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Paired carbon and nitrogen metabolism by ammonia-oxidizing bacteria and archaea in temperate forest soils

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Citation: Norman, J. S., L. Lin, and J. E. Barrett. 2015. Paired carbon and nitrogen metabolism by ammonia-oxidizing bacteria and archaea in temperate forest soils. Ecosphere 6(10):176. http://dx.doi.org/10.1890/ES14-00299.1

Abstract. Nitrification is a biologically mediated nutrient transformation, which influences the availability of inorganic nitrogen to other microorganisms and plants and mediates mobility of nitrogen in the environment. Ammonia oxidation, the rate-limiting step of nitrification, is performed by two groups of microbes: ammonia-oxidizing archaea (AOA) and bacteria (AOB) that couple this process with the chemoautotrophic fixation of carbon. Due to the energetic constraints on these organisms, both AOA and AOB likely oxidize large amounts of ammonia to fix relatively small amounts of carbon in natural environments. Here we sought to investigate paired carbon and nitrogen metabolism by AOA and AOB in forest soils. To accomplish this objective, we used quantitative polymerase chain reaction (qPCR) to quantify changes in AOA and AOB *ammonia monooxygenase subunit A* (*amoA*) genes during in situ incubations. We then used qPCR data alongside AOA and AOB community profiles at each site to convert changes in *amoA* gene copy number to carbon accumulation by each group. Finally, we regressed group-specific carbon accumulation values against observed values of NO_3^- accumulation to establish cross-site relationships between ammonia oxidation and carbon accumulation by each group. By this procedure we estimated that forest soil AOA oxidized 59.8 μg of ammonia-N to add 1 μg of carbon to biomass, while forest soil AOB oxidized 58.2 μg of ammonia-N to add 1 μg of carbon to biomass. These findings represent the first field-based estimates of paired carbon and nitrogen metabolism by these organisms, and could be used to inform microbially explicit models of nitrification in forest soils.

Key words: ammonia oxidation; archaea; bacteria; forest soil; nitrification.

Received 2 September 2014; revised 19 January 2015; accepted 30 March 2015; **published** 16 October 2015. Corresponding Editor: M. F. Allen.

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INTRODUCTION

Ammonia oxidation is the rate-limiting step of nitrification, a microbially mediated nutrient transformation that serves as a bottleneck for nitrogen loss in terrestrial ecosystems (Vitousek et al. 1979). Ammonia-oxidizing bacteria (AOB) were first isolated in the late 1800s and were long thought to be the only organisms capable of oxidizing ammonia in natural systems. However, the first ammonia-oxidizing archaeon (AOA) was isolated in 2005 (Könneke et al. 2005) and these organisms were soon found to co-occur with AOB in soils from a range of temperate biomes (Leininger et al. 2006). Both AOA and AOB use the energy they acquire from ammonia oxidation to fuel chemoautotrophic growth. The low energy yield of this process likely means that these organisms must oxidize large quantities of ammonia to fuel small amounts of carbon

fixation. Here we sought investigate the explicit links between carbon and nitrogen metabolism in natural temperate forest soil AOA and AOB during in situ net nitrification incubations. Estimating how much carbon each group accumulates from ammonia oxidation in the field allowed us to assess the relative importance of each group to this ecosystem-level process and investigate group-level differences in paired carbon and nitrogen metabolism in temperate forest soils.

Several studies have investigated paired carbon and nitrogen metabolism of AOA and AOB by incubating soils in the lab, and comparing observed rates of ammonia oxidation to either growth of AOA and AOB as estimated by qPCR (e.g., Di et al. 2009, 2010, Jia and Conrad 2009, Schauss et al. 2009, Gubry-Rangin et al. 2010, Onodera et al. 2010, Zhang et al. 2010), or inorganic carbon assimilation by AOA and AOB as measured by stable isotope probing (SIP; e.g., Jia and Conrad 2009, Zhang et al. 2010, 2012, Pratscher et al. 2011). Both approaches rely on incubations conducted at constant temperature, moisture, and oxygen concentrations, which are not characteristic of actual field conditions. Furthermore, many of these studies often include the addition of ammonium, which likely favors the growth of AOB (Martens-Habbena et al. 2009). Here we estimated carbon accumulation by AOA and AOB along with nitrate accumulation during buried-bag incubations, in which unamended soils are divided into polyethylene bags and incubated in the ground from which they were collected (sensu Eno 1960), in several long-term temperate forest study sites at the Coweeta Hydrological Laboratory, in Otto, NC, USA. This field-based approach ensured that AOA and AOB were exposed to environmentally relevant temperature regimes, nitrogen concentrations, moisture conditions, and dissolved oxygen concentrations during incubation. We then used multiple linear regression and a model selection procedure to link carbon accumulation by each group to the total amount of nitrate accumulation across incubations.

