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Meta-analysis reveals ammonia-oxidizing bacteria respond more strongly to nitrogen addition than ammonia-oxidizing archaea

Chelsea J. Carey [a, b, c, *], Nicholas C. Dove [b], J. Michael Beman [c, d], Stephen C. Hart [c, d], Emma L. Aronson [a]

[a] Department of Plant Pathology and Microbiology, 900 University Avenue, University of California Riverside, Riverside, CA 92521, USA
[b] Environmental Systems Graduate Group, 5200 North Lake Road, University of California Merced, Merced, CA 95343, USA
[c] Life and Environmental Sciences, 5200 North Lake Road, University of California Merced, Merced, CA 95343, USA
[d] Sierra Nevada Research Institute, 5200 North Lake Road, University of California Merced, Merced, CA 95343, USA

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ABSTRACT

Shifts in microbial communities driven by anthropogenic nitrogen (N) addition have broad-scale ecological consequences. However, responses of microbial groups to exogenous N supply vary considerably across studies, hindering efforts to predict community changes. We used meta-analytical techniques to explore how *amoA* gene abundances of ammonia-oxidizing archaea (AOA) and bacteria (AOB) respond to N addition, and found that N addition increased AOA and AOB abundances by an average of 27% and 326%, respectively. Responses of AOB varied by study type, ecosystem, fertilizer type, and soil pH, and were strongest in unmanaged wildland soils and soils fertilized with inorganic N sources. Increases in nitrification potential with N addition significantly correlated with only AOB. Our analyses suggest that elevated N supply enhances soil nitrification potential by increasing AOB populations, and that this effect may be most pronounced in unmanaged wildland soils.

1. Introduction

Humans have rapidly and fundamentally transformed the global nitrogen (N) cycle by combusting fossil fuels, fertilizing agricultural lands, and cultivating legumes (Vitousek et al., 1997; Fowler et al., 2013; Liu et al., 2013). Inputs of available N to terrestrial systems have more than doubled as a result of these activities, with approximately 210 Tg N fixed anthropogenically each year (Fowler et al., 2013). Much of this fixed N is used to fertilize agroecosystems, which are regularly supplied with up to 400 kg N ha^{-1} y^{-1} (average 180 kg N ha^{-1} y^{-1}; Rosenstock et al., 2013). Atmospheric N deposition has increased concomitantly, such that current global rates average 105 Tg N y^{-1} (Galloway et al., 2008) and some hotspots of N deposition reach 90 kg N ha^{-1} y^{-1} (Fenn et al., 2003, 2010). Because N often limits plant and microbial growth (Vitousek and Howarth, 1991; Hart and Stark, 1997; LeBauer and Treseder, 2008), the effects of enhanced N supply can cascade through an ecosystem, altering plant composition (Bobbink et al., 2010), net primary productivity (LeBauer and Treseder, 2008), and processes such as decomposition, nitrification, and denitrification (Barnard et al., 2005; Vivanco and Austin, 2011; Frey et al., 2014).

A number of meta-analyses have examined the effects of elevated N supply on plant community dynamics (LeBauer and Treseder, 2008; Xia and Wan, 2008) and N pools and transformations (Barnard et al., 2005; Knorr et al., 2005; Lu et al., 2011; Aronson and Allison, 2012). Findings from these syntheses indicate that exogenous N inputs increase soil inorganic N pools, rates of nitrification, and N_2O fluxes, while inhibiting organic matter decomposition under some conditions. However, meta-analyses that assess how soil N-cycling microbial populations and communities respond to N additions are sparse, and those that exist have focused on fungi or total microbial biomass (Treseder, 2004, 2008). Our ability to mechanistically understand how ecosystems respond to enhanced N supply requires that the sensitivity of other N-cycling microorganisms—such as key bacterial and archaeal taxa—be investigated in a similarly comprehensive way. In particular, predictions of ecosystem dynamics in areas that receive elevated N inputs may be improved by explicitly including trait-

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[*] Corresponding author. Department of Plant Pathology and Microbiology, 900 University Avenue, University of California Riverside, Riverside, CA 92521, USA.
E-mail address: chelsea.carey@ucr.edu (C.J. Carey).

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based microbial data, such as niche preferences for N availability, in predictive biogeochemical models (McGuire and Treseder, 2010; Treseder et al., 2011; Nazaries et al., 2013).

Within the N cycle, ammonia oxidizers are a functionally important group of microorganisms that perform the first and rate-limiting step of nitrification. Although recent evidence reveals the capacity for some *Nitrospira* to perform complete nitrification (Daims et al., 2015), aerobic chemoautotrophic nitrification has historically been considered a two-step process whereby ammonia-oxidizing bacteria ('AOB' from β- and Υ- classes of *Proteobacteria*) and archaea ('AOA' from *Thaumarchaeota* phylum) oxidize ammonia (NH_3) to nitrite (NO_2^-); nitrite is then rapidly oxidized by nitrite oxidizing bacteria to nitrate (NO_3^-). The occurrence of heterotrophic nitrification, where organic N or ammonia is sequentially oxidized by heterotrophic microorganisms, has also been suggested as important in some soils (Hart et al., 1997; Zhu et al., 2014; Chen et al., 2015); however, the chemoautotrophic nitrification pathway generally dominates (Barraclough and Puri, 1995; Islam et al., 2007). Further, the development of techniques to examine the *amoA* functional gene, which encodes the α-subunit of the ammonia monooxygenase enzyme in AOA and AOB (Leininger et al., 2006), has resulted in greater molecular assessment of the chemoautotrophic pathway. This approach is often used to assess the genetic potential for ammonia oxidation in soils and provides a way of exploring how ammonia-oxidizing microbial communities respond to environmental change (e.g., Hawkes et al., 2005; Szukics et al., 2010).

