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Bacterial and archaeal *amoA* gene distribution covaries with soil nitrification properties across a range of land uses

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Summary

Ammonia-oxidizing bacteria and ammonia-oxidizing archaea are commonly found together in soils, yet the factors influencing their relative distribution and activity remain unclear. We examined archaeal and bacterial *amoA* gene distribution, and used a novel bioassay to assess archaeal and bacterial contributions to nitrification potentials in soils spanning a range of land uses (forest, pasture, cultivated and long-term fallowed cropland) along a 10 km transect. The assay, which quantifies the extent to which acetylene-inactivated soil nitrification potential recovers (RNP) in the presence of bacterial protein synthesis inhibitors, indicated a significant archaeal contribution to the nitrification potentials of the pasture and long-term fallowed soils. Archaeal *amoA* gene abundance did not vary significantly among the soils, but bacterial *amoA* gene abundance did, resulting in archaeal : bacterial *amoA* abundance ratios ranging from 1.1 ± 0.8 in cultivated soils to 396 ± 176 in pasture soils. Both archaeal and bacterial *amoA* gene compositions were heterogeneous across the landscape, but differed in their patterns of variability. Archaeal *amoA* gene distributions were distinct among each of the three main land-use types: forest, pasture and cropland soils. In contrast, bacterial *amoA* gene composition was distinct in forest and in cultivated cropland, while pasture and long-term fallowed cropland soils were similar. In both pasture and long-term fallowed cropland soils, one phylotype of *Nitrosospira* cluster 3a was highly abundant. This distinct bacterial *amoA* gene fingerprint correlated with significant contributions of archaea to RNP of both soils, despite differences in archaeal *amoA* gene composition between the pasture and fallowed soils. This observation suggests that the factors driving the development of ammonia-oxidizing bacteria community composition might influence the extent of archaeal contribution to soil nitrification.

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Introduction

Nitrification, the microbially mediated transformation of ammonium (NH_4^+) to nitrate (NO_3^-), is one of the most important components of the nitrogen (N) cycle. The rate-limiting step in this process is the oxidation of ammonia to nitrite (NO_2^-). Ammonia oxidation by bacteria has been studied for many years, and takes place throughout terrestrial and aquatic environments. Recently, thaumarchaeal isolates from marine (*Candidatus* Nitrosopumilus maritimus) and soil (*Candidatus* Nitrosopumilus viennensis) have been shown to grow lithotrophically via ammonia oxidation in culture (Konneke *et al*., 2005; Tourna *et al*., 2011). Furthermore, several culture-independent soil studies have detected expression of the archaeal ammonia monooxygenase gene (*amoA*), suggesting that soil-borne archaea are capable of ammonia oxidation *in situ* (Treusch *et al*., 2005; Leininger *et al*., 2006; Chen *et al*., 2008; Nicol *et al*., 2008; Mertens *et al*., 2009). Whereas *amoA* genes from ammonia-oxidizing thaumarchaea (AOA) tend to be numerically dominant over ammonia-oxidizing bacteria (AOB) in marine environments (Santoro *et al*., 2010), soil environments are more variable with the ratio between archaeal and bacterial *amoA* genes ranging between 1 and 1000 (Francis *et al*., 2005; Beman and Francis, 2006; Leininger *et al*., 2006; Coolen *et al*., 2007; He *et al*., 2007; Adair and Schwartz, 2008; Boyle-Yarwood *et al*., 2008). Several studies have shown that population size and community structure of archaeal, as well as bacterial, *amoA* genes are related to environmental conditions such as pH, salinity or N fertilization history (He *et al*., 2007; Hansel *et al*., 2008; Mosier and Francis, 2008; Nicol *et al*., 2008; Shen *et al*., 2008; Hallin *et al*., 2009; Schauss *et al*., 2009); however, to our knowledge there have been no direct comparisons of both AOA and AOB *amoA* gene community composition, population

sizes and nitrification activities in different soil types with varied land-use histories.

The factors affecting AOA and AOB distribution and their activities in the environment may be different (Erguder *et al*., 2009). Generally, *Nitrosospira spp.* are the most common type of AOB found in soils. Vegetation type (Mintie *et al*., 2003; Hawkes *et al*., 2005) or N fertilization level (Avrahami *et al*., 2003; Horz *et al*., 2004) can affect the relative abundance of specific *Nitrosospira* taxa, and distinct *Nitrosospira* clades have been associated with soils expressing different nitrification properties (Webster *et al*., 2005). Archaeal *amoA* genes exhibit phylogenetic structure definable by predominant habitat type ('soil' or 'marine') (Prosser and Nicol, 2008), and soil AOA abundance and activity has been shown to depend on pH and ammonium availability (Nicol *et al*., 2008; Di *et al*., 2010). In addition to pH, AOA community structure may also vary with fertilization practice (He *et al*., 2007; Tourna *et al*., 2008) or vegetation type (Boyle-Yarwood *et al*., 2008; Chen *et al*., 2008). Although it is now clear that some members of the Group 1.1a Thaumarchaea can oxidize ammonia, fix CO_2, and grow in soil (Offre *et al*., 2009; Zhang *et al*., 2010; Verhamme *et al*., 2011), soil-borne AOB usually proliferate more extensively than AOA in response to large additions of exogenous ammonia- or urea-N fertilizer (Di *et al*., 2009; Jia and Conrad, 2009; Mertens *et al*., 2009; Pratscher *et al*., 2011; Verhamme *et al*., 2011). Based on a novel assay that measures the extent of recovery of nitrification potential (RNP) over a 24–48 h incubation in the presence of bacterial protein synthesis inhibitors after irreversibly inactivating ammonia monoxygenase with acetylene, AOB and AOA were implicated as the dominant contributors to the nitrification potential of cropped and pasture soils respectively (Taylor *et al*., 2010). However, it remains unknown whether population sizes, community composition or edaphic properties control the relative contribution of AOA or AOB to nitrification activity. In order to understand the controls over contributions of AOA and AOB to *in situ* soil nitrification rates, it is necessary to identify factors that link diversity, abundance and activity of the AOA and AOB that coexist in field soils.

In this study, we investigated archaeal and bacterial *amoA* gene abundance (using Q-PCR), community composition (using T-RFLP and clone libraries), and nitrification activity in soils representing a range of land-use types (coniferous and actinorhizal forest, improved and unimproved pasture, actively cultivated and long-term fallowed cropland) from the Willamette Valley, Oregon, USA. Our goal was to identify patterns of coexistence of archaeal and bacterial *amoA* genes in these soils, and to determine if any relationship existed between these patterns and the relative contributions of archaea and bacteria to the RNP. A better understanding of the field distribution of *amoA* genes and nitrification activity in relation to varied soil management strategies will help to predict the relative importance of bacterial and archaeal nitrification across the landscape.

Results and discussion

Soil properties, nitrification potential and amoA *gene abundance*

Soils spanned a narrow pH range (6.0–6.7), a fourfold range in total C, a twofold range in total N and a sixfold range in net N mineralization rates (Table 1). Nitrification potential rates (NP) were high and variable, and were highest in the annually urea-N-fertilized cropped soils and lowest in the pasture soils, although this difference was

Table 1. Soil characteristics, N cycling parameters, and *amoA* gene abundance and richness.