We predicted that AOA would be the dominant ammonia oxidizers in temperate forest soil ecosystems, because these soils are generally acidic and contain low levels of ammonium; conditions that have been shown to favor AOA rather than AOB activity (e.g., Nicol et al. 2008, Martens-Habbena et al. 2009). Furthermore, we predicted that we would find group level differences in paired carbon and nitrogen metabolism between AOA and AOB due to group-level differences in ammonia uptake affinities and mechanisms for ammonia capture (e.g., Martens-Habbena et al. 2009, Gorman-Lewis et al. 2014).

Methods

Site selection

This study was conducted at Coweeta Hydrologic Laboratory, a Long Term Ecological Research site located in the Appalachian Mountains of North Carolina, USA. Coweeta is a humid, temperate forest system, with a history of experimental watershed-level manipulations (Swank and Vose 1997). This variation in land-use history has contributed to significant variation in soil chemistry and watershed-level nitrogen export within the Coweeta Basin (Swank and Vose 1997; Adams et al. 2014).

Three sampling positions were located along stream-to-hillslope transects within each of four experimental watersheds (WS) at Coweeta, for a total of 12 sampling sites. We chose sites within the following watersheds at Coweeta: WS18, a reference watershed that has no history of disturbance since 1927; WS7, which was clear cut in 1977 using cable-logging; WS6, which was clear-cut, burned, limed, and fertilized from 1965-1967; and WS17, which was clear cut, then replanted in white pine in 1956. These differences in land use history have resulted in differences in nitrogen export as well (Swank and Vose 1997), with WS 18 exhibiting the lowest level of nitrogen export (0.09 kg inorganic nitrogen-N/hectare $\times$ year), followed by WS 7 (0.64 kg inorganic nitrogen-N/hectare $\times$ year), WS 17 (1.00 kg inorganic nitrogen-N/hectare $\times$ year), and finally WS 6 with the highest level of nitrogen export (7.09 kg inorganic nitrogen-N/hectare $\times$ year). By performing incubations in these watersheds we hoped to sample the breadth of nitrogen availability and soil pH in this system; changes in these conditions were expected to contribute to differences in habitat suitability for AOA and AOB (sensu Nicol et al. 2008, Martens-Habbena et al. 2009).

In this paper sampling site names are expressed as a combination of a number and letter (for example 7A). The number in the site name refers to the watershed in which the sampling site was located as discussed above. The letter refers to the position of the site along the stream-to-hillslope sampling transect as follows: A refers to 5 m from the stream, B refers to 10 m from the stream, C refers to 25 m from the stream.

Buried-bag incubations

At each site, we collected approximately 1 kg of soil from the top 5 cm of the soil profile. The soil was then sieved to remove small rocks and fine roots, and the sieved soil was then divided into sterile Whirl-Pak bags containing ~100 g soil each. Three replicate bags from each site were stored at 4°C prior to processing and analysis of day 0 soil conditions (day 0 soils), and three replicate bags were incubated in the ground for approximately 1 month (day 30 soils) to estimate net rates of total ammonia oxidation by NO_3^- accumulation (sensu Eno 1960) along with growth of AOA and AOB by AOA and AOB carbon accumulation. Day 30 bags from one site in WS 7 were damaged in transport, and this site (7C) was excluded from further analysis.

NO_3^- accumulation measurements

NO_3^- accumulation was measured as an index of ammonia oxidation in each bag. While other fluxes including heterotrophic nitrification, denitrification, and nitrate uptake could have affected NO_3^- accumulation rates during incubation, buried-bag estimates of nitrification assume that these fluxes are minor in comparison to the actions of nitrifying microorganisms. The methods we applied to measure NO_3^- capture soil NO_2^- as well and therefore NO_3^- accumulation serves as an estimate of ammonia oxidation regardless of the activity of nitrite oxidizing bacteria.

We measured soil water content along with the concentration of NO_3^- from each day 0 and day 30 bag and then used the numbers to calculate NO_3^- accumulation rates per gram dry weight (dw) of soil as follows. Soil water content was measured as weight loss from 10 g of soil after overnight incubation at 105°C. Inorganic nitrogen, including NO_3^-, was extracted from soils in 2M KCl (Bundy and Meisinger 1994). Extracts were filtered, and NO_3^- was measured using a Lachat Quickchem flow injection autoanalyzer (Lachat Instruments, Loveland, Colorado, USA). Values were reported as μg NO_3-N/g dw soil, respectively. NO_3^- accumulation was calculated per g dw of soil in each day 30 bag by Eq. 1.

