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Ammonia oxidizer populations vary with nitrogen cycling across a tropical montane mean annual temperature gradient

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Abstract. Functional gene approaches have been used to better understand the roles of microbes in driving forest soil nitrogen (N) cycling rates and bioavailability. Ammonia oxidation is a rate limiting step in nitrification, and is a key area for understanding environmental constraints on N availability in forests. We studied how increasing temperature affects the role of ammonia oxidizing archaea (AOA) and bacteria (AOB) in soil N cycling and availability by using a highly constrained natural mean annual temperature (MAT) elevation gradient in a tropical montane wet forest. We found that net nitrate (NO_3^-) bioavailability is positively related to MAT ($r^2 = 0.79$, $P = 0.0033$), and AOA DNA abundance is positively related to both NO_3^- availability ($r^2 = 0.34$, $P = 0.0071$) and MAT ($r^2 = 0.34$, $P < 0.001$). In contrast, AOB DNA was only detected in some soils across the gradient. We identified three distinct phylotypes within the AOA which differed from one another in abundance and relative gene expression. In addition, one AOA phylotype increased in abundance with MAT, while others did not. We conclude that MAT is the primary driver of ecosystem N availability across this gradient, and AOA population size and structure appear to mediate the relationship between the nitrification and N bioavailability. These findings hold important implications for nutrient limitation in forests and feedbacks to primary production under changing climate.

Key words: ammonium monooxygenase; amoA; *biogeochemistry; climate change; gene expression; nitrification; nutrient limitation; qPCR; RNA; tropical elevation gradient.*

Introduction

Ecosystem responses to global change are greatly influenced by the cycling and availability of nutrients. Nitrogen (N) availability often limits primary production and influences terrestrial ecosystem structure and function (Vitousek and Howarth 1991, Thomas et al. 2013). The bioavailability of N (i.e. accessibility for root and microbial uptake) is therefore closely tied to ecosystem carbon (C) accumulation and cycling (Giardina et al. 2001, 2003). Nitrification is a critical step in the N cycle that transforms ammonium (NH_4^+) to nitrate (NO_3^-). Following nitrification, N can be immobilized in microbial biomass, taken up by roots, or lost through hydrologic or gaseous pathways (Kaye and Hart 1997, Vitousek et al. 1997). Nitrification is mediated by several microbial functional groups (De Boer and Kowalchuk 2001, Isobe et al. 2011). Community composition and relative gene expression (i.e. quantity of RNA transcripts produced per DNA gene copy) of these groups can modulate N cycling dynamics (Wilmes and Bond 2006, Blazewicz et al. 2013, Bowen et al. 2014), and may influence ecosystem C cycling as abiotic conditions change (Litton and Giardina 2008). Despite evidence that specific microbial functional groups are key regulators of N cycling rates (Webster et al. 2005, Baldrian et al. 2012, Taylor et al. 2012, Litchman et al. 2015) no research to date has generated a mechanistic understanding of how functional groups and related N fluxes respond to environmental change *in situ* (Bissett et al. 2013, Sundqvist et al. 2013).

Natural environmental gradients have proven useful in understanding ecosystem responses to global change (Vitousek and Matson 1991, Fukami and Wardle 2005, Malhi et al. 2010). Natural covariation of ecophysiological drivers (e.g. moisture; precipitation; temperature; pH) across gradients has caused researchers to rely on laboratory and field manipulations to study variables in isolation (Melillo et al. 2002, Horz et al. 2004, Bowen et al. 2014). Despite the insights these approaches have provided (Torsvik and Øvreås 2002, Fuhrman 2009, Schimel 2016), the unique roles of environmental drivers in gene-flux linkages under natural conditions remain unclear. To address ecosystem responses to temperature, well-constrained elevation gradients have become a

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common tool (Vitousek et al. 1988, Raich et al. 1997, Knoepp and Swank 1998, Schuur and Matson 2001, Salinas et al. 2011, Girardin et al. 2013, Giardina et al. 2014), but the influence of mean annual temperature (MAT) has been difficult to parse from its covariates, such as precipitation, plant diversity, and soil age (Austin and Sala 2002, Litton et al. 2011, Sundqvist et al. 2013, Giardina et al. 2014, McCalley et al. 2014).

To overcome the common issues facing elevation gradients, this study takes advantage of a unique tropical wet montane elevation gradient with a 5°C range in MAT, where abiotic and biotic variables besides MAT remain constant (Litton et al. 2011). This uniformity is possible because tephra-derived parent material from a single volcanic event along the gradient allows for uniform soil type and age (Litton et al. 2011). The isolation of the Hawaiian islands has allowed for consistent vegetation composition along the gradient, in addition to constant soil pH and water content (Litton et al. 2011, Table 1). The Hawaii MAT gradient provides a means of holding the major ecophysiolgical drivers of N cycling constant (e.g. climate, plant community composition and edaphic properties) (Carney et al. 2004, Booth et al. 2005, Wallenstein et al. 2006, Petersen et al. 2012), while providing a context in which the positive relationship between MAT and C cycling has been well characterized (Litton et al. 2011, Iwashita et al. 2013, Bothwell et al. 2014, Giardina et al. 2014, Selmants et al. 2014).

