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Links among Nitrification, Nitrifier Communities, and Edaphic Properties in Contrasting Soils Receiving Dairy Slurry

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Links among Nitrification, Nitrifier Communities, and Edaphic Properties in Contrasting Soils Receiving Dairy Slurry

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Soil biotic and abiotic factors strongly influence nitrogen (N) availability and increases in nitrification rates associated with the application of manure. In this study, we examine the effects of edaphic properties and a dairy (*Bos taurus*) slurry amendment on N availability, nitrification rates and nitrifier communities. Soils of variable texture and clay mineralogy were collected from six USDA–ARS research sites and incubated for 28 d with and without dairy slurry applied at a rate of $\sim 300 \text{ kg N ha}^{-1}$. Periodically, subsamples were removed for analyses of 2 M KCl extractable N and nitrification potential, as well as gene copy numbers of ammonia-oxidizing bacteria (AOB) and archaea (AOA). Spearman coefficients for nitrification potentials and AOB copy number were positively correlated with total soil C, total soil N, cation exchange capacity, and clay mineralogy in treatments with and without slurry application. Our data show that the quantity and type of clay minerals present in a soil affect nitrifier populations, nitrification rates, and the release of inorganic N. Nitrogen mineralization, nitrification potentials, and edaphic properties were positively correlated with AOB gene copy numbers. On average, AOA gene copy numbers were an order of magnitude lower than those of AOB across the six soils and did not increase with slurry application. Our research suggests that the two nitrifier communities overlap but have different optimum environmental conditions for growth and activity that are partly determined by the interaction of manure-derived ammonium with soil properties.

BEST MANAGEMENT PRACTICES for application of manure should take into account the linkages among N cycling, edaphic factors, and microbial communities that affect the fate of plant available N from organic fractions. Manure N excreted from animal production systems in the United States exceeds 28 million metric tons annually, representing a valuable source of nutrients (USDA–ERS, 2001). Less than 40% of manure N is recycled to cropland, while 11 million metric tons of N fertilizer is purchased annually (USDA–NASS, 2004). Therefore, accurate predictive measures of manure N transformations could potentially reduce input costs and excess nutrient loading to the environment.

High clay content can increase the amount of total soil N by physically protecting soil organic matter (SOM) (Bosatta and Agren, 1998). Greater concentrations of SOM have been shown to support larger populations of microorganisms that foster increased potential for mineralization and possibly nitrification (Accoe et al., 2004). Gross N mineralization rates have been correlated with total soil N (TSN) content (Accoe et al., 2004). Soils containing vermiculitic and smectitic clays can fix a portion of ammonium applied in fertilizers and manures due to the presence of expanding interlayers in these clays (Bajwa, 1982; Meunier and Velde, 2004). Kaolinite does not allow for fixation of NH_4^+ because the tetrahedral and octahedral sheets do not allow for expansion. Chloritic clays do not fix NH_4^+ due to the presence of a hydroxide sheet in their interlayers.

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Abbreviations: AOA, ammonia-oxidizing archaea; AOB, ammonia-oxidizing bacteria; BD, bulk density; CEC, cation exchange capacity; L_NE, loam, Newport soil; PCR, polymerase chain reaction; PVC, polyvinyl chloride; PVP, polyvinylpyrrolidone; qPCR, quantitative polymerase chain reaction; S_VA, sand, Valentine soil series; SIC_CA, silty clay loam, Catlin soil series; SIC_SH, silty clay, Sharpsburg soil series; SICL_BR, silty clay loam, Brooksville; SIL_CA, loam, Caribou soil series; SIL_LO, silt loam, Loyal soil series; SIL_RO, silt loam, Roshalt soil series; SL_AD, sandy loam, Adkins soil series; SOC, total soil organic carbon; SOM, soil organic matter; TSN, total soil nitrogen; VR, vermiculite; WFPS, water-filled pore space.

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Therefore, research that emphasizes clay mineralogy, as well as textural class, is needed to improve estimates of the wide range of plant-available N and nitrification rates that occur when a particular manure is applied to soils of differing mineralogy.

Nitrification potential is the maximum capacity of nitrifying bacteria and archaea to transform $\text{NH}_4\text{-N}$ to $\text{NO}_3\text{-N}$ via a two-step process in which ammonium is oxidized and converted to nitrite and nitrite is converted to nitrate. Oxidation of ammonium to nitrite is the rate-limiting step and is catalyzed by the ammonia monooxygenase enzyme (*amoA*). Until recently, ammonia-oxidizing bacteria (AOB) were thought to be the sole group of organisms in marine and soil systems capable of converting ammonia to nitrite chemolithoautotrophically. Probes designed to isolate the gene encoding a subunit of the enzyme ammonia monooxygenase (*amoA*) revealed the presence of *amoA* in Archaea (Leininger et al., 2006; Wuchter et al., 2006). The use of quantitative polymerase chain reaction (qPCR) further revealed that archaea containing the *amoA* gene inhabit a range of environments that include marine and fresh water, terrestrial soil systems, and wastewater treatment facilities (Leininger et al., 2006; You et al., 2009).

Edaphic properties, land-use management, and ecosystem characteristics select for different ratios of AOB to ammonia-oxidizing archaea (AOA) and influence the size of each nitrifier community that can be supported, leading to potential variations in nitrification rates (Lehtovirta et al., 2009; Nicol et al., 2004; Taylor et al., 2010; Webster et al., 2005). Ammonia-oxidizing bacteria can proliferate in environments at higher NH_4^+ concentrations and typically respond to ammonia additions. Although archaea containing the *amoA* gene have been isolated from wastewater treatment facilities (You et al., 2009), most archaea containing the *amoA* gene have been isolated from oligotrophic environments (Martens-Habbena et al., 2009).

Nitrifying AOA are often found in higher numbers relative to AOB in grassland systems that are often N limited, resulting in decreased nitrification rates. Grasslands can select for higher AOA numbers and have been shown to support higher AOA activity relative to agronomic managements that are dominated by AOB activity (Taylor et al., 2010). The

presence and activity of AOA in six managed grasslands did not vary with ammonia additions, whereas AOB copy numbers and activity increased with additions (Di et al., 2009). Nitrifying archaea are present in high numbers in agronomic systems receiving higher N inputs than natural systems. However, AOA growth in agricultural soils has been shown to remain constant after addition of ammonia (Di et al., 2010; Jia and Conrad, 2009). Previous studies showed that application of pig manure, inorganic fertilizer, or sheep urine to soils stimulated AOB growth but had little effect on AOA growth (Nicol et al., 2004; Schauss et al., 2009).