		Total C	Total N					Arch-*amoA*†	Bact-*amoA*†			
	pH	(%)		C : N	Nmin μg N g^{-1}	NP soil d^{-1}	RNP-kan† (ratio)	Copies × 10^6 g^{-1} soil		Arch : Bact-*amoA*†	Arch-*amoA* T-RFs	Bact-*amoA* T-RFs
F-F	6.5	5.3^{a}	0.27^{abc}	23.0^{a}	2.2^{b}	5.8	na	9.32	0.51^{ab}	13.7^{b}	5^{ab}	11^{ab}
	(0.1)	(0.2)	(0.01)	(0.9)	(0.1)	(0.4)		(6.5)	(0.2)	(7.5)	(0.9)	(0.6)
F-A	6.7	5.2^{a}	0.28^{ab}	21.6^{a}	4.4^{a}	7.0	0.0^{c}	6.30	1.85^{a}	10.6^{b}	3^{b}	12^{a}
	(0.1)	(0.9)	(0.05)	(0.2)	(0.7)	(5.0)		(1.4)	(0.9)	(8.6)	(0.3)	(0.1)
P-L	6.0	3.6^{abc}	0.26^{abc}	16.2^{b}	2.0^{b}	1.8	na	11.1	0.08^{b}	74.2^{ab}	8^{a}	6^{c}
	(0.05)	(0.4)	(0.02)	(0.6)	(0.2)	(0.6)		(10.7)	(0.07)	(39)	(0.3)	(1.7)
P-H	6.0	4.5^{ab}	0.33^{a}	15.8^{bc}	1.5^{b}	2.9	0.9^{a}	41.73	0.35^{ab}	396^{a}	7^{a}	9^{abc}
	(0.3)	(0.8)	(0.05)	(0.2)	(0.4)	(2.5)	(0.2)	(21.2)	(0.3)	(176)	(0.3)	(1.5)
C-F	6.4	2.1^{bc}	0.17^{bc}	13.9^{bc}	1.1^{b}	4.6	0.5^{b}	3.88	1.02^{ab}	3.3^{b}	6^{ab}	7^{bc}
	(0.1)	(0.05)	(0.005)	(0.1)	(0.3)	(0.6)	(0.05)	(2.7)	(0.5)	(1.3)	(0.1)	(0.5)
C-C	6.3	1.4^{c}	0.12^{c}	13.4^{c}	0.68^{b}	23.9	0.1^{c}	0.92	0.82^{ab}	1.1^{b}	5^{ab}	11^{ab}
	(0.2)	(0.04)	(0.003)	(0.4)	(0.2)	(15.6)	(0.03)	(0.7)	(0.2)	(0.8)	(1.0)	(0.1)

†. Modified from Taylor *et al.* (2010); Data are mean (SE), and superscripts denote differences based on one-way ANOVA ($P < 0.05$, Tukey *post hoc*).

F-F, Douglas-fir forest; F-A, alder forest; P-L, lightly grazed pasture; P-H, heavily grazed pasture; C-F, fallowed cultivated; C-C, cropped cultivated; Nmin, 30 day mineralizeable N; Arch, archaeal; Bact, bacterial; na, not available.

not statistically significant. Recovery of NP (RNP) in the presence of bacterial protein synthesis inhibitors after acetylene inactivation varied from 0% to 104%, and was not correlated to the initial NP. Bacterial *amoA* gene abundance ranged over 20-fold, archaeal *amoA* gene abundance ranged almost 50-fold and the archaeal : bacterial *amoA* ratio spanned two orders of magnitude. Differences in putative ammonia-oxidizer population sizes between land-use types were driven by bacterial *amoA* gene abundance ($P = 0.023$) and archaeal : bacterial *amoA* ratio ($P = 0.016$). Pasture soils had the lowest bacterial *amoA* gene abundance and highest archaeal : bacterial *amoA* ratio, while forest, cultivated and long-term fallowed soils had the lowest archaeal : bacterial *amoA* ratio (Tukey *post hoc* comparisons, $P < 0.05$).

Despite considerable differences among land-use types in nitrification rates (NP, RNP) and bacterial and archaeal *amoA* gene abundances, there were no statistically significant relationships between NP, RNP and either bacterial, archaeal or bacterial + archaeal *amoA* gene abundance or the archaeal : bacterial *amoA* ratio. Perhaps this result is to be expected given the limited knowledge of links between environmental *amoA* gene distribution and ammonia oxidizer activity. For example, it is well known that *in situ* gross rates of nitrification in whole soils range between 10% and 60% of the NP, implying that a variable fraction of soil ammonia oxidizers are NH_4^+ limited, starved or inactive in whole soil situations (Norton, 2008). There are a few examples of pure cultures of AOB that, after NH_4^+ starvation for between 1 and 5 weeks, can produce NO_2^- immediately upon addition of NH_4^+ (Johnstone and Jones, 1988; Bollmann *et al.*, 2005; Berube *et al.*, 2007). However, within heterogeneous soil populations of AOB and AOA, the factors controlling recovery of ammonia oxidation after starvation remain unknown.

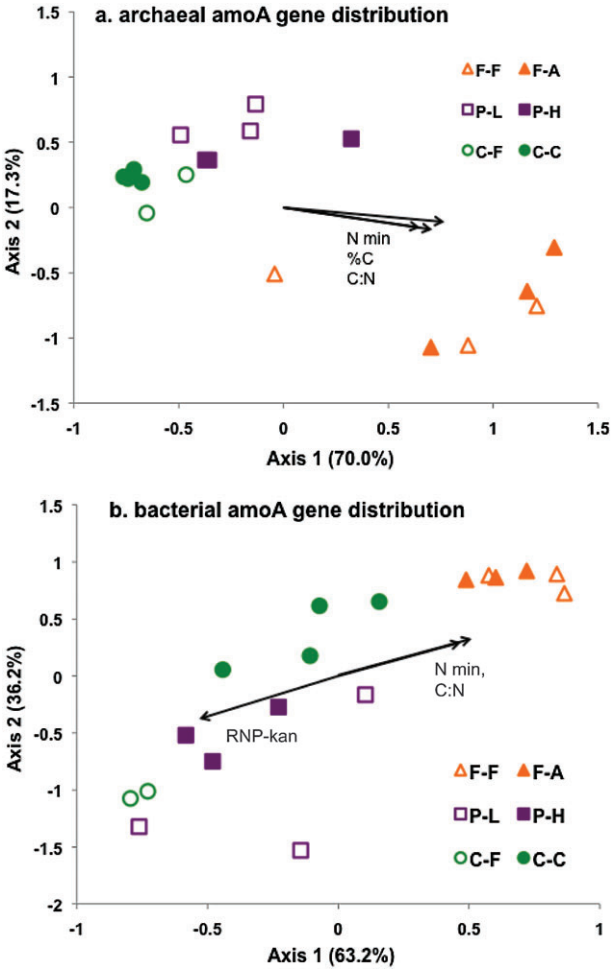

Fig. 1. Nonmetric multidimensional scaling ordinations of *amoA* gene T-RF relative abundance for (A) archaeal and (B) bacterial *amoA* gene communities. Archaeal model parameters are stress = 3.182, instability = 0.011; bacterial model parameters are stress = 2.409, instability < 0.001. Site abbreviations are F-F, Douglas-fir forest; F-A, alder forest; P-L, lightly grazed pasture; P-H, heavily grazed pasture; C-F, fallowed cultivated; C-C, cropped cultivated. Significant correlations between community composition and environmental metrics (see Table 1 for abbreviation definitions) are shown by the vector overlay ($P < 0.005$ with either axis).