$$NO_3^- \text{ accumulation} = \text{day 30 } NO_3^-/\text{g dw soil} - \text{avg. day 0 } NO_3^-/\text{g dw soil} \quad (1)$$

Carbon accumulation by AOA and AOB

By excluding plant demand for ammonia generated by mineralization, conditions created during buried-bag incubations can increase the abundance of ammonia oxidizers, even without nutrient amendment (Norman and Barrett 2014). AOA carbon accumulation and AOB carbon accumulation were estimated as metrics of net primary production by each group. We calculated AOA and AOB carbon accumulation by extracting DNA from soils, estimating the abundance of group-specific ammonia monooxygenase subunit A (*amoA*) genes in DNA extracts from each day 0 and day 30 bag using quantitative PCR (qPCR), converting these numbers to estimates of biomass carbon based on community structure data and culture-based estimates, and using these numbers to estimate rates of carbon accumulation as follows.

DNA extraction and qPCR

We extracted DNA from each bag of soil using PowerSoil DNA isolation kits (MO BIO Laboratories, California, USA). Kit protocols were followed, except that the final elution step altered to include two separate additions of 75 μL of solution C6, designed to maximize DNA extraction efficiency. A Nanodrop spectrophotometer (ThermoFisher Scientific, Waltham, Massachusetts, USA) was used to measure the 260/280 ratio of each extract. This ratio exceeded 1.8 in every extract, indicating that DNA was of high quality with low humic contamination.

AOA and AOB *amoA* abundances were estimated in day 0 and day 30 samples using SYBR-green based qPCR with Domain-specific primers, including *amoA-1F* (Stephen et al. 1998) and *amoA-2R* (Rotthauwe et al. 1997) for AOB, and *CrenamoA23f* and *CrenamoA616r* (Tourna et al. 2008) for AOA. All samples were run in triplicate and gene copy number was estimated in each

sample by comparing average threshold cycle values to a standard curve ranging from 10^1–10^6 copies of each gene ligated into a TOPO TA cloning vector (Invitrogen, ThermoFisher Scientific, Waltham, Massachusetts, USA), which was linearized prior to use as a qPCR standard. Plasmids used as AOB standards contained a 491 bp sequence of the *amoA* sequence from *Nitrosomonas europaea* (McTavish et al. 1993), while plasmids used as AOA standards contained a 628 bp sequence identical to soil fosmid 54d9 (Treusch et al. 2005). Standard curve r^2 values ranged between 0.998 and 0.999 for AOB and 0.984 and 0.995 for AOA; standard curve efficiency values ranged between 81% and 85% for AOB and 84% and 89% for AOA. Standard curve efficiency values could not be improved without increasing the amplification of non-specific products in some samples as assessed by melt curve analysis and verified by agarose gel electrophoresis.

Fifty microliter reactions contained 25 μL of Quantitect SYBR green PCR mix (Qiagen, Venlo, Netherlands), 0.2 mg/mL BSA, and 0.5 μM of each primer for AOB *amoA* enumeration, or 1.5 μM of each primer for AOA *amoA* enumeration. We initially added 50 ng of template DNA in both AOA and AOB reactions, but had to re-run all AOB reactions for one site with 250 ng template DNA in order to achieve a detection limit of >10 copies per reaction, and had to re-run all AOA reactions for another site with 1.25 ng of template DNA in order to eliminate the formation of non-specific PCR products. Though we did not intentionally test for PCR inhibition in samples or differences in reaction efficiency between unknown samples and standards (as recommended most-recently by Hargreaves et al. 2013), we obtained similar estimates of *amoA*/g dw of soil within samples during testing for optimization of template concentrations across the ranges of template addition tested (1.25–250 ng per reaction) when samples were not below detection. This indicates both a lack of PCR inhibition and that sample reaction efficiencies were similar to unknown reaction efficiencies. The use of 50 μL reactions (the maximum volume allowed in the thermocycler) was also designed to dilute out any PCR inhibitors that soil DNA extracts may have contained. The thermal protocol for both AOA and AOB was 95°C for 15 minutes, followed by 40 cycles of 95°C for 15 seconds, 53°C for 30 seconds, 72°C for 1 minute. All values were reported as either AOA or AOB *amoA*/g dw soil.

