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Research paper

Ammonia-uptake kinetics and domain-level contributions of bacteria and archaea to nitrification in temperate forest soils

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ABSTRACT

Ammonia-oxidizing bacteria and archaea (AOA and AOB) perform the rate-limiting step of nitrification, a biogeochemical process that controls the availability of inorganic nitrogen in terrestrial and aquatic ecosystems. We sought to investigate field values of AOA and AOB ammonia-uptake kinetics along with domain-level contributions to ammonia oxidation in temperate forest soils. To accomplish this goal, we constructed an ecosystem model that simulates ammonia oxidation in temperate forest soils based only on inorganic nitrogen pools and AOA and AOB population dynamics observed during *in situ* incubations. The model used Bayesian Markov chain Monte Carlo procedure to choose the most likely combination of *in situ* ammonia-uptake parameters for AOA and AOB, including $K_{m,AOA}$, $K_{m,AOB}$, $V_{max,AOA}$, and $V_{max,AOB}$. Domain-level contributions to ammonia oxidation were extracted from the best-fit solution and the model-selected values indicate that AOB was responsible for 70.0% of the simulated ammonia oxidation across sites, while AOA was responsible for the remaining 30.0%. We believe that the approach we demonstrate here can be applied to microbially-mediated biogeochemical fluxes in other elemental cycles as well.

1. Introduction

Nitrification, the oxidation of ammonium to nitrate, is a biogeochemical process primarily controlled by chemoautotrophic microbes. The high mobility of nitrate in soils, along with its role as a substrate for denitrification, make nitrification a critical mediator of nitrogen availability and loss in terrestrial and aquatic ecosystems alike. Ammonia-Oxidizing Bacteria (AOB) and Archaea (AOA) coexist in high numbers in a variety of terrestrial environments (Leininger et al., 2006) and both groups likely contribute to ecosystem-scale ammonia oxidation, the rate limiting step of nitrification. However, studies designed to estimate AOA and AOB contributions to ammonia oxidation in terrestrial systems fail to give quantitative estimates of domain-level contributions to this important flux; rather these studies typically give qualitative conclusions that either AOA (*e.g.* Di et al., 2009; Gubry-Rangin et al., 2010; Zhang et al., 2010, 2012) or AOB (*e.g.* Jia and Conrad, 2009; Onodera et al., 2010) are responsible for the majority of ammonia oxidation, or that both AOA and AOB have some level of contribution to the process (*e.g.* Pratscher et al., 2011; Norman et al., 2015), in a given environment. Furthermore, while ammonia-uptake kinetics for AOA and AOB are quite different in optimal lab conditions (*i.e.* Martiens-Habbena et al., 2009) little is known about the uptake kinetics of ammonia oxidizers in natural ecosystems.

Here we used an ecosystem modeling approach to estimate the contributions of AOA and AOB to ammonia oxidation and gain insight into domain-level ammonia-uptake kinetics in temperate forest soils. The biogeochemical model we constructed simulates nitrification based on AOA and AOB population dynamics observed during *in situ* incubations conducted at the Coweeta Long Term Ecological Research site in Otto, North Carolina U.S.A. (hereafter Coweeta). The model fit was optimized by varying AOA and AOB uptake parameters and comparing simulated rates of nitrification to those observed in the field. Once a best-fit solution was achieved, we extracted domain-level contributions to ammonia oxidation from the model and we interpreted the best-fit parameters as system-specific *in situ* Michaelis-Menton ammonia-uptake parameters for AOA and AOB. Finally, we conducted a global sensitivity and uncertainty analysis (*sensu* Saltelli, 2002) of the resulting model to evaluate which model inputs, including those related to AOA and AOB, had the biggest effects on model outputs, including both total

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simulated ammonia oxidation and simulated ammonia oxidation by AOA and AOB.

Soils at Coweeta are acidic and low in inorganic nitrogen (Norman et al., 2015), conditions that have been shown to favor AOA over AOB activity (*e.g* Di et al., 2009; Nicol et al., 2008; Gubry-Rangin et al., 2010; Zhang et al., 2012). We therefore predicted that AOA would have a greater role in nitrification than AOB across the sites that we sampled. Furthermore, AOA isolates reach maximum oxidation rates at lower substrate concentrations than AOB in pure culture (*i.e.* AOA have lower Km values than AOB) and maximum cellular oxidation rates of AOA are lower than AOB isolates in pure culture as well (Martiens-Habbena et al., 2009); we predicted that these patterns would be observed in soil systems as well.

2. Methods

2.1. Buried bag incubations, DNA extraction, and quantitative PCR

What follows is a brief description of our field and laboratory methods, the details of which are reported in Norman et al., 2015. We conducted triplicate buried-bag incubations (hereafter soil incubations) of ~100 g of soil at 12 sites in Coweeta following methods presented by Eno (1960). Three sampling sites, labeled A, B, and C in alphabetical order of increasing distance from a stream, were chosen in each of four experimental watersheds (WS) at Coweeta: WS 6, which was clear cut, limed, and fertilized; WS 7, which was clear cut and allowed to regrow; WS 17, which was replanted in pine; WS 18, which has remained undisturbed since 1927 (Swank and Vose, 1997). We describe sites using a combination of a number and letter: for example site 17A was collected near the stream in watershed 17. Site 7C was excluded from further analysis because samples from this site were damaged by animals during the incubation. We therefore based our model on data from soil incubations conducted at 11 sites at Coweeta. Note that the raw data used to create this model is publicly-available through the Coweeta LTER website (coweeta.uga.edu).

