

ORIGINAL ARTICLE

Modeling of soil nitrification responses to temperature reveals thermodynamic differences between ammonia-oxidizing activity of archaea and bacteria

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Soil nitrification potential (NP) activities of ammonia-oxidizing archaea and bacteria (AOA and AOB, respectively) were evaluated across a temperature gradient (4–42 °C) imposed upon eight soils from four different sites in Oregon and modeled with both the macromolecular rate theory and the square root growth models to quantify the thermodynamic responses. There were significant differences in response by the dominant AOA and AOB contributing to the NPs. The optimal temperatures (T_{opt}) for AOA- and AOB-supported NPs were significantly different ($P < 0.001$), with AOA having $T_{opt} > 12$ °C greater than AOB. The change in heat capacity associated with the temperature dependence of nitrification (ΔC_p^\ddagger) was correlated with T_{opt} across the eight soils, and the ΔC_p^\ddagger of AOB activity was significantly more negative than that of AOA activity ($P < 0.01$). Model results predicted, and confirmatory experiments showed, a significantly lower minimum temperature (T_{min}) and different, albeit very similar, maximum temperature (T_{max}) values for AOB than for AOA activity. The results also suggested that there may be different forms of AOA AMO that are active over different temperature ranges with different T_{min} , but no evidence of multiple T_{min} values within the AOB. Fundamental differences in temperature-influenced properties of nitrification driven by AOA and AOB provides support for the idea that the biochemical processes associated with NH_3 oxidation in AOA and AOB differ thermodynamically from each other, and that also might account for the difficulties encountered in attempting to model the response of nitrification to temperature change in soil environments.

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Introduction

Both ammonia-oxidizing archaea (AOA) and bacteria (AOB) co-habit diverse soils. In some situations, AOA outnumber AOB by two to three orders of magnitude, and in other cases, their abundances are more similar (Leininger *et al.*, 2006, Adair and Schwartz, 2008, Schauss *et al.*, 2009, Taylor *et al.*, 2012). Considerable debate has occurred about the relative contributions of AOA and AOB to soil nitrification, and the factors that may influence those contributions (Schleper, 2010, Hatzenpichler, 2012, Prosser and Nicol, 2012). There is direct and indirect evidence that factors such as increased CO_2 , N concentration and form, pH, and temperature differentially influence AOA and AOB contributions to

nitrification (Hu *et al.*, 2015, Liu *et al.*, 2015, Sun *et al.*, 2015). For example, growth of pure cultures of AOB isolates is usually optimal ≤ 30 °C (Jiang and Bakken, 1999), and one AOB, *Nitrosomonas cryotolerans*, can grow at 4–5 °C (Jones *et al.*, 1988). These observations corroborate several studies that showed the temperature optimum of soil nitrification is often ≤ 30 °C (Malhi and McGill, 1982; Dalias *et al.*, 2002; Avrahami *et al.*, 2003; Fierer *et al.*, 2003), and soil nitrification has been measured at temperatures as low as 2 °C (Cookson *et al.*, 2002).

However, a few reports have described nitrification in soils from western United States and Australia with temperature optima of 35–40 °C (Myers, 1975; Stark and Firestone, 1996). The isolation of the AOA *Nitrososphaera gargensis* and *Nitrosocaldus yellowstonii* from geothermal sources with temperature optima of 46 and 65–72 °C, respectively (de la Torre *et al.*, 2008; Hatzenpichler *et al.*, 2008), and the discovery of AOA soil isolates, *Nitrosotalea devanattera* Nd1, ‘*Candidatus* Nitrosocosmicus franklandus’ and *Nitrososphaera viennensis*, growing optimally at 35–40 °C (Tournia *et al.*,

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2011; Lehtovirta-Morley *et al.*, 2014; Lehtovirta-Morley *et al.*, 2016), led us to speculate that AOA might be primarily responsible for soil nitrification $\geq 30^\circ\text{C}$. In a field study, we previously observed increases of AOA numbers, and evidence of their activity, in cropped soils under late summer/early fall conditions when soil temperatures reached $30\text{--}35^\circ\text{C}$ (Taylor *et al.*, 2012), and studies from a UK agricultural soil incubated at temperatures between 10 and 30°C showed that the thaumarchaeal composition (determined by analysis of the relative abundance of 16S ribosomal RNA) shifted during incubation at 25 and 30°C , whereas there was no change in the AOB community composition (Tourna *et al.*, 2008; Offre *et al.*, 2009). In contrast, AOA were enriched from Arctic soils incubated at 20°C , and rates of nitrification were measured in three Arctic soils incubated at 15°C , where AOB *amoA* numbers were below detection (Alves *et al.*, 2013).

In previous studies, we showed that the relative contributions of AOB and AOA to nitrifying activity varied among four pairs of noncropped and cropped soils sampled throughout the state of Oregon (Taylor *et al.*, 2013, 2015; Giguere *et al.*, 2015). In this study, the AOA and AOB contributions to the nitrification potential (NP) of the above-mentioned soils were evaluated over a temperature range that they annually experience ($4\text{--}42^\circ\text{C}$). We hypothesized that AOA and AOB would exhibit different characteristic responses to temperature, and to quantify these differences we used two models: the square root growth model (SQRT) that has been used to relate soil respiratory and growth processes to temperature (Birgander *et al.*, 2013; van Gestel *et al.*, 2013), and the macromolecular rate theory model (MMRT) utilized by Schipper *et al.* (2014) to model the temperature response of a variety of microbial activities, including nitrification.

Materials and methods

Chemicals

Vanadium chloride, 1-octyne (C_8) and NH_4Cl were obtained from Sigma-Aldrich (St Louis, MO, USA). Acetylene was obtained from Airgas (Radnor, PA, USA).

Collection of soils

In the spring of 2014, soil samples, representing different soil types from different climate regions of Oregon, were collected from cropped fields and noncropped locations at four locations (three replicates of each) at Oregon State University Agricultural Experimental Stations located in Corvallis, Pendleton, Madras and Klamath Falls, Oregon (Taylor *et al.*, 2013; Giguere *et al.*, 2015). Four to five soil samples were recovered to a depth of 10 cm from each replicate site via a random walk process. A composited sample was prepared for each replicate site and

brought to the laboratory where they were sieved $<4.75\text{ mm}$. The soils were stored at 4°C before experimentation.