Converting gene copy number estimates to carbon accumulation

We estimated AOA and AOB carbon accumulation by each group by converting values of AOA and AOB *amoA* for day 0 and day 30 soils to AOA and AOB carbon using literature-based estimates, and then calculating carbon accumulation in each day 30 bag by Eqs. 2 and 3.

$$\text{AOA carbon accumulation} = (\text{day 30 AOA carbon/g dw soil}) - (\text{avg. day 0 AOA carbon/g dw soil}) \quad (2)$$

$$\text{AOB carbon accumulation} = (\text{day 30 AOB carbon/g dw soil}) - (\text{avg. day 0 AOB carbon/g dw soil}) \quad (3)$$

The factors we used to convert *amoA* values obtained by qPCR to estimates of AOA and AOB carbon relied on three key pieces of information for each group: (1) the average number of *amoA* copies per cell, (2) average cell volume, and (3) average cell carbon content. Since these values likely vary among AOA and AOB taxa, it was necessary to examine AOA and AOB community structure prior to estimating carbon conversion factors. We therefore measured community structure of AOA and AOB at each site by pyrosequencing of *amoA* PCR amplicons from each site using group specific primers (pyrosequencing and sequence-processing methods are outlined in the Appendix) and used these findings to inform group-specific *amoA* to carbon conversions.

Greater than 90% of the AOB sequences we recovered belonged to operational taxonomic units (OTUs) most closely related to isolates of the genus *Nitrosospira*. We therefore based our AOB *amoA* to AOB carbon conversion factor on available information for isolates from the genus *Nitrosospira* including *Nitrosospira multiformis* and *Nitrosospira briensis*. *N. multiformis* is the only member of the genus *Nitrosospira* for which we could find genomic data and this organism has 3

copies of *amoA* in its genome (Norton et al. 2008). We therefore assumed that the number of AOB cells/g dw soil was equal to 1/3 the number of AOB *amoA*/g dw soil. We based AOB cell size estimates on *N. briensis*, an AOB common to soil which Watson (1971) described as tightly coiled spirals, best represented as cylinders 1.5–2.5 μm in length and 0.8–1.0 μm in width. Using an average of these dimensions, we assumed an AOB cell volume of 1.27 μm^3.

Of the AOA *amoA* sequences we recovered across sites, 85% belonged to a single operational taxonomic unit (OTU) most closely related to *Nitrosotalea devanterra*, an acidophilic soil AOA isolate (Lehtovirta-Morley et al. 2011), and we therefore based AOA *amoA* to AOA carbon conversions on *N. devanterra* when information for this isolate was available. While the number of *amoA* amplicons in the *N. devanterra* genome remains unknown, genome sequences from other AOA isolates show a single copy of *amoA* per cell. We therefore assumed that AOA *amoA* copy number per gram of soil was equivalent to the number of AOA cells per gram of soil. Lehtovirta-Morley et al. (2011) described *N. devanaterra* cells as straight rods with an average length of 0.89 μm and an average width of 0.33 μm. We used this information to an estimate average AOA cell volume of 0.067 μm^3.

No data were available for biovolume to carbon conversions for single AOA and AOB taxa, nor were these data available for archaea in general. All biovolume to carbon conversions were therefore based on Eq. 4, which is a biovolume to carbon conversion formula for general bacteria as suggested by Romanova and Sazhin (2010)

$$\text{fg carbon per cell} = (133.754) \times (\text{cell biovolume}^{0.438}). \quad (4)$$

Using the aforementioned biovolume estimates for AOA and AOB with Eq. 4 yielded respective AOA and AOB carbon contents of 40.9 femtograms (fg) carbon/cell and 148.5 fg carbon/cell. Since AOA were assumed to have one *amoA* gene copy per cell, we assumed a final *amoA* gene copy to carbon conversion value of 40.9 fg carbon/AOA *amoA*. Since AOB were assumed to have three *amoA* genes per cell, we assumed a final *amoA* gene copy to carbon conversion factor of 49.5 fg carbon/AOB *amoA* copy, which represents 1/3 of the AOB carbon/cell estimate given above. We therefore converted AOA and AOB *amoA*/g dw soil estimates generated by qPCR to estimates of AOA and AOB carbon/g dw soil by Eqs. 5 and 6.

$$\text{AOA carbon/g dw soil} = (40.9 \text{ fg carbon/AOA } amoA) \times (\text{AOA } amoA/\text{g dw soil}) \quad (5)$$

$$\text{AOB carbon/g dw soil} = (49.5 \text{ fg carbon/AOB } amoA) \times (\text{AOB } amoA/\text{g dw soil}) \quad (6)$$

Model selection

We used multiple linear regression to explore the links between observed NO_3^- accumulation and AOA and AOB carbon accumulation across sites. We converted negative carbon accumulation values to zeros prior to performing this analysis since these values represent net cell death, which should not contribute to nitrate production by either organism. The equation from this analysis was used to assess paired carbon and nitrogen metabolism by each group across sites as explained in the discussion section of this paper. We assessed the validity of the resulting multiple linear regression equation using a stepwise model selection procedure based on the Akaike information criterion (AIC) values (function "stepAIC," library "MASS," the R Foundation for Statistical Computing, Vienna, Austria). This was done to determine whether carbon accumulation by both AOA and AOB was necessary to explain patterns in NO_3^- accumulation, or whether NO_3^- accumulation was related to the carbon accumulation by a single group in particular.