Archaeal and bacterial amoA *gene T-RF clustering among soils*

An examination of the relative abundances of soil bacterial and archaeal *amoA* gene terminal restriction fragment (T-RF) revealed diverse, heterogeneous archaeal and bacterial *amoA* gene distributions within and among land-use types (Figs 1 and 2). Although there was some overlap in individual T-RF occurrence, distinct archaeal *amoA* gene compositions were found in forest, pasture and cropland (including the long-term fallowed) soils (Figs 1A and 2A; MRPP *post hoc*: $P < 0.002$, $T < -4.5$, $A > 0.21$). In contrast, bacterial *amoA* gene composition was distinct in forest soils (Figs 1B and 2B; MRPP *post hoc*: $P < 0.001$, $T < -6.0$, $A > 0.30$) and in cropped soils (MRPP: $P = 0.010$, $T = -3.4$, $A = 0.43$), but was similar in both pasture and long-term fallowed soils ($P = 0.131$, MRPP: $T = -1.0$, $A = 0.06$). These results imply that whereas bacterial *amoA* gene composition has shifted from that of a cultivated soil to that of a pasture soil since the cessation of active cultivation (no N fertilization or tillage), the archaeal *amoA* gene composition has not changed significantly.

To identify soil properties and N cycling variables that were related to *amoA* gene composition, vectors representing the strongest correlations between the NMDS distance matrix and other study metrics were plotted (Fig. 1). C : N and N mineralization rate were correlated with both archaeal and bacterial *amoA* gene community structure, suggesting a commonality between those factors: in fact, the forest soils drove this relationship, being highest in C : N and N mineralization, and having

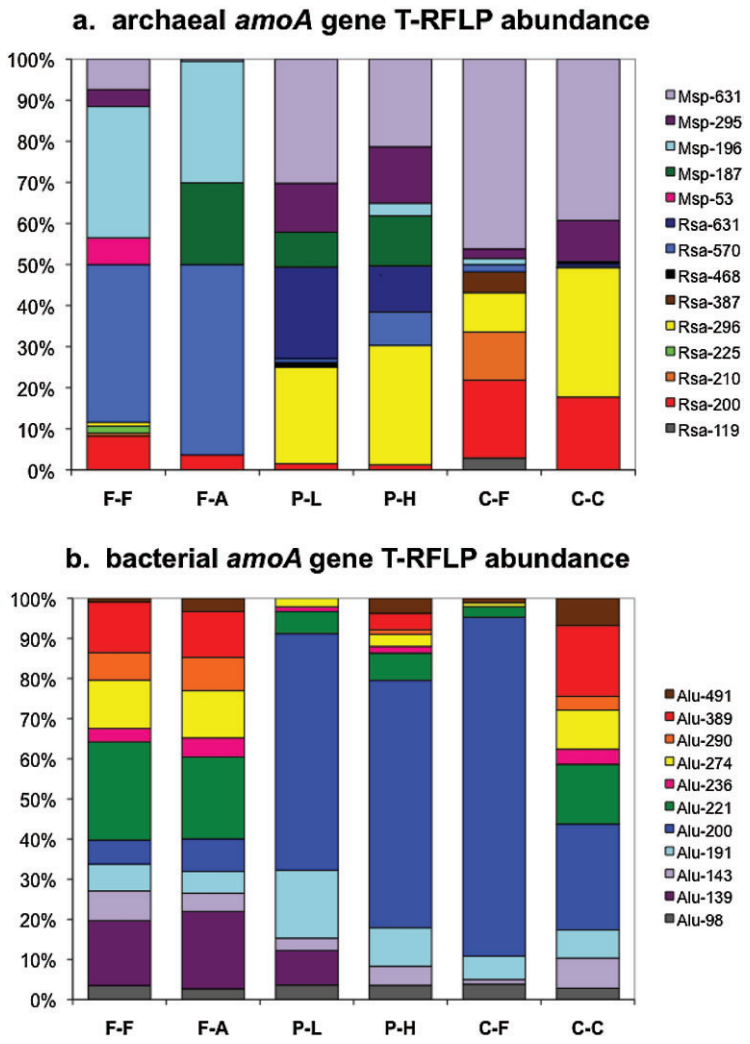

Fig. 2. Bar graphs of individual T-RF abundances at each sampling site (mean of field replicates, $n = 3$ for all except C-F: $n = 2$ and C-C: $n = 4$) for (A) archaeal and (B) bacterial *amoA* gene communities. Site abbreviations are F-F, Douglas-fir forest; F-A, alder forest; P-L, lightly grazed pasture; P-H, heavily grazed pasture; C-F, fallowed cultivated; C-C, cropped cultivated. T-RF abundance is scaled to relative abundance, with 100% set as the sum of all T-RF peak areas for the bacterial *amoA* gene and 50% set as the sum of all T-RF peak areas for each restriction enzyme on the archaeal *amoA* gene.

both unique bacterial and archaeal *amoA* gene compositions. On the other hand, only the bacterial *amoA* gene composition was correlated to RNP + kanamycin (the fraction of RNP that is assumed to be attributable to AOA).

Archaeal amoA *gene T-RF and sequence composition*

Specific archaeal taxa were found to make up large percentages of the T-RF total fluorescence in forest, pasture and cropland soils (Fig. 2A). For example, on average, Rsa570 comprised 85% of the forest soil T-RF total fluorescence, Msp295 and Rsa631 constituted 25% and 33%, respectively, of the pasture soil T-RF fluorescence, and Rsa296 and Rsa200 contributed 47% and 38%, respectively, of the cropland soil T-RF fluorescence. Although vegetation has been suggested to influence soil archaeal community composition via rhizosphere effects (Chen *et al.*, 2008; Bomberg and Timonen, 2009), the archaeal *amoA* gene composition in forest soils was similar in both Douglas-fir and red alder stands, which comprise different rhizosphere habitats. Additionally, archaeal *amoA* gene composition from this study showed no taxonomic overlap with the composition of archaeal *amoA* genes from Douglas-fir and red alder stands located on an acidic (pH 4) andisol about 100 km distant (Boyle-Yarwood *et al.*, 2008). All soils in this study had similar pH; however, they differed a great deal in age, texture, mineralogy and parent material (Parsons *et al.*, 1970; Taylor *et al.*, 2010). Although it is difficult to constrain and identify any one soil physical property as a driver of microbial community composition, soil texture determines pore space distribution and water dynamics, which can affect dispersal patterns and substrate availability for both microbial cells and microbial predators. Given the patterns we observed, it seems that vegetation type *per se* may be less important than edaphic factors as a driver of differences in archaeal *amoA* distribution among soils from differing land-use type.