Numerous individual experimental studies have measured if and how AOA and AOB abundances respond to elevated N supply. While some general trends and their underlying physiological mechanisms have been summarized in qualitative reviews—for example, the idea that AOA seem to prefer oligotrophic conditions (Gubry-Rangin et al., 2011; Hatzenpichler, 2012; He et al., 2012; Prosser and Nicol, 2012)—the magnitude and direction of change with N varies among studies (Cavagnaro et al., 2008; Chen et al., 2013; Fan et al., 2011a; Habteselassie et al., 2013; Kelly et al., 2011; Levičnik-Höfferle et al., 2012) and therefore limits our ability to discern meaningful patterns across ecosystems, management scenarios, and depositional loads. A quantitative synthesis of the literature will help to illuminate underlying reasons for variable response patterns within and among these studies.

Several different factors may be important in determining the magnitude and direction of the response of these microbial groups to N additions. For example, whether bacterial and archaeal *amoA* gene abundances change following exogenous N additions may depend on whether the N is derived from organic or inorganic sources (Levičnik-Höfferle et al., 2012). Indeed in recent work by Ouyang et al. (2016), AOB abundances tended to show larger increases when fertilized with ammonium sulfate than composted manure. Supplying co-nutrients (e.g., phosphorus; P) along with N (Norman and Barrett, 2014) may also alter how these groups generally respond. However, differences in physiologies, habitat preferences, and metabolism within the AOA and AOB (Offre et al., 2014; Taylor and Bottomley, 2006; Webster et al., 2005) may introduce meaningful variation in how individual AOA and AOB taxa are affected by these and other environmental modifiers (Martiny et al., 2015).

Soil pH may be another important factor influencing the outcome of fertilization (He et al., 2012; Zhang et al., 2012). Some AOA are obligatory acidophilic and can only grow at low pH conditions (pH 4–5.5) (Lehtovirta-Morley et al., 2011), while other AOA prefer circumneutral conditions (Tourna et al., 2011) like their cultivated AOB counterparts (Prosser and Nicol, 2012). In addition to directly selecting-for acidophilic or neutrophilic ammonia oxidizers, pH can affect the availability of NH_3 (the substrate for ammonia oxidation; Suzuki et al., 1974; Stempfhuber et al., 2015) through protonation. More acidic soils have higher NH_4^+ to NH_3 ratios and therefore lower NH_3 availability for a given N concentration or addition rate—and most cultivated AOA tend to have higher NH_3 affinities than AOB (Prosser and Nicol, 2012). Further, there is circumstantial evidence that AOB can tolerate higher NH_3 concentrations than AOA (Erguder et al., 2009; Park and Bae, 2009; Prosser and Nicol, 2012). Therefore, in addition to maximum specific growth rates (Prosser and Nicol, 2012), the responses of ammonia oxidizers will be regulated by soil pH, overall NH_3/NH_4^+ concentrations, and particular affinities of AOA and AOB within the community. Altogether, this suggests that the amount, duration, and total fertilization load are all factors that could influence how AOA and AOB *amoA* gene abundances change with elevated N supply.

Using a meta-analytical approach, we combined results of 33 individual studies to elucidate general trends in the response of AOA and AOB abundances to elevated N supply—and to identify the consequences for potential nitrification activity of soils. In addition, we used this approach to reveal possible explanations for variability among studies by examining whether N source (organic or inorganic), amount, fertilization duration, soil pH, and co-fertilization with P or potassium (K) affect how ammonia oxidizers respond to N additions across contrasting ecosystem types. We hypothesized that N addition would increase *amoA* gene abundances of AOA up to a point (i.e., a particular fertilization rate), after which abundances would remain stable or decline. In contrast, we hypothesized that AOB would continue to increase with N supply rate. We further predicted that the response of these two groups would be modified by soil pH, ecosystem, fertilizer type, and co-fertilization with other nutrients. Finally, we hypothesized that increases in *amoA* gene abundances would positively relate to potential nitrification activity of soils.

2. Material and methods

2.1. Data collection

We used ISI Web of Knowledge, Google Scholar, and cross-referencing to search for relevant studies. Key search terms were: ammonia-oxidizing, amoA, AOA, *or* AOB, *and* elevated N, N addition, N deposition, fertilization, *or* fertilizer. Studies were included if (1) they measured AOB, AOA, or both using qPCR of the *amoA* functional gene; (2) treated soil was compared to an untreated control; (3) means, standard deviations, and replicate numbers were reported or could be determined; and (4) N application rate was provided or could be estimated by assuming a bulk density (i.e., 1 Mg m^{-3}). Data were not excluded based on study type: laboratory, greenhouse, and field studies were all included. We excluded studies where other treatments, such as mowing, were applied in addition to fertilizer.

In order to take full advantage of published results, multiple experimental treatments from the same study were included in our analyses (e.g., treatments that varied by fertilizer application rate). However, only one measurement from each experimental replicate was included to maximize independence among measurements. For example, in studies where *amoA* gene abundances were measured multiple times from the same experimental unit, we restricted our analyses to the longest time point. In addition, when multiple soil depths were assessed, we used only the shallowest depth.

