas aeruginosa* 16S rRNA sequence. As an alternative, we also used the abundances of unique phylotypes, with a phylotype representing any group of sequences that were 97% similar, to quantify the distance between AOB communities by examining the relative abundances of distinct taxa at this specific level of taxonomic resolution. For this phylotype-based approach, we used the proportional abundance of each phylotype as input into Primer (version 5, Primer-E Ltd., Plymouth, UK) with similarity between communities calculated using the Bray–Curtis similarity metric (the Jaccard presence/absence similarity metric yielded nearly identical results). Unlike the UniFrac distance metric, this phylotype-based approach only compares the relative abundances of the AOB phylotypes in each soil; therefore, the degree of similarity between communities is estimated without considering the phylogenetic distance between taxa.

Statistical Analyses

Regression analyses examining correlations between community diversity metrics (Faith's PD or number of unique

phylotypes) and measured soil/site characteristics were conducted using SYSTAT [34]. To determine correlations between environmental variables and the estimated degree of similarity in AOB communities, we used both the UniFrac distance matrix and the phylotype-based distance matrix as input into the Primer software package. Mantel tests (the "Relate" function) were used to compare the environmental distance of individual parameters to the "distance" in AOB community composition with model selection conducted using the stepwise "BVSTEP" function. This function builds a similarity matrix of normalized Euclidean distances for each environmental variable listed in Table 2 and selects the variable yielding the highest correlation coefficient with either of the community distance matrices, adding the remaining abiotic variables in a forward selection process until there is no improvement in the correlation coefficient. For clarity, we also provide univariate correlations between soil/site characteristics and AOB community distances.

Table 2 Spearman rank correlation coefficients (ρ) between measured site/soil characteristics (Euclidean distances) and community "distances" estimated by UniFrac or by measuring the proportional abundances of OTUs across samples (97% cutoff, Euclidean distance of proportional abundances)

	UniFrac distance	OTU distance
Mean annual temp.	0.40**	0.42**
Mean annual precip.	0.13	0.04
Soil moisture deficit	0.03	0.05
Mean monthly temp.	0.12	0.25*
% H_2O	0.1	0.03
% organic C	−0.08	0.05
% N	−0.16	0.02
C/N ratio	0.28*	0.29*
% silt + clay	0.28*	0.18
soil pH	0.16	0.01
NH_4^+ concentrations (extractable)	0.05	0.09
DOC (extractable)	−0.17	0.03
DON (extractable)	−0.13	−0.06
DIN (extractable)	−0.07	−0.07
C mineralization rate (per g soil)	−0.002	0.09
C mineralization rate (per g organic C)	−0.02	−0.03
Net N mineralization rate (per g soil)	−0.11	−0.01
Microbial biomass	−0.15	0.01

DOC dissolved organic carbon, *DON* dissolved organic nitrogen, *DIN* dissolved inorganic nitrogen

DIN is the sum of extractable NH_4^+ and NO_3^- concentrations. Net C and N mineralization rates were estimated from 50-day lab incubations and provide a relative index of microbial C and N availability across the soils sampled. See Fierer and Jackson [22] for details on the measurement of the soil and site characteristics. Using a stepwise regression procedure, we found that a univariate model with mean annual temperature provided the best fit to the two distance metrics; however, we have reported all univariate correlations here for completeness. These *P* values were not corrected for multiple comparisons, and since these variables are not necessarily independent of one another, these results do not represent tests of specific hypotheses, and the individual correlations should be interpreted with care

*$P<0.05$ and **$P<0.001$

Results and Discussion

Diversity of Soil AOB

Out of the 602 sequences identified as close matches to bacterial ammonia oxidizers, there were 425 unique sequences at the 100% similarity level. At the 99%, 97%, and 95% sequence similarity levels, we identified a total of 161, 24, and three unique phylotypes across all of the 23 soils examined (Fig. 1). If we assume that the 97% sequence similarity level roughly corresponds to the "species" level [35], these results suggest that across a wide range of ecosystem and soil types, we found only 24 unique phylotypes ("species") of AOB residing in soil. This relatively low level of AOB diversity may be a result of our primer sets failing to capture the full extent of AOB diversity in soil. An amoA-based survey may reveal more phylogenetic diversity (or at least more diversity at finer levels of taxonomic resolution) than our 16S rRNA-based survey, particularly considering that certain clusters of *Nitrosospira* can only be identified from amoA-based phylogenies [36]. However, although there are differences in methodological approaches and sequence analysis procedures that render direct comparisons difficult, a number of other studies have also found relatively low levels of sequence diversity in soil AOB communities [9, 14, 37, 38].