It is notable that although several archaeal *amoA* gene T-RFs and sequences were detected in the study

soils, none corresponded with the T-RFLP profile or phylogenetic placement of '*Candidatus* N. maritimus' (Msp631/Rsa119), '*Candidatus* Nitrososphaera gargensis' (Msp631/Rsa196) or '*Candidatus* N. viennensis' (Msp196/Rsa200) (Figs 2A and S1). However, an interesting pattern emerges when comparing the results of this study with others of archaeal *amoA* gene phylogenetic composition. Unlike any sequences collected in this study, the dominant archaeal *amoA* sequences identified by Boyle-Yarwood and colleagues (2008) in an acidic forested andisol fell into the same phylogenetic clades (iv and v) that were described in a Scottish agricultural loamy sand soil [Fig. S1; (Nicol *et al*., 2008)]. In the latter study, archaeal *amoA* genes from clades iv and v were not only more abundant, but were also expressed to greater levels in acid soil (pH 4.3) than in the same soil that had been limed to maintain pH 6.9 (Nicol *et al*., 2008). In addition, the dominant archaeal *amoA* gene phylotype that dominated the pH 6 forest soils of our study, T-RF Rsa570, fell into the same phylogenetic clade (iii) as sequences from the same Scottish agricultural soil (Fig. S1) that were more strongly expressed at pH 6.9 than 4.3 (Nicol *et al*., 2008). The complimentary results of these three studies clearly suggest that pH is a soil factor affecting the abundance of certain phylotypes of archaeal *amoA* genes, regardless of soil type, local climate, vegetation or land use.

Bacterial amoA *gene T-RF and sequence composition*

The long-term fallowed and pasture soils had a bacterial *amoA* gene composition dominated by one phylotype (T-RF Alu200, 56% and 78%, respectively, of total fluorescence), unlike the forested and cropped soils, which contained a more even distribution of taxa (Fig. 2B). In addition, ordination analysis revealed that the long-term fallowed and pasture soils displayed a greater archaeal contribution to the RNP (RNP + kanamycin; Fig. 1B) than the cropped soils. Therefore, we should consider the possibility of a link between the factors that result in the dominance of T-RF Alu200 in the bacterial *amoA* gene community and the detection of a significant archaeal contribution to the RNP.

Although we cannot determine how many years without cultivation activity passed before the apparent shift of the bacterial *amoA* gene composition took place, the long-term fallowed soils had not been tilled, cropped, or N-fertilized for 18 years at the time of sampling. In calcareous grassland soils in the Netherlands, Kowalchuk and colleagues (2000) showed that the AOB community composition shifted noticeably following cessation of N fertilization over time periods ranging between 10 and 24 years. These shifts corresponded with the time needed to see substantial declines in net N mineralization rates, suggesting that the ammonia oxidizers in non-fertilized soils were better adapted to living with lower NH_4^+ availability. The Alu200 phylotype is related to sequences from *Nitrosopira* Cluster 3a (Table S1, Fig. S1). Previous work from the UK showed that unimproved, unfertilized pasture soils tend to contain *Nitrosospira* Cluster 3a, and these soils showed a trend for a significant delay in nitrification response to added N (Webster *et al*., 2005). Culture-based studies confirmed that growth of representative isolates of *Nitrosospira* Cluster 3a is inhibited by high NH_4^+ concentrations (Tourna *et al*., 2010). Perhaps the dominance of the Alu200 phylotype in pasture and long-term fallowed soils, and the delay of *Nitrosospira* Group 3a isolates to respond to added NH_4^+ is somehow linked to the high archaeal contribution to the RNP of these soils. Clearly, further studies are needed to identify the specific factors controlling the relative contributions of archaea and bacteria to nitrification in the long-term fallowed and pasture soils.

The contrasting patterns of soil archaeal and bacterial activity and *amoA* abundance and distribution suggest that environmental factors that vary at a landscape scale may affect AOA differently than AOB. The concept of niche differentiation among AOB phylotypes has been established for several years, following observations that soil AOB phylotypic composition differs in response to N availability (Bollmann *et al*., 2002; Avrahami *et al*., 2003; Tourna *et al*., 2008), plant community composition (Hawkes *et al*., 2005) temperature (Avrahami *et al*., 2003), soil moisture (Avrahami and Bohannan, 2007), oxygen tension (Kowalchuk *et al*., 1998), ammonia uptake kinetics and/or inhibition (Webster *et al*., 2005; Tourna *et al*., 2010). The range of factors that may influence AOB composition reflect the many possible niches that may exist for individual AOB phylotypes. In addition, recent studies suggest that soil AOA communities have different response patterns than co-located AOB to pH and temperature changes (Nicol *et al*., 2008; Tourna *et al*., 2008). In this study, there were no significant differences in archaeal *amoA* abundance or composition between 'high' and 'low' N situations within any land-use type, suggesting that N supply was less important to AOA distribution than AOB. AOA abundance and diversity have shown no or inconsistent responses to N fertilization in other studies (He *et al*., 2007; Shen *et al*., 2008; Di *et al*., 2009; Hallin *et al*., 2009; Jia and Conrad, 2009; Schauss *et al*., 2009; Wang *et al*., 2009), supporting the hypothesis that landscape-scale factors might mediate and/or dominate the influence of N amendment on soil AOA composition. In contrast, bacterial *amoA* gene composition was quite different in long-term fallowed soils than in adjacent cultivated and N-fertilized soils, thus N fertilization remains a potential driver of AOB distribution. Most impor-

tantly, our study suggests that differences in distribution patterns of archaeal and bacterial *amoA* genes may have ramifications for each group's respective role in soil nitrification across the landscape. Future research should further dissect the role of soil properties and patterns of land use on the distribution and activity of terrestrial nitrifying microbes.

Experimental procedures

Sites and sampling approach

We established a 10 km transect that spans the major landforms in the Willamette Valley, Oregon, USA, and represents the dominant land uses (i.e. vegetation and land management practices) and soil types. Three locations were selected near Corvallis, Oregon, USA (44.7°N 123.3°W), on lands owned and managed by Oregon State University: McDonald Forest (forest = F), Soap Creek Ranch (pasture = P) and Hyslop Farm (cropland = C). At each location, we selected two sites with histories of higher or lower N inputs. Soil chemical and N cycling properties for all soils are reported in Table 1.