Temperature limits microbial N turnover kinetics (Zak et al. 1999), nitrifier community structure (Avrahami et al. 2003), and nitrifier function in diverse environments (Osborne et al. 2015). Ammonia oxidation (AO), the conversion of ammonia (NH_3) to nitrite (NO_2^-), is the rate limiting step of nitrification and is performed by ammonia oxidizing bacteria (AOB) and archaea (AOA) (Kowalchuck and Stephen 2003). Ammonia oxidation is performed by obligate aerobes and is a pH-sensitive, metabolic process (Francis et al. 2007). Its sensitivity may have important implications for N bioavailability to primary producers as well as N mobility in soils (Hallin et al. 2009). Ammonia oxidation is regulated by the gene *amoA*, which encodes the ammonia monooxygenase small alpha subunit (Rotthauwe et al. 1997). The *amoA* gene is ubiquitous in terrestrial and aquatic systems (Rotthauwe et al. 1997, Fierer et al. 2009), and because it is phylogenetically constrained within the Betaproteobacteria and Proteoarchaeota, it is a tractable target in environmental samples (Purkhold et al. 2000). Many studies have targeted *amoA* in soils, but most have relied on artificial warming to understand the role of temperature in AO and nitrification regulation via *amoA* (Avrahami et al. 2003, Horz et al. 2004, Tourna et al. 2008, Osborne et al. 2015). The relationship between temperature and microbial AO regulation has not been clearly predictable because while AOA appear more responsive to temperature than AOB in some cases (Tourna et al. 2008, Taylor et al. 2012), these responses may depend on phylogenetic clustering, possibly due to phenotypic adaptation to environment, within and among AOA clusters (Avrahami and Conrad 2003, Horner-Devine and Bohannan 2006, Tourna et al. 2008). Short-term warming approaches may obscure the importance of long-term, in situ differences in mean annual temperature (MAT) that may shape microbial communities and their potential activity (Osborne et al. 2015).

We aimed to answer the following questions: Does N availability change systematically across the elevation (MAT) gradient? Do the abundance and expression of AOA and AOB *amoA* in soils vary predictably across MAT? Do *amoA* abundance and expression predict the availability and cycling of N with MAT? Studies at this MAT gradient site have shown that plant belowground carbon (C) flux increases with MAT (Giardina et al. 2014). This suggests that increased rhizosphere C flux with MAT may differentially stimulate microbial activity along the gradient (Kuzyakov et al. 2007, Brzostek et al. 2013, Finzi et al. 2015). Further, litter N loss

TABLE 1. Elevation, mean annual temperature (MAT), mean annual precipitation (MAP), and soil properties measured in eight plots along a mean annual temperature gradient along the northeast slope of the Mauna Kea volcano on the Big Island of Hawai'i, USA. Mean (± SD) values for soil properties from 0–10 cm depth encompassing the Oe-Oa horizons. All values presented represent subsamples taken from a pool of five soil cores at each plot. Three plots were excluded from potential net N mineralization and nitrification measurements because representative samples were destroyed in transport. Soils were collected in October 2014 and August 2015.

Plot	Elevation (m a.s.l.)	MAT (°C)	MAP (mm)	Soil pH	Microbial biomass N (μg-N g dry soil^{-1} d^{-1})	Soil N stock (0–10 cm; g N m^{-2})	Soil C stock (0–10 cm; g C m^{-2})	Potential net N mineralization (μg-N g dry soil^{-1})	Potential net nitrification (μg-N g dry soil^{-1})
SPE800	800	18.2	4204	4.1	NA	162.6 (75.0)	4066.7 (1382.3)	NA	NA
SPE934	934	17.3	4133	4.2	493.186	291.2 (105.0)	4400.1 (1581.0)	−1.349	2.126
SPE1024	1024	16.5	4043	3.7	407.787	152.5 (88.2)	2562.4 (1248.0)	−3.714	0.528
SPE1116	1116	16.1	3988	3.8	NA	157.3 (108.0)	2662.8 (1923.1)	NA	NA
WPL1204	1204	15.5	3521	3.7	368.976	192.1 (102.9)	4139.4 (2577.6)	−3.007	−2.147
WPL 1274	1274	15.1	3448	3.9	NA	29.0 (4.6)	3674.2 (719.1)	NA	NA
HAK1468	1468	13.8	3488	4.2	354.644	151.6 (12.1)	2958.3 (326.5)	−5.181	−7.109
HAK1600	1600	13	3195	4.1	339.448	311.9 (283.4)	5666.0 (5020.1)	−2.489	−2.426

Notes: NA = data not available for this measurement.

rates during decomposition increase with MAT along this gradient (Bothwell et al. 2014), supporting the notion that N cycling may vary predictably with MAT (Rustad et al. 2001, Bai et al. 2013). We hypothesized that N bioavailability and rates of potential net nitrification and net N mineralization in soils would increase with MAT (Melillo et al. 2011, Bai et al. 2013). Following this, we hypothesized that the abundance and normalized gene expression (cDNA:DNA) of AOB and AOA *amoA* increase with MAT, given the positive effects of warming on microbial metabolic efficiency (Price and Sowers 2004, Allison et al. 2010) and transcriptional activity (Avrahami et al. 2003, Tourna et al. 2008). Finally, we hypothesized that there would be no significant difference between the responses of AOA and AOB communities to MAT in the field.

Methods

Study site

The elevation gradient is comprised of nine 20 × 20 m plots forming an 800 m gradient that corresponds to a ~5.2°C MAT gradient (13.0 to 18.2°C). This gradient is located on the northeastern slope of the Mauna Kea Volcano on the Island of Hawaii (Table 1). Seven plots are located in the Hawaii Experimental Tropical Forest (HETF; 19°56′41.3″ N, 155°15′44.2″ W; 600–1800 m.a.s.l) and two high elevation plots are located in the Hakalau Forest National Wildlife Refuge (HFNWR; 19°50′31.3″ N, 155°17′35.2″ W; 600–2000 m.a.s.l) (Litton et al. 2011). For this study, we sampled from eight of nine plots because of limited time and capacity. All plots are located within tropical montane wet forests characterized as *Metrosideros polymorpha* Gaudich.–*Acacia koa* A. Gray forests. *M. polymorpha* and *Cheirodendron trigynum* (Gaudich.) A. Heller dominate the canopy and midstory, respectively, across all plots (84–97% of basal area excluding tree ferns), while tree ferns (*Cibotium* spp.) make up approximately half of total stand basal area in all plots (Litton et al. 2011).