We also collected measurements of nitrification potential in order to compare responses of potential activity with *amoA* gene abundances, which are often used to approximate ammonia-oxidizing population sizes. When nitrification potential measurements were reported, authors commonly used the chlorate inhibition method (Kurola et al., 2005) and the shaken soil slurry method (Hart et al., 1994). Other reported methods included those from Hayatsu and Kosuge (1993) and Fan et al. (2011b). All of these approaches incubated recently collected soil under ideal conditions for nitrification and measured NO_2^- or NO_3^- production over time.

In addition to examining the overall effects of N addition on AOA and AOB *amoA* gene abundances, a major goal of our meta-analysis was to determine whether particular experimental approaches or environmental settings modify how AOA and AOB *amoA* respond to N addition. Therefore, when possible, categorical and continuous variables were collected from each study to partition the variability among *amoA* gene responses. The categorical variables were: (1) taxonomy (bacterial or archaeal *amoA*); (2) experiment type (laboratory or field); (3) fertilizer type (organic [manure, urine, compost], inorganic [synthetic urea, $(NH_4)_2SO_4$, NH_4NO_3, KNO_3], or both); (4) ecosystem type (i.e., wildland [unmanaged forest, grassland, desert, marsh], pastoral [grazed grassland], or agricultural [croplands]); (5) co-fertilization (P added; K added; both P and K added); and (6) soil pH (<6, 6–7, 7–8, >8). Biomes were grouped into a single category (unmanaged wildand) because there were not enough studies to conduct robust analyses for forests, grasslands, and other ecosystem types. The continuous variables were: (1) N application rate (presented as or converted to kg N ha^{-1} y^{-1}); (2) duration of fertilization (years); (3) total N load (determined by multiplying the N application rate by total number of applications); and (4) time since last fertilization (days).

In order to determine whether ecosystems varied in their sensitivity to rates of N addition, we partitioned rates into low (≤100 kg N ha^{-1} y^{-1}), medium (100–500 kg N ha^{-1} y^{-1}) and high (≥500 kg N ha^{-1} y^{-1}), and subsequently assessed the influence of ecosystem type for each application level. Bins were partitioned with these particular cutoffs in order to balance realism (e.g., the low cutoff is just above maximum known N deposition rates (Fenn et al., 2010)); while still retaining enough observations in each bin for a robust analysis.

2.2. Statistics

The natural log of the response ratio (ln R) was used to evaluate the effects of N fertilization on *amoA* gene abundances:

$$lnR = ln\left(\frac{T}{C}\right),$$

where lnR is the effect size, T is the fertilized treatment mean, and C is the unfertilized control mean (Gurevitch and Hedges, 1993). We used MetaWin 2.1 software (Rosenberg et al., 2000) to calculate the mean effect size and variance using a weighted random effects approach. Bias-corrected 95% confidence intervals (CIs) were produced by bootstrapping, and were considered significantly different from zero ($\alpha < 0.05$) if the 95% CI did not overlap zero. Similarly, responses among categorical variables were considered to be significantly different when the 95% CIs did not overlap. For each categorical variable (taxonomy, experiment type, fertilizer type, ecosystem, and soil pH), total heterogeneity (Q_T) was partitioned into within-group (Q_W) and between-group (Q_b) heterogeneity. The Q statistic follows a chi-square distribution with $k - 1$ degrees of freedom, where k is the number of paired means between the N fertilized and unfertilized control for a particular categorical variable. We considered a particular categorical variable to have a significant impact on the response ratio when Q_b was larger than the critical value (Gurevitch and Hedges, 1993), and we examined this significance using P_{random} values (produced from randomized tests with 999 permutations and sample size as the weighting function). Where Q_b was significant ($\alpha < 0.05$), categorical data were subdivided to partition the variation by levels within that category. We also used regression analysis to test for the effects of continuous variables on AOA and AOB *amoA* gene abundance (duration of fertilization and time since last fertilization). Response ratios of AOA and AOB followed a normal distribution; however, the continuous modifiers did not. We therefore natural log transformed all continuous modifiers prior to analysis. In addition, (Mendum et al., 1999) was excluded from the analysis of duration and total N load because their site was fertilized for 147 years (>3 SD from the mean) and was therefore considered an outlier. Finally, to facilitate interpretation of the meta-analytical results, we performed a one-way ANOVA followed by a Tukey HSD post-hoc analysis on *amoA* gene abundances of unmanipulated control soil with ecosystem type as the explanatory variable (to provide an idea of 'background' AOA and AOB abundances; *amoA* copy numbers were log transformed).

Of the 215 observations (from 33 studies) included in this meta-analysis, 98 measured the response of archaeal *amoA* and 117 measured the response of bacterial *amoA* gene abundances (see Appendices S1 and S2). Of those, 154 were field studies and 61 were laboratory studies. In addition, 123 observations (57%) were from agricultural settings, 19 (9%) were from pastures, and 73 (34%) from unmanaged wildlands. One hundred fifty nine observations (74%) were in response to inorganic N addition, 44 (20%) were in response to organic N addition, and 12 (6%) were in response to a combination of inorganic and organic N. Organic fertilizers tended to be applied at higher rates than inorganic fertilizers; 100% of N applications below 100 kg N ha^{-1} y^{-1} were from inorganic fertilizers, while 76% of applications above 500 kg N ha^{-1} y^{-1} were from organic fertilizers (Appendix S1).