The forest is dominated by Douglas-fir (*Pseudotsuga menziesii*) interspersed with stands of actinorhizal red alder (*Alnus rubra*) along water courses; sample points were located at ~ 350 m elevation, in soils of primarily a Jory-Gelderman complex (basalt-derived Haplohumults). The pasture is grazed land in a mid-elevation stream valley (elevation ~ 125 m), with soils of a Dixonville-Gellatly-Witham complex (basalt-derived Argixerolls and Haploxerolls). The cultivated location in the Willamette River floodplain (elevation ~ 85 m) has been the site of cropping trials since 1929, and includes actively cropped cereal (wheat, barley) fields and adjacent long-term fallowed fields that have been uncultivated and unfertilized since 1990 and are colonized by fescue grass (planted 1999), volunteer grasses and forbs. The soil is classified as a Woodburn silt loam (glaciolacustrine-derived Argixerolls). All sites have a Mediterranean climate (cool wet winters, warm dry summers), receive an annual mean precipitation of 90–130 cm and have an annual mean temperature of 10–12°C.

Soil N properties also differ among the three land-use types, ranging from high to low amounts of net mineralizable N along the continuum of forest, pasture and cultivated soils (Table 1). In order to sample soils representing a range of N inputs within each land-use type, we established paired sampling plots at each location. From the forest, we sampled soil in three Douglas-fir stands (F-F) and three adjacent stands of N_2-fixing red alder (F-A). At the pasture, we sampled soil from three lightly grazed, upslope pastures (P-L) and three heavily grazed and manured bottomland pastures (P-H). At the cultivated location, we collected soil from four cropped and fertilized fields (single application of 90–180 kg urea-N ha^{-1} $year^{-1}$), two planted in wheat and two planted in barley (cropped sites were combined for statistical analysis, C-C), and two long-term fallow fields that had not been cultivated or fertilized for 18 years (C-F). Thus, for all parameters measured, field replicates were collected and analysed separately [$n = 3$ except C-C ($n = 4$) and C-F ($n = 2$)].

Soils analysed for DNA, total C and N and pH were collected in late July 2008. Soils analysed for NP, RNP and N mineralization (N min) assays were collected in late July 2009. We collected soils from the same plots at the same time of year in order to gather supplemental data on soil properties under similar field conditions. Although summer climate is generally stable at the study sites, temporal heterogeneity may affect variability in the data. At each of the 18 field plots, 4–6 soil cores (2.5 cm diameter by 10 cm depth) were gathered from points across each plot and homogenized for further analysis. Soils were carried back to the lab on ice and immediately sieved (2 mm) and roots removed. Soils were stored at either 4°C (soil properties, NP, RNP) or –20°C (DNA) until analysis.

Soil chemistry and nitrogen cycling parameters

Soil pH was measured using standard methods (Klute, 1994; Sparks, 1994). Total organic C and N were measured on a Leco CNS-2000 Macro Analyzer (St. Joseph, MI, USA). N mineralization (N min) was calculated as the amount of inorganic N ($NH_4^+ + NO_2^- + NO_3^-$ extractable in 0.5 M KCl) accumulated during a 30 day incubation at 30°C and 60% water holding capacity. NP was measured using a modified shaken soil slurry method (Hart *et al*., 1994) as the amount of $NO_2^- + NO_3^-$ formed over a 24 h incubation period (Taylor *et al*., 2010). To measure RNP, assay slurries were held in an acetylene atmosphere for 6 h to completely inactivate ammonia monoxygenase, degassed and aerated, then incubated similarly to the NP assays for 48 h. Inferred archaeal (non-bacterial) contribution to RNP was assessed by adding kanamycin at the beginning of the recovery phase (Taylor *et al*., 2010).

Nucleic acid analysis

DNA was extracted from 0.25–0.30 g dry soil using a MoBio PowerSoil (Carlsbad, CA, USA) extraction kit, and quantified using a NanoDrop ND-1000 UV-Vis Spectrophotometer (Wilmington, DE, USA). PCR of the *amoA* gene was performed in 50 μl reaction volumes using approximately 50 ng of template DNA, 1 × PCR buffer (Promega Colorless GoTaq Reaction Buffer, Madison, WI, USA), 0.2 mM dNTPs, 1.2 Units Promega GoTaq (Madison, WI, USA), 0.04–0.12% BSA, and 0.2 μM each of either the bacterial *amoA*_1R and *amoA*_2F primers (Rotthauwe *et al*., 1997; Mintie *et al*., 2003; Boyle-Yarwood *et al*., 2008), or the archaeal Arch-*amoA*-F and Arch-*amoA*-R primers (Francis *et al*., 2005; de la Torre *et al*., 2008). Thermalcycler protocols followed existing programs for archaeal (Francis *et al*., 2005; Boyle-Yarwood *et al*., 2008; de la Torre *et al*., 2008) and bacterial *amoA* PCR (Mintie *et al*., 2003).

For T-RFLP assays, 5′ end 6-FAM-labelled forward primers were used to produce PCR products, which were purified using a QIAquick PCR Purification Kit (QIAGEN, Valencia, CA, USA), and restricted with TaqI and AluI for the bacterial *amoA* gene analysis (Horz *et al*., 2000; Mintie *et al*., 2003), and with RsaI and MspI for the archaeal *amoA* gene analysis (Boyle-Yarwood *et al*., 2008). The digests were purified and fragment lengths and relative abundances were analysed

using an ABI 3100 capillary sequencer and Genotyper 3.7 (Foster City, CA, USA). Technical replicates were analysed on a subset (33%) of samples to ensure profile reproducibility. For each sample, any fragments comprising < 3% relative abundance of total fluorescence were removed from subsequent analysis. The relative fluorescence abundances of unique terminal restriction fragments (T-RFs) were exported for further analysis.

Cloning and sequencing was performed on both combined archaeal and bacterial PCR products from all samples; i.e. two 96-well plate libraries were constructed, one for each gene. A ligation of each batch of PCR products was produced using the TOPO TA kit (Invitrogen, Carlsbad, CA, USA), and submitted to the Genome Center at Washington University (St Louis, MO, USA). 96 clones per library were sequenced on an ABI 3730 capillary sequencer (Foster City, CA, USA) using both M13F and R primers. Sequences were trimmed and culled to remove all data with a *phred* value of < 20. Using CodonCode software (Dedham, MA, USA), remaining sequences were aligned and assembled to 2 × coverage and operational taxonomic units (OTUs) for the archaeal and bacterial *amoA* gene were defined at a sequence identity level of 99%. The highest quality representative sequence of each OTU was exported for phylogenetic analysis.

Q-PCR of the archaeal and bacterial *amoA* genes was performed using the Brilliant II SYBR Green Master Mix (Stratagene, La Jolla, CA, USA) and an ABI 7500 Real Time PCR System (Foster City, CA, USA). We used ~10 ng template DNA, a 25 µl reaction volume, and published primers and thermalcycler protocols for both bacterial [*amoA*_1R and *amoA*_2F primers (Rotthauwe *et al*., 1997; Mosier and Francis, 2008)] and archaeal [Arch-*amoA*F and Arch-*amoA*R primers (Francis *et al*., 2005; Mosier and Francis, 2008)]. Standard curves were constructed with 2.0×10^{1} to 2.0×10^{-4} ng *Nitrosomonas europaea* genomic DNA (bacterial *amoA*, efficiency = 91–94%, $R^2 = 0.996$) or 1.25×10^{1} to 1.25×10^{-3} ng of a TOPO plasmid containing a *Nitrosopumilis maritmus amoBAC* gene insert (archaeal *amoA*, efficiency = 95–100%, $R^2 = 0.994$-0.995). Each reaction was run in triplicate, and replicates were analysed for a subset (33%) of samples to ensure quantitative accuracy. *AmoA* gene abundances were standardized by the mass of DNA recovered per g dry soil and $\log_{10}$ transformed before statistical analysis.