Results

AOA and AOB amoA abundances and general soil characteristics

Day 0 and day 30 estimates of AOA and AOB *amoA*/g dw of soil are presented in Fig. 1a and b, respectively. Soils ranged in pH from 4.77 to 5.95, with an average value of 5.27. Day 0 ammonia concentrations ranged from 6.71 to 25.2 μg NH_4-N/g dw soil and averaged 12.80 μg NH_4-N/g dw soil.

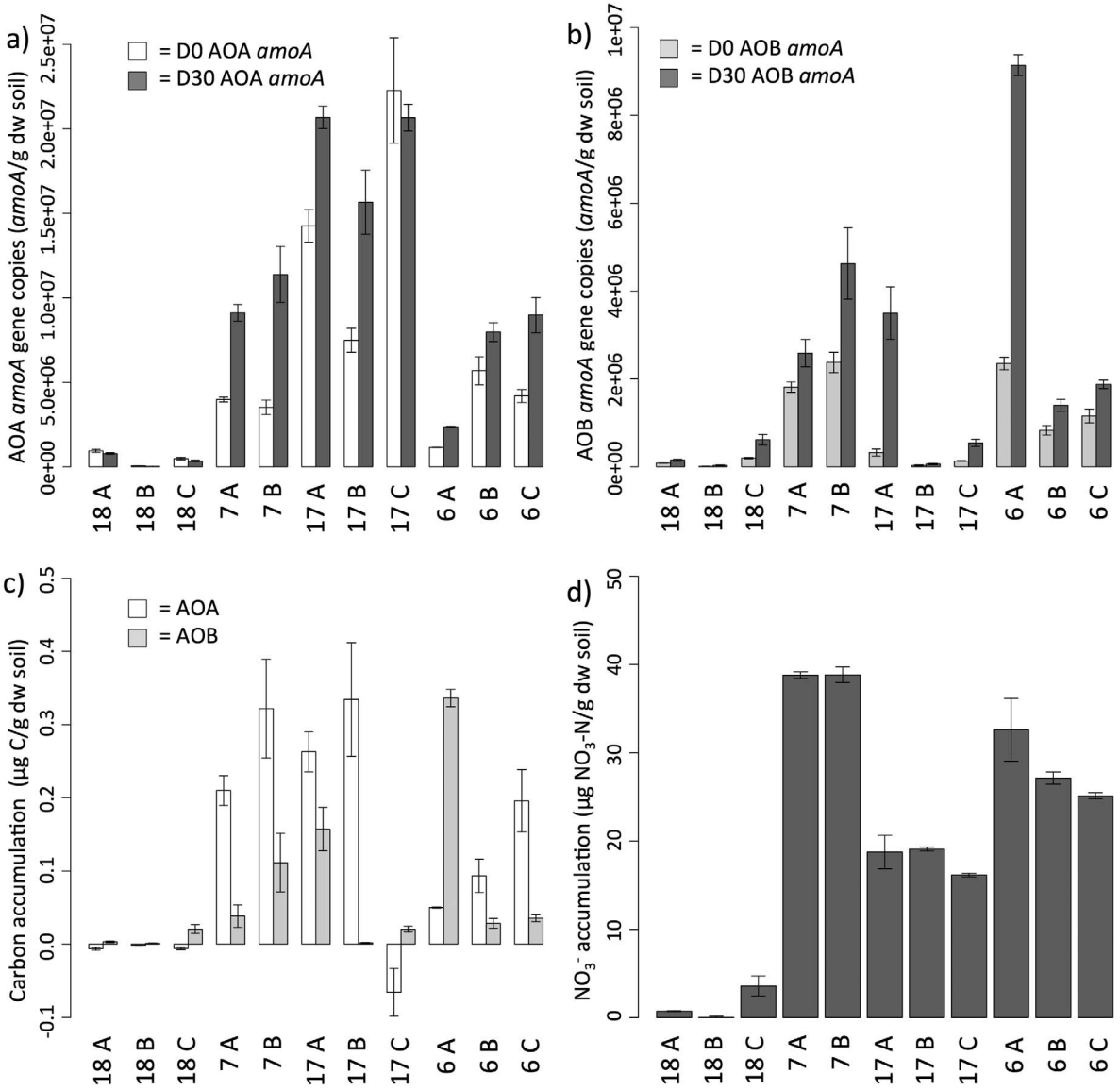

Fig. 1. (a) Day 0 and day 30 AOA *amoA* values and (b) day 0 and day 30 AOB *amoA* values. Error bars represent standard error between replicate incubations. Note that error between replicate qPCR reactions is not shown here. (c) Average AOA carbon accumulation (white bars) and AOB carbon accumulation (grey bars) values from replicate incubations at each sampling site as calculated by Eqs. 2 and 3. Error bars represent standard error between replicate incubations. (d) Average NO_3^- accumulation values from replicate incubations at each sampling site as calculated by Eq. 1. Error bars represent standard error between replicate incubations.