Few if any studies have been conducted to address the role of AOA relative to AOB in the nitrification process across a range of soils of varying edaphic properties in agronomic systems. This research examines changes in nitrification rates and AOA, AOB gene copy numbers per gram of soil resulting from manure application to nine soils of differing clay mineralogy, total soil organic C (SOC), TSN, and fertility under controlled conditions. Adding manure to previously unamended soils with no N input for several years allowed for the comparison of soil properties by eliminating the effects of recent management. Microbial community shifts were expected to be more dramatic after manure additions to soils with limited nitrogen. The inclusion of a range of soils from several regions in the United States enabled us to assess which chemical, physical, and biological characteristics of a given soil had the strongest influence on nitrifiers, nitrification, and N cycling. We correlated measurements of nitrification potentials with AOA, AOB copy numbers, and soil properties after application of manure N to evaluate the subsequent effects of this N source on the nitrification processes. An additional objective was to evaluate whether potential niches of AOA and AOB communities are consistent across a range of manure-amended soils.

Materials and Methods

Study Sites

Field samples were collected from USDA-ARS research centers across a range of soil series with differing clay mineralogy in the United States (Tables 1 and 2). Soil abbreviations

Table 1. Soil series and selected management information for soils used in this study.

State	Soil series abbreviation	Soil series description	Vegetation	Previous cropping history	Tillage
Maine	Caribou SIL_CA	fine-loamy, mixed, frigid Typic Haplorthod	weedy fallow	7 yr since last crop of potatoes	discing, mowed
	Newport L_NE	unnamed variant of a Bangor series; coarse loamy, mixed frigid Typic Haplorthod	annual sod	none	none
Nebraska	Sharpsburg SIC_SH	fine, montmorillonitic, mesic, Typic Argiudoll	wheat stubble	soybean	no tillage
	Valentine S_VA	mixed, mesic, Typic Ustipsamment	native short grass prairie	grazed	none
Oregon	Adkins SL_AD	coarse-loamy, mixed, mesic, Xerollic Camborthid	sagebrush	none	none
Wisconsin	Loyal SIL_LO	fine-loamy, mixed, superactive frigid Oxyaquic Glossudalfs	corn	Corn	chisel
	Roshalt SIL_RO	coarse-loamy, mixed, superactive, frigid Haplic Glossudalf	corn	soybean/corn	chisel
Mississippi	Brooksville SICL_BR	fine, smectitic, thermic Aquic Hapludert	grass border of field	not cropped for at least 5 yr	none
Illinois	Catlin SIC_CA	fine-silty, mixed, superactive mesic Oxyaquic Arguidoll	grass border of field	not cropped for at least 5 yr	none

were derived from the Soil Survey textural class abbreviations and the first two letters of the soil series: SICL_BR—silty clay loam, Brooksville; SIC_CA—silty clay loam, Catlin soil series; SIC_SH—silty clay, Sharpsburg soil series; L_NE—loam, Newport soil; S_VA—sand, Valentine soil series; SIL_CA—loam, Caribou soil series; SIL_LO—silt loam, Loyal soil series; SIL_RO—silt loam, Roshalt soil series; SL_AD—sandy loam, Adkins soil series (Table 1). The soil series designation is used to distinguish soils of the same textural class from one another. Soil samples were collected between 29 Apr. and 8 Aug. 2005. All soil samples were taken in areas that had received no N inputs for 5 yr and no manure for 10 yr. Three sites were located on grasslands. The Valentine series was sampled from a native short grass prairie, Adkins was sampled from sagebrush, and Newport was in annual sod. The remaining sites were previously cropped or on the border of a field (Table 1).

Sample Collection

Soils at each location were randomly sampled by collecting 12 cores within a 2-m² area to 15-cm depth using polyvinyl chloride (PVC) inserts of 689-cm³ volume and a sliding drop hammer. The inserts prevented soil compaction and allowed us to measure soil bulk density. Each PVC insert containing field moist soil was capped at both ends, placed in a cooler, and shipped to the New England Plant, Soil and Water Laboratory in Orono, ME. The Catlin soil samples from Illinois were collected to an approximate 15-cm depth using a shovel, and field moist soil were transported at ambient temperature for 1 wk.

Each of the 12 field moist soil cores from a given soil series was pooled, sieved through a 2-mm sieve, and weighed. Three replicate 10-g subsamples were collected from each sieved core for determination of gravimetric water content. Bulk density (BD) was calculated for each core (Blake and Hartge, 1986). Bulk soil was stored at 4°C in the laboratory before the start of the experiment. Disturbance resulting from sampling often causes a 2-wk initial flush of mineralization that may interfere with estimates of potentially mineralizable N (Stanford and Smith, 1972; Curtin and Campbell, 2008). Because of the differences in the size of the potential flush among soil series,

soils were preincubated. All soils, except for the Catlin, were preincubated at 25°C for 2 wk. The week the Catlin soil was in transport was considered week 1 of a 2-wk preincubation.

Soil Properties

Soil samples were analyzed for SOC and TSN. A set of soil samples was prepared for dry combustion by pulverization in a Wig-L-Bug amalgamator/mixer (Crescent, Elgin, IL). Total soil organic C and TSN were measured via dry combustion on a LECO CN-2000 (Leco Corporation, St. Joseph, MI). The pH of soils was measured via the method of McLean (1982). Cation exchange capacity (CEC, cmol_c kg⁻¹) was estimated as the Σ (Ca + Mg + K + Na) (Ross, 1995). Exchangeable acidity was negligible.

Mineralogy

Mineralogical data from the clay fraction (<2 μm diameter) was processed at the USDA–NRCS National Soil Survey Center Soil Survey Laboratory. The clay fraction was separated from other dispersed soil separates via centrifugation (Moore and Reynolds, 1997). Chlorite, goethite, illite, kaolinite, quartz, smectite, and vermiculite were determined by standard X-ray diffraction techniques (Whittig and Allardice, 1986). Continuous, locked coupled scans were obtained at 0.0545-s time steps at 0.02° 2-theta and a slit of 1.000° 2-theta. A quartz standard was used to measure peak height drift and periodically corrected with time-step adjustments. In addition, a soil standard was included in each set of scans as a check for constant behavior of clay mineral patterns. Counts per second values were used in the statistical analysis. Each mineral was also assigned to one of five semiquantitative classes used for soil survey classification based on peak height above background (counts s⁻¹). Classes include 5 (very large, >1800 counts s⁻¹), 4 (large, 1120–1800 counts s⁻¹), 3 (medium, 360–1120 counts s⁻¹), 2 (small, 110–360 counts s⁻¹), and 1 (very small, <110 counts s⁻¹) (Burt, 2004). We divided the illite class by the sum of all mineral classes for a given soil to approximate the potential for illite to adsorb NH₄ at selective sites (“fixed” NH₄). The quantity of selective sites was estimated as 3% of the

Table 2. Biotic, abiotic and chemical properties of soils used to assess nitrification, cumulative inorganic N and ammonia oxidizer (AOB) gene copy number g⁻¹ soil in soils with and without dairy slurry applications.