Statistical analysis

To test for differences in quantitative variables (soil characteristics, N min, NP, RNP, T-RF richness, archaeal and bacterial *amoA* gene abundances) between land-use type, we used ANOVA with Tukey's *post hoc* analysis. Ordinations of community composition were constructed based on the relative abundance of T-RFs at all sites: nonmetric multidimensional scaling (NMS) scores were calculated from Sørenson's distances, a robust distance measure for datasets with many null values (Legendre and Gallagher, 2001). Highly significant correlations between NMS scores and edaphic properties or N cycling parameters ($P < 0.005$ with either axis) were graphed using vectors overlaid on each NMS plot. Multiple response permutation procedure (MRPP) was used to test for treatment differences (land use, N input) in community composition. All ANOVA and correlation analyses were performed using SPSS 11 for Mac, and all multivariate analyses (NMS, MRPP) were performed using PC-ORD (McCune and Mefford, 1999).

The archaeal and bacterial *amoA* gene clone library sequence data were compared with the known sequences in the NCBI library and analysed phylogenetically to determine their taxonomic affiliations. The representative sequence from each OTU was compared with the NCBI nr/nt database using BLAST, and the GenBank number and percent sequence similarity of the closest match was recorded. A phylogenetic analysis was performed on all representative sequences from this study plus selected sequences from known isolates and environmental clones using ARB (Ludwig *et al*., 2004) and PAUP 4.0 (Swofford, 2003). Phylogenetic trees were built using the neighbour-joining algorithm in ARB, and confirmed by PAUP with the general time-reversible model of nucleotide evolution as recommended by ModelTest (Posada and Crandall, 1998). Trees were bootstrapped in PAUP by resampling 1000 times using stepwise addition and tree bisection replacement. Finally, all sequences included in the phylogenetic analysis were restricted *in silico* with the same enzymes used for T-RFLP analysis of soil amplicons using CodonCode, and these results were used to designate phyla represented by the T-RFs. Sequences are stored in GenBank with accession numbers HM113498-HM113519.

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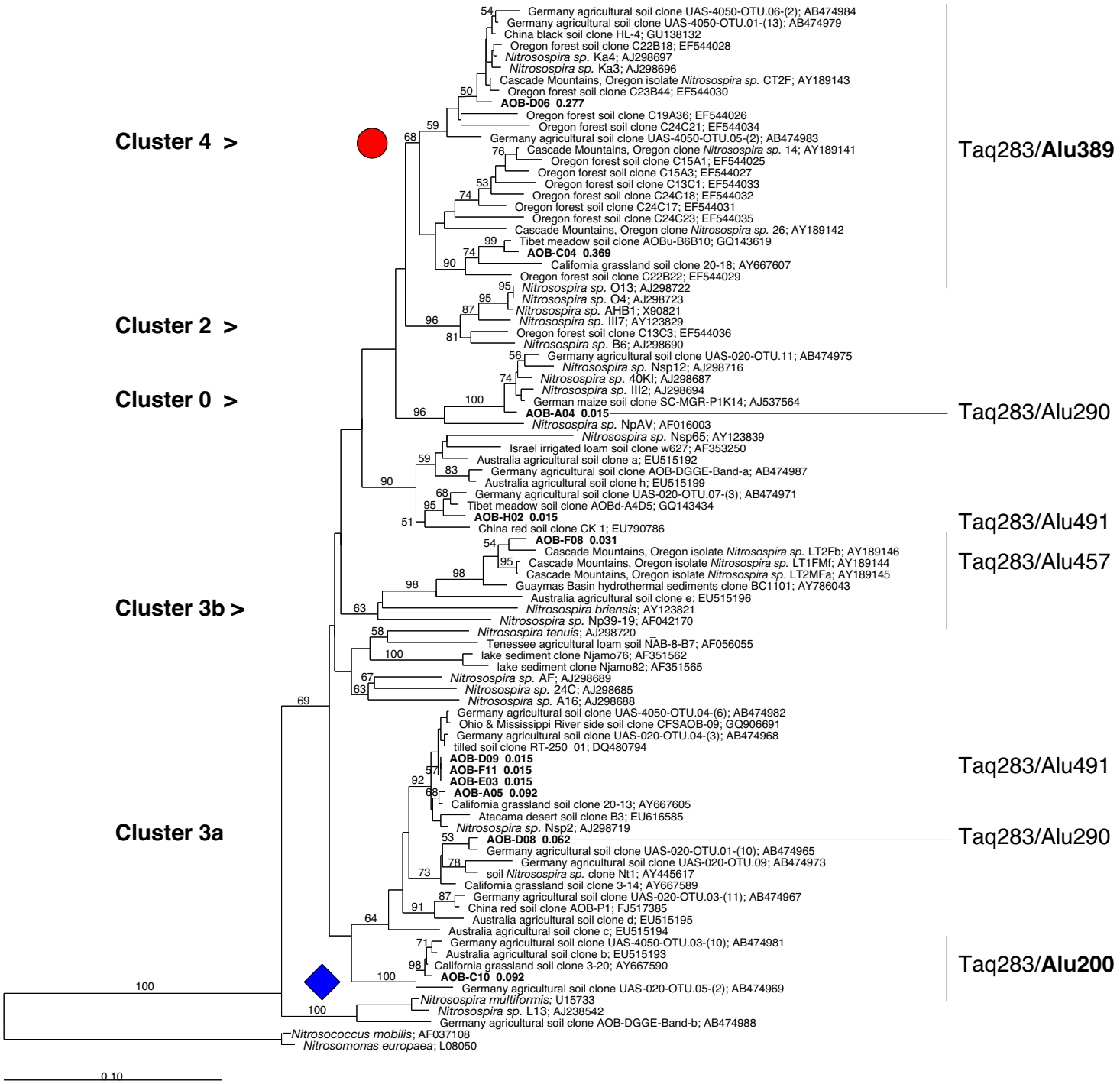
Supporting information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Neighbour-joining phylogenetic tree of Thaumarchaeal *amoA* gene sequences from this study (99% OTU representatives marked in bold, number represents that OTU's abundance in the clone library), cultured isolates and other environmental sequences. *In silico* determinations of T-RFs for all sequences are noted to the right of the phylogram. T-RFs that represent monophyletic taxonomic groups are highlighted in bold/italic type, and the corresponding clade is marked with a unique symbol.