NO_3^- accumulation and carbon accumulation by AOA and AOB

We found values of NO_3^- accumulation that ranged between 0.031 and 38.84 µg NO_3-N/g dw soil across all sites examined at Coweeta (Fig. 1c). These values correspond to net nitrification rates of between 0.001 and 1.339 µg NO_3-N/g dw soil × day. We estimated values of AOA carbon accumulation that ranged from −0.066 to 0.334 µg C/g dw soil and values of AOB carbon accumulation that ranged from 0.001 to 0.336 µg C/g dw soil (Fig. 1d). We found negative

values of AOA carbon accumulation at 4 sites, though these values were close to zero (<0.01 μg C/g dw soil) at all four sites except for 17C, which had an AOA carbon accumulation value of −0.066 μg C/g dw soil.

Paired C and N metabolism by AOA and AOB

We used multiple linear regression to investigate the relationship between NO_3^- accumulation and carbon accumulation by AOA and AOB in order to look at the links between carbon and nitrogen metabolism by each group. We found a significant relationship ($P < 0.05$, $R^2 = 0.54$) between AOA and AOB carbon accumulation and NO_3^- accumulation (Fig. 2), which is described by Eq 7.

$$\begin{aligned} &NO_3^- \text{ accumulation} \\ &= (59.8 \times \text{AOA carbon accumulation}) \\ &\quad +(58.2 \times \text{AOB carbon accumulation}) \\ &\quad +8.1 \end{aligned} \tag{7}$$

The validity of this relationship was confirmed by a stepwise selection process based on AIC scores: the multiple linear regression presented in Eq. 7 had a lower AIC score (AIC = 54.7) than linear regressions that predicted NO_3^- accumulation based carbon accumulation by AOA (AIC = 58.6) or AOB (AIC = 56.2) alone. However, we did find a significant positive linear relationship between NO_3^- accumulation and AOA carbon accumulation ($P < 0.05$, $R^2 = 0.30$), while no such relationship existed between NO_3^- accumulation and AOB carbon accumulation.

Discussion

Here we used buried-bag incubations to investigate paired carbon and nitrogen metabolism by ammonia oxidizing microbes in temperate forest soils. Buried-bag incubations are often used to estimate background rates of nitrification by excluding plant demand for NO_3^- and thereby allowing for NO_3^- accumulation; indeed we saw positive values of NO_3^- accumulation at all 11 sites at which we were able to acquire data. However, we suspected that unamended net nitrification incubations would lead to growth of AOA and AOB as well due to the increased ammonium availability to these organisms (Norman and Barrett 2014 showed that this occurred in one soil from a reference watershed at Coweeta). Consistent with this prediction, we saw increased gene copy numbers of AOB *amoA*/g dw soil at every site and increased numbers of AOA *amoA*/g dw soil at 7 out of 11 sites that we sampled.

Interestingly, our results show that AOB are capable of growth during buried-bag incubations in acidic low ammonium soils, which is surprising since AOB isolates do not readily grow in these conditions in pure culture (Hatzenpichler 2012 and references therein). However, detectable AOB activity has been shown in soils below pH 5 (Nicol et al. 2008), and AOB growth during buried-bag incubations has been shown in a similar soil from Coweeta previously (Norman and Barrett 2014). Soils are likely heterogeneous at the microscopic scale, and it is possible that the AOB growth we observed occurred in less-acidic microsites with high ammonia availability. Furthermore, many AOB express urease, which allows them to obtain substrate in acidic soils (Pommerening-Röser and Koops 2005), and form biofilms that serve as a means of pH protection (Allison and Prosser 1993).

Converting *amoA* gene copy estimates to μg carbon allowed us to assess the relationships between carbon accumulation by AOA and AOB, a proxy for primary production by each group, and NO_3^- accumulation, which serves as a proxy for ammonia oxidation by both groups. Since there is no way to determine which proportion of the NO_3^- accumulation at each site was due to by activity of each group, we used a model selection procedure to assess statistical relationships between carbon accumulation by both groups and NO_3^- accumulation across sites. Our model selection procedure showed that carbon accumulation by both AOA and AOB best explained variation in NO_3^- accumulation across sites and we interpret this finding to show that both groups have a role in ammonia oxidation in the forest soils we sampled in line with our predictions.