3. Results

3.1. Ammonia-oxidizing archaea

When combined across all observations, archaeal *amoA* gene abundances responded positively to N addition (Fig. 1; 27% average increase, 95% CI range 7–49%). This response was consistent among studies, as demonstrated by a non-significant Q_T value ($Q_T = 67.54$, $P = 0.99$). We found no significant differences in response ratios of AOA across most of the modifying categorical variables ($P > 0.05$; Table 1), including experiment type (Appendix S3), fertilizer type (Fig. 1), soil pH (Fig. 2), and co-fertilization (Fig. 2). However, responses of AOA to N addition depended on ecosystem type (Table 1): the effects of N addition were greater in agricultural soils than pasture soils, and this trend was most dramatic at high fertilization levels (Fig. 3). Although the response ratios of AOA across categorical variables did not usually differ from each other, some modifiers showed significant responses compared to the unfertilized control while others did not. In particular, response ratios of AOA were significantly different from zero only in soils where the pH was greater than 7 (Fig. 2). In addition, inorganic, but not organic, fertilizers stimulated a significant response in AOA compared to the unfertilized controls (Fig. 1), and this response to inorganic N occurred only when K was added in addition to N (Fig. 2).

Across all observations, responses of AOA were not significantly related to fertilization rate ($R^2 < 0.00$, $P = 0.77$; Appendix S4). This remained true when estimated fertilization rates were excluded from the analysis (23.4% were estimated values; data not shown). Similarly, AOA response ratios were not affected by the number of years fertilized ($R^2 = 0.04$, $P = 0.12$), or time since last fertilization ($R^2 = 0.001$, $P = 0.79$). However, response ratios of AOA were positively but weakly related to total N load ($R^2 = 0.05$, $P = 0.02$).

ANOVA and Tukey post-hoc analysis of control soil *amoA* gene abundances revealed greater background abundances of AOA in agricultural soils than wildland and pastoral soils ($P < 0.05$; data not shown).

3.2. Ammonia-oxidizing bacteria

Bacterial *amoA* gene abundances also responded positively to N addition when combined across all observations (Fig. 1), but to a substantially greater degree (325% average increase, 95% CI range 232–458%) than that of AOA ($Q_b = 57.74$, $P < 0.001$). The AOB responses were heterogeneous among studies, as demonstrated by a significant Q_T value ($Q_T = 155.05$, $P < 0.01$). While these response ratios were always greater than or equal to zero (with the exception of pasture soils that were fertilized with low application rates; Fig. 3), the magnitude depended on study type, ecosystem, fertilizer type, and soil pH (Table 1). Responses of AOB to N addition were stronger in field studies (Appendix S3) and in studies where inorganic fertilizers were used (Fig. 1) or where soils had a pH between 7 and 8 (Fig. 2). In addition, AOB of wildland soils were more responsive to N addition than those of agricultural soils, a trend that was driven largely by studies with medium and high application rates (Fig. 3). This variation in response to categorical modifiers was captured primarily by field studies, rather than laboratory microcosm studies (Appendix S3).

We found no significant relationship between N application rate and responses of AOB when collapsed across all ecosystem types ($R^2 = 0.01$, $P = 0.33$; Appendix S5). This remained true when estimated fertilization rates were excluded from the analysis. *amoA* gene abundances of AOB were also not significantly related to time since last fertilization ($R^2 = 0.03$, $P = 0.12$). Response ratios increased with total N load ($R^2 = 0.06$, $P = 0.01$), although this relationship was only marginally significant when total loads calculated from estimated rates were excluded ($R^2 = 0.03$, $P = 0.08$), and the relationship explained little variation in the data. Finally, response ratios of AOB were significantly influenced by duration of fertilization, with responses peaking at around 20 years of fertilization and then declining thereafter ($R^2 = 0.13$, $P = 0.005$). ANOVA and Tukey post-hoc analysis of control soil *amoA* gene abundances revealed greater background abundances of AOB in agricultural and pastoral soils than wildland soils ($P < 0.01$; data not shown).

3.3. Nitrification potentials

Fourteen studies, totaling 107 observations, measured N effects on nitrification potential in addition to *amoA* gene abundances. Across these studies, response ratios of AOB and nitrification potential were significantly and positively correlated ($NP[\ln R] = 0.20 \times AOB[\ln R] + 0.56$, $R^2 = 0.12$, $P = 0.006$; Fig 4). In contrast, response ratios of AOA did not correlate significantly with nitrification potential ($NP[\ln R] = -0.06 \times AOA[\ln R] + 0.72$, $R^2 < 0.00$, $P = 0.73$; Fig 4).

4. Discussion

Both AOA and AOB *amoA* gene abundances responded positively to N addition, suggesting that elevated N supply generally increases soil ammonia-oxidizing microbial abundance. However, across all studies, AOB mean log response ratios to N additions were over 6 times greater than those of AOA. This indicates that AOB abundances are substantially more responsive to increases in N availability, although because of differences in cell sizes and specific activities (Prosser and Nicol, 2012) this may not necessarily reflect comparable changes in activity between AOA and AOB. We primarily attribute this overall finding to the different affinities for NH_3 typically observed between these two groups. Most research to date indicates that AOA have higher NH_3 affinities (lower K_m values) than AOB (Martens-Habbena et al., 2009; reviewed in Prosser and Nicol, 2012), making AOA more effective competitors at lower substrate concentrations. Ammonia-oxidizing bacteria, in contrast, have been found to maintain high levels of ribosomal content (Hatzenpichler et al., 2008; Wagner et al., 1995) and to have relatively high K_m values (Koops et al., 2006; Hatzenpichler, 2012), which would allow this group to readily respond to higher N concentrations. These and potentially other physiological or metabolic differences between AOA and AOB can lead to niche differentiation (Prosser and Nicol, 2012), as evidenced in our meta-analysis by a much greater response of AOB *amoA* gene abundances to N additions than their archaeal counterparts.