Fig. S2. Neighbour-joining phylogenetic tree of bacterial *amoA* gene sequences from this study (99% OTU representatives marked in bold, number represents that OTU's abundance in the clone library), characterized *Nitrosospira sp.* isolates and other environmental sequences. *In silico* determinations of T-RFs for all sequences are noted to the right of the phylogram. T-RFs that represent monophyletic taxonomic groups are highlighted in bold/italic type, and the corresponding clade is marked with a unique symbol.

Table S1. Archaeal and bacterial *amoA* gene clone library sequence information. AOB taxonomic identity based on a sequence grouping in a clade with known isolates (Fig. S2); cluster designation based on Purkhold and colleagues (2000).


Cluster 4 >
Cluster 2 >
Cluster 0 >
Cluster 3b >
Cluster 3a
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Taq283/Alu290
Taq283/Alu491
Taq283/Alu457
Taq283/Alu491
Taq283/Alu290
Taq283/Alu200
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Germany agricultural soil clone UAS-4050-OTU.01-(13); AB474979
China black soil clone HL-4; GU138132
Oregon forest soil clone C22B18; EF544028
Nitrosospira sp. Ka4; AJ298697
Nitrosospira sp. Ka3; AJ298696
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Oregon forest soil clone C23B44; EF544030
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Oregon forest soil clone C19A36; EF544026
Oregon forest soil clone C24C21; EF544034
Germany agricultural soil clone UAS-4050-OTU.05-(2); AB474983
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Oregon forest soil clone C15A1; EF544025
Oregon forest soil clone C15A3; EF544027
Oregon forest soil clone C13C1; EF544033
Oregon forest soil clone C24C18; EF544032
Oregon forest soil clone C24C17; EF544031
Oregon forest soil clone C24C23; EF544035
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Tibet meadow soil clone AOBu-B6B10; GQ143619
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California grassland soil clone 20-18; AY667607
Oregon forest soil clone C22B22; EF544029
Nitrosospira sp. O13; AJ298722
Nitrosospira sp. O4; AJ298723
Nitrosospira sp. AHB1; X90821
Nitrosospira sp. III7; AY123829
Oregon forest soil clone C13C3; EF544036
Nitrosospira sp. B6; AJ298690
Germany agricultural soil clone UAS-020-OTU.11; AB474975
Nitrosospira sp. Nsp12; AJ298716
Nitrosospira sp. 40KI; AJ298687
Nitrosospira sp. III2; AJ298694
German maize soil clone SC-MGR-P1K14; AJ537564
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Israel irrigated loam soil clone w627; AF353250
Australia agricultural soil clone a; EU515192
Germany agricultural soil clone AOB-DGGE-Band-a; AB474987
Australia agricultural soil clone h; EU515199
Germany agricultural soil clone UAS-020-OTU.07-(3); AB474971
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China red soil clone CK 1; EU790786
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Cascade Mountains, Oregon isolate Nitrosospira sp. LT2MFa; AY189145
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Nitrosospira sp. Np39-19; AF042170
Nitrosospira tenuis; AJ298720
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Nitrosospira sp. 24C; AJ298685
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Australia agricultural soil clone d; EU515195
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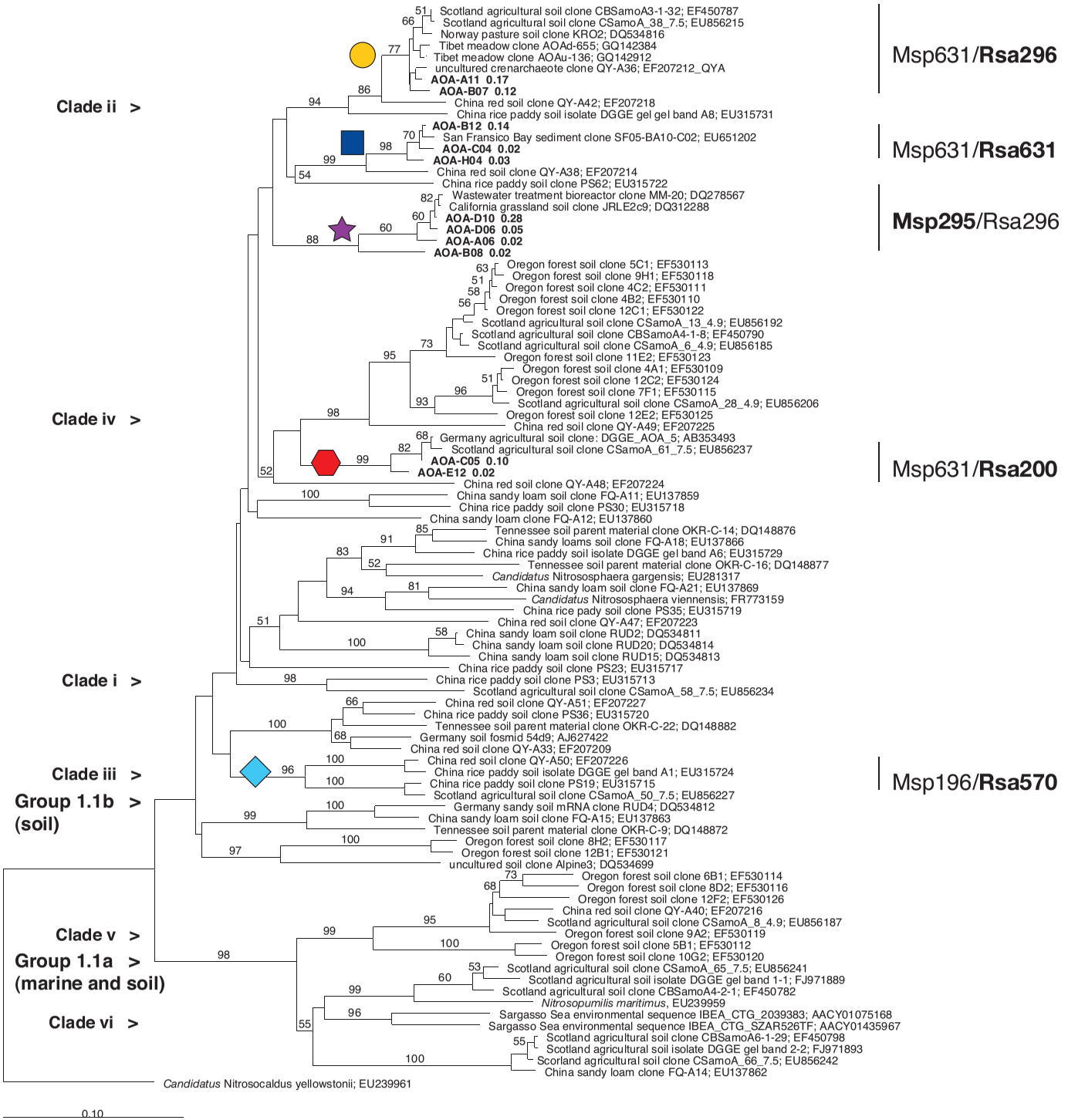
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Tibet meadow clone AOAd-655; GQ142384
Tibet meadow clone AOAu-136; GQ142912
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AOA-H04 0.03
China red soil clone QY-A38; EF207214
China rice paddy soil clone PS62; EU315722
Wastewater treatment bioreactor clone MM-20; DQ278567
California grassland soil clone JRLE2c9; DQ312288
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Oregon forest soil clone 4C2; EF530111
Oregon forest soil clone 4B2; EF530110
Oregon forest soil clone 12C1; EF530122
Scotland agricultural soil clone CSamoA_13_4.9; EU856192
Scotland agricultural soil clone CBSamoA4-1-8; EF450790
Scotland agricultural soil clone CSamoA_6_4.9; EU856185
Oregon forest soil clone 11E2; EF530123
Oregon forest soil clone 4A1; EF530109
Oregon forest soil clone 12C2; EF530124
Oregon forest soil clone 7F1; EF530115
Scotland agricultural soil clone CSamoA_28_4.9; EU856206
Oregon forest soil clone 12E2; EF530125
China red soil clone QY-A49; EF207225
Germany agricultural soil clone: DGGE_AOA_5; AB353493
Scotland agricultural soil clone CSamoA_61_7.5; EU856237
AOA-C05 0.10
AOA-E12 0.02
China red soil clone QY-A48; EF207224
China sandy loam soil clone FQ-A11; EU137859
China rice paddy soil clone PS30; EU315718
China sandy loam clone FQ-A12; EU137860
Tennessee soil parent material clone OKR-C-14; DQ148876
China sandy loams soil clone FQ-A18; EU137866
China rice paddy soil isolate DGGE gel band A6; EU315729
Tennessee soil parent material clone OKR-C-16; DQ148877
Candidatus Nitrososphaera gargensis; EU281317
China sandy loam soil clone FQ-A21; EU137869
Candidatus Nitrososphaera viennensis; FR773159
China rice pady soil clone PS35; EU315719
China red soil clone QY-A47; EF207223
China sandy loam soil clone RUD2; DQ534811
China sandy loam soil clone RUD20; DQ534814
China sandy loam soil clone RUD15; DQ534813
China rice paddy soil clone PS23; EU315717
China rice paddy soil clone PS3; EU315713
Scotland agricultural soil clone CSamoA_58_7.5; EU856234
China red soil clone QY-A51; EF207227
China rice paddy soil clone PS36; EU315720
Tennessee soil parent material clone OKR-C-22; DQ148882
Germany soil fosmid 54d9; AJ627422
China red soil clone QY-A33; EF207209
China red soil clone QY-A50; EF207226
China rice paddy soil isolate DGGE gel band A1; EU315724
China rice paddy soil clone PS19; EU315715
Scotland agricultural soil clone CSamoA_50_7.5; EU856227
Germany sandy soil mRNA clone RUD4; DQ534812
China sandy loam soil clone FQ-A15; EU137863
Tennessee soil parent material clone OKR-C-9; DQ148872
Oregon forest soil clone 8H2; EF530117
Oregon forest soil clone 12B1; EF530121
uncultured soil clone Alpine3; DQ534699
Oregon forest soil clone 6B1; EF530114
Oregon forest soil clone 8D2; EF530116
Oregon forest soil clone 12F2; EF530126
China red soil clone QY-A40; EF207216
Scotland agricultural soil clone CSamoA_8_4.9; EU856187
Oregon forest soil clone 9A2; EF530119
Oregon forest soil clone 5B1; EF530112
Oregon forest soil clone 10G2; EF530120
Scotland agricultural soil clone CSamoA_65_7.5; EU856241
Scotland agricultural soil isolate DGGE gel band 1-1; FJ971889
Scotland agricultural soil clone CBSamoA4-2-1; EF450782
Nitrosopumilis maritimus, EU239959
Sargasso Sea environmental sequence IBEA_CTG_2039383; AACY01075168
Sargasso Sea environmental sequence IBEA_CTG_SZAR526TF; AACY01435967
Scotland agricultural soil clone CBSamoA6-1-29; EF450798
Scotland agricultural soil isolate DGGE gel band 2-2; FJ971893
Scorland agricultural soil clone CSamoA_66_7.5; EU856242
China sandy loam clone FQ-A14; EU137862
Candidatus Nitrosocaldus yellowstonii; EU239961
0.10
Clade ii >
Clade iv >
Clade i >
Clade iii >
Group 1.1b > (soil)
Clade v >
Group 1.1a > (marine and soil)
Clade vi >
Msp631/Rsa296
Msp631/Rsa631
Msp295/Rsa296
Msp631/Rsa200
Msp196/Rsa570