Sample code	Texture	Mineralogy†‡	Sand	Silt	Clay	SOC§	TSN§	pH 0.01 M CaCl ₂	BD§	CEC§
			— g kg ⁻¹ —			— g kg ⁻¹ soil —			g cm ⁻³	cmol _c kg ⁻¹
SIL_CA	silt loam	KK 2 IL 2 S 2	370	510	120	20	1.7	5.2	1.2	6.62
L_NE	loam	KK 2 IL 3 S 3	430	430	140	25	2.0	5.2	1.2	4.75
SIC_SH	silty clay	KK 2 IL 2 VR 2	30	570	400	19	1.7	5.4	1.3	21.9
S_VA	sand	KK 2 IL 2 S 2	930	40	30	5.0	0.5	5.4	1.4	3.47
SL_AD	sandy loam	KK 1 IL 2 S 2	730	210	60	5.8	0.6	6.4	1.5	10.1
SIL_LO	silt loam	KK 2 IL 1 VR 2	120	720	160	23	2.1	6.2	1.4	13.5
SIL_RO	silt loam	KK 3 IL 1 VR 2	300	600	100	13	1.2	4.5	1.5	4.62
SICL_BR	silty clay loam	KK 4 S 3	100	580	320	23	2.1	6.2	1.3	31.0
SIC_CA	silty clay	KK 2 S 3 IL 2 VR 1	190	410	400	35	2.5	7.0	1.3	25.4

† Mineral interpretation: KK, kaolinite; IL, illite; S, smectite; VR, vermiculite.

Mineralogical data provided by the USDA–NRCS National Soil Survey Center Soil Survey Laboratory.

‡ Relative peak size: 5, very large; 4, large; 3, medium; 2, small; 1, very small. Measured via X-ray diffraction.

§ SOC, soil organic carbon sample depth 0–15 cm 2005; TSN, total soil nitrogen sample depth 0–15 cm 2005; BD, soil bulk density and BD of soil in incubation vessels; CEC, cation exchange capacity, Σ(Ca + Mg + K + Na) from the Mehlich III extract—exchangeable acidity and Na were negligible.

average CEC of illite $30 \text{ cmol}_c \text{ kg}^{-1} - 0.9 \text{ cmol}_c \text{ kg}^{-1}$ (Meunier and Velde, 2004).

Soil Incubations and Nitrogen Analyses

A portion of the soil collected from each soil series was used in a 28-d laboratory incubation. Each soil series contained five time points (Day 0, 7, 14, 21, and 28), four replicates, and two treatments, soil amended with dairy slurry and an unamended control soil. The pH of the dairy slurry was measured by the method of Wolf (2003). A pH electrode was immersed into a mixture of 40 mL of deionized H_2O and 20 mL of dairy slurry. The pH of the slurry was 7.2. Field-moist soil, equal to 50 g of dry soil, was weighed into 120-mL specimen vials (53 mm diam., 60 mm height). The soil height in the specimen vials was approximately 35 mm. Soils were packed to the equivalent BD of each soil series at the time of field sampling. Two grams of dairy slurry were applied directly to the surface of soils receiving the slurry. The slurry moved into the soil from the surface within a few minutes and migrated approximately 15 mm, after which the top 1 to 2 cm of soil were turned over and the BD restored. This operation simulated incorporation of the dairy slurry and minimized ammonia volatilization. Incubations were conducted at 25°C and 60% of water-filled pore space (WFPS); following initial manure additions, distilled H_2O was added to bring soil in incubation vials up to 60% of WFPS. Once a week, soil samples were aired and weighed and water added to maintain WFPS at 60%. Specimen vials were then rearranged in the incubator to account for potential variability within the incubator. Eight specimen vials, four replicates of each treatment, were removed per soil series at each sampling. Subsamples (20 g) were taken from each specimen vial for inorganic N analysis (NH_4 , NO_2 , and NO_3)-N, nitrification potential, and qPCR measurements, respectively. Each subsample was taken as a wedge from the center of the specimen vial to the edge of the vessel and included soil from 0 to 35 mm.

Inorganic N, (NH_4 , NO_2 , and NO_3)-N, was extracted with 100 mL of 2 M KCl. Aliquots were run on an automated, continuous flow OI Analytical ALPKEM Flow Solution IV Auto-Analyzer (OI Analytical, College Station, TX) to determine ammonium N using the salicylate-nitroprusside method (Mulvaney, 1996) and the cadmium reduction method for combined nitrate N plus nitrite-N (Gavlak et al., 1994). Nitrite- and nitrate-N were not determined separately because nitrite concentrations are considered negligible ($<0.5\%$ of $\text{NO}_3 + \text{NO}_2$) under conditions comparable to our experiment (Smith et al., 1997).

The potential rate of nitrification was determined using 15-g subsamples of soil via the shaken slurry method (Hart et al., 1994) for all soils except the Valentine sand. The coarse sand fraction of this soil settled too quickly to obtain a consistent soil-to-media ratio. Nitrate from the centrifuged supernatant was measured via an automated OI Analytical ALPKEM Flow Solution IV Auto-Analyzer as previously described.

Ammonia Volatilization

The dairy slurry contained inorganic N in the form of NH_4^+ that could be lost as NH_3 . Therefore, a separate incubation experiment was conducted to measure the amount of ammo-

nia volatilized from each soil series. Twenty-four grams of dairy slurry ($\sim 300 \text{ kg N ha}^{-1}$) were added to 200 g of soil maintained at 60% of WFPS and 25°C for 7 d with three replications. Ammonia was captured on acid traps consisting of filter paper treated with oxalic acid ($3 \text{ g oxalic acid L}^{-1}$ acetone) suspended in each incubation vessel following the procedure of Russell et al. (2004). Upon removal of traps, NH_4^+ was extracted with 100 mL of 2 M KCl and run on an automated OI Analytical ALPKEM Flow Solution IV Auto-Analyzer as previously described. Internal controls consisted of varying concentrations of ammonium persulfate, $(\text{NH}_4)_2\text{S}_2\text{O}_8$, dissolved in cold filtered H_2O . Magnesium oxide (MgO) was used as an alkali to drive off ammonium as ammonia gas (NH_3).

Quantification of Ammonia-Oxidizing Archaea and Ammonia-Oxidizing Bacteria by qPCR

We selected soil series with contrasting soil properties and nitrogen parameters (SIC_CA, SIL_CA, SIC_SH, SL_AD, SIL_RO, and SIL_LO) for molecular analyses. The Valentine soil (S_VA) was not included because it was too coarse a sand to be analyzed via nitrification potential (see "Materials and Methods," Soil Incubations and Nitrogen Analyses), preventing correlations of nitrification rates with AOA, AOB copy numbers. The Newport soil (L_NE) and Brooksville soils (SICL_BR) contained soil components that inhibited qPCR analysis. These samples were treated with a polyvinylpyrrolidone (PVP) P6755 (Sigma-Aldrich) suspension, in sterile water, 10% w/v. Quantitative polymerase chain reaction reactions amplified successfully after the additional clean-up step with PVP. But copy numbers of AOB and AOA were lower than typically reported in the literature. The additional PVP step may have removed a portion of the AOA, AOB genomic DNA, and inhibition of polymerase chain reaction (PCR) reactions did not appear to be completely eliminated. Therefore, due to inhibition and the difference in treatment of genomic DNA from L_NE and SICL_BR, these samples were not included in the data analysis. The AOB copy numbers were higher than AOA copy numbers in both soils.