Surprisingly, even though N additions clearly resulted in greater population sizes of ammonia oxidizers, the characteristics of these N additions were not strong predictors of the *amoA* gene responses.

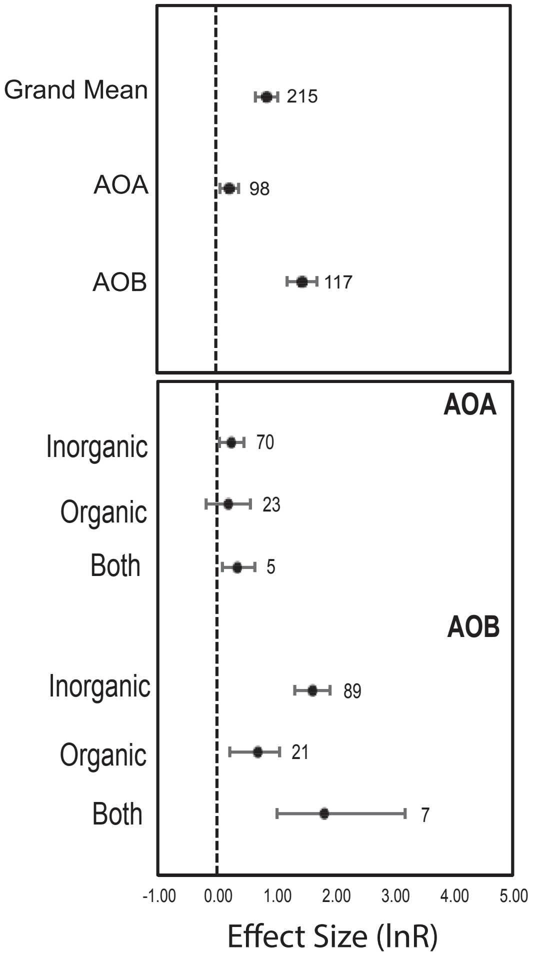

Fig. 1. Mean response ratios (lnR) and bootstrapped 95% Confidence Intervals (CI) for the effects of nitrogen (N) addition on ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) across all observations (upper panel), and partitioned by fertilizer type (lower panel). Means and confidence intervals include all ecosystem types and both microcosm and field studies. lnR = natural log of the response ratio (treatment/control); $\ln R > 0$ indicates an increase in amoA gene abundance with N addition. If 95% CI do not overlap zero, then amoA gene abundances of fertilized soils differed significantly compared to unfertilized soils. Inorganic = studies that applied fertilizer as synthetic urea, $(NH_4)_2SO_4$, NH_4NO_3, or KNO_3; organic = studies that applied N from manure, urine, or compost; both = studies where inorganic and organic fertilizers were applied in combination. Number to the right of symbols refers to the number of observations (n) in that group.

Table 1
Between-group heterogeneity (Q_b) illustrating the effects of N additions on ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) across categorical modifiers. Q_B/Q_T ($Q_{Between}/Q_{Total}$) describes the proportion of total variation explained by each modifier. The *P*-value is the probability value for randomization tests (999 permutations) with sample size as the weighting function, calculated only for the Q_b values; P-value * < 0.05; ** < 0.01.

Modifier	Comparison	AOA		AOB	
		Q_b	Q_b/Q_T	Q_b	Q_b/Q_T
Study type	Field, Microcosm	1.24	0.02	6.66*	0.04
Ecosystem type	Agriculture, Pasture, Wildland	5.97*	0.09	17.96**	0.12
Fertilizer type	Inorganic, Organic, Both	0.09	<0.00	9.52*	0.06
Co-fertilization	Phosphorus, Potassium, Both	0.73	0.01	0.79	0.01
Soil pH	<6, 6–7, 7–8, >8	2.41	0.04	11.19*	0.07