Soil water balance is relatively constant across all plots because annual precipitation and evapotranspiration declines with increasing elevation (Litton et al. 2011). Substrate in all plots is derived from ~20 ky (14–65 ky) weathered tephra (Giardina et al. 2014). Soils are moderate to well-drained hydrous, ferrihydritic/amorphic, isothermic/isomesic Acrudoxic Hydrudands of the closely related Akaka, Honokaa, Maile, and Piihonua soil series (Soil Survey Staff 2010). Mean soil pH is 3.9 and varies little with MAT (±0.3; Table 1). Base saturation and estimated mean cation exchange capacity are 32.4(±10.56) % and 11.9(±2.75) cmol kg^{-1} (measured to 9.5 cm depth), respectively (Litton et al. 2011). Mean bulk density across plots is 0.21(±0.07) g cm^{-3} (Litton et al. 2011), mean surface organic matter (Oa-Oe horizons) N content is 29.59(±11.09) Mg N ha^{-1} and mean C content is 1499.68 (±1626.17) Mg C ha^{-1}.

Nitrogen bioavailability

Bioavailable inorganic N was measured using Plant Root Simulator (PRS™) resin probes (WesternAg Innovations, Saskatoon, SK). Three pairs of cation and anion-adsorbing probes were buried to 8 cm depth at random locations in the plots to measure total bioavailable NH_4^+ and NO_3^- (Western Ag Innovations, PRS™ probe Operations Manual). Probes were collected after an average of 17 d, cleaned with distilled water to remove soil and shipped to the manufacturer for extraction. Ions adsorbed to the resin probes were analyzed colorometrically and the net rate of nutrient supply is expressed here in mg m^{-2} d^{-1}. Probes were grouped at the plot level and extracted together. Individual values represent the pooled extractions for three probes from each plot (See Fig. 1).

Potential net nitrogen mineralization and net nitrification

Total inorganic N (TIN), potential net N mineralization (PM) and potential net nitrification (PN) were determined for five of eight MAT gradient plots sampled in this study (Table 1) using the chloroform fumigation-incubation method (Jenkinson and Powlson 1976, Durán et al. 2013). In October 2014, five replicate soil cores (5 cm wide × 25 cm deep, OiOe-OaA) were removed from randomized locations in eight MAT gradient plots and were stored in plastic bags on ice in the field. Within 2 h, these cores were taken to the lab and stored at 4°C for approximately 3 d until processing. Cores from each plot were then carefully homogenized by hand to remove roots, woody debris and rocks, and pooled into one sample per plot. From each pooled sample, soil gravimetric water content was determined on a subsample by drying at 60°C for 5 d. An initial 7 g soil subsample from the homogenized pool was shaken with 30 mL 2 M KCl for 1 h and filtered using Whatman #1 filters. Filtrate was stored at 4°C. A 10 ± 0.05 g subsample of the homogenized soil from each plot was then fumigated with chloroform in a dessicator for 12 h to lyse microbial cells and the fumigated samples were then inoculated with 0.2 ± 0.05 g of fresh soil from the remaining unfumigated homogenized soil. Inoculated samples and 10 ± 0.05 g unfumigated control samples were incubated for 10 d at 16°C. After incubation, samples were shaken with 10 mL of 2 M KCl for 2 h and filtered using Whatman #1 filters. TIN (NH_4^+ + NO_3^-) was quantified from KCl extracts colorimetrically using a flow injection analyzer (Lachat QuickChem 8100). The amount of NH_4^+ released during the 10 d incubation was assumed to be directly proportional to the amount of microbial biomass in the original (unfumigated) sample; however, no correction was applied for the representation of relative microbial biomass N (Durán et al. 2013). PM was calculated as the accumulation of TIN over the 10-d incubation of unfumigated samples and PN was calculated as the accumulation of NO_3^- over the 10 d incubation.

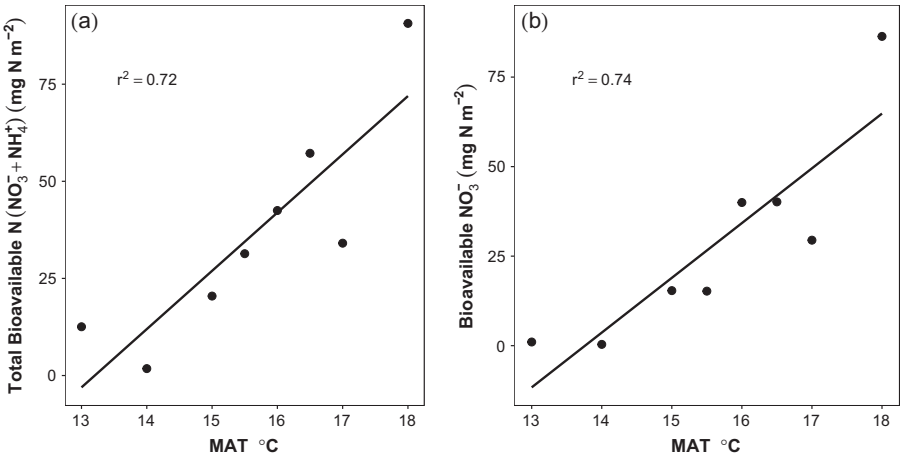

FIG. 1. The positive relationship between NO_3^- bioavailability and MAT in situ (1a; $r^2 = 0.72$, $P = 0.003$) drives the relationship between bioavailable N ($NO_3^- + NH_4^+$) and MAT across the elevation/mean annual temperature (MAT) gradient (1b; $r^2 = 0.74$, $P = 0.047$).