Genomic DNA was extracted with a MoBio Power Soil DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA) from soil subsamples taken on Days 0 and 28 of the incubations from control and manure-amended soils. Genomic DNA was frozen at -80°C for use in qPCR analyses.

Quantitative PCR assays were conducted to quantify beta-proteobacterial AOB-specific 16S rRNA gene sequences and the AOA-specific *amoA* gene encoding for the *ammonium monooxygenase* enzyme. Primers specific for AOB were used to amplify a 116-bp DNA fragment in the V2 region of the 16S ribosomal DNA: CTO 189fA/B GGAGRAAAGCAGGGGATCG, CTO 189fC GGAGGAAAGTAGGGGATCG, RT1r CGTCCTCTCAGACCARCTACTG, and the probe, TMP1 CAACTAGCTAATCAGRCATCRGCCGCTC (Hermansson and Lindgren, 2002) labeled at the 5' terminus with fluorochrome 6-carboxyfluorescein (FAM) and a quencher dye tetra-methylcarboxyrhodamine (TAMRA) at the 3' terminus. The primers and TaqMan probe were synthesized by Applied Biosystems, Inc. (Foster City, CA). Use of the CTO primers diagnostic for the 16S rRNA gene containing a single region

specific to ammonia oxidizers of the beta subdivision of *Proteobacteria* (Aakra et al., 2000) reducing the potential bias caused by the presence of variable copy numbers of the *amoA* operon in the genomes of different AOB strains (Norton et al., 2002) reduced the bias caused when the multicopy *amoA* gene is targeted instead (Aakra et al., 2000). Primers specific for archaeal *amoA* were used to amplify a 635-bp DNA fragment: Arch-amoAF STAATGGTCTGGCTTAGACG and Arch-amoAR GCGGCCATCCATCTGTATGT (Taylor et al., 2010). The primers were synthesized by Applied Biosystems Inc. Each reaction contained a total volume of 25 μ L that included Universal PCR Master Mix (Applied Biosystems Inc.). The AOB qPCR reactions contained 7.5 pmol of each forward primer CTO 189fA/B and CTO189fC, 7.5 pmol of the reverse primer RT1r, and 3 pmol of TaqMan probe TMP1. The AOA qPCR reactions contained 3.75 pmol of forward Arch-amoAF and reverse Arch-amoAR primer. Standard curves for AOB were generated using five different genomic DNA concentrations extracted from pure culture *Nitrosomonas europaea* ATCC 19718 ranging from 0.4 pg to 4 ng per qPCR reaction. Standard curves for AOA were generated using six different DNA concentrations of TOPO vector containing the *Nitrosopumilus maritimus* SCM1 strain genes *amoBAC*. DNA concentrations ranged from 4.4 fg to 44 pg per reaction. The TOPO vector containing *Nitrosopumilus maritimus* SCM1 strain *amoBAC* gene insert (EU239959) was kindly provided by Daniel Arp and David Myrold (Oregon State University, Corvallis, OR). The Arp laboratory received the original glycerol stocks containing the *Nitrosopumilus maritimus* TOPO vector (Invitrogen, Carlsbad, CA) from David Stahl, University of Washington, Seattle, WA. Genomic DNA template equivalent to 4 ng (AOB) and (AOA) 0.4 ng per reaction was used for each environmental sample. Reaction mixtures were amplified and measured in an ABI Prism 7500 (Applied Biosystems Inc.). Quantitative PCR reactions for AOB were performed using a default thermocycling profile that consisted of 2 min at 50°C, 10 min at 95°C, followed by 40 cycles of 15 s at 95°C and 1 min at 60°C; and for AOA, 10 min at 95°C, followed by 40 cycles of 15 s at 95°C, 30s at 55°C, and 30s at 72°C for AOA. The initial 2-min step at 50°C inactivates the AmpErase UNG contained in the master mix. AmpErase UNG is added to degrade PCR product contaminations that may be present when the PCR reactions are initially setup. The AmpErase UNG is activated at 50°C, then inactivated at the first cycle at 95°C before amplification of the *amoA* AOA gene.

Genomic DNA from environmental samples used in qPCR reactions was amplified and cloned to verify that the sequences obtained were 98% similar to AOA sequences. Polymerase chain reaction products were purified with a QIAquick PCR purification kit (Qiagen, Valencia, CA) and ligated into a pCR 2.1-TOPO cloning vector (Invitrogen, Carlsbad, CA) then heat-shocked into One Shot TOP10 chemically competent *E. coli* cells (Invitrogen). Resultant transformants were grown on LB amp 50 agar. Fifteen colonies were selected and grown in 5 mL of LB amp50. The 600-bp insert contained in the plasmids was isolated via digestion with *EcoRI* (Promega, Madison, WI). Sequencing reactions were set up using primers M13F: GTAAAACGACGGCCAG or M13R: CAGGAAACAGCTATGAC. Resultant sequences were run

through a National Center for Biotechnology Information Blast search (www.ncbi.nlm.nih.gov/BLAST/) and aligned with a 98% max identity to “uncultured crenarchaeote *amoA* gene for ammonia monooxygenase subunit A” accession number AB545944.1.

Statistical Analyses

We analyzed changes in nitrification potentials, cumulative inorganic N, ammonia volatilization, and AOB, AOA copy number per gram of soil as a function of manure application and time across a range of soil series via a completely randomized three factor factorial analysis of variance using SAS PROC MIXED (SAS Institute, 1997). Means were considered significantly different at the Bonferroni adjusted *p*-value of 0.01. Spearman's rank correlation was used to test for monotonic trends among edaphic factors and cumulative inorganic N mineralized, nitrification potentials and AOB copy number per gram of soil measured at Day 28 of the N incubation using SAS PROC CORR. A Spearman's rank correlation coefficient and *p*-value were generated for each pair of variables. Soil properties included pH, TSN, SOC, pH, CEC, BD, the quantity (g g^{-1}) of sand, silt and clay, clay mineralogy, and AOB, AOA gene copy number per gram of soil. Minerals used in the Spearman's correlation were present in three or more soils and included kaolinite, illite, smectite, and vermiculite.

Results

Nitrogen Mineralization and Nitrification

The amount of N mineralized (NH_4 , NO_2 , and NO_3)-N and net nitrification were dependent on specific soil properties. Inorganic N on Day 28 in soils without dairy slurry was positively correlated with SOC, TSN, CEC, and kaolinite. The manure-applied treatment revealed a positive correlation between cumulative inorganic N mineralized and CEC (Table 3). The S_VA soil had the lowest net nitrification values with or without dairy slurry application (4.5 and 9.4 kg ha^{-1}) on Day 28 (Tables 4 and 5). Spearman's correlation coefficients for cumulative inorganic N mineralized were not positively correlated with illite and smectite, the dominant minerals in S_VA (Table 3). Soil series with the highest net nitrification, Sharpsburg silty clay (SIC_SH), Roshalt (SIL_RO), and Brooksville (SICL_BR), also contained vermiculite or smectite (Table 2) that can retain and release ammonium.