Diversity was highly variable across the individual soil samples examined. The number of unique phylotypes (as defined at the 97% sequence similarity level) found in each soil ranged from one to nine (Fig. 1b, Fig. 2), and the estimated phylogenetic diversity within each community (Faith's PD) varied from 0.02 to 0.14 (Fig. 2). Although the individual libraries may not have captured the full extent of AOB diversity in each sample (Fig. 1b), these results clearly demonstrate a high level of variability in AOB diversity between individual soils. Qualitatively, it is apparent that no individual ecosystem type had higher levels of phylogenetic diversity than any other broadly defined ecosystem type (Fig. 2, Table 1). Likewise, the diversity of AOB communities, whether estimated by the number of unique phylotypes or by Faith's PD, was not correlated with any of the measured soil and site characteristics listed in Table 2 ($r^2<0.15$ and $P>0.3$ in all cases).

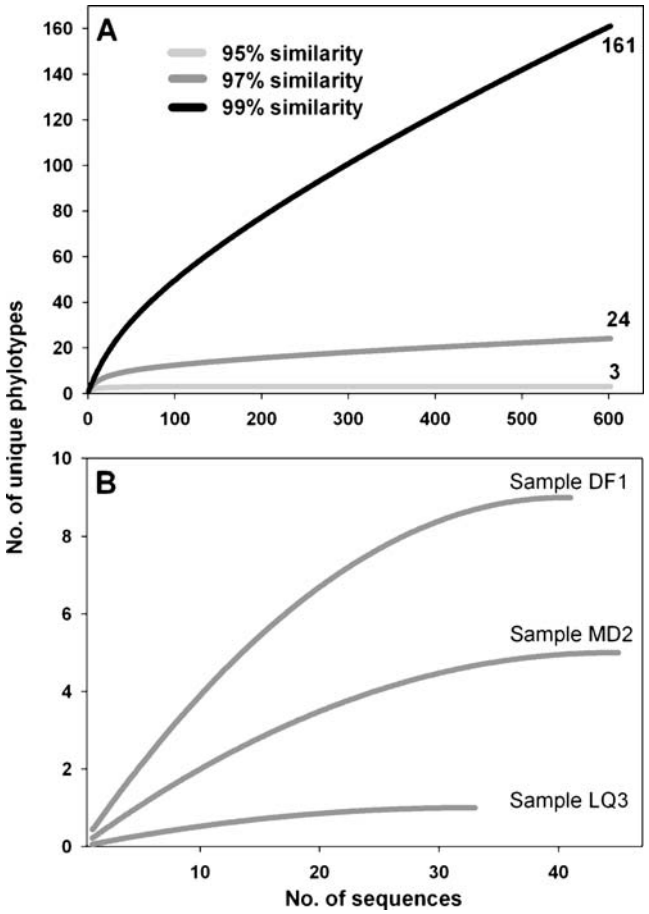

Figure 1 Rarefaction curves constructed using FastGroupII and EstimateS (see "Methods") for all libraries combined (**A**) and individual libraries (**B**). **A** The sequences from all 23 soil samples were analyzed together, and the *numbers at the end of the lines* indicate the cumulative number of phylotypes at each of the three different levels of sequence similarity. **B** We show the rarefaction curves for individual soil samples representative of those soil samples with relatively low (*LQ3*), medium (*MD2*), and high (*DF1*) levels of phylotype richness with richness estimated at only the 97% sequence similarity level. See Table 1 for specific information on these three individual soils

Together, these results indicate that although levels of AOB phylogenetic diversity are not equivalent across all of the soil samples included in this study, the observed patterns are not predictable based on the measured edaphic and site characteristics. AOB diversity may be driven by stochastic processes or factors (or combinations of factors) not measured here or there may simply be no common factor identifiable as regulating AOB diversity across such a broad range of soil types.