Soil sampling and DNA/RNA extraction

A 10 g subsample was taken from the homogenized soil pool for each of eight MAT plots for DNA extraction and stored on ice in the field. Within 12 h of collection, each core from each plot was separately homogenized by hand into bulk samples and a subsample was stored at −22°C until extraction. DNA was extracted from ~2.3 g soil subsamples using the ZR Soil Microbe DNA Midiprep Kit according to manufacturer instructions (Zymo Research Corporation, Irvine, CA). Four spatially independent (~10 m separation) soil samples were taken from 0 to 10 cm depth, placed on ice, stored in liquid nitrogen within 1 h of collection, and then at −80°C in the laboratory until RNA extraction. Soil RNA was extracted from ~200 mg subsamples using the ZR Soil/Fecal RNA MicroPrep kit according to manufacturer's instructions (Zymo Research Corporation, Irvine, CA). cDNA was synthesized in duplicate from RNA samples (diluted to 10% in nuclease free H_2O) using the SuperScript® III First Strand Synthesis System for RT-PCR according to manufacturer's intructions (Life Technologies, Carlsbad, CA).

PCR amplification and amoA *sequencing*

PCR amplification of archaeal and bacterial *amoA* genes was conducted using general published primer sequences from Francis et al. (2005) and Rotthauwe et al. (1997), respectively (see Supplemental Material). Initial PCR reaction volumes were 25 μL (see Appendix S1 for reaction mixture). PCR products were then separated using gel electrophoresis and PCR products were cloned in the laboratory and sequenced at the Cornell Bioinformatics Facility (see Supplemental Material). We confirmed the identities of the *amoA* gene sequences against the non-redundant protein databases at NCBI using the BLASTn algorithm (www.ncbi.nlm.nih.gov/blast, Altschul et al. 1997). We then designed qPCR primers and probes around based on recovered sequences from three phylotypes most highly represented in clone libraries (Appendix S1: Table S1).

Quantitative PCR with DNA and cDNA

Quantitative PCR (qPCR, "real time PCR") was performed with DNA and cDNA templates extracted from soils to estimate abundance of *amoA* copies in each plot. Quantification was based on the fluorescent probe SYBR-Gold (Molecular Probes). We used AOB and AOA *amoA* primers designed based on sequences amplified by previously published primers (Rotthauwe et al. 1997, Francis et al. 2005). Reactions were performed using a StepOnePlusTM Real-Time PCR system (Applied Biosystems). Standards for AOA *amoA* and AOB *amoA* were prepared from synthesized oligonucleotide standards and were analyzed in a serial dilution over eight orders of magnitude to generate a standard curve. StepOnePlus (v.2.3) software automatically calculated the cycle threshold (C_T) values for all reactions (see Appendix S1) (Applied Biosystems). In order to isolate dominant AOA phylotypes, PCR products from each site were then cloned using the pGEM®-T vector system and vectorized *amoA* DNA fragments were extracted and sequenced.

Phylogenetic analysis

Sequences were first trimmed of primers and vector and alignment was performed by ClustalW. They were then compared by BLASTn against the non-redundant database at NCBI (Madden 2013). From there, the closest cultivated and uncultivated relatives were imported into the alignment. The tree was constructed using neighbor joining and UPGMA in ClustalX, where tree topology was bootstrapped with 1,000 iterations. The tree was then viewed and edited in TreeViewX. The tree

is rooted to the closest archaeal cultivated representative, *Nitrosopumilus marinus*. The primers and probes for qPCR used to target the dominant AOA *amoA* phylotypes in soil samples were designed in Primer3 (Koressaar 2007) and were synthesized by Eurofins Genomics.

Statistical analysis

Relationships among MAT, bioavailable NH_4^+ and NO_3^- from resin probes, PM and PN rates from laboratory incubations, and *amoA* abundance and relative expression were determined using Spearman correlation and linear regression models. Diagnostic tests for data conformance to assumptions of normality were performed and data were log transformed as needed. A partial *F* test was used to compare a full model using all measured variables to predict bioavailable NO_3^- to reduced models to determine the main drivers of NO_3^- availability. Bartlett's test and the Fligner-Killeen test were applied to determine the equality of variances in mean gene copy numbers from qPCR with DNA and cDNA among different *amoA* phylotypes. Analyses of variance were then conducted to determine significant differences among phylotype abundance, expression and response to MAT.

Results

Soil nitrogen availability and cycling

Soil NO_3^- availability strongly increased with MAT ($r^2 = 0.75$, $P = 0.0033$, Fig. 1a). The estimated rate of total nitrogen ($NO_3^- + NH_4^+$) bioavailability increased with MAT across the gradient ($r^2 = 0.42$, $P = 0.047$; Fig. 1b), whereas soil NH_4^+ availability was not correlated with MAT ($P = 0.26$). Potential net N mineralization and PN showed no relationship to increasing MAT (Table 1). Potential net N mineralization was positively related to TN bioavailability ($P = 0.041$), and PN was not related to TN bioavailability (Table 1).

Ammonia oxidizer abundance and expression

The AOA *amoA* gene was PCR amplified from soils from all the MAT gradient plots and could be quantified via qPCR and RTqPCR in all soil samples. The AOB *amoA* gene could only be PCR amplified in a small subset of samples, and in these samples, AOB genes were found in very negligible quantities via qPCR. Thus, AOA appear to be the dominant domain of ammonia oxidizers in these tropical wet montane forest soils. Our results suggest that MAT and AOA *amoA* abundance are the primary drivers of NO_3^- bioavailability in this system. That is, the abundance of AOA *amoA* gene copies increased linearly with MAT ($P = 0.00024$, Fig. 2a) and was also strongly correlated with total NO_3^- availability ($P = 0.0071$, Fig. 2b). In contrast, the absolute expression of the AOA *amoA* gene was not related to MAT ($P = 0.106$). A partial *F* test revealed that NO_3^- bioavailability was best predicted by a reduced linear model only including the interaction between MAT and AOA *amoA* abundance variables ($P < 0.05$), while PN and normalized *amoA* expression were not significantly ($P = 0.194$) correlated with MAT across the gradient. Further, MAT was not predictive of the normalized expression of AOA *amoA* across the gradient ($P = 0.573$).