Nitrification rates varied between soils and increased significantly with manure application. Nitrification potentials taken a few minutes after application of dairy slurry (time zero) ranged from 2 to 10 $\text{mg kg}^{-1} \text{d}^{-1}$ and were not significantly different in manured and unmanured treatments within a soil series (Table 6). Nitrification potentials did not differ among time intervals in soil samples without dairy slurry application but varied significantly between soil series ranging from 2 to 11 $\text{mg kg}^{-1} \text{d}^{-1}$. Nitrification potentials increased between time of initial manure application and Day 7 (6 to 20 $\text{mg kg}^{-1} \text{d}^{-1}$) and remained constant in the majority of the amended treatments throughout the remainder of the 28-d incubation (Table 6). Spearman's correlation coefficients in soils with and without dairy slurry applications revealed

Table 3. Spearman correlations between edaphic properties and ammonia-oxidizing bacteria (AOB) copy number per gram of soil taken from Day 28 of a N incubation.†

	AOB_C	NP_D	NP_C	InOrgND	InOrgNC	SOC	TSN	pH	CEC	Sand	Silt	Clay	KK	IL	S	VR
AOB_D	0.71**	0.39	0.69**	-0.53	0.71**	0.69**	0.60*	-0.06	-0.06	-0.07	0.41	0.17	0.67*	-0.76**	0.48	0.39
AOB_C		0.41	0.86**	-0.73**	0.55	0.85**	0.78**	0.08	0.31	-0.23	0.07	0.58*	0.24	-0.35	0.60	-0.39
NP_D			0.66*	-0.64*	-0.26	0.66*	0.64*	0.88**	0.66*	-0.03	-0.31	0.31	-0.11	0.03	0.82**	-0.40
NP_C				-0.55	0.31	0.99**	0.98**	0.37	0.60*	-0.49	0.20	0.75**	0.26	-0.42	0.86*	-0.40
InOrgND					-0.23	-0.55	-0.44	-0.40	0.15*	-0.32	0.45	-0.15	0.20	-0.06	-0.50	0.40
InOrgNC						0.31**	0.20**	-0.66*	0.21**	0.08	0.49	-0.06	0.83**	-0.77**	-0.50	0.60
SOC							0.98**	0.37	0.60*	-0.48	0.20	0.75**	0.25	-0.43	1.00**	-0.40
TSN								0.40	0.70**	-0.61*	0.23	0.84**	0.20	-0.40	1.00**	-0.40
pH									0.77**	-0.03	-0.54*	0.28	-0.60*	0.43	0.50	-0.40
CEC										-0.60*	-0.20	0.81**	-0.48	0.31	0.50	-0.80*

* Significant at $P < 0.05$.

**Significant at $P < 0.01$.

† AOB_D, AOB copy number per gram soil manured treatment; AOB_C, AOB copy number per gram soil unmanured treatment; NP_D, nitrification potential manured treatment; NP_C, nitrification potential unmanured treatment; InOrgND, cumulative inorganic N (NH_4^+ , NO_3^- , NO_2^-)-N manured treatment; InOrgNC, cumulative inorganic N (NH_4^+ , NO_3^- , NO_2^-)-N unmanured treatment; KK, kaolinite; IL, illite; S, smectite; VR, vermiculite; SOC, total soil organic carbon; TSN, total soil nitrogen; CEC, cation exchange capacity.

Table 4. The effect of incubation time, soil series, and no dairy slurry on cumulative inorganic N (NH_4^+ , NO_2^- , and NO_3^-)-N kg ha^{-1} during a 28-d incubation.

Sample code	Soil series	Nitrate and nitrite (NO ₃ ⁻ + NO ₂)–N					Ammonium (NH ₄ ⁺)–N				
		Time interval†					Time interval				
		0 d	7 d	14 d	21 d	28 d	0 d	7 d	14 d	21 d	28 d
kg ha ⁻¹											
SIL_CA	Caribou	21 e‡	40 j	52 m	65 n	82 o	2.6 bc	2.2 b	6.6 d	2.8 bc	2.6 bc
L_NE	Newport	8.6 b	19 de	29 gh	41 jk	52 m	2.9 bc	2.2 b	4.3 c	2.3 b	2.9 bc
SIC_SH	Sharpsburg	19 de	24 f	28 g	28 g	32 hi	5.9 d	1.4 a	1.8 ab	1.5 ab	1.4 ab
SL_AD	Adkins	9.8 b	13 c	18 de	24 f	29 gh	2.2 b	1.5 ab	1.1 ab	1.4 ab	0.99 a
SIL_LO	Loyal	18 d	27 g	34 i	42 jk	49 i	1.8 ab	2.0 ab	1.9 ab	1.4 a	1.6 ab
SIL_RO	Roshalt	9.6 b	22 ef	32 hi	43 k	47 i	1.9 ab	1.6 ab	1.8 ab	1.3 ab	2.8 bc
SICL_BR	Brooksville	23 f	31 h	35 i	40 j	41 jk	5.4 cd	1.6 ab	1.5 b	3.4 c	1.6 ab
SIC_CA	Catlin	16 cd	21 ef	29 gh	31 h	35 i	4.0 c	1.8 ab	1.5 a	1.3 a	1.2 a
S_VA	Valentine	4.0 a	2.6 a	4.1 a	4.4 a	4.5 a	6.5 d	14 e	22 f	24 f	24 f

† Time interval of the incubation in days.

‡ Values followed by a different letter designated nitrate and nitrite or ammonium are significantly different at $p = 0.01$. Calculated via a completely randomized three factor factorial analysis of variance in SAS Proc Mixed to determine the effect of dairy slurry management \times soil series \times time of incubation during a 28-d incubation.

Table 5. The effect of dairy slurry application, soil series, and incubation time on cumulative inorganic N (NH_4^+ , NO_2^- , and NO_3^-)-N kg ha^{-1} , during a 28-d incubation.

Sample code	Soil series	Nitrate and nitrite (NO ₃ ⁻ + NO ₂ ⁻)-N					Ammonium (NH ₄ ⁺)-N				
		Time interval†					Time interval				
		0 d	7 d	14 d	21 d	28 d	0 d	7 d	14 d	21 d	28 d
kg ha ⁻¹											
SIL_CA	Caribou	22 bc‡	37 d	56 ef	98 h	86 gh	127 f	37 b	7.0 a	2.9 a	2.4 a
L_NE	Newport	7.8 ab	22 bc	42 dc	38 d	45 dc	142 g	53 c	7.6 a	3.2 a	3.2 a
SIC_SH	Sharpsburg	19 bc	65 f	100 h	112 i	113 i	145 g	52 c	5.6 a	2.3 a	1.5 a
SL_AD	Adkins	11 ab	21 bc	72 fg	85 gh	91 h	113 e	55 c	2.3 a	1.2 a	1.1 a
SIL_LO	Loyal	183 i	83 d	121 ef	136 f	122 ef	174 k	6.5 ab	3.3 ab	1.4 a	1.4 a
SIL_RO	Roshalt	18 bc	50 e	111 i	154 j	149 j	160 h	81 d	6.5 a	1.8 a	1.7 a
SICL_BR	Brooksville	25 c	84 gh	94 h	101 hi	101 hi	123 ef	32 b	1.7 a	2.0 a	1.4 a
SIC_CA	Catlin	15 bc	61 f	66 f	62 f	80 g	139 fg	2.0 a	1.7 a	1.4 a	1.1 a
S_VA	Valentine	30 cd	15 bc	14 b	12 b	9.4 ab	159 h	79 d	72 d	62 cd	50 c

† Time interval of the incubation in days.