Response of soil NPs to temperature

NPs were determined on soil samples as described previously (Taylor *et al.*, 2010b), except slurries were shaken at a range of temperatures ($4\text{--}42^\circ\text{C}$) in deionized water containing 1 mM NH_4^+ . Some NP treatments included octyne ($4\text{ }\mu\text{M}$) to distinguish between AOA and AOB activities, using a procedure described by Taylor *et al.* (2013). AOA are octyne resistant, and AOB are octyne sensitive (total nitrification – nitrification by octyne-resistant AOA). NP controls were comprised of soil suspensions to which acetylene was added ($10\text{ }\mu\text{M}$) to evaluate the possibility of acetylene-resistant heterotrophic nitrification, and also to assess the significance of NO_3^- consumption due to assimilation or denitrification. Each treatment had three replicates. Accumulation of $\text{NO}_2^- + \text{NO}_3^-$ was monitored over a 3-day interval in incubations performed at 4 , 10 and 16°C , whereas 1 day was sufficient for incubations at higher temperatures. The accumulation of $\text{NO}_2^- + \text{NO}_3^-$ in the NPs was considered to be the rate of nitrification. NO_2^- accumulated to various fractions of the total $\text{NO}_2^- + \text{NO}_3^-$ in actively nitrifying soil slurries over the temperature range, and generally increased as temperature increased but did not change the overall rate of $\text{NO}_2^- + \text{NO}_3^-$ accumulation. The accumulation of $\text{NO}_2^- + \text{NO}_3^-$ in the 1- or 3-day NPs was linear at all temperatures, indicating that there was no change in the nitrifier abundance or adaptation/recovery of NP activity over the course of the NP assays. There was no significant NO_3^- consumption or production in plus acetylene controls (data not shown), indicating all $\text{NO}_2^- + \text{NO}_3^-$ accumulation was likely due to NH_4^+ -dependent nitrification, and that alternate sinks for NO_2^- or NO_3^- were not significant under NP conditions.

Modeling of the nitrification response to temperature

Each replicate of the NP temperature response data was modeled using two methods. The first was the SQRT developed by Ratkowsky *et al.* (1983) to describe bacterial growth response to temperature, but has been used more recently to describe soil respiratory and growth processes to temperature (Birgander *et al.*, 2013; van Gestel *et al.*, 2013). Here it was used to model the NP response to temperature:

$$\sqrt{\text{Bacterial growth}} = a(T - T_{\min})(1 - e^{b(T - T_{\max})}) \quad (1)$$

Four unknown parameters were fit to this model in MATLAB (The MathWorks Inc., Natick, MA, USA): (i) ' a ' is a parameter associated with the temperature response of activity at temperatures below the temperature at which the highest nitrification rates

were obtained (T_{opt}); (ii) T_{min} represents a theoretical constant describing the x -intercept, and is referred to as the apparent minimum temperature at which activity is measured; (iii) ' b ' describes the decrease in nitrifying activity above T_{opt} ; and (iv) T_{max} represents the maximum temperature where activity may be detected. T_{opt} is described by the Equation

$$-a \left((b(T - T_{\text{min}}) + 1) e^{(b(T - T_{\text{max}}))} - 1 \right) = 0 \quad (2)$$

The second model used was MMRT utilized by Schipper *et al.* (2014) to model the temperature response of a variety of microbial activities, including nitrification, and is described by the Equation

$$\ln(k) = \ln\left(\frac{k_B T}{h}\right) - \frac{\Delta H_{T_o}^\ddagger + \Delta C_P^\ddagger(T - T_o)}{RT} + \frac{\Delta S_{T_o}^\ddagger + \Delta C_P^\ddagger(\ln T - \ln T_o)}{R} \quad (3)$$

where k is the rate constant, T_{naught} (T_o) is the reference temperature at which the fitting process is initiated, k_B is Boltzmann's constant, h is Planck's constant and R is the universal gas constant ($8.314 \text{ J K}^{-1} \text{ mol}^{-1}$). Three unknown parameters were fit to the model using SigmaPlot (Systat Software, San Jose, CA, USA): (i) the change of enthalpy of AOA or AOB supported nitrification ($\Delta H_{T_o}^\ddagger$), (ii) the change of entropy of the AOA or AOB supported nitrification ($\Delta S_{T_o}^\ddagger$), and (iii) the change in heat capacity associated with temperature dependence of $\text{NO}_2^- + \text{NO}_3^-$ accumulation attributed to AOA or AOB (ΔC_P^\ddagger). The temperature for optimum activity T_{opt} is described by:

$$T_{\text{opt}} = \frac{\Delta H_{T_o}^\ddagger + \Delta C_P^\ddagger T_o}{-\Delta C_P^\ddagger - R} \quad (4)$$

The temperature where the AOA- or AOB-supported $\text{NO}_2^- + \text{NO}_3^-$ accumulation rate shows maximum sensitivity to changes in temperature ($T_{s_{\text{max}}}$) is described by:

$$T_{s_{\text{max}}} = \frac{T_{\text{opt}}}{1 + \sqrt{\frac{k}{\Delta C_P^\ddagger}}} \quad (5)$$

From the modeling activities, we obtained information that predicted there were different temperature responses of AOA- and AOB-driven activities. As a consequence, a series of experiments were conducted to (i) gain further insights into how well the recovery of acetylene-inactivated $\text{NO}_2^- + \text{NO}_3^-$ production activity in both AOA- and AOB (biosynthetic competence) matched $\text{NO}_2^- + \text{NO}_3^-$ production activity across the temperature range, (ii) confirm the predicted T_{min} and T_{max} of AOA and AOB activity, and (iii) determine whether $T_{s_{\text{max}}}$ values reflected simply a thermodynamic response of preexisting activity potential, or whether they were influenced

by the recruitment/activation of nitrifying activity of subpopulations with different temperature profiles. This series of experiments was conducted in Pendleton noncropped soils where both AOA and AOB consistently demonstrated their highest rates of $\text{NO}_2^- + \text{NO}_3^-$ production activity.

The effect of temperature on acetylene inactivation of soil slurries

(i) Does biosynthetic competence match $\text{NO}_2^- + \text{NO}_3^-$ production activity in AOA and AOB? The ability of acetylene to irreversibly denature NH_3 monooxygenase (AMO) depends upon the enzyme being potentially active and capable of using acetylene as a substrate under the experimental conditions; recovery of AMO activity is dependent upon the ability to carry out *de novo* protein synthesis under the same conditions. Therefore, we used the recovery of nitrification potential (RNP) assay as a surrogate to assess the protein synthesis (biosynthetic) potential of the AOB and AOA at a range of temperatures (10, 16, 23, 30 or 37 °C) in Pendleton noncropped soil. Details of the RNP assay have been described previously (Taylor *et al.*, 2010b). Soils were preincubated at field capacity for 2–3 days at specific temperatures of 10, 16, 23 or 30 °C, or for a shorter duration (18 h) at 37 °C. Then, the soils were slurried in deionized water containing 1 mM NH_4^+ , exposed to acetylene ($10 \mu\text{M C}_{\text{aq}}$) for 6 h at each of the specific temperatures, after which acetylene was removed by evacuation under vacuum, and RNP determined plus and minus bacterial protein synthesis inhibitors (RNP_{ab}, 800 $\mu\text{g ml}^{-1}$ kanamycin plus 400 $\mu\text{g ml}^{-1}$ spectinomycin) at the specific inactivation temperatures. At 10 °C, soil was allowed to recover activity for 90 h, whereas the magnitude of recovery at other temperatures was determined after 48–72 h. Controls included (a) no alkyne amendment, (b) plus acetylene ($10 \mu\text{M C}_{\text{aq}}$) and (c) plus octyne ($4 \mu\text{M C}_{\text{aq}}$) treatments to establish the AOA- and AOB-dependent activities at each temperature.

(ii) Effect of temperature on the magnitude of acetylene inactivation of optimal nitrification activity to experimentally determine T_{min} and T_{max} . Noncropped Pendleton soil was preincubated at temperatures ranging from 4 to 42 °C as described above. Soils were slurried in distilled H_2O containing 1 mM NH_4^+ and exposed to acetylene ($10 \mu\text{M C}_{\text{aq}}$) for 6 h at each specific preincubation temperature. Then, acetylene was removed by vacuum, and soil slurries were incubated plus and minus $4 \mu\text{M C}_{\text{aq}}$ octyne with shaking at 16 or 37 °C (the optimal temperatures for AOB or AOA activity in this soil, respectively, see Figure 1). Controls included no alkyne amendment, plus acetylene ($10 \mu\text{M C}_{\text{aq}}$) and plus octyne ($4 \mu\text{M C}_{\text{aq}}$) treatments to evaluate the effect of the temperature inactivation step on activity at the optimal temperatures. Maximum RNP_{AOA} at 37 °C in this soil normally occurred between 24 and 48 h after acetylene removal, and maximum RNP_{AOB}

at 16 °C occurred between 48 and 72 h after acetylene removal. Accumulation of $\text{NO}_2^- + \text{NO}_3^-$ after ≤ 12 h of incubation was considered to be residual NH_3 -oxidizing activity that had escaped acetylene

inactivation. The octyne-resistant residual activity was considered to be residual AOA activity, whereas residual AOB activity was calculated as the difference between the plus and minus octyne treatments.

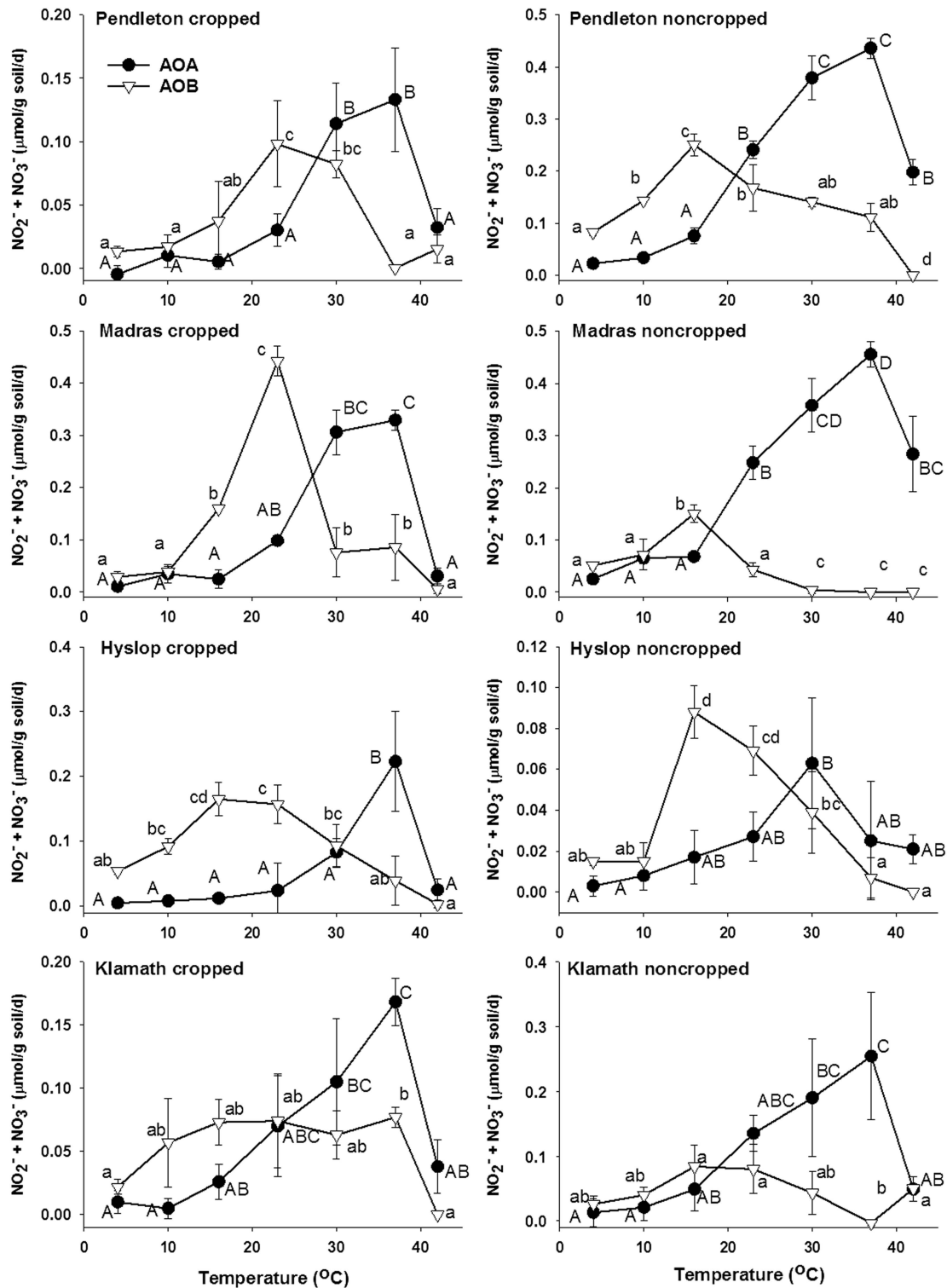


Figure 1 Nitrification potential response of AOA and AOB in cropped and noncropped soils to a range of temperatures (4–42 °C). Symbols with error bars represent the s.d. of the average of three replicate measurements. Upper case letters indicate significant differences ($P < 0.05$) in rates of nitrification by AOA at each temperature. Lower case letters indicate significant differences ($P < 0.05$) between rates of nitrification by AOB at each temperature. Temperatures that have letters in common were not significantly different.

To confirm that 6 h of incubation in the presence of acetylene was sufficient to completely inhibit all NH_3 -oxidizing activity occurring at temperatures of 10, 23 and 30 °C, a longer exposure time (10 h) was evaluated. There was no significant difference ($P > 0.1$) between the residual activity after the two periods of acetylene inactivation.

Statistical analysis

To determine the significant differences ($P < 0.05$) in AOA or AOB NP activities over the 4–42 °C range, analysis of variance (ANOVA) tests with the Tukey–Kramer adjustment were performed using the three replicates in Statgraphics Version 17.1.06 (Statpoint Technologies, Warrenton, VA, USA) for all pair-wise comparisons among temperatures within each soil. With the sites as replicates, ANOVA using the default Type III Sum of Squares was performed in Statgraphics on the average of the mean of each of the model coefficients to determine the statistical significance of each factor. ANOVA using the Holm–Sidak method was used for pair-wise comparisons in SigmaPlot (Systat Software, San Jose, CA, USA) to determine whether there were significant differences in AOA or AOB activity after acetylene treatment.

Results

AOA and AOB contributions to $\text{NO}_2^- + \text{NO}_3^-$ production over a temperature profile

The soil AOA and AOB contributions to the NPs of four pairs of adjacent cropped and noncropped soils were evaluated over a temperature range of 4–42 °C using the octyne method to discriminate between AOA and AOB activities (Figure 1). Regardless of soil origin or cropping history, AOA consistently expressed their maximum NP rates between 30 and 37 °C where they contributed virtually all of the NP. In contrast, AOB expressed their maximum NP rates at either 16 or 23 °C, contributing most of the total NP in cropped and noncropped soils. The experiments were repeated with soils collected from the same sites in the summer and fall of 2014 and the same trends were observed (data not shown).

To further quantify the differences in temperature response of AOA and AOB driven activities, two complementary models that have been previously used to describe the response of soil processes to temperature, MMRT and SQRT, were applied to the data (Figure 2; Supplementary Tables 1 and 2). Based on estimates of least squared error, the SQRT model fit the temperature response better than the MMRT model (0.03 ± 0.02 and 1.77 ± 1.40 for SQRT and MMRT); however, there was not a significant difference in how well each model fit either the AOA or AOB temperature response ($P > 0.2$). Conversely, there was no difference between the SQRT and MMRT models on how well a regression of the model outcome approximated the experimental data; the models yielded coefficients of determination (R^2)

with an average of 0.75 ± 0.13 for MMRT and 0.77 ± 0.15 for SQRT models. In addition, there was no significant difference of any of the MMRT or SQRT model parameters between cropped and noncropped soils ($P > 0.05$).

When the AOA and AOB activity responses to temperature were compared, several model parameters were highly significantly different (Figure 3a). Both models identified T_{opt} values (the optimal temperature at which activity is achieved) for AOA that were significantly greater than estimates of T_{opt} for AOB ($P < 0.000$, Figure 3a), with the T_{opt} derived from the SQRT model being significantly greater (4.7 ± 2.6 °C) than that derived from the MMRT model ($P < 0.000$, Supplementary Tables 1 and 2). The SQRT model estimated a T_{opt} for AOA activity that was 12.8 ± 4.4 °C higher than that of AOB activity, and the T_{opt} for AOA activity estimated by the MMRT model was 13.1 ± 6.3 °C higher than that of AOB.

The SQRT model generated a theoretical apparent minimum temperature of activity (T_{min}) for AOB that was significantly lower than that of AOA (-8.8 ± 5.3 and 1.4 ± 3.5 °C for AOB and AOA, respectively, $P < 0.001$, Figure 3a). In addition, SQRT yielded a maximum temperature of activity, T_{max} , for AOA that was significantly greater than that of AOB (43.5 ± 1.1 and 39.7 ± 3.7 °C, $P < 0.01$). The temperature interval between T_{opt} and T_{max} ($T_{\text{max-opt}}$) was significantly greater for AOB than AOA (14.9 ± 1.5 and 5.9 ± 2.6 °C, $P < 0.000$), which was in agreement with the significant difference in the b parameter (Supplementary Tables 1 and 2, $P < 0.01$) that describes the much steeper decline in AOA activity above T_{opt} compared with AOB. The MMRT model yielded a significant difference in the temperature at which the rate of nitrification showed maximum sensitivity to changes in temperature ($T_{\text{s,max}}$, $P < 0.000$) between AOA and AOB activity (20.9 ± 3.0 and 11.1 ± 2.4 °C for AOA and AOB), and also in the heat capacity term ΔC_p^\ddagger (Supplementary Tables 1 and 2, $P < 0.01$).

The curvature of the $\ln(\text{rate})$ vs temperature plots of both AOA and AOB activities (Figures 2a–h) indicated that enthalpy and entropy ($\Delta H_{T_0}^\ddagger$ and $\Delta S_{T_0}^\ddagger$, respectively) of the overall nitrification processes were temperature-dependent (Equation (3)), and the significant difference in ΔC_p^\ddagger pointed to potential differences in thermodynamic properties of AOA and AOB. A large negative ΔC_p^\ddagger of an enzyme reaction is associated with a lower T_{opt} (Hobbs *et al.*, 2013; Schipper *et al.*, 2014). When the relationship between the response of T_{opt} and ΔC_p^\ddagger of soil AOA and AOB activities was compared (Figure 3b), larger negative ΔC_p^\ddagger values correlated with lower T_{opt} across the spectrum of soils ($R^2 = 0.6201$); and there was a clear trend for the ΔC_p^\ddagger of soil AOB-driven activity to be more

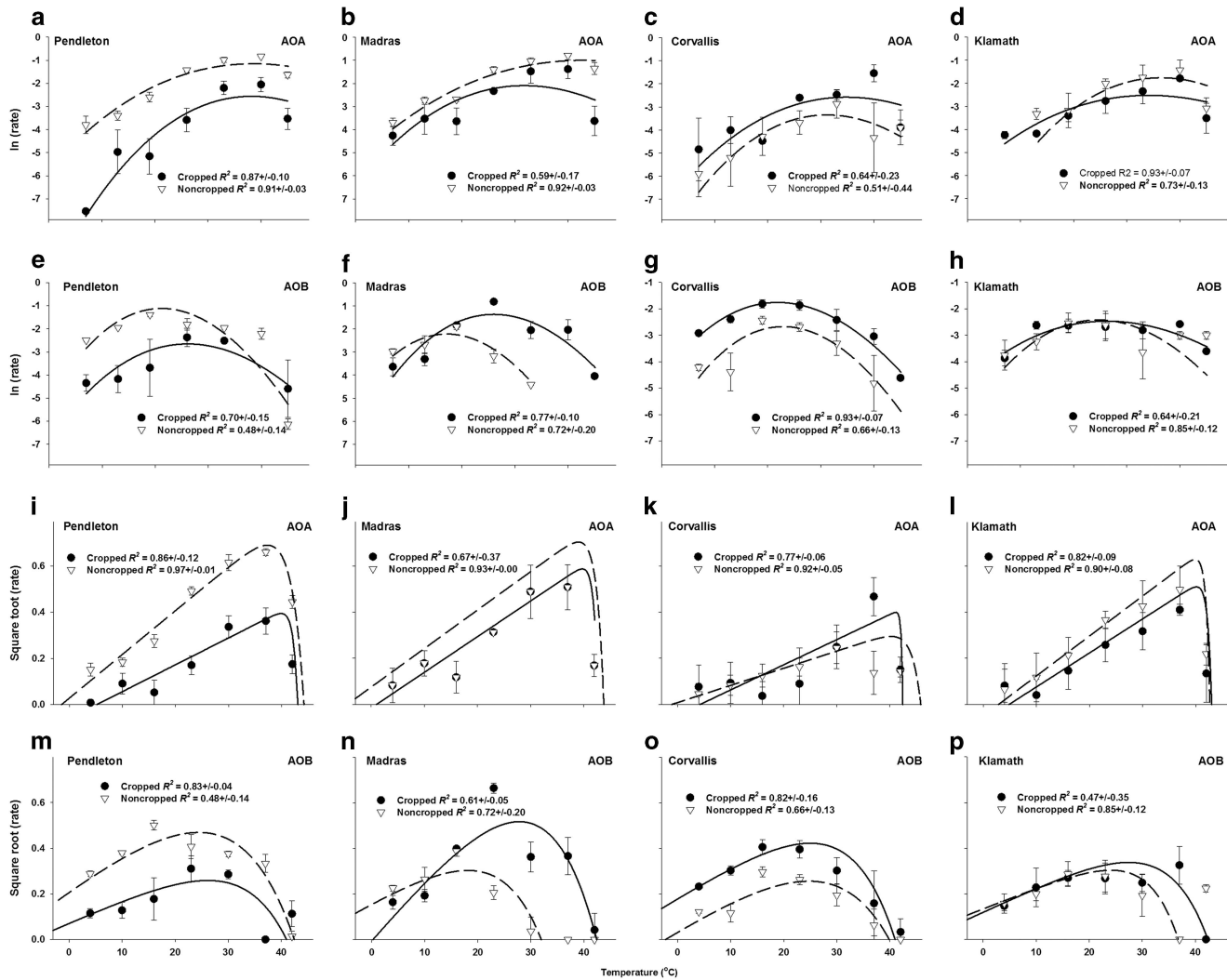


Figure 2 The fit of the MMRT (a–h) and SQRT (i–p) models to rates of $\text{NO}_2 + \text{NO}_3$ production by AOA and AOB in cropped (solid lines) and noncropped (broken lines) using the average of the model fit from each replicate. Symbols with error bars represent the s.d. of the average of the experimental data.

negative than of soil AOA-driven activity, implying differences in the thermodynamic properties of $\text{NO}_2 + \text{NO}_3$ production supported by the two groups of nitrifiers.

Experimental validation of insights into AOA and AOB activity differences gained from modeling of temperature-influenced parameters

A series of experiments were conducted to gain further insights into (i) protein biosynthetic competence of AOA and AOB across a temperature profile, (ii) how the predicted cardinal values of T_{\min} and T_{\max} reflect AOA and AOB activity, and (iii) how properties of $T_{s_{\max}}$ of AOA and AOB activity might be influenced by recruitment of AMO activity exhibiting different temperature profiles. These experiments were carried out in Pendleton non-cropped soils because the high rates of both AOA

and AOB activities expressed in this soil allowed for the execution of these detailed experiments.

Evaluation of protein biosynthetic potential in soil across a temperature profile. Using the RNP assay as a surrogate to assess protein synthesizing potential of AOA and AOB, the NP and RNP were compared across the temperature range in Pendleton noncropped soil (Figure 4). AOA demonstrated RNP (RNP_{AOA}) at 10–37 °C, illustrating their effective protein synthesis potential at temperatures where significant nitrifying activity occurred (Figure 4a). However, at 37 °C, RNP_{AOA} was significantly less than the NP_{AOA} and RNP_{AOA} did not occur at all at 42 °C, suggesting that high temperatures that support short-term rates of $\text{NO}_2 + \text{NO}_3$ producing activity might not necessarily support biosynthetic potential and growth of all AOA population members. In contrast, AOB demonstrated RNP_{AOB}

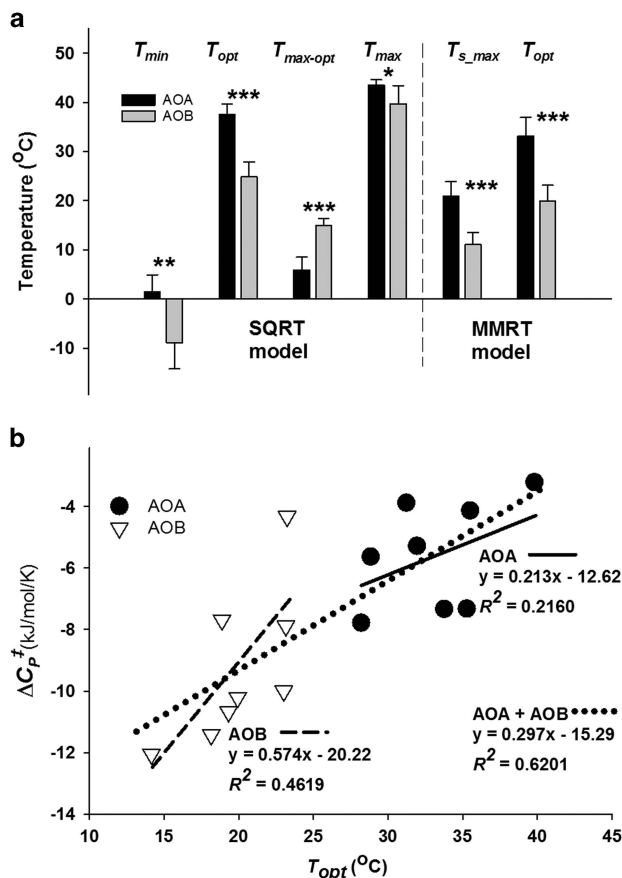


Figure 3 Significant outcomes from modeling the NP response to temperature in eight Oregon soils. (a) SQRT and MMRT model parameters that had significantly different values for $\text{NO}_2^- + \text{NO}_3^-$ production by soil AOA and AOB. The pairs of bars represent the average of the mean AOA or AOB value of a given parameter, and error bars represent the s.d. The asterisks assign the level of significance to each pair of bars ($*P < 0.01$, $**P < 0.001$, $***P < 0.0001$). (b) Comparison of the $\Delta C_p^\ddagger - T_{opt}$ -relationships of AOA and AOB in soil. Symbols represent the values for individual soils. The dotted regression line demonstrates the $\Delta C_p^\ddagger - T_{opt}$ -relationship for all AOA and AOB values across the entire temperature range. The solid and dashed regression lines are for the AOA and AOB $\Delta C_p^\ddagger - T_{opt}$ relationship, respectively.

only at 16, 23 and 30 °C (Figure 4b) despite expressing substantial $\text{NO}_2^- + \text{NO}_3^-$ producing activity over a wider temperature range (4–37 °C), suggesting a much narrower temperature range of protein synthesis and growth potential than for $\text{NO}_2^- + \text{NO}_3^-$ producing activity *per se*.

Experimental validation of T_{min} and T_{max} , and evidence for different and overlapping contributions to soil nitrification by different groups of ammonia oxidizers. We considered the possibility that subgroups of AOA and AOB might occupy different temperature niches within the overall range of temperatures and would possess different T_{min} and T_{max} . It is known that acetylene is an irreversible inactivator of AMO of both AOA and AOB during

active substrate turnover (Hyman and Wood, 1985; Taylor *et al.*, 2013; Vajjala *et al.*, 2014), and that *de novo* protein synthesis is required for activity to resume. Our hypothesis was that if the AMO of AOA or AOB are not actively turning over substrate at specific temperatures, they should not be inactivated by acetylene. We also hypothesized that we would observe heat inactivation of $\text{NO}_2^- + \text{NO}_3^-$ accumulating activity above their modeled T_{max} . To evaluate these hypotheses, Pendleton noncropped soil was inactivated with acetylene at a range of temperatures from 4 to 46 °C, and the residual activities after acetylene exposure measured at 16 °C (temperature of optimal AOB activity) and 37 °C (temperature of optimal AOA activity).

The SQRT model predicted T_{max} of 44.2 and 42.4 °C for AOA and AOB, respectively, in Pendleton noncropped soil. To determine whether both AOA and AOB were capable of oxidizing NH_3 or enzyme turnover at temperatures > 40 °C, Pendleton noncropped soil was exposed to acetylene at 42, 44 or 46 °C, and then transferred to 16 or 37 °C. After acetylene exposure of soil at 42 °C, there was no residual AOA- or AOB-driven activity significantly greater than zero over 12 h of incubation (Figures 4c and d). These data indicate that both AOA and AOB were capable of expressing sufficient AMO activity for acetylene to inactivate all $\text{NO}_2^- + \text{NO}_3^-$ production activity, even though AOB-driven $\text{NO}_2^- + \text{NO}_3^-$ accumulation could not be measured at 42 °C over 24 h. Both AOA and AOB retained all their $\text{NO}_2^- + \text{NO}_3^-$ production activities when returned to 16 or 37 °C, respectively, in non-acetylene exposed controls after incubation at 42 °C, demonstrating there was no irreversible heat inactivation of cellular functions at this temperature. After acetylene exposure at 44 °C, there was no significant residual post-acetylene exposure AOA activity, again indicating that AOA expressed sufficient AMO turnover at 44 °C for acetylene to inactivate all activity, but in the non-acetylene controls all activity was retained. In contrast, there was no residual AOB activity in either the non-acetylene or acetylene treatments exposed to 44 °C, suggesting that the upper limit of AOB thermal stability had been exceeded. Incubations at 46 °C heat-inactivated both AOA and AOB activity regardless of acetylene treatment. Despite the close similarity of T_{max} values for AOA and AOB activities, the outcome of the experiment confirmed the model prediction.

The SQRT model also predicted significant differences in T_{min} of AOA and AOB, and when acetylene inactivation was carried out at 4 °C and the soil moved to 37 °C, all of the octyne-resistant AOA activity was immediately expressed, indicating that none of the AOA AMO activity was actively turning over substrate during acetylene exposure at 4 °C, and that T_{min} of the active soil AOA must be > 4 °C. In contrast, there was no residual AOB activity significantly greater than zero at 16 °C after acetylene exposure at 4 °C (Figure 4d) verifying the model

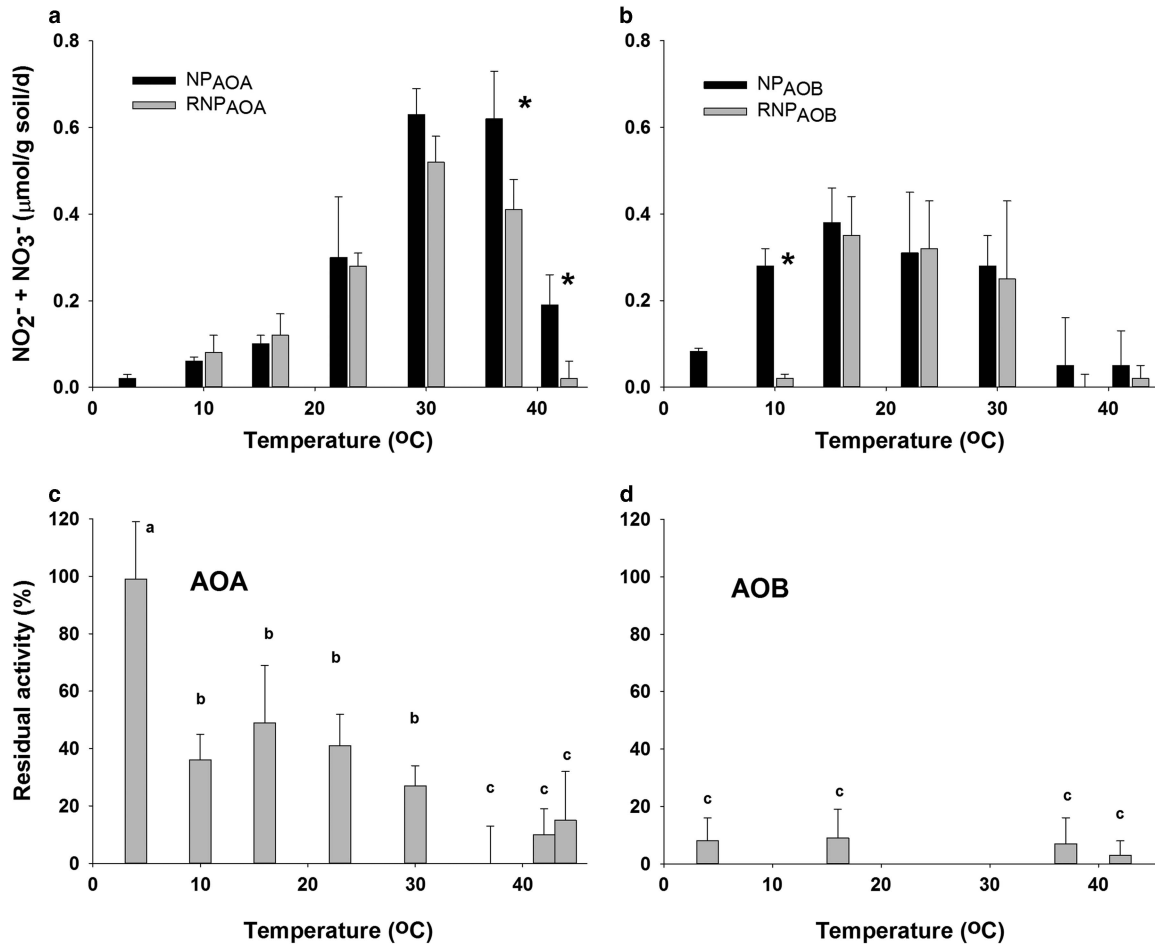


Figure 4 The effect of acetylene exposure on AOA and AOB in Pendleton noncropped soil. Evaluation of biosynthesis potential (RNP) of AOA (a) or AOB (b) in comparison with potential nitrification activity (NP) over a range of temperatures in Pendleton noncropped soil. Soils were inactivated and activity recovered at the same temperature. Error bars represent the s.d. of three replicates. The asterisks (*) represent the NP and RNP that were significantly different at the same temperature ($P > 0.05$). The effect of acetylene on the residual T_{opt} activity of AOA at 37 °C (c) or AOB at 16 °C (d). Soil was acetylene-inactivated at 4 through 46 °C for 6h, degassed, and transferred to 37 °C for measurement of AOA residual activity, or 16 °C for measurement of AOB residual activity. Residual activity measured between 0 and 12 h was considered to be due to AMO that was not acetylene-inactivated. Error bars represent the s.d. of three replicates. Lower case letters indicate significant differences between residual activities ($P < 0.01$). Bars with the same letter are not significantly different.

prediction that the soil AOB were active at 4 °C and possess a lower T_{min} than the soil AOA.

When acetylene inactivation of Pendleton noncropped soil was performed at 10 °C, however, and the slurries subsequently transferred to 37 °C, only a fraction of the non-acetylene exposed octyne-resistant activity (27–49%) was immediately expressed, indicating that a fraction (51–63%) of the AOA potential activity had become active at the lower incubation temperatures between 10 and 30 °C and inactivated by acetylene. Longer exposures to acetylene at ≤ 30 °C did not increase the fraction of activity that was inactivated. The response to acetylene inactivation across the temperature range suggested that there may be different types of AMO within the AOA population with one response type having a T_{min} between 4 and 10 °C, and another AMO response type with a T_{min} that must be higher than 30 °C. Evidence of different temperature responsive AOA

AMO activities could indicate that $T_{s,max}$ of AOA might be explained by different AOA AMO types with different T_{min} values active across different ranges of temperature.

In the case of AOB, there was no significant residual nitrifying activity at 16 °C after acetylene exposure at any temperature, indicating that the soil AOB expressed sufficient AMO activity across the whole temperature range to be inactivated by acetylene. This indicates that the AOB actively contributing to the measured $NO_2^- + NO_3^-$ production in the Pendleton noncropped soil may have AMO possessing the same temperature response profile, and that $T_{s,max}$ describes the thermodynamic response of AOB AMO to temperature increase.

Discussion

We have measured the temperature response of the AOA and AOB actively contributing to $NO_2^- + NO_3^-$

production in eight Oregon soils, and collected data demonstrating that soil AOA and AOB possess different temperature-dependent characteristics and contribute differentially to soil nitrification over a range of temperature. The temperature responses were evaluated in well-aerated soil slurry NPs containing saturating NH_4^+ . Although we readily concede that our results might not necessarily extrapolate to all soils, and *in situ* whole soil nitrification, nonetheless, several interesting points have arisen from this work that are worthy of comment and further study. For example, because it appears that AOA and AOB AMO possess different responses to temperature, it is perhaps not surprising that it has been challenging to model the response of soil nitrification to temperature (Stark, 1996), and for Schipper *et al.* (2014) to determine the parameters T_{opt} and ΔC_p^{\ddagger} when the temperature range of their data set only extended from 5 to 20 °C—a range below the T_{opt} of AOA in the soils used in our study. Similarly, a trait-based modeling approach to nitrification was limited by both the lack of data obtained with AOA and AOB laboratory isolates, and a comprehensive data set with which to test the model (Bouskill *et al.*, 2012). By distinguishing between AOA and AOB contributions to soil NPs with the octyne method over a range of temperatures, and then evaluating those data using models with either empirical or thermodynamic underpinnings, we were able to parameterize some of the characteristics of nitrification driven by AOA and AOB, respectively. Our results agree with, and in part explain what has previously been observed, but also contribute new insights into how AOA and AOB may contribute differentially to nitrification in soils. The discovery of *Nitrospira* capable of complete nitrification (comammox) potentially adds further complexity to the interpretation of the AOA and AOB contributions in soil (Daims *et al.*, 2015; van Kessel *et al.*, 2015). Nonetheless, limited data imply that comammox is highly sensitive to octyne (Daims, personal communication) and, therefore, should not interfere with any conclusions drawn about octyne-resistant AOA activity. How important the contributions of comammox to nitrification are in soils will remain unknown until tools to discriminate between comammox activity and that of AOA and AOB have been developed.

Significance of the model results

The SQRT and MMRT models were in agreement that T_{opt} for AOA activity was significantly greater than that of AOB. This observation was true for soils that had never been cropped or N fertilized, as well as for adjacent cropped soils of the same soil series that are regularly cultivated and N fertilized. Although it is unknown if the mechanism of octyne inhibition may itself be temperature-dependent, the temperature niche separation determined with the octyne method agrees with previous studies that

found differences in AOA and AOB *amoA* gene abundances or differential labeling with $^{13}\text{CO}_2$ in response to different incubation temperatures (Avrahami *et al.*, 2011; Wu *et al.*, 2013; Zeng *et al.*, 2014), and with the isolation of soil AOA that grow optimally at 35–40 °C (Tourna *et al.*, 2011; Kim *et al.*, 2012; Lehtovirta-Morley *et al.*, 2014, 2016). Some of these AOA isolates were obtained at an initial cultivation of 37 °C (Lehtovirta-Morley *et al.*, 2011, 2016; Tourna *et al.*, 2011); however, another isolate was initially enriched at 25 °C (Kim *et al.*, 2012). Virtually, all AOB isolates grow best at ≤ 30 °C (Jones and Morita, 1985; Stein and Arp, 1998; Jiang and Bakken, 1999; Norton *et al.*, 2008). Examples of the response of specific rates of activity by AOB isolates to comprehensive temperature ranges are rare in the literature (Jiang and Bakken, 1999), and the influence of initial cultivation or growth temperatures on the cultures' specific rates of activity in response to temperature are uncertain. Four AOB strains that were grown at 22 °C had their highest rates of activity at 25–30 °C (Jiang and Bakken, 1999), and the AOB *Nitrosomonas* sp. 4W30 (initially isolated at 10 °C) had highest specific rates of activity at 20 and 32 °C when cultivated at 5 and 25 °C, respectively (Jones and Morita, 1985). The temperature response of only a few AOA isolates has been examined, and in these cases, the response of specific growth rates, not activities, to different temperatures were measured (Jung *et al.*, 2011; Kim *et al.*, 2012; Lehtovirta-Morley *et al.*, 2014, 2016). Comparing coefficients generated using growth data of isolates may not be a fair comparison with the specific rates of soil-nitrifying activity that we measured in this study. For example, when the T_{opt} values for respiratory (glucose mineralization) and growth (leucine incorporation) processes were compared in a soil, the T_{opt} for respiration was >20 °C higher than the T_{opt} of growth (Birgander *et al.*, 2013), suggesting that the thermodynamic properties of growth and respiration can differ among mixed soil populations. In this context, we showed that, although the temperature range of AOB activity was wide (4–42 °C), the ability of AOB to conduct de novo protein synthesis to replace acetylene-inactivated AMO covered a much narrower temperature range. Clearly, more comparative studies are needed with AOA and AOB isolates, taking both growth and NH_3 -oxidizing activity into consideration.

The SQRT model predicted significantly higher T_{max} values for AOA- and AOB-driven nitrification ($P < 0.01$). We experimentally demonstrated in Pendleton noncropped soil that both AOA and AOB (T_{max} estimates of 44.2 ± 0.9 °C and 42.4 ± 0.1 °C) had sufficient rates of NH_4^+ turnover at 42 °C for their respective AMO to be irreversibly inactivated by acetylene, resulting in no residual activity upon subsequent incubation at their respective T_{opt} values. We also found that their heat tolerance was very similar, with AOB withstanding temperatures ≤ 44 °C, and AOA ≤ 46 °C. Perhaps it makes sense that AOA

and AOB in the same soil should have similar thermal stability. Previous studies have shown that changes in the temperature response of soil microbes, using ^{14}C leucine incorporation occurred as a result of 1–2-month incubations of soil at elevated temperatures (Barcenas-Moreno *et al.*, 2009; Birgander *et al.*, 2013). As a result, the highest temperature in an environment, even transiently over seasons, can select for organisms that have similar heat tolerance (van Gestel *et al.*, 2013; Schipper *et al.*, 2014). With the acetylene inactivation approach, we confirmed the model predictions of a significantly lower T_{\min} value for AOB than AOA. Through this approach, we also obtained evidence that raising the soil temperature to 10°C resulted in appearance of some activity of AOA AMO, but not all of it, suggesting the existence in the AOA population of different temperature responsive forms of AMO. This result contrasts with the lack of evidence for different temperature responsive forms of AMO among the AOB. With regard to model parameters, these results could suggest different interpretations of the $T_{s,\max}$ parameter for AOA and AOB. Because the AOB demonstrated a single temperature response, $T_{s,\max}$ could indicate a purely thermodynamic response of NH_3 -oxidizing activity to temperature increase, whereas the higher $T_{s,\max}$ for AOA could be due to recruitment of additional AMO types within the AOA population with different thermodynamic response profiles.

Although the ΔC_p^\ddagger parameter modeled from the MMRT model should be considered as an integration of multiple biochemical reactions that define nitrification, it is intriguing to consider ΔC_p^\ddagger in context with specific properties of AMO. For example, Hobbs *et al.* (2013) demonstrated through site-directed mutagenesis studies with the enzyme α -glucosidase₁ (MaLL) that enzymes with larger negative ΔC_p^\ddagger values have more conformational states than enzymes possessing less negative ΔC_p^\ddagger values. The soil AOB in our study expressed larger negative ΔC_p^\ddagger values than AOA ($P < 0.01$), suggesting a conformationally more flexible AOB AMO, which according to the model should be less restrained at its active site. This ‘flexibility’ might correspond with the broad substrate range of AMO of AOB (Hyman *et al.*, 1988), and correlate with T_{opt} and $T_{s,\max}$ values that were consistently lower than the corresponding values ascribed to their soil AOA counterparts. By comparison, a less negative ΔC_p^\ddagger for AOA might extrapolate to a more rigid and constrained version of AMO that may correspond with a more limited substrate range as shown by Taylor *et al.* (2015). This property might also explain the inability of acetylene to inactivate a fraction of AOA AMO at lower temperatures. It is well documented, albeit for unknown reasons, that the efficacy of acetylene as a substrate for both alkene and aromatic monooxygenases varies considerably (Hyman *et al.*, 1988; Ensign *et al.*, 1992; Yeager *et al.*, 1999; Taylor *et al.*, 2010a). With a less negative ΔC_p^\ddagger , optimal AOA

activity could be constrained to higher soil temperatures, closer to T_{\max} , resulting in a much narrower $T_{\max-\text{opt}}$ than the AOB (6 ± 3 and 15 ± 2 for AOA and AOB, respectively), and a significantly steeper decline ($'b'$ parameter) in AOA-dependent activity above T_{opt} ($P < 0.01$). The significant difference in ΔC_p^\ddagger for AOA and AOB might also provide support for the idea that the two groups of NH_3 oxidizers utilize different biochemical mechanisms for NH_3 oxidation to NO_2^- (Walker *et al.*, 2010; Martens-Habbena *et al.*, 2015; Kozłowski *et al.*, 2016).

It is of some importance to consider how the ΔC_p^\ddagger values we have determined in this study fit into context with other data on temperature dependence of soil processes. The ΔC_p^\ddagger values of soil AOB in this study ($-9.3 \pm 2.5 \text{ kJ mol}^{-1} \text{ K}^{-1}$) fell within the same range as ΔC_p^\ddagger values determined from a study of soil nitrification evaluated between 5 and 20°C ($-10.1 \pm 2.9 \text{ kJ mol}^{-1} \text{ K}^{-1}$; Russell *et al.*, 2002; Schipper *et al.*, 2014). In our study, $\text{NO}_2^- + \text{NO}_3^-$ production activity over this temperature range was dominated by AOB. ΔC_p^\ddagger values were also modeled from a study on temperature dependency of soil methane oxidation and values obtained were also very similar to that of AOB-driven soil nitrification ($-7.3 \pm 4.7 \text{ kJ mol}^{-1} \text{ K}^{-1}$; Schipper *et al.*, 2014). It is well known that AMO of AOB is genetically similar to particulate methane monooxygenase, and that both enzymes are capable of oxidizing both substrates (Holmes *et al.*, 1995; Semrau *et al.*, 1995; Arp and Stein, 2003), whereas we do not know if CH_4 is a substrate for AOA AMO. It remains to be seen if ΔC_p^\ddagger values of soil nitrification from other soil environments will yield confirmatory results, and how ΔC_p^\ddagger values based on rates of activity of NH_3 -oxidizing isolates will add to our understanding of the biochemical mechanisms behind this process and how they respond to temperature change.

Value of application of SQRT and MMRT models to environmental data

By modeling the rates of $\text{NO}_2^- + \text{NO}_3^-$ production activity by AOA and AOB in soil, we obtained parameters that reflect the response of the AOA and AOB actively contributing to nitrification, and not just the response of the few that can be isolated into culture. This approach allowed us to determine that key characteristics (T_{\min} , T_{\max} , T_{opt} , $T_{s,\max}$ and ΔC_p^\ddagger) within each of the two groups of NH_3 oxidizers were consistent across the study sites, and are perhaps intrinsic attributes of these groups. The modeled and experimental results yielded a clear trend for differences in T_{\min} , T_{opt} , $T_{s,\max}$ and ΔC_p^\ddagger of soil AOA and AOB activities, implying fundamental differences in temperature-influenced properties of nitrification driven by the two groups of NH_3 oxidizers. These thermodynamic parameters could be utilized in trait-based modeling systems to gain understanding into how past

climates have shaped the current communities of NH₃ oxidizers, and predict future response to climate change.

Conflict of Interest

The authors declare no conflict of interest.

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