Community structure

Among the AOA *amoA* sequences detected, three unique phylotypes (described here as Phylotype 1, Phylotype 2, and Phylotype 3) (Fig. 3) were detected. Phylotype 1 and Phylotype 3 were most abundant, and

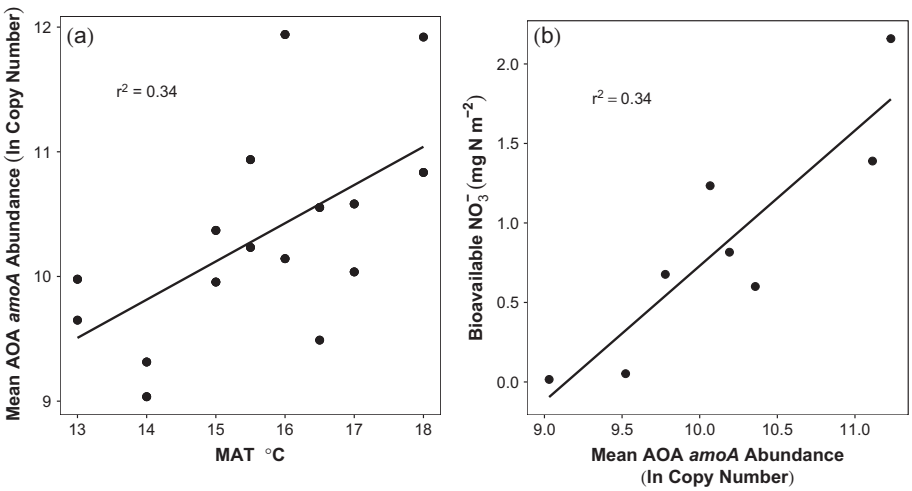

FIG. 2. Abundance of ammonia oxidizing archaea (AOA) *amoA* gene (log transformed copy number) is positively correlated with mean annual temperature (MAT) (2.a.; $P = 0.00024$, $r^2 = 0.34$). AOA *amoA* abundance is also positively correlated with total bioavailable NO_3^- (2.b.; $P = 0.0071$, $r^2 = 0.34$).