‡ Values followed by a different letter designated nitrate and nitrite or ammonium are significantly different at $p = 0.01$. Calculated via a completely randomized three factor factorial analysis of variance in SAS Proc Mixed to determine the effect of dairy slurry management \times soil series \times time of incubation during a 28-d incubation.

that nitrification potentials were positively correlated with SOC, TSN, CEC, and smectite (Table 3).

The presence or absence of specific clay minerals as well as texture influenced nitrification rates among soil series. The SL_AD soil, which had the lowest nitrification potentials in the control treatments (Table 6), contained the greatest quantity of sand (730 g kg⁻¹) and lowest clay content (60 g kg⁻¹) (Table 2). Rates of nitrification were lower in soils high in sand and silt (Table 6). Nitrification potentials in the manured SL_AD significantly increased from time 0 to 21 d (Table 6). Between Day 21 and 28, nitrification rates dropped significantly in the manured SL_AD treatment. This was the only soil on which nitrification potentials decreased during the 28 d after manure was applied.

Soil series high in clay that contained smectite (SICL_BR and SIC_CA) had elevated nitrification potentials. The nitrification rates taken from the SICL_BR and SIC_CA soils without manure had significantly higher nitrification potentials and high total clay content (320 and 400 g kg⁻¹) (Tables 2 and 6). Smectite, a mineral that can retain and rerelease ammonium due to basal surface exchange, was the dominant or one of the dominant minerals in the SICL_BR and SIC_CA soils (Table 2). Smectite was significantly positively correlated ($p = 0.01$) with nitrification potential in both the dairy slurry and control treatments (Table 3).

The SIC_SH soil contained a similar quantity of total clay to SICL_BR and SIC_CA, but vermiculite (VR) was the dominant mineral and the pH was lower (Table 2). There was no significant correlation between VR and nitrification potentials (Table 3). The SIC_CA soil contained a mixture of clays (illite, smectite, and vermiculite) with high CEC and the potential to retain NH₄⁺. This soil had the highest nitrification rates in manured treatments and lowest pH (Tables 2 and 6). Higher pH across soil series was positively correlated with nitrification potentials (Table 3). Nitrification potentials in the SIC_CA and SICL_BR soil series were not significantly different in the absence of manuring (Table 6). The addition of manure to SIC_CA significantly increased nitrification potentials for Days 7 through 28 relative to the SICL_BR soil.

Ammonia Volatilization

As expected for soils with neutral to acidic pH values, ammonia volatilization was minimal in all soil series, ranging from 2.5 to 18 kg NH₄⁺-N ha⁻¹. The highest losses occurred in soils containing high sand concentrations; i.e., the SL_AD treatment lost 17 NH₄⁺-N kg ha⁻¹, and the S_VA treatment lost 18 NH₄⁺-N kg ha⁻¹, equivalent to approximately 10% of the NH₄⁺ applied in dairy slurry. Dairy slurry additions (pH 7.2) raised the pH of all soils by 0.1 pH unit or less.

Quantification of Ammonia-Oxidizing Archaea and Ammonia-Oxidizing Bacteria

The use of a standard curve based on known concentrations of *Nitrosomonas europaea* ATCC 19718 provided R^2 values of 0.99 to 0.98 for all curves. Quantitative PCR assays for AOB contained a significant three-way interaction among soil series \times dairy slurry management \times time of incubation that resulted in different AOB gene copy numbers per gram of soil ($p = 0.01$) (Table 7). Average AOB copy numbers in soils incubated for 28 d with and without dairy slurry tended to increase with incubation time and slurry applications. The trend of increasing AOB copy numbers was statistically significant in the no-slurry control treatments only in the SIL_CA soils (0 d, 4.73×10^6 vs. 28 d, 1.69×10^7) and was a result of time of incubation. The copy numbers of AOB decreased after 28 d of incubation in only one no-slurry treatment the SIL_RO soil (0 d, 8.30×10^6 vs. 28 d, 6.31×10^5). Copy numbers of AOB were significantly higher on Day 28 relative to time zero in the unamended SIL_CA soil (Table 7).

Ammonia-oxidizing bacteria copy numbers were highly correlated with several nitrogen parameters and negatively correlated in manured treatments with illite, a mineral with edge and interlayer sites that sorb and potentially fix NH₄⁺. Spearman's correlation coefficients indicated that AOB gene copy numbers per gram of soil were positively correlated with nitrification potential and clay content in control treatments and SOC and TSN in manured and unmanured soils (Table 3). The AOB gene copy numbers per gram of soil in manured plots were positively correlated with cumulative inorganic N and kaolinite and negatively correlated with illite (Table 3).

Table 6. Nitrification potentials for a given time interval and soil series with and without dairy slurry applications.

Sample code	Soil series	Control nitrification potentials					Dairy slurry applied nitrification potentials				
		Time interval†					Time interval				
		0 d	7 d	14 d	21 d	28 d	0 d	7 d	14 d	21 d	28 d
mg kg ⁻¹ d ⁻¹											
SIL_CA	Caribou	5 bc‡	5 bc	4 b	5 bc	5 bc	3 ab	11 ef	11 ef	11 ef	11 ef
L_NE	Newport	4 b	4 b	4 b	4 b	4 b	3 ab	9 de	11 ef	11 ef	11 ef
SIC_SH	Sharpsburg	4 b	4 b	4 b	4 b	4 b	3 ab	6 c	9 de	8 de	8 de
SL_AD	Adkins	2 ab	2 ab	3 ab	2 ab	2 ab	2 ab	8 d	13 g	17 h	12 f
SIL_LO	Loyal	6 c	6 c	6 c	6 c	7 cd	5 bc	15 gh	15 gh	15 gh	15 gh
SIL_RO	Roshalt	3 ab	4 b	4 b	4 b	4 b	1 a	6 c	6 c	7 cd	7 cb
SICL_BR	Brooksville	10 ef	11 ef	10 ef	11 ef	11 ef	10 ef	11 ef	12 f	13 g	14 g
SIC_CA	Catlin	10 ef	10 ef	10 ef	9 de	10 ef	10 ef	20 i	19 i	17 h	19 i

†Time interval of the incubation in days.