We extracted NH_4^+ and NO_3^- from pre-incubation (day 0) and post-incubation (day 30) soils in 2 M KCl. Concentrations of N-NH_4^+ and N-NO_3^- were measured in each KCl extract using a Lachat Autoanalyzer (APHA 1Autoanalyzer (APHA, 1999) and were reported per gram dry weight of soil (g dw soil). We extracted DNA for day 0 and day 30 soils using MO-BIO Powersoil kits and measured the abundance of AOA and AOB *amoA* genes by qPCR in each sample, using primer sets *amoA-1F* (Stephen et al., 1998) and *amoA-2R* (Rotthauwe et al., 1997) to amplify the *amoA* gene of AOB, and *CrenamoA23f* and *CrenamoA616r* (Tourna et al., 2008) to amplify the *amoA* gene of AOA. Gene copy numbers were converted to estimates of AOA and AOB abundance per g dw soil by assuming one *amoA*/cell for AOA (Hatzenpichler, 2012) and three *amoA*/cell for AOB. The AOB conversion number was based on the amount of copies per cell in the genome of *Nitrosospira multiformis* (Norton et al., 2008) since the AOB community at Coweeta is dominated by members of the genus *Nitrosospira* (Norman et al., 2015).

2.2. Model description and parameterization

Using data from our soil incubations (presented in Fig. 1), we constructed an ecosystem model designed to simulate NO_3^- and NH_4^+ dynamics at each of our 11 sampling sites based entirely on population dynamics of AOA and AOB. A conceptual outline of this model is presented in Fig. 2. No environmental factors such as soil moisture, temperature, or pH were explicitly included in our model; since the observed growth patterns of AOA and AOB were likely affected by these environmental variables, the influence of environmental variables is implicitly included by incorporating organismal growth into the model parameterization procedure we describe below.

2.2.1. Simulating growth and population sizes of AOA and AOB

As described above, we measured pre- and post-incubation population sizes of AOA ($AOA_{initial}$, AOA_{end}) and AOB ($AOB_{initial}$, AOB_{end}) during buried bag incubations by qPCR; we then used these data to simulate daily population sizes of AOA and AOB between sampling dates. The model presented here accomplishes this by assuming that growth of both groups was exponential and that growth of each group was governed by growth rates specific to each site (i) for AOA ($\lambda_{AOA,i}$) and AOB ($\lambda_{AOB,i}$), all of which remained constant for each group at each site during the course of the incubation. We believe this to be the most parsimonious growth assumption for the incubations we conducted (reasons for this further are outlined in the discussion section of this paper).

Site-specific daily growth rates for AOA ($\lambda_{AOA,i}$) and AOB ($\lambda_{AOB,i}$), during incubations in which positive population growth was observed were calculated by Eqs. (1) and (2):

$$\lambda_{AOA,i} = \frac{ln\left(AOA_{end,i}\right) - ln\left(AOA_{initial,i}\right)}{t_{end,i} - t_{initial,i}}, \quad (1)$$

$$\lambda_{AOB,i} = \frac{ln\left(AOB_{end,i}\right) - \ln\left(AOB_{initial,i}\right)}{t_{end,i} - t_{initial,i}}, \quad (2)$$

where t_{inital} and t_{end} are the initial and end time points for each incubation in days (incubation times varied between 29 and 31 days). It is important to note that our approach for calculating growth of AOA and AOB makes no assumptions about which energy or carbon sources AOA and AOB were accessing during these incubations; we merely incorporated empirical growth data from each site into the model to give a realistic assessment of the numbers of organisms present during each day of our incubation.

Site specific growth rates (*i.e.*, $\lambda_{AOA,i}$ and $\lambda_{AOB,i}$) were then used to simulate AOA and AOB population sizes at each time point (t), by Eqs. (3) and (4).

$$AOA_{t+1,i} = AOA_{t,i} + AOA_{t,i} \times \lambda_{AOA,i} \times \Delta t \quad (3)$$

$$AOB_{t+1,i} = AOB_{t,i} + AOB_{t,i} \times \lambda_{AOB,i} \times \Delta t \quad (4)$$

The time step (Δt) used in Eqs. (3) and (4), as well as subsequent equations, was equal to 1 day.

We observed net decreases in cell numbers of AOA at 3 sites, and the negative growth (*i.e.* death, represented here by the symbol φ) of AOA was simulated as linear at these sites by Eq. (5):

$$\varphi_{AOA,i} = (AOA_{end,i} - AOA_{initial,i})/(t_{end,i} - t_{initial,i}) \quad (5)$$

Daily population sizes of AOA were therefore simulated in the three sites with net decreases in cell numbers of AOA by Eq. (6):

$$AOA_{t+1,i} = AOA_{t,i} + (\varphi_{AOA,i} \times \Delta t) \quad (6)$$

2.2.2. Simulating ammonia uptake, oxidation, and assimilation by AOA and AOB

AOA and AOB affected NH_4^+ pools in the model through an uptake flux that was divided into both domain-specific daily assimilation and domain-specific daily ammonia oxidation activity once NH_4^+ was acquired by each group. Simulated AOA and AOB ammonia uptake during each time step was governed by Michalis-Menten kinetics according to Eqs. (7) and (8):

$$AOA\ ammonia\ uptake_i = AOA_{t,i} \times \frac{V_{max,AOA} \times \left[NH_4^+\right]_{t,i}}{K_{m,AOA} + \left[NH_4^+\right]_{t,i}} \times \Delta t\ , \quad (7)$$