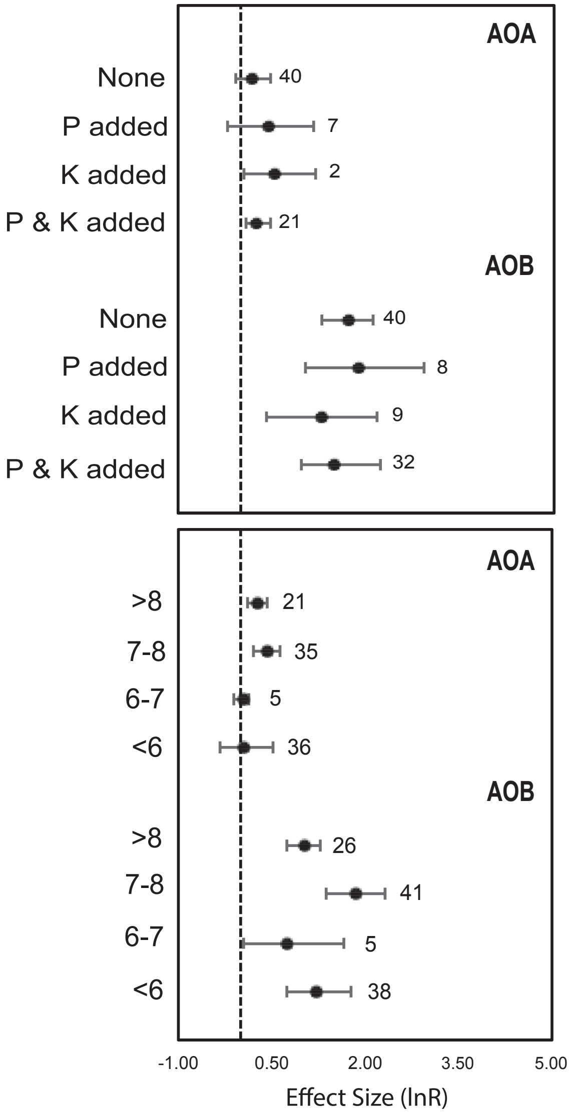

Fig. 2. Mean response ratios (lnR) and bootstrapped 95% Confidence Intervals (CI) for the effects of nitrogen (N) addition on ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB). Responses are partitioned by co-fertilization (upper panel), and soil pH (lower panel). For co-fertilization, only studies of inorganic N were included as organic N sources contain (unknown) amounts of other nutrients. lnR = natural log of the response ratio (treatment/control). None = only inorganic N was supplied; phosphorus (P) added = inorganic N and P were supplied; potassium (K) added = inorganic N and K were supplied; P & K added = inorganic N, P, and K were supplied. pH was binned into four categories; analysis of pH included all observations, however, not all studies reported pH (AOA = 97 observations included; AOB = 110 observations included).

For instance, the magnitude of response was not significantly influenced by time since last fertilization or N application rate when analyzed across all studies. Only total fertilizer load significantly explained some (about 5.2%) of the variation in response of AOA *amoA* gene abundance to N addition. Similarly, only total N load and the duration of N addition significantly explained some of the response of AOB *amoA* gene abundance to N addition (about 5.6% and 12.5% of the variation, respectively). As total N load increased, so did AOA and AOB *amoA* gene abundances. These results contrast with a previous meta-analysis, which synthesized the effects of N addition on soil total microbial biomass, total fungi, and total bacteria (Treseder, 2008). In that study, response ratios of the soil total microbial biomass and the fungal biomass alone were both negatively correlated to the duration of N additions and total N load, and the response ratio of the fungal biomass was positively related to N application rate. Moreover, bacterial biomass as a whole did not change with N addition, regardless of the fertilization rate or duration. The contrasting findings between our study and Treseder (2008), which together show that soil microbial groups respond differently to changing patterns in soil N supply, illustrate why it is so difficult to predict responses of belowground communities to global changes. It further highlights the importance of measuring and synthesizing how exogenous N inputs influence microorganisms across varying taxonomic levels and functional groups.

Fertilizer type modified the response of AOB, but not AOA, to N addition. A number of studies have reported greater growth of AOA in soils fertilized with organic rather than inorganic NH_3 (Kelly et al., 2011; Levičnik-Höfferle et al., 2012), a pattern that has been speculatively attributed to NH_3 inhibition or mixotrophic tendencies. However, the results of our meta-analysis indicate that the source of NH_3 is not an important modifier for AOA, such that AOA responded consistently across all fertilizer types. In contrast to AOA, the source of N significantly influenced how AOB responded to fertilization. Despite typically being applied at lower rates (and potentially causing soil acidification in the case of ammonium fertilizers) (Barak et al., 1997), inorganic fertilizers stimulated a greater response in AOB than organic fertilizers. This is presumably because the NH_3 in inorganic fertilizers does not need to be mineralized from organic material, and is therefore quickly and readily oxidized. The notion that organic fertilizers can minimize NO_3^- leaching from agroecosystems is long-standing, and is supported by studies that demonstrate higher denitrification activity in organically-amended soils (Kramer et al., 2006). Our results further confirm that organic fertilization could mitigate NO_3^- loss by suppressing the growth and activity of AOB when compared to traditional inorganic fertilization techniques.

Many agricultural management practices co-fertilize with P and/or K, given that plant productivity is co-limited by these nutrients in addition to N (Fay et al., 2015). Co-fertilization can have coincident effects on soil microbial communities (Liu et al., 2012; Turner and Wright, 2013)—including some evidence for