Composition of the Soil AOB Communities

Across all 23 soils, only 20% of the sequences obtained were close matches to the *Nitrosomonas*-like group of AOB (Fig. 2), with all of these *Nitrosomonas*-like sequences clustering within the *N. communis* lineage defined by Purkhold et al. [5]. These results are consistent with other studies that have shown the dominance of *Nitrosospira* AOB in terrestrial environments [4, 10, 18, 39, 40]. If we examine the proportional occurrence or abundance of *Nitrosomonas*-like AOB versus *Nitrosospira*-like AOB in each clone library, we find that nitrosomonads were particularly abundant in the desert soils (MD2, MD3, MD5) where they represented >75% of the sequences in these clone libraries (Fig. 2). The abundance of nitrosomonads in desert soils may also be related to soil salinity (which was not measured but which is often higher in desert than in non-desert soils), given that nitrosomads are often halophilic [41]. Although it has been hypothesized that nitrosomonads are likely to become relatively more abundant in soils with high N availability [10, 42], our results do not support this hypothesis; we found no significant correlations between the proportional representation of nitrosomonads in the libraries and any of the measured soil and site parameters listed in Table 2 ($\rho<0.2$, $P>0.4$ in all cases), including those estimates of soil N availability (extractable NH_4^+ concentrations, total dissolved inorganic N concentrations, and net N mineralization rates). However, it is important to note that nearly all of our sites are nonagricultural and have not received high fertilizer inputs so the N availability levels in our soils may be far lower than in soils analyzed in previous studies.

Although there are a number of published exceptions, *Nitrosospira* cluster 2 is often considered to dominate in acidic soils [4, 18, 37]. However, while *Nitrosopira* cluster 2 was relatively abundant in three forest soil libraries (BF1, BZ2, BZ3) with reasonably low pHs (4.05–5.36), there was no overall correlation between the relative abundances of cluster 2 in the libraries and soil pH ($\rho=0.15$, $P=0.3$) suggesting that cluster 2 AOB do not necessarily have higher relative abundances in acidic soils. Likewise, results from previous studies [14–16, 20] have contributed to the hypothesis that representatives of *Nitrosospira* cluster 3 are likely to be relatively more abundant in soils with higher levels of N availability. If we examine the proportional abundances of *Nitrosospira* cluster 3, we see no correlations with our measured indices of N availability (N mineralization rates and extractable NH_4^+ or the sum of NH_4^+ plus NO_3^-concentrations, $P>0.4$ in all cases). Either we did not quantify a specific N pool or flux these organisms are sensitive to, or it is not valid to assume that N availability drives the relative abundance of *Nitrosospira* cluster 3 in soil AOB communities across such a broad range of different ecosystem types. Together, it is clear that hypotheses based on previous surveys of AOB communities in one to a few individual soil types did not hold when compared to our results from 23 soils. This demonstrates the limitations associated with trying to make broad