Supplemental Table 1.

	Closest BLAST match				
Clone name	**GenBank #**	**% Identity**	**Clone lib. %**	**T-RFLP profile**	**Taxonomic ID* (AOB only)**
AOA-A11	EF207212	99	20.8	Msp631/Rsa296	.
AOA-B07	GQ142912	98	13.9	Msp631/Rsa296	.
AOA-B12	EU651202	99	13.9	Msp631/Rsa631	.
AOA-C04	EU651202	99	1.4	Msp631/Rsa631	.
AOA-H04	EU651202	97	2.8	Msp631/Rsa468	.
AOA-D10	DQ312288	99	29.2	Msp295/Rsa296	.
AOA-D06	DQ312288	99	4.2	Msp295/Rsa296	.
AOA-A06	DQ278567	98	1.4	Msp295/Rsa296	.
AOA-B08	GQ142384	91	1.4	Msp631/Rsa336	.
AOA-C05	AB353493	99	8.3	Msp631/Rsa200	.
AOA-E12	AB353493	96	1.4	Msp631/Rsa200	.
AOB-D06	DQ480827	98	27.7	Taq283/Alu389	*Nitrosospira* sp. Ka3/Ka4 Cluster 4
AOB-C04	GQ143619	99	36.9	Taq283/Alu389	*Nitrosospira* sp. uncultured Cluster 4
AOB-C10	AY667590	99	9.2	Taq283/Alu200	*Nitrosospira* sp. uncultured Cluster 3a
AOB-A05	AY667605	99	9.2	Taq283/Alu491	*Nitrosospira* sp. Nsp2 Cluster 3a
AOB-D09	DQ480794	99	1.5	Taq283/Alu491	*Nitrosospira* sp. Nsp2 Cluster 3a
AOB-E03	DQ480794	99	1.5	Taq283/Alu491	*Nitrosospira* sp. Nsp2 Cluster 3a
AOB-F11	DQ480794	99	1.5	Taq283/Alu491	*Nitrosospira* sp. Nsp2 Cluster 3a
AOB-D08	AY667589	97	6.2	Taq283/Alu290	*Nitrosospira* sp. uncultured Cluster 3a
AOB-F08	DQ480823	98	3.1	Taq283/Alu290	*Nitrosospira briensis* Cluster 3b
AOB-A04	AJ537564	98	1.5	Taq283/Alu290	*Nitrosospira* sp. 40KI/Nsp12 Cluster 0
AOB-H02	GQ143434	97	1.5	Taq283/Alu491	*Nitrosospira* sp. Nsp65