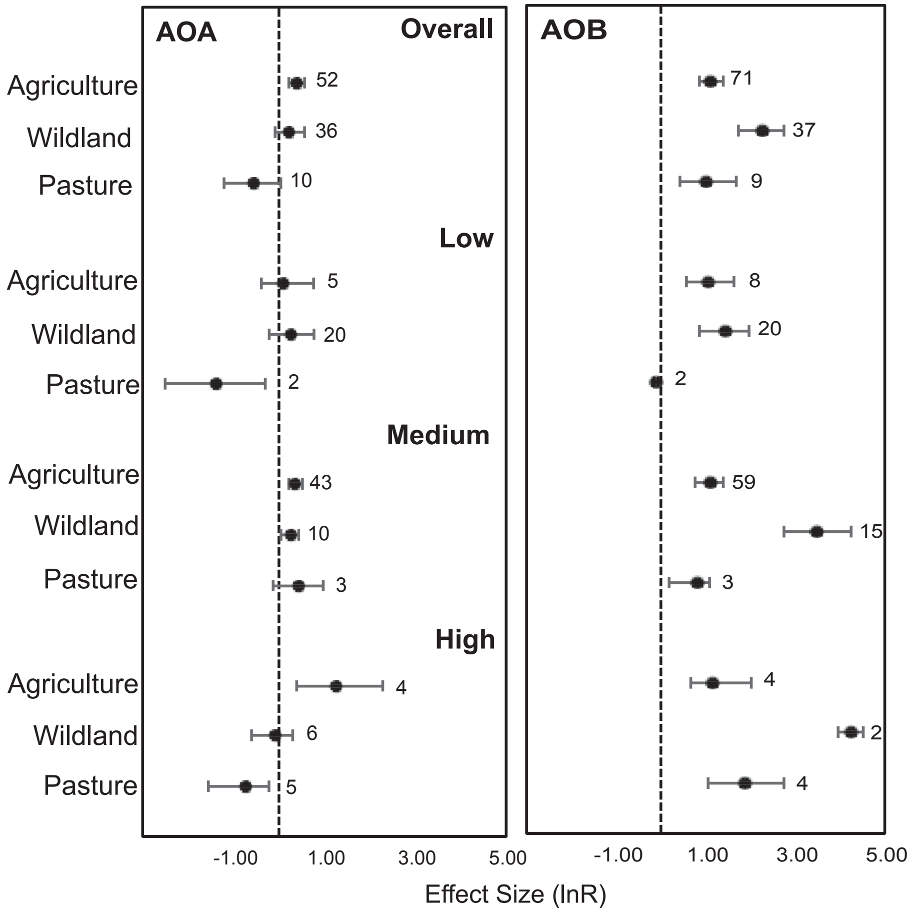

Fig. 3. Mean response ratios (lnR) and bootstrapped 95% Confidence Intervals (CI) for the effects of nitrogen (N) addition on ammonia-oxidizing archaea (AOA; left panel) and ammonia-oxidizing bacteria (AOB; right panel) by ecosystem type and fertilization level. lnR = natural log of the response ratio (treatment/control). Overall = the response of AOA or AOB *amoA* gene abundances partitioned by ecosystem but including all fertilization levels. Low = response of AOA and AOB across ecosystems when fertilized with low rates of N (<100 kg N ha^{-1} y^{-1}); Medium = response when fertilized with medium rates (100–500 kg N ha^{-1} y^{-1}); High = response when fertilized with high rates (>500 kg N ha^{-1} y^{-1}).

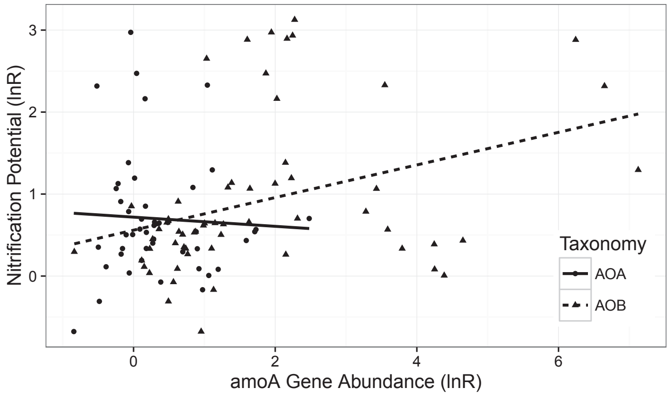

Fig. 4. Relationship between response ratios (lnR) of nitrification potential and response ratios (lnR) of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB). lnR = natural log of the response ratio (treatment/control). Line is the best-fit regression, where AOA ~ Nitrification Potential is the solid line and AOB ~ Nitrification Potential is the dashed line. Each symbol represents one observation; circles = AOA, triangles = AOB. Nitrification potential was significantly and positively correlated with AOB (P < 0.01) but not AOA. NP[lnR] = 0.20 × AOB[lnR]+ 0.56, $R^2 = 0.12$, P = 0.006; NP[lnR] = −0.06 × AOA[lnR]+0.72, $R^2 < 0.00$, P = 0.73.

stimulatory effects on AOA and AOB (Norman and Barrett, 2014). Our meta-analysis, however, did not find AOA or AOB *amoA* gene abundances to be greater in soils that were supplied with P and K in addition to N, suggesting that AOA and AOB growth is not co-limited by these nutrients. Lack of co-limitation likely stems from the fact that the N demand for ammonia oxidizers is considerably higher than the demand for other nutrients, and higher than the N demand of other organisms. The underlying reason for this is that, for AOA and AOB, the vast majority of N is used to generate energy via oxidation rather than for assimilation (Bock and Wagner, 2006). Significant co-fertilization effects should therefore only be prominent in soils with exceptionally high N:P:K ratios, or where P and K indirectly increase N availability.

In contrast to co-fertilization, soil pH significantly affected the response of AOB to exogenous N addition. Soil pH modifies overall microbial community composition and diversity (Lauber et al., 2009), and is known in individual studies to have strong effects on ammonia oxidizer communities specifically (e.g., AOA/AOB ratio and composition) (Nicol et al., 2008; Stempfhuber et al., 2015; Zhang et al., 2012). In our meta-analysis, responses of AOB to N addition were greatest in soils with a pH between 7 and 8, suggesting that elevated N supply influences bacterial ammonia oxidation and growth most dramatically in circumneutral soils. This finding is not surprising given that at low pH most of the added N remains in the form of NH_4^+—a form that requires active transport (Burton and Prosser, 2001)—and is therefore not as readily taken up by ammonia oxidizers as NH_3. In agreement with our findings, all cultivated AOB to date have been neutrophilic, the growth rate and activity of many isolates peak at pH 7.5 (Jiang and Bakken, 1999), and previous studies have found nitrification of fertilizer to be greatest in soils with pH 7.5–8 (e.g., Kyveryga et al., 2004). By extension this finding suggests that soil acidification, which can occur with chronic ammonium fertilization, may mitigate the degree to which AOB *amoA* gene abundances increase with N supply over time (Song et al., 2016).