Eq. 7 was generated from the aforementioned multiple linear regression procedure and is illustrated by the regression plane in Fig. 2. When solved for NO_3^- accumulation (as presented in Eq. 7), this regression equation essentially predicts NO_3^- accumulation values based on AOA and AOB carbon accumulation. The coef-

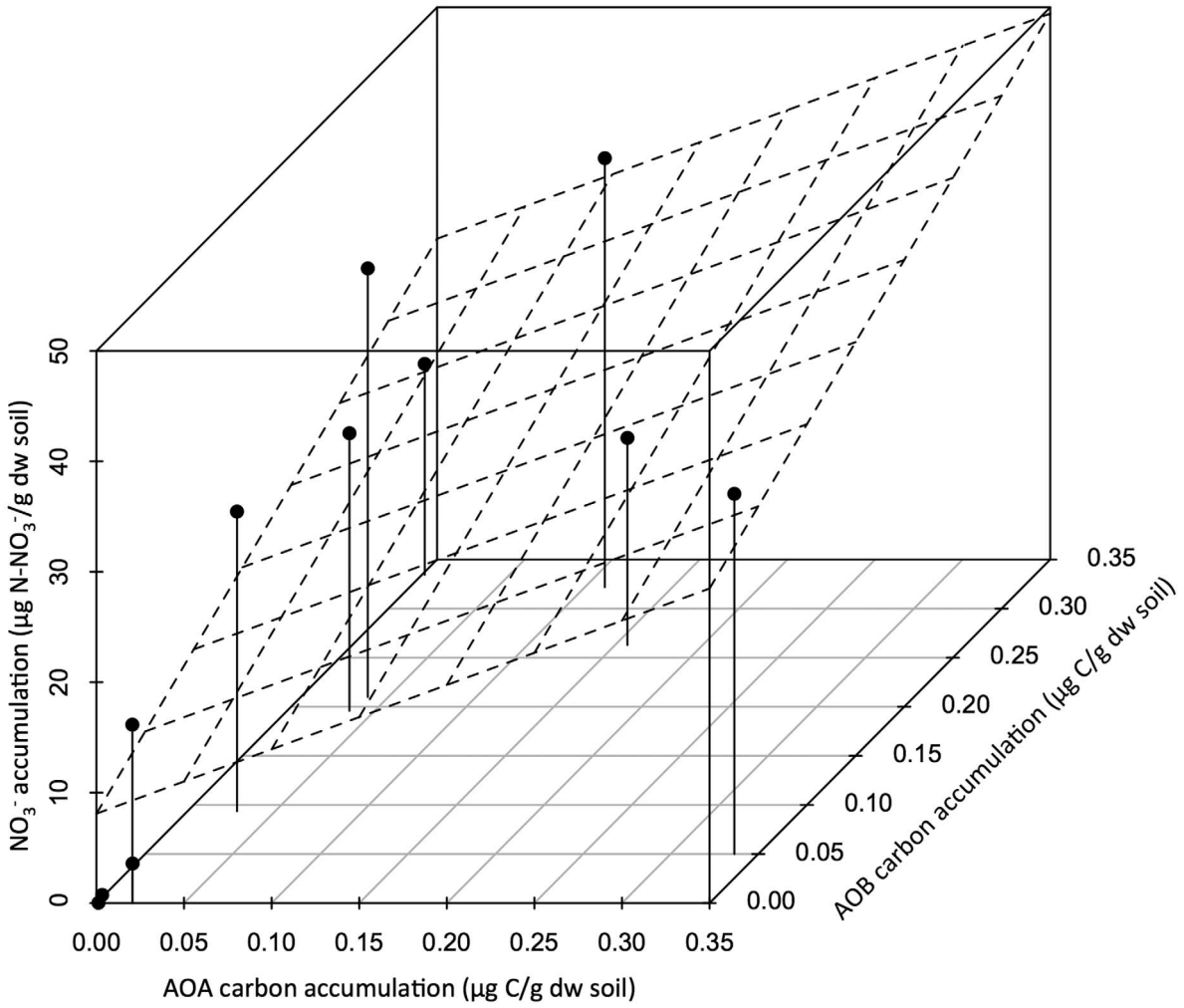

Fig. 2. Average NO_3^- accumulation values vs. average AOA and AOB carbon accumulation values across sampling sites. Plane represents a significant multiple linear regression ($P < 0.05$, $R^2 = 0.54$) with the equation $z = 59.8(x) + 58.2(y) + 8.1$. This equation is identical to Eq. 7 in this manuscript.

