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The Ammonia Oxidisers Activity and Growth Inhibition By Simvastatin

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Abstract: *The quest to understand and distinguish the contributions of ammonia oxidising archaea (AOA) and ammonia oxidising bacteria (AOB) to nitrification in the soil has increased the search for compounds that can selectively inhibit either of them. Several AOA specific inhibitors used in pure cultures loss their potency in soil due to interaction with complex ecosystem. This study is aimed to investigate selective inhibition of AOA, in pure cultures and soil, by simvastatin and to determine the effect of selective inhibition of AOA and AOB on NH3 oxidation activity and growth of AOA and AOB in the soil based on the hypotheses that: a) simvastatin will selectively inhibit AOA in soil and pure cultures; and b) AOA and AOB will be more active when competition for NH4 + is prevented using selective inhibitors. In pure cultures, simvastatin selectively inhibited all tested AOA isolates at concentrations between 8 and 100 µM. In soil microcosms incubated with 1 mM of simvastatin at low and high NH3 concentrations for 28 days, simvastatin selectively inhibited AOA but not AOB in soil. Simvastatin is a selective AOA inhibitor in culture and soil. AOA and AOB growth in soil were detected both at low and high NH4+concentration. Therefore, AOA and AOB are more active when competition for NH4+ in the soil is relieved using selective inhibitors.*

Keywords: *simvastatin, ammonia oxidising archaea, ammonia oxidising bacteria, inhibition*.

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I. Introduction

Both ammonia oxidising bacteria (AOB) and ammonia oxidising archaea (AOA) are involved in ammonia oxidation[26,31]and there is evidence for niche differentiation associated with ammonium (NH_4^+) concentration and supply[7,8,14,42,48]. Another factor differentiating niche between AOA and AOB is that AOA are more sensitive to drought than AOB[2]. The initial discovery that AOB prefer highly fertilised soils, while AOA prefer soils receiving low rates of ammonia supply was questioned by recent discoveries; the isolation of *Ca*. Nitrosocosmicus genus of AOA[19,16,36]and the detection of ammonia oxidation and growth of AOA in soil amended with low and high NH_4^+ concentration[12,13] coupled with the fact that AOA contributed to ammonia oxidation activity in highly fertilised soil[2]. This latter discovery clearly suggests that differences in competition for ammonia by AOA and AOB in soil supplied with different concentrations of NH_4^+ are the differentiating factors between AOA and AOB rather than the differential inhibition of AOA and AOB by high ammonia concentration.

Previous studies by Gubry-Rangin *et al.*(2010) detected ammonia oxidation activity and growth of AOA with no recorded growth of AOB even though their presence was detected in two soils with different pH (4.5 and 6.0) and low ammonia concentration. The presence of AOB in acidic soil was supported by the detection of AOB phylotypes [29, 42,32]and isolation of Gamma-proteobacteria AOB (strain TAO100) from acidic soil [11]. However, information on the contribution of AOB to nitrification in acidic soil and in soil with low ammonia concentration is limited due to lack of a potent selective AOA inhibitor. The need to study the contributions of different groups of ammonia oxidisers (AOA and AOB) to ammonia oxidation in soil at different ammonia concentrations and pH lead to the use of compounds that can selectively inhibit ammonia oxidation activity of AOA or AOB. Although several chemicals inhibitors such as nitrapyrin, dicyandiamide, allylthiourea, carbon disulphide-based inhibitors, 3,4-dimethylpyrazol-phosphate, 2-amino-4-chloro-6-methylpyrimidine and acetylene have been characterised and used as inhibitors of soil nitrification with a view to their commercial use to increase the efficiency of ammonia-based fertilisers [43,39,31]. The details of the mechanisms of actions of these chemicals are described by Subbarao *et al.*(2006).

Previous studies have shown that ammonia oxidation activity of AOA and AOB is inhibited by acetylene [28,21]and AOB are selectively inhibited by octyne[44,13]. The ammonia oxidation inhibitory effect of trolox, methylene blue hydrate, caffeic acid and curcumin [37] and 2-phenyl-4,4,5,5,-tetramethylimidazoline-1-oxyl-3-oxide (PTIO)[23] on pure cultures of AOA (*Ca.* N. maritimus,AOA-G6 and AOA-6f) was recently investigated. The ammonia oxidation activity in the tested AOA were selectively inhibited by the inhibitors mentioned above

at different concentrations. However, the potency of these AOA inhibitors in natural (soil and aquatic) ecosystems decreases after several days due to the potential interactions of inhibitors with complex environments [30,23,37]. There is little or no evidence of specific inhibitors, particularly specific AOA inhibitors that are effective in soil in relevant studies.

Simvastatin popularly known as "statin", is a drug classified as 3-hydroxyl-3-methylglutaryl coenzyme A reductase (HMG-coA) inhibitor[40,6]. It is a secondary fungal compound that inhibits HMG-coA reductase, which catalyses the synthesis of isoprenoid in archaea. Isoprenoid is the main component of the side chain of the archaeal pseudomurein lipid layer, which gives archaea a characteristic unique lipid cell membrane component compared to bacteria [9,3,38]. Inhibition of growth of several archaea by simvastatin has been demonstrated in previous studies, which make simvastatin a suitable drug for potential inhibition of ammonia oxidising archaea [15,50,34,35,25,4,18,24,6]. The aim of this study is to investigate the inhibitory effect of simvastatin on AOA in pure culture and to determine the effect of selective inhibition of AOA and AOB on NH_3 oxidation activity and growth of AOA and AOB in soil. It is hypothesised that: a) AOA will be selectively inhibited by simvastatin both in soil and pure cultures; b) prevention of competition using selective inhibitor will enhance the NH_3 oxidation activity and growth of AOA and AOB in soil.

II. Materials and methods

2.1**Growth in liquid medium**

The inhibitory effect of simvastatin on AOA and AOB was assessed by batch growth in liquid medium containing different concentrations of simvastatin and inoculated with pure cultures of four AOA (*Candidatus* Nitrosotaleadevanaterra, *Candidatus* Nitrosotalea sinensis, *Candidatus* Nitrosocosmicus franklandus[19,20]and *Nitrososphaera viennensis* [46]) and three AOB (*Nitrosomonas europaea*(ATCC 19718), *Nitrosospira multiformis*(ATCC 25196) and *Nitrosomonas eutropha*). Simvastatin (Sigma-Aldrich, UK) was dissolved in 100% dimethyl-sulfoxide (DMSO) before used. DMSO was chosen as solvent because simvastatin is not water-soluble compound. *Ca.* N. sinensis and *Ca.* N.devanaterra were grown in freshwater medium (FWM) at pH 5.0 as described by Lehtovirta-Morley *et al.*(2011), modified by adding 2.5 mM final concentration 2-(N-morpholino)ethane sulfonic acid buffer (MES buffer)(pH 5.35) and 4 mM sodium bicarbonate. *Ca.* N. franklandus was grown in freshwater medium (FWM) at pH 7.5 as described by Lehtovirta-Morley *et al.*(2016) while *Nitrososphaera viennensis* was grown in FWM according to Tourna *et al.*(2011). *N. europaea*, *N. multiformis* and *N. eutropha* were cultivated in Skinner and Walker medium at pH 7.9 [41]. Inhibition was studied by supplementing the final concentrations between 8 and 100 µM of simvastatin without simvastatin (water) and DMSO being used as controls. Batch culture growth was investigated in 100-ml Duran bottles containing 10 ml FWM and inoculated with 2% (v v^{-1}) of exponentially growing cells of both AOA or AOB, except for *N. viennensis* which was inoculated with 10% (v v^{-1}). AOA and AOB were incubated in the dark without shaking at 35 ^{o}C for *Ca.* N. sinensis and *Ca.* N.devanaterra, 40 ^{o}C for *Ca.* N. franklandus and *N. viennensis* and 28 ^{o}C for all AOB, and samples (100 µl) were taken daily for chemical analysis. Potential contamination by heterotrophs was assessed by inoculating the culture on 5% nutrient agar medium, incubated under the same conditions as the liquid cultures.

2.2**Construction and incubation of microcosms**

Microcosms were constructed using agricultural soil samples (0 – 15 cm depth) with pH 6.5. Details of the sampling site and soil characteristics are described by Kemp *et al.*(1992) and Bartram *et al.* (2014). The soil samples were air-dried at 25 ^{o}C for 4 days, sieved (3.35 mm mesh) and stored at 4 ^{o}C for 5 weeks. The moisture content and soil pH were determined as described by Nicol *et al.* (2005). Soil microcosms were established in 250-ml, sterile glass bottles containing 40 g equivalent dry soil and sufficient sterile distilled water to achieve an initial moisture content of 30% (g water g^{-1} dry soil). Microcosms were divided into two sets; the first was amended with 100 µg N g^{-1} NH_4Cl(high NH_4^+ concentration), while the second with water only (low NH_4^+ concentration). All the microcosms were tightly capped with butyl rubber stoppers and metal crimp tops and divided into four equal groups with each set containing an equal number of microcosms with low and high NH_4^+ concentrations. An AOB inhibitor, 1-octyne, prepared as described by Taylor *et al.* (2013), was added to a set of microcosms amended with high and low NH_4^+ concentration according to Hink *et al.* (2016). Acetylene (0.01%) was prepared and applied to another set of microcosms according to Offre *et al.*(2009), while 1 mM final concentration of simvastatin was applied to the third set of microcosms. Inhibitor was not added to the last set of microcosms serving as control. The final concentration of simvastatin (1 mM), which was ten times (x10) the concentration of simvastatin that inhibited AOA in pure culture, was used because previous studies of other ammonia oxidiser inhibitors (e.g., octyne, PTIO and acetylene) applied similar higher concentrations of inhibitor to soil microcosm [28,44,23]. The last set of microcosms received no inhibitor, while triplicate microcosms without ammonia amendment received 100 µl of 100% DMSO only as the second control, which was equivalent to 0.83% v v^{-1} of total soil moisture content. Microcosms were incubated at 30 ^{o}C for 28 days. All the microcosms

were aerated twice in a week for 5 to 10 minutes and non-destructive samples (2 g soil) were taken and analysed to determine NH_4^+ and NO_3^- concentrations during the incubation period. Microcosms in which NH_4^+ concentration decreased where amended with NH_4Cl to maintain high NH_4^+ concentration, according to Verhamme *et al.* (2011). Equal volume of sterile distilled water was added to microcosms with low NH_4^+ concentration only, which was determined by calculation of NH_4^+ concentration that was added during spiking. All microcosms were immediately re-capped after aeration at their respective sampling times and partial pressures of 1-octyne and acetylene were re-established. Triplicate microcosms were repeatedly sampled during incubation at 0, 4, 7, 11, 14, 18, 21, 25 and 28 days to determine the $NH_4^+/NH_3$and NO_3^- concentrations, while samples (1 g) taken at 0, 7, 14, 21 and 28 days were stored at -80 ^oC for molecular analysis.

2.3**Chemical analysis**

NO_2^- concentration was determined in samples of liquid growth medium. NO_2^- concentration was measured colourimetrically as described by Lehtovirta-Morley *et al.* (2011) and values of NO_2^- concentration (µM)*vs*. time (hours) were plotted to determine the inhibitory effect of simvastatin on AOA and AOB. Soil solution was extracted from 1 g soil with 5 ml 1 M KCl and centrifuged at 3000 rpm for 15 minutes. NH_4^+/NH_3 and NO_3^- concentrations were determined colourimetrically in 96-well plates[5]. The minimum and maximum nitrification rates were determined in microcosms incubated with low and high NH_4^+ concentration, respectively as the slope of linear regression of NO_3^- concentration *vs*. time during incubation.

2.4**Nucleic acid extraction and quantification of amoA genes**

Deoxyribonucleic acid (DNA) was extracted from 0.5 g of soil according to Thion and Prosser (2014) and concentration and purity of the extracted DNA were measured using a Nanodrop ND-2000 UV-Vis Spectrophotometer (NanoDrop Technologies, Wilmington, DE). The abundances of AOA and AOB in soil were estimated by quantitative PCR (qPCR) of *amoA* genes using the primers CrenamoA-23F/CrenamoA-616R [47] and amoA-1F/amoA-2R [33] respectively with 5 µl of 2 ng $µl^{-1}$ DNA template. AOA and AOB *amoA* standards were prepared using *Ca.