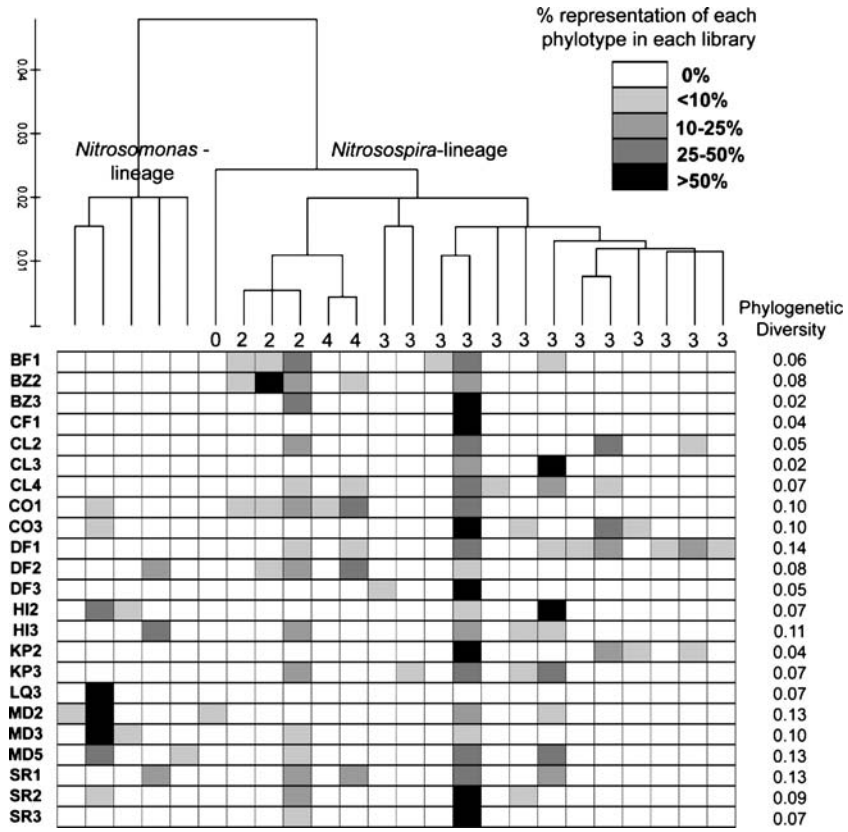

Figure 2 Relative abundances of the 24 different phylotypes in each of the 23 soil samples with the relationships between phylotypes shown via a neighbor-joining tree. The phylogenetic tree was generated using MEGA [33] and was linearized assuming equal evolutionary rates in all lineages. The evolutionary distances are in the units of the number of base substitutions per site. The numbers at the tips of the *Nitrosospira* lineages denote the 16S rRNA-based *Nitrosospira* clusters as defined by Purkhold et al. [5]. All of the *Nitrosomonas* sequences were close matches to the *Nitrosomonas oligotropha* lineage. The *rightmost column* denotes the relative levels of phylogenetic diversity within each of the 23 soil samples with diversity estimated using Faith's index of phylogenetic diversity [29]. Note that this index of phylogenetic diversity does not necessarily equate directly with the number of unique phylotypes in a given sample

generalizations about AOB taxa from individual studies examining only a limited diversity of soil types. At the same time, it is important to recognize that methodological differences (particularly differences in primer choice) make it difficult to quantitatively compare results between studies. Although there are undoubtedly some biases associated with our choice of primer sets and our specific methods, such biases should be reasonably consistent across all soils, and any examination of the changes in AOB community composition should still be robust.

We found few representative sequences from AOB groups that are commonly found in aquatic environments, including *Nitrosospira* cluster 1 and other *Nitrosomonas* lineages besides the *N. oligotropha* lineage that dominated the soils included in this study (Fig. 2). This simply confirms previous observations that soils are a unique habitat for AOB and they harbor distinct AOB communities [4, 38]. When we examined the specific types of AOB identified (Fig. 2), we found that most of the AOB sequences were close matches to those AOB frequently found in soils. In terms of sequence representation in the libraries, the most abundant group of ammonia oxidizers found in the soil samples was *Nitrosospira* cluster 3. Other studies have also found that this group is common in the soil environment [18, 38, 43]. One specific phylotype, most similar to *Nitrosospira* sp. str. Nsp17 (AY123804) within the *Nitrosospira* cluster 3 lineage, was particularly abundant in nearly all of the samples (Fig. 2). Given that we used a primer set targeting the 16S rRNA gene, we could not identify those clusters of Nitrosospiras only defined through amoA gene phylogenies (e.g., clusters 9–12) [18, 36]. This highlights one of the key limitations associated with comparing results from AOB surveys based on the analysis of the 16S rRNA gene versus the amoA gene.

Factors Correlated with the Observed Patterns in AOB Biogeography

The composition of AOB communities was highly variable across the 23 soil samples included in this study (Figs. 2

and 3). The clustering patterns between communities were very similar regardless of whether we used the UniFrac algorithm (Fig. 3) or the phylotype-based approach (data not shown) to calculate pairwise distances as the two distance estimates were strongly correlated with one another, Spearman's ρ=0.91, P<0.001). The factors driving the observed spatial variability in AOB communities were not immediately apparent (Figs. 2 and 3). Although it is difficult to make robust generalizations about the differences in AOB communities across ecosystem types with only 23 soils, we found that similar ecosystem types did not necessarily harbor phylogenetically similar AOB communities (Fig. 3). This is qualitatively apparent given that soils from temperate coniferous forests did not necessarily harbor similar AOB communities (e.g., CF1, DF1, CL3 in Fig. 3) nor did those soils collected from temperate grasslands (e.g., KP2, SR3, CL4 in Fig. 3). Likewise, soils that were collected in close proximity to one another, but under different vegetation types, often had phylogenetically distinct AOB communities (e.g., the "DF," "KP," and "CL" samples; Fig. 3).