ficients from this equation represent conversions between carbon accumulation and NO_3^- accumulation for each group: AOA carbon accumulation at a given site will be multiplied by 59.8 to estimate NO_3^- accumulation by AOA, while AOB carbon accumulation at a given site will be multiplied by 58.2 to estimate NO_3^- accumulation by AOB. While the estimates of AOA and AOB NO_3^- accumulation generated by Eq. 7 may not be particularly accurate for a given site, we believe that the aforementioned coefficients from this equation provide insight into cross-site patterns of paired carbon and nitrogen metabolism by AOA and AOB. If one assumes that the carbon accumulation observed by each group results from carbon fixation driven by ammonia oxidation, these coefficients can be thought of as group-specific cross-site estimates of ammonia oxidation efficiency; that is, these coefficients represent the amount of ammonia oxidation each group must accomplish to fix 1 μg of carbon. Using the coefficients from Eq. 7, we can therefore estimate that AOA must oxidize 59.8 μg of ammonia-N to add 1 μg of carbon to biomass, while AOB must oxidize 58.2 μg of ammonia-N to add 1 μg of carbon to biomass in this system. Using molar mass conversions for nitrogen and carbon, we can similarly estimate

that AOA must oxidize ~51 molecules of ammonia for every molecule of carbon they add to biomass, while AOB must oxidize ~50 molecules of ammonia for every molecule of carbon they add to biomass.

What is most striking about these estimates is how similar they are despite the physiological differences, including the use of different carbon fixation pathways and the large phylogenetic distance, between AOA and AOB (Hatzenpichler 2012). Perhaps conditions in temperate soil environments dictate certain energetic constraints requiring similar ratios between ammonia oxidation and carbon fixation by each group. Winogradsky (1890) estimated that *Nitrosomonas europaea* oxidized 35 molecules of ammonia per molecule of carbon fixed in pure culture. Painter (1970) summarized known values of economic coefficients, defined as the weight of dried cells produced per weight of nitrogen oxidized, for ammonia oxidizing bacteria ranging from 0.04 to 0.13. This given range of economic coefficients corresponds to values of ~52 and ~16 molecules of ammonia oxidized per carbon added to biomass by fixation, respectively. The estimate we provide of ~50 molecules of ammonia oxidized per carbon added to biomass by AOB therefore falls within the range observed in pure culture. This estimate is on the low end of observed efficiency for AOB in pure culture, which is unsurprising due to the additional stresses associated with growth in the field rather than in the laboratory, including imperfect environmental factors and competition for ammonia.

However, certain assumptions went into the production of our model, the accuracy of which cannot be verified from our data. For instance, as no field data were available, we relied on cell biovolume estimates from culture-based studies. Cell biovolumes may differ in field conditions and accurate estimates of AOA and AOB cell volumes would improve carbon accumulation estimates in the future. Also, we assumed that all carbon accumulation was due to chemoautotrophc carbon fixation, while both groups could be using organic carbon to supplement their biomass carbon demand. Furthermore, we assumed that positive carbon accumulation values represented only AOA and AOB growth rather than the difference between growth and death. However, while AOA and AOB cells likely died during buried-bag incubations, our qPCR assay could still detect dead cells as long as the DNA from these cells had not degraded. Also, as with all nitrification estimates from buried-bag incubations, we assumed that the NO_3^- accumulation values were primarily due to the activity of nitrifying microorganisms, and were not affected by other processes such as NO_3^- uptake or denitrification.

Despite these caveats, we feel that this study provides important information about ammonia oxidation as a process in temperate forest soils. Evidence from our model selection procedure suggests that both AOA and AOB are involved in ammonia oxidation at the landscape scale, though participation by each group varies from site to site. Furthermore, we believe that this is the first study to explicitly investigate paired carbon and nitrogen metabolism by ammonia oxidizers in a field setting. We give remarkably similar estimates for the amount of ammonia oxidation each group must accomplish to add carbon biomass across sites but we feel that this is merely a first step for research on paired carbon and nitrogen metabolism in AOA and AOB. Future work on this matter could employ stable isotope-based techniques to explicitly track carbon fixation by each group in addition to growth, and investigate whether estimates of paired carbon and nitrogen cycling by AOA and AOB are constant at the group level or taxon level, whether they differ by environment, and whether individual organisms can change their efficiency in response to ammonia availability or other environmental factors.

Acknowledgments

The authors would like to thank several members of the Virginia Tech Stream Team, most notably Jackson Webster, who gave advice on sampling at Coweeta and reviewed portions of this manuscript, and Bobbie Niederlehner, who performed much of the analytical chemical analyses described here. This work was funded by two National Science Foundation grants (NSF DEB #083293; NSF DEB #1210607) and a grant from the Fralin Life Science Institute at Virginia Tech.

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Supplemental Material

Ecological Archives

The Appendix is available online: http://dx.doi.org/10.1890/ES14-00299.1.sm