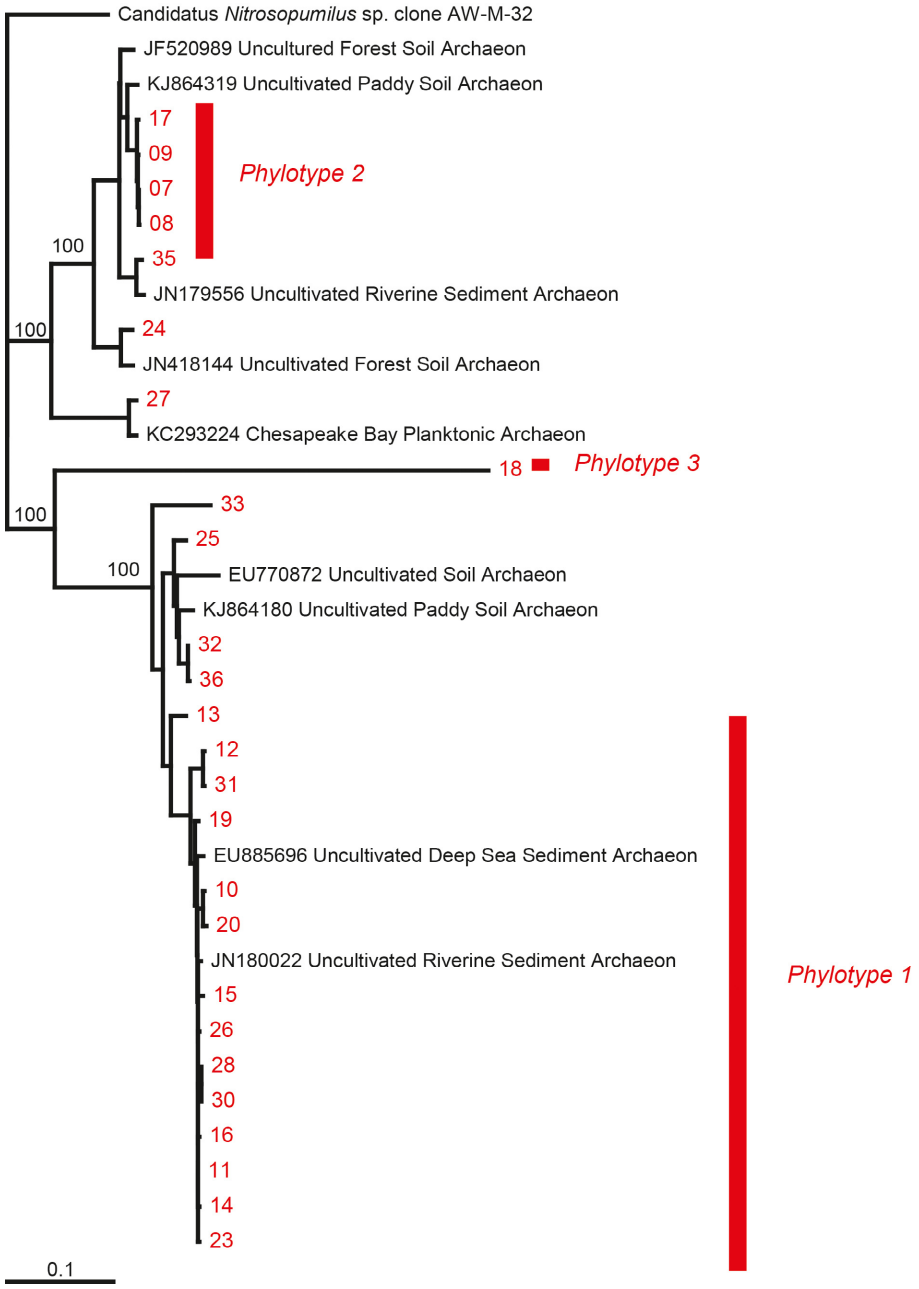

FIG. 3. Phylogeny of AOA *amoA* DNA sequences from soils sampled at nine sites along the Mauna Kea, Hawaii MAT gradient, sampled in August 2014. [Color figure can be viewed at wileyonlinelibrary.com]

TaqMan qPCR primers and probes were designed around these two major phylotypes and one additional clone to understand abundance across sites (see Supporting Information). Quantitative PCR with DNA revealed differences ($P = 0.0019$) in DNA abundance among the three dominant phylotypes detected. Only Phylotype 3 showed differences in DNA abundance across MAT ($P = 0.0017$, $r^2 = 0.39$). Relative expression among the three phylotypes differed (Fig. 4b; $P = 0.029$).

DISCUSSION

Nitrogen cycling and bioavailability across the MAT gradient

We found clear evidence that soil N availability increases with MAT in this tropical montane wet forest. In particular, both total inorganic N and NO_3^- availability increased significantly with MAT (Fig. 1). Patterns of N cycling and bioavailability across natural MAT

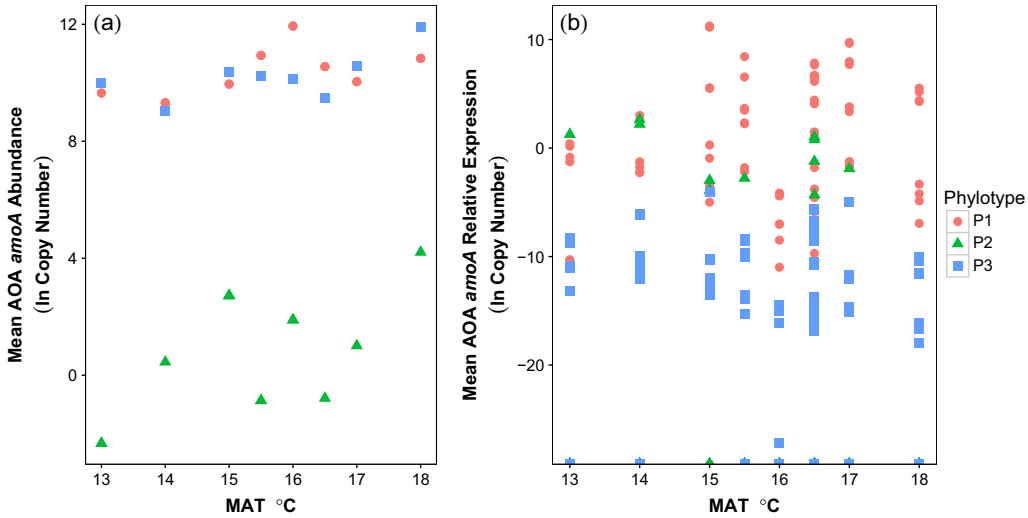

FIG. 4. Abundance of AOA *amoA* quantified using DNA (3a) and relative expression of AOA *amoA* (cDNA:DNA) (3b). DNA abundances (3a) differ significantly ($P = 0.0019$) among Phylotype 1 (circles), Phylotype 2 (triangles), and Phylotype 3 (squares). Relative expression (3b) differs significantly ($P = 0.029$) among the three observed phylotypes. [Color figure can be viewed at wileyonlinelibrary.com]

gradients have been previously investigated (Martinson et al. 2012, Durán et al. 2016), but the patterns observed have been inconsistent, likely due to differences among field sites and experimental design. Interactions among MAT, mean annual precipitation (MAP) (Idol et al. 2007), vegetation composition (Fissore et al. 2008), and soil characteristics (Smith et al. 2002), among other variables, can confound results pertaining to the specific effects of MAT. Previous studies which have focused on identifying mechanisms driving the relationship between N bioavailability and MAT (Bohlen et al. 2001, Groffman et al. 2009, Fisk et al. 2010, Martinson et al. 2012, Durán et al. 2016) have suggested that plant N demand increases with MAT, leading to decreased N bioavailability. An alternative to this hypothesis suggests that increasing MAT influences reaction kinetics in microbial processes, thereby increasing N cycling and bioavailability (Price and Sowers 2004). These alternative hypotheses imply MAT has a generalizable effect on biogeochemical processes, rather than a specific effect on ammonia oxidizers. The work presented here provides evidence of a microbial source of variation in N bioavailability across MAT, and illustrates the significance of responses in ammonia oxidizer community structure.

Ammonia oxidizer community abundance

We hypothesized that bacterial and archaeal ammonia oxidizer abundance and expression of *amoA* genes would increase linearly with MAT. This hypothesis is predicated on the observations from this gradient that primary productivity, total belowground C allocation and microbial N metabolism all increase with temperature (Litton et al. 2011, Bothwell et al. 2014, Giardina et al. 2014). N recycling through the detrital food web would thus be expected to increase with temperature, resulting in a higher supply of mineral N to nitrifiers. Our findings partially supported this hypothesis. We observed that AOA *amoA* gene abundance was positively related to MAT (Fig. 2a), which, to our knowledge, is the first finding of its kind along a natural elevation gradient. Moreover, *amoA* abundance was strongly correlated with soil nitrate availability. The relationship between *amoA* abundance and soil PN was weaker than that between *amoA* abundance and nitrate availability, possibly due to the low sample size for PN measurements ($n = 5$). While soil moisture and pH are important PN drivers, these variables are fairly constant across this MAT gradient (Table 1). We suggest that factors which interact to alter soil C:N, such as MAT and litter C:N at this site (Litton et al.2011, Bothwell et al.2014), or mycorrhizal colonization across temperature (Monz et al. 1994, Heinemeyer and Fitter 2004), may dampen the response of PN to MAT.