‡Values followed by a different letter are significantly different at $p = 0.01$. Calculated via a completely randomized three factor factorial analysis of variance in SAS Proc Mixed to determine the effect of dairy slurry management \times soil series \times time of incubation during a 28-d incubation.

Table 7. The effect of dairy slurry management and time since slurry application on ammonia oxidizing bacteria (AOB) gene copy number per gram soil across a range of soil series.

Sample code	Soil series	Control		Dairy slurry	
		0 d†	28 d	0 d	28 d
SIL_CA	Caribou	4.73×10^6 bc‡	1.69×10^7 a	1.82×10^7 a	2.40×10^7 a
SIC_SH	Sharpsburg	2.95×10^6 bc	4.76×10^6 bc	3.55×10^6 bc	3.60×10^6 bc
SL_AD	Adkins	3.83×10^6 bc	2.85×10^6 c	8.23×10^6 ab	6.17×10^6 b
SIL_LO	Loyal	5.37×10^6 bc	3.48×10^6 bc	7.54×10^6 ab	5.47×10^6 bc
SIL_RO	Roshalt	8.30×10^6 ab	6.31×10^6 d	3.24×10^6 bc	9.50×10^6 ab
SIC_CA	Catlin	7.47×10^6 ab	1.62×10^7 a	2.59×10^7 a	1.85×10^7 a

† Time interval of the incubation in days.

‡ Values followed by a different letter are significantly different at $p = 0.01$. Calculated via a completely randomized three factor factorial analysis of variance in SAS Proc Mixed to determine the effect of dairy slurry management \times soil series \times time of incubation during a 28-d incubation.

Application of dairy slurry did not affect AOA gene copy numbers per gram of soil (Table 8). The use of a standard curve based on known concentrations of a TOPO vector containing the *Nitrosopumilus maritimus* SCM1 *amoBAC* gene insert provided R^2 values of 0.98 to 0.99 for all curves. Ammonia-oxidizing archaea gene copies ranged from 8.12×10^4 to 2.59×10^7 g⁻¹ soil (Table 8). The length of incubation \times soil series had a significant effect on AOA gene copy numbers in two soils. Incubating soil for 28 d significantly increased AOA copy numbers in the SIL_CA soil (0 d, 1.01×10^5 e vs. 28 d, 1.56×10^6 c) and decreased AOA copy numbers (0 d, 3.77×10^5 d vs. 28 d, 8.12×10^4 e) in the SL_AD soil. All other soils were unaffected by the incubation. On average, AOB gene copies were an order of magnitude greater than those of AOA (Table 7 and 8). Ammonia-oxidizing archaea copy numbers did not correlate with dairy slurry application and most edaphic properties. Therefore, correlations of AOA copy numbers are not presented.

Discussion

Fate of Manure-Applied NH₄⁺

Mineralization of organic N and the concentration of ammonium in soil have a direct effect on plant-available N and the process of nitrification. Mineralized N in the form of ammonium derived from soil and dairy slurry can be nitrified, immobilized by microbial biomass, incorporated into organic N, fixed by clay minerals, held on exchange sites, or lost as

NH₃ and N₂O (derived from nitrification and denitrification). Previous research showed that the majority of changes in net N transformations occur during the first 28 d following manure application (Griffin et al., 2005; Honeycutt et al., 2005; Sistani et al., 2008). Ammonium concentrations in our study were negligible after 7 d in all soils except the sand (S_VA). Increases in nitrification rates and their subsequent stabilization after 7 d coincided with the disappearance of NH₄⁺.

Incubation conditions during this study favored immobilization of inorganic N in microbial biomass or retention of inorganic N by mineral components. We chose conditions that minimized gaseous losses of NH₃ and denitrification by control of soil water content and aeration. Ammonia volatilization in our study was equivalent to 10% or less of the amount of ammonium applied in the dairy slurry. Loss of ammonia from surface-applied animal slurries can be 40 to 60% of the NH₄⁺ content of slurries (Thompson et al., 1990).

Upon termination of our 28-d incubation, KCl-extractable inorganic N ranged from 42 to 131 kg ha⁻¹ in treatments amended with dairy slurry. Our data suggest the potential for fixation of NH₄⁺ on mineral exchange sites. Differences in the amount of ammonium that can interact with exchange sites and be rereleased or fixed by a clay is dependent on the amount and types of exchange sites present in a particular mineral (Sawhney, 1972). Illite is a more effective fixer of NH₄⁺ than smectite or vermiculite and may reduce the availability of NH₄⁺. Therefore, we estimated the amount of NH₄⁺ that could be adsorbed to selective sites on illite. Although only an approximation, it gives an idea of the potential “fixed” NH₄⁺ that would not be extractable by KCl and only slowly available to microorganisms. Because most fixation by clays occurs in the first days of incubation (Drury and Beauchamp, 1991), we estimated the fraction of nonextractable NH₄⁺ fixed by illite at Day 7. The fractions ranged from 0% for SICL_BR, which had 320 g clay kg⁻¹ but no illite or vermiculite, to 44% for SIC_CA with 400 g clay kg⁻¹ containing both illite and vermiculite. The potential for vermiculite fixation of NH₄⁺ could not be determined because no average data are available for the quantity of selective sites in vermiculite.

Clay minerals with high CEC and frayed edges and/or basal sites, such as smectite, can provide a consistent source of exchangeable NH₄⁺ ions (Sawhney, 1972) that will support populations of nitrifiers found in biofilms on clay surfaces (Powell and Prosser, 1991). Fluctuations in nitrification potential among time intervals and soil series may be due to

Table 8. The effect of soil and time of incubation on ammonia oxidizing archaea (AOA) gene copy number per gram of soil across a range of soil series.

Sample code	Soil series	Time interval†	
		0 d	28 d
SIL_CA	Caribou	1.01×10^5 e‡	1.56×10^6 c
SIC_SH	Sharpsburg	3.26×10^6 bc	3.06×10^6 c
SL_AD	Adkins	3.77×10^5 d	8.12×10^4 e
SIL_LO	Loyal	1.90×10^7 a	2.59×10^7 a
SIL_RO	Roshalt	1.43×10^5 de	1.42×10^5 de
SIC_CA	Catlin	9.42×10^6 b	1.24×10^7 b

† Time interval of the incubation in days.

‡ Values followed by a different letter are significantly different at $p = 0.01$. Calculated via a completely randomized three factor factorial analysis of variance in SAS Proc Mixed to determine the effect of dairy slurry \times soil series \times time of incubation during a 28-d incubation. There was a significant two-way interaction between soil series \times time of incubation.

the fixation by illite or retention of NH_4^+ by smectite and vermiculite and rerelease of NH_4^+ from smectite. Other researchers have shown fixation and retention of NH_4^+ by clay minerals to be an important source of crop N (Mamo et al., 1993). The lower pH on the SIC_SH, and the dominance of illite and vermiculite, likely contributed to lowered nitrification potentials in this particular soil. Nitrifiers have been shown to inhabit surfaces of clays to protect themselves from, and minimize the effects of, hydrogen ions, allowing for nitrification to occur for longer periods of time under suboptimal conditions (Powell and Prosser, 1991).

Can Process Level Functions such as Nitrification Be Linked to Ammonia-Oxidizing Archaea and Ammonia-Oxidizing Bacteria Gene Copy Numbers?

Ammonia-oxidizing bacteria gene copy numbers and nitrification rates were influenced by manuring, management history and edaphic properties such as soil texture, mineralogy, TSN, and SOC. The current study revealed correlations among AOB copy number, nitrification potentials, SOC, TSN, smectite, kaolinite, and illite. Smectite that potentially releases exchangeable N was positively correlated with increased nitrification potential, and illite that fixes NH_4^+ was negatively correlated with AOB copy number. Positive correlations between TSN, SOC, and CEC are likely associated with increases in NH_4^+ ions, the substrate for nitrifiers and the nitrification process. Increases in each of these edaphic properties will often increase NH_4^+ . Previous measurements of microbial community characteristics such as substrate utilization and fatty acid methyl ester profiles taken during a separate study of the SIL_CA, SIC_CA, and L_NE soils with and without manure were also highly correlated with soil properties (Larkin et al., 2006).

Ammonia-oxidizing archaea copy numbers were equal to or less than AOB copy numbers in all soils except the SIL_RO, the soil with the lowest nitrification potential. The AOB gene copies were highest in SIL_CA and SIC_CA soils. Dairy slurry applications had no effect on AOA copy numbers and did not always result in an increase in AOB gene copies despite increases in nitrification potential with dairy slurry application. These results indicated that increases in cell activity could explain a portion of the change in nitrification potential. Reported cell activities for AOB are higher than those of AOA (Herrmann et al., 2008), and AOA are reported to be more active when ammonium is limited (Taylor et al., 2010). Manure additions were shown to increase the activity per cell during a 29-d incubation at 28°C (Laanbroek and Gerards, 1991) and are known to reduce or have no effect on AOA populations (Nicol et al., 2004). Potential nitrification activity in the former study increased due to higher specific activities of nitrifying bacteria, but the quantity of nitrifiers was not significantly different in manured and unmanured treatments (Laanbroek and Gerards, 1991). An additional study determined that rapid changes in NH_4^+ -N concentrations had an effect on the rate of substrate oxidation and bicarbonate uptake in several species of AOB (Belser, 1984). Rates were independent of nitrifier growth rates. High concentrations of NH_4^+ and bicarbonate from liquid manure applied to a 20-d incubation increased the extent and duration of nitrification (Petersen et al., 1991).

Application of dairy slurry may have had a similar effect during our incubation.

Prior land-use management may have had an important effect on nitrification and AOB copy numbers. The SIC_CA soil had one of the highest initial numbers of AOB gene copies before application of slurry. This soil had been cropped, but not for the past 7 yr. Prior fertilizer applications may have caused a change in AOB community composition and/or biomass such that the AOB population could respond immediately to NH_4^+ inputs without an increase in gene copies. A study from Rothamsted revealed that despite low AOB activity before fertilization, application of ammonium nitrate caused an immediate response to NH_4^+ additions, resulting in increased nitrification in wheat plots (Mendum et al., 1999). In the Rothamsted study, nitrifier populations had maintained a high metabolic potential despite previous static conditions. Ammonia-oxidizing bacteria population growth rates were 0.5 to 1 cellular division per week (Mendum et al., 1999). Gene copy numbers of AOB remained constant or increased slightly in all soils receiving manure with the exception of the SIL_RO soil. The SIL_RO soil had low nitrification potentials even in the presence of manure NH_4^+ , the least number of AOB and the highest number of AOA. These results suggest that AOA in this soil may inhabit niches unfavorable to AOB.

The current and continuing debate over the role of AOA in terrestrial N cycling is important. Estimates of oxidation rates per cell in fertilized and no-input systems indicate the potential for an additional community of nitrifiers. Estimates of oxidation rates per AOB cell in microcosms amended with 1.5 and 7.5 mM NH_4^+ ranged from 0.5 to 25 fmol of NH_4^+ h⁻¹ cell⁻¹ (Okano et al., 2004). These rates and estimates used by Boyle-Yarwood et al. (2008) indicate that the majority of nitrification that occurs in soil can be attributed to AOB populations. Our research indicates that AOA gene copy numbers tended to be lower than those of AOB and did not respond to manure N additions. Reported cell activities for AOB are higher than those of AOA (Herrmann et al., 2008). Therefore, equal numbers of AOB and AOA copies would not contribute equally to nitrification rates, which provides further evidence that AOB is likely to be the predominant nitrifier in our soils. Our data indicate that soil properties such as mineralogy, TSN, SOC, CEC, and cumulative inorganic N, as well as manure management, affect AOB copy numbers and nitrification rates due to their impact on environmental conditions and the quantity and duration of the ammonium supplied to nitrifiers.

Despite such findings, nitrification rates from our study and others would allow for a potential contribution of AOA. Recent work conducted across fallow, pasture, and forest systems suggests that in certain niche ecosystems, AOA may be the sole contributor or the dominant source of nitrification (Taylor et al., 2010). Nitrifying archaea are found in agro-nomic systems that receive higher N inputs than grassland and forest systems (Taylor et al., 2010). However, activity of AOA in agricultural soils has not been shown to respond to, or to remain constant, after ammonia addition (Di et al., 2010; Jia and Conrad, 2009). In contrast to AOB, which are obligate chemoautotrophs (Koops and Pommerening-Röser, 2001), research conducted by Zhang et al. (2010) indicates that AOA isolated and cultured from soil have the ability to grow on an

organic source of C, pyruvate, and grow more effectively in mixtures of other microorganisms. This research thus indicates that soil AOA may not be strict autotrophs that use CO₂ only. For the soils described in this study, our data indicate that nitrification rates were controlled by AOB.

Conclusions

This study provides new information with respect to the microbial ecology of nitrifiers and their niche across a range of manure-amended soils of varying edaphic properties. Currently, authors can find no other studies that compare AOA and AOB communities across as wide a range of soils under laboratory or in situ conditions. Our data indicate that soil properties such as mineralogy, TSN, SOC, and CEC, as well as manure management, affect AOB copy numbers and nitrification rates due to their impact on environmental conditions and the quantity and duration of the ammonium supplied to nitrifiers. Smectite, which has the potential to release exchangeable N, was positively correlated with increases in nitrification rates, and illite, with the potential to fix NH₄⁺, was negatively correlated with AOB copy number. Ammonia-oxidizing archaea gene copies were lower or equal to those of AOB and did not increase after dairy slurry additions. The one soil in which AOA copy numbers were higher than AOB had the lowest nitrification potential. Our research suggests that AOB are the dominant nitrifiers in these predominantly fallow agricultural soils. These two nitrifier communities inhabit the same environment but respond differently to soil properties and vary in their growth conditions and potential contribution to nitrification rates.

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