Unlike AOB, AOA were not significantly influenced by pH ($Q_b > 0.05$), although AOA in N-fertilized soils with a pH > 7 showed a positive effect size compared to the unfertilized control. Many studies suggest AOA dominate ammonia oxidation in acidic soils (Prosser and Nicol, 2012; Zhang et al., 2012) due to the acidophilic nature of some AOA (Lehtovirta-Morley et al., 2011) and the ability of AOA to function under low NH_3 availability (high NH_4^+/NH_3 ratio) (Lu et al., 2012). In support of this, a recent use of the octyne inhibition technique demonstrated that AOA can be responsible for nearly 100% of the recovered nitrification potential of exogenous N in soils with pH below 5 (Song et al., 2016). However, our results indicate that when generalized across many studies this dominance is not reflected in changes to *amoA* gene abundances with N fertilization, and may instead be mediated by changes in specific activity or community composition within the AOA.

Ecosystem type significantly mediated the extent to which ammonia oxidizers responded to exogenous N additions. Overall, AOA showed a greater response to fertilization in soils derived from agricultural settings, while AOB showed a greater response to fertilization in soils derived from wildlands. However, the rate of N addition also interacted with ecosystem type to influence AOA and AOB *amoA* gene abundances. For example, when supplied with low rates of N that mimic levels received from atmospheric deposition (<100 kg N ha^{-1} y^{-1}), AOB *amoA* gene abundance in wildland soils increased by 317% compared to the unfertilized control, and this response was amplified by an order of magnitude when supplied with rates more typical of fertilization in agroecosystems. These findings suggest that AOB abundances in unmanaged wildland soils increase considerably with N additions that simulate current and projected N deposition rates (Fenn et al., 2003), and that they continue to increase with greater rates of N addition. In contrast, AOB abundances in agricultural soils respond just as much under low N supply as high N supply (but this response was always less than AOB of wildland soils). It is possible that these intensively managed soils are more saturated with N than their unmanaged wildland counterparts, that AOB are consequently relieved from N limitation, and that additional N is therefore less important to those AOB communities. In support of this, the background population size of AOB in unmanipulated agricultural control soils was significantly higher than that of wildland control soils ($2.55 \times 10^7 \pm 4.65 \times 10^7$ and $2.39 \times 10^6 \pm 4.59 \times 10^6$, respectively). It is also possible that AOB communities of agricultural soils are adapted, and therefore more resistant or resilient, to repeated fertilization events (Griffiths and Philippot, 2013).

Increases in *amoA* gene abundances may result in a greater potential for soils to nitrify. We therefore hypothesized that there would be a significant relationship between *amoA* gene abundances and soil nitrification potential. In partial support of this hypothesis, soil nitrification potential increased concomitantly with AOB *amoA* gene abundances, but not AOA. Other recent studies not focused on fertilization effects have also reported stronger correlations between (potential and gross) rates of nitrification and bacteria than archaea (Bernhard et al., 2010). For example, in semi-arid agricultural soils of southern Australia, Banning et al. (2015) found the abundance of AOB but not AOA to positively correlate with gross nitrification rates across the soil profile. However, when regressing the log response ratios of nitrification potential with AOB in our meta-analysis, significant variation remained unexplained (AOB $R^2 = 0.12$), which could possibly be accounted for by metabolic and physiological heterogeneity within the AOB (e.g., Alves et al., 2013). Ammonia oxidizer community composition may therefore be an important factor to consider when explaining variation in potential activity (e.g., Yao et al., 2013).

The non-significant relationship between AOA and nitrification potential found in our study does not necessarily suggest that AOA are unimportant for ammonia oxidation in soils. On the contrary, AOA have been shown to drive gross nitrification of some unmanaged soils (Huang et al., 2011; Isobe et al., 2015), with their greatest contribution likely occurring in N-limited scenarios. The lack of a relationship between AOA and nitrification potential may instead be an artifact of the nitrification potential assay conditions, where NH_4^+ is excessive (~1.5 mM) and pH is neutral (7.2) (Hart et al., 1994). Because many AOA are adapted to low pH conditions (i.e., have a pH optima below 7; Hatzenpichler, 2012), and some may be mixotrophic (Lehtovirta-Morley et al., 2014; Tourna et al., 2011) or inhibited by high NH_3 concentrations, these conditions of the nitrification potential assay could promote the activity of AOB over AOA. Indeed, Ouyang et al. (2016) found that 82–91% of NO_3^- produced during 1 mM NH_4^+ nitrification potentials resulted from AOB while only ~20% resulted from AOA. However, the contribution of these groups to potential nitrification can vary based on initial environmental and management conditions (Lu et al., 2015; Taylor et al., 2012). Additional studies using inhibitory techniques (Taylor et al., 2010), DNA stable isotope probing (Zhang et al., 2012), potential assays with varied conditions to account for contrasting physiologies of AOA and AOB, and manipulations of microbial community composition will help to further elucidate underlying microbial mechanisms regulating nitrification under elevated N conditions.

5. Conclusions

Our meta-analysis demonstrates that N additions increase both AOA and AOB *amoA* gene abundances. However, AOB responded more dramatically and showed a significant positive relationship with nitrification potential. Additionally, responses of AOB to increasing rates of N application were significantly stronger in wildand than agricultural and pastoral soils. Taken together, these results suggest that AOB populations are more dynamic when faced with enhanced N supply, and may be more responsive to changes in land-use or soil management than AOA. The identification of consistent patterns in niche separation for AOA and AOB based on N availability should help incorporate ammonia-oxidizing microbial dynamics into predictive biogeochemical models. For both management and modeling, increased AOA and AOB abundances following fertilization may change the dynamics of N cycling in soils, as larger population sizes may promote higher maximum rates of ammonia oxidation, and subsequently change the availability of oxidized forms of N and thus N mobility in soil.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2016.05.014.

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