Our results are consistent with previous studies of temperature effects on PN and ammonia oxidizer community dynamics, which have shown that while PN rates, AOA abundance, and AOA community structure change with increasing temperature, AOB abundance and community structure remain unchanged (Tourna et al.2008, Avrahami and Bohannan 2009). These and other temperature manipulation studies suggest that AOA are more responsive to increasing temperature while AOB are less well acclimated to warming environments and become less involved in nitrification as temperatures increase. Habitat pH, nutrient deposition, dissolved oxygen, and soil texture determine influence the relative dominance of AOA vs. AOB, and phylogenetically structure these communities (De Boer and Kowalchuk 2001, Schmidt et al. 2007, Hansel et al. 2008, Erguder et al. 2009). Variability among AOB responses to temperature

may result from methodological differences between laboratory and field warming studies (Ågren and Bosatta 2002), or may result from differences among AOB microhabitats among studies.

In this study, AOB *amoA* gene amplification was only detectable in a small number of soil samples in a subset of gradient plots (SPE800, SPE934, SPE1024, SPE1116; see Table 1), and were not quantifiable via qPCR. This suggests that AOB are not the dominant ammonia oxidizers in this ecosystem, or that the AOB *amoA* sequences in these soils are significantly different from those targeted by published primers (Rotthauwe et al. 1997). Low or absent AOB copy numbers have been reported for other undisturbed terrestrial (Leininger et al. 2006, Adair and Schwartz 2008, Prosser and Nicol 2008, Zhang et al. 2012) and aquatic (Bowen et al. 2014) environments, and several studies point to AOA as the dominant ammonia oxidizers in most natural environments (Leininger et al. 2006, Nicol and Schleper 2006).

Biogeographic differences between AOB and AOA may explain why we did not observe quantifiable AOB populations in this tropical wet montane forest system, where the coldest site studied experiences 13°C MAT. Avrahami and Conrad (2003) hypothesized that AOB may dominate cold environments, and others have shown that under certain habitat conditions, AOB may be minimally involved in nitrification, and therefore unresponsive to environmental change (Jordan et al. 2005). Our observations are in line with those of Tourna et al. (2008) which show that AOA, and not AOB, are responsive to increasing temperature. Certain compounds, such as acetylene, anthropogenic pollutants and secondary plant metabolites, can inhibit heterotrophic and autotrophic AOB nitrification, and may explain their apparent lack of temperature response in some environments (Pedersen et al. 1999, Kowalchuk and Stephen 2001). The abundance and structure of AOB communities appears to strongly relate to MAT at larger geographic scales (Fierer et al. 2009). This observation lends further support to our hypothesis that MAT is the primary abiotic factor structuring AO communities and their ecosystem function. Taken together, the findings in this study and others suggest that there may be broader, biogeographic niche specialization between AOA and AOB related to MAT (Fierer et al. 2009), and habitat-scale niche specialization within these groups at different MAT (Nicol and Schleper 2006, Erguder et al. 2009, Fierer et al. 2009, Martens-Habbena et al. 2009).

We identified three phylotypes of AOA in soils across the gradient (Fig. 3). Phylotypes 1 and 3 are sister taxa, and are most related to uncultivated archaea from paddy soils and forest soils. Other relatives to Phylotype 1 include aquatic sediment archaea (Fig. 3). The Phylotype 2 outgroup is most closely related to uncultivated archaea from paddy soils and forest soils. The closest identified relative to the archaea in these phylotypes is in the genus *Candidatus* Nitrosopumilus (Fig. 3). From this, we conclude that organisms targeted in this study are chemolithoautotrophic ammonia oxidizers of the phylum Thaumarchaeota (Nicol and Schleper 2006). We observed differences in abundance among the three phylotypes across MAT (Fig. 4a). Previous studies have shown similar responses in ammonia oxidizer community structure with changing temperature (Avrahami and Conrad 2003, 2011, Tourna et al. 2008). The elevated abundance of AOA and certain AOA phylotypes, as seen in Phylotype 3 abundance across MAT (Fig. 5), has been attributed to differences in the maximum specific growth rate and saturation constant of different taxa within the AOA group, in addition to the generally greater sensitivity of AOA to increasing temperature (Prosser and Nicol 2008, Tourna et al. 2008, Litchman et al. 2015). The response of Phylotype 3 to MAT, and lack of significant response to N bioavailability, also support our suggestion that AOA experience within-group phylogenetic niche specialization at the habitat scale in response to temperature. While our results suggest that AOA abundance may drive N bioavailability across MAT, mixotrophy in AOA may allow for changes in abundance without reflecting changes in ammonia oxidation activity (Prosser and Nicol 2008, Tourna et al. 2008).