More importantly, both community distance estimates indicated that mean annual temperature was significantly correlated with the degree of community similarity across the 23 samples (Table 2). When we ran the stepwise regression procedure using the variables listed in Table 2, we found that a univariate model with mean annual temperature provided the best fit to the data and the addition of other variables in multivariate models did not significantly improve the correlation coefficients. For completeness, we ran other univariate models to explore the dataset and found that mean monthly temperature, soil C/N ratio, and soil texture (percentage silt + clay) were also correlated with community similarity, but the correlations were weaker than for mean annual temperature (Table 2). The relationship between site mean annual temperature and the composition of AOB communities in each of the soils is evident in Fig. 3, where we see evidence for some degree of community clustering when the AOB communities found in each soil are divided into broadly defined mean annual temperature categories. Even though the correlation was highly significant (P<0.001), it is important to recognize that mean annual temperature only explained some of the variability in AOB communities (Spearman's ρ=0.40; Table 2). However the strength of this correlation is still noteworthy given that a single factor could, to some degree, predict differences in AOB community composition across a range of soils collected from very different ecosystem

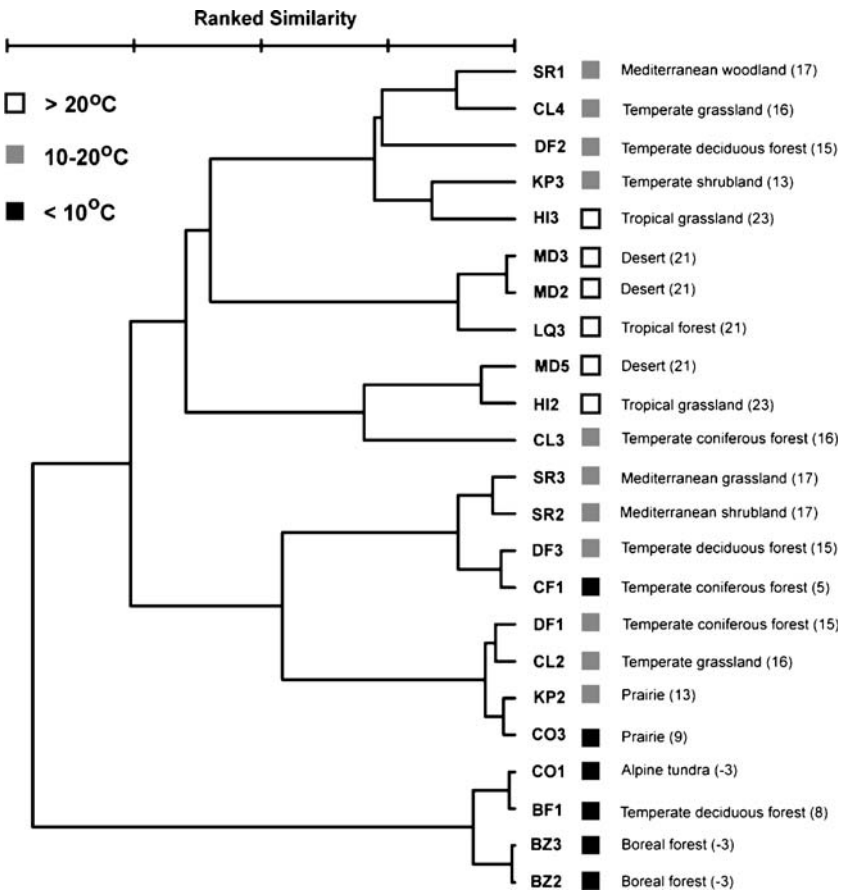

Figure 3 UPGMA dendrogram showing the ranked similarities (a unitless index) between AOB communities in each of the soil samples. Phylogenetic similarity was estimated using UniFrac. Soil codes are identical to those in Table 1. The *square symbols* identify the mean annual temperature (MAT) category, with MAT values (in °C) provided *in parentheses*. A general description of the ecosystem type represented by each soil is also provided with additional details on soil and site characteristics available in Table 1

types. Future studies which utilize different primer sets to survey the AOB, measure more soil and site characteristics, or assess interactions between bacterial and archaeal ammonia oxidizers may lead to models that are more effective at predicting the biogeographical patterns exhibited by soil AOB.

Although pH, soil moisture levels, and nitrogen availability can have a strong influence on AOB activities and community composition [4, 10, 13], none of these factors were important determinants of differences in AOB community structure across the range of soils examined at these large inter-site spatial scales (Table 2). Such factors may be important in individual soils, across particular gradients, or across specific experimental treatments, but they do not appear to be the environmental factors most important in regulating AOB community composition across the larger spatial scales examined here. The qualitative observation that sites in close proximity do not necessarily harbor similar AOB communities (Fig. 3) is confirmed by the weak correlations between geographic and phylogenetic distances between pairs of samples measured using the UniFrac algorithm (Spearman's ρ=0.21, P=0.04) and the phylotype-based approach (Spearman's ρ=0.18, P=0.06). It is possible that there may be some dispersal limitations that influence AOB community composition at these spatial scales, but this study was not designed to explicitly test for such effects. Increased sampling across a range of spatial scales and higher resolution phylogenetic analyses are necessary in order to document the potential impacts of dispersal limitation on AOB community structure [44]. Instead, we can conclude that differences in soil environmental conditions, namely differences in mean annual temperature seem to have important predictable effects on the composition of AOB communities in the soils included in this study. This result is consistent with other studies in soil and aquatic environments where a strong temperature influence on AOB community composition has also been observed [13, 18, 45, 46].

The meta-analysis by Avrahami and Conrad [18] suggests that *Nitrosospira* cluster 2 may be more common in cold-temperate soils than in warmer soils. This hypothesis is consistent with our results; those soils from sites with lower mean annual temperature (see Fig. 2) had a higher proportion of sequences that closely matched *Nitrosospira* cluster 2 (e.g., sites CO1, BF1, BZ2 and BZ3 in Fig. 2), and there was a significant negative correlation between the relative abundance of *Nitrosospira* cluster 2 and MAT (Spearman's ρ=0.6, P<0.001). However, we must also note that *Nitrosospira* cluster 2 does not appear to be restricted to colder soils, having also been found in many of the soils from warmer sites (Fig. 2). Together, these results suggest that there is an important influence of temperature in shaping AOB community composition, and this pattern is evident whether we look across a range of ecosystem types (this study, [18]) or if individual soils are incubated under different temperature regimes in the laboratory [13, 36, 46]. The question we must then ask is: why does temperature appear to be the best predictor of AOB community composition? The results from this study do not provide the answer, but we can hypothesize that distinct phylogenetic groups may have distinct temperature optima. This has been demonstrated with *Nitrosospira* strains grown in pure culture [47] and with AOB communities from different soils [48]. If these observations are generalizable and there is minimal functional redundancy between taxa (i.e., phylogenetically distinct communities have distinct temperature sensitivities), it may explain why the temperature responses in nitrification activity can vary so widely across different soils [49, 50] or sediment samples [51]. More broadly, if distinct AOB communities do indeed have different temperature responses, it would suggest that information on the spatial variability in AOB communities could be integrated into models of soil nitrogen dynamics in order to better predict how soil nitrogen dynamics may be affected by changes in climatic conditions.

Conclusion

Given their key role in the soil nitrogen cycle, the soil AOB represent a tractable system in which to integrate microbial ecology into models of soil processes. Soil AOB communities are not phylogenetically homogeneous across larger spatial scales and the observed biogeographical patterns are, to some degree, predictable across ecosystems. We found that community composition appears to be most strongly influenced by temperature, a result consistent with other studies of AOB. The next step is to ascertain if the distinct communities are also functionally distinct in order to determine if microbial biogeography may, in the case of soil AOB, be directly relevant to understanding soil biogeochemistry. However, it is important to note that we did not examine the biogeographic patterns exhibited by ammonia-oxidizing archaea (AOA) in this study. Given that such archaea are relatively abundant in soil and may play a key role in ammonia oxidation [6, 7], it is clearly important to study both AOA and AOB in order to gain an integrated understanding of how the composition of these communities may influence ammonia oxidation rates in soil.

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