Ammonia oxidizer gene expression and N cycling

We observed no significant change in the relative expression of AOA and AOB *amoA* genes in soils across the MAT gradient and found. Contrary to our hypotheses, this result suggests that MAT may not drive variation in the transcription of AOA *amoA* in soils despite the observed increase in the abundance of AOA *amoA* with MAT (Fig. 2a). This is surprising in light of experimentally demonstrated effects of temperature on AOA *amoA* gene transcription (Tourna et al. 2008), which have been explained in terms of higher growth rate at elevated

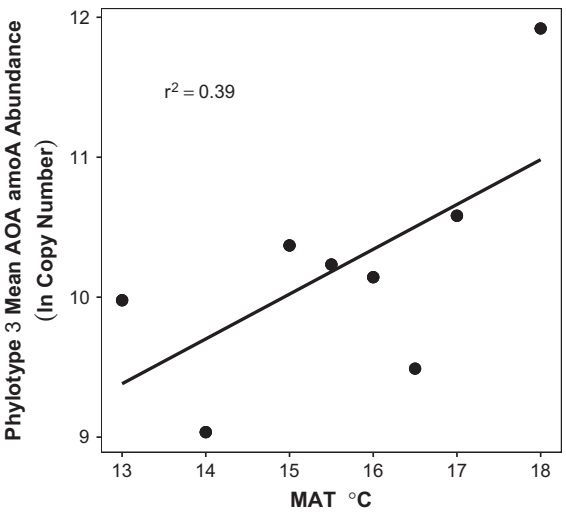

FIG. 5. AOA *amoA* in Phylotype 3 showed a significant ($P = 0.0017$), positive relationship to MAT in the field while Phylotypes 1 and 2 did not respond to MAT.

temperatures and selective growth of certain phylogenetic groups of ammonia oxidizers. In this study, the overall lack of significant response in relative *amoA* expression to increasing MAT, despite the increase in *amoA* abundance with MAT, suggests that in situ, MAT may select for population size and structure, but does not significantly drive *amoA* transcription in the general AOA community. Relative *amoA* expression differed among phylotypes, however, with Phylotype 1 displaying the highest levels of relative expression across the gradient (Fig. 4b, $P = 0.002$). This suggests that only one group of AOA increase transcription per gene copy in response to MAT, and implies a shift in Phylotype 1 transcription efficiency, possibly due to genotypic and phenotypic changes across MAT. It should be noted that soils for DNA and RNA extraction were sampled in August 2014 and October 2015, respectively, and thus it is possible that differences in DNA abundance between these times could influence relative expression results. Our observations may also support the aforementioned potential for metabolic shifts among mixotrophic AOA, as more favorable electron acceptors may become available with increasing MAT (Zogg et al. 1997, Prosser and Nicol 2008).

Implications for ecosystem function

The positive relationships among AOA *amoA* abundance, soil NO_3^-, availability and MAT observed in this study provide strong evidence that MAT is the primary driver of N cycling in this system; however, the causal chain underlying this relationship is not entirely clear. Higher temperatures at lower elevations result in higher forest productivity (Litton et al. 2011, Giardina et al. 2014), but the maintenance of high rates of production requires a larger nitrogen supply (Treseder and Vitousek 2001, Fernández-Martínez et al. 2014). Giardina et al. (2014) demonstrated that belowground carbon flux increases strongly with temperature along this gradient, presumably reflecting increased demand for soil resources in the rhizosphere, including N. Higher temperatures also lead to faster litter decomposition (Bothwell et al. 2014), accelerated forest floor turnover (Giardina et al. 2014), and a lower ratio of detrital C to total soil C along this gradient (Selmants et al. 2014). Thus, recycling of N through the detrital food web increases with MAT in this system. Our results suggest that this increased N recycling is coincident with, and may be influenced by, increased AOA abundance and, consequently, elevated ecosystem capacity for ammonia oxidation and nitrification at higher temperatures.

The next steps in this work are to test the sensitivity of microbial functional assemblages to MAT, identify how organic N inputs change with elevated MAT, and determine how MAT affects the coupling of forest N cycling to other biogeochemical cycles. The work presented here illustrates that N availability and limitation are governed by both microbial functional dynamics as well as biotic demand across MAT (Kuzyakov and Xu, 2013). To apply these observations to broader ecosystem contexts, future work must address in greater detail how community structure within microbial functional groups impacts forest nutrient regimes. Further, while MAT appears to increase the bioavailability and turnover (Bothwell et al. 2014) of N in this system, it remains unclear whether this trend is linked to the size of organic N pools. As MAT increases, N substrate for ammonia oxidation and other downstream N processes may enter forests through increased biological N fixation (BNF), and numerous studies have illustrated the dominance of BNF in tropical forests compared to temperate forest systems (Houlton et al. 2008, Vitousek et al. 2013). The potentially concomitant responses of BNF and nitrification to MAT may therefore differ between forest type, with implications for interpreting global responses to rising MAT. These nuances are also important for coupling between the N, P and C cycles, and modeling approaches will be critical to assessing the conditions under which new biogeochemical steady states may arise as N bioavailability increases with MAT.

Our study concludes that with all else being equal, ammonia oxidizer populations increase with rising MAT at this site. In tandem, NH_4^+ and NO_3^- supplies to terrestrial primary producers also increase. Similar patterns have been observed along elevation gradients in other forest types, suggesting that increasing MAT may be a driver of internal N fluxes in many forest systems (Vitousek et al. 1988, Knoepp and Swank 1998, Johnson et al. 2000, Liu and Wang 2010, Averill and Finzi 2011, Salinas et al. 2011). If soil NH_4^+ supplies increase in the future, we predict from our results that nitrifier populations will continue to increase nitrification rates, thus impacting forest N demand and rates of primary production (Raich et al. 1997, Nemani et al. 2003, Giardina et al. 2014). Further, the ability for nitrifier populations to sustain increased nitrification as MAT increases may alter N mobility in soils as MAT increases (Nemani et al. 2003, Groffman et al. 2009). This study shows that the abundance of ammonia oxidizers in soils may be a useful parameter in predicting future biogeochemical changes in forests under climate change.

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Supporting Information

Additional supporting information may be found in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/ecy.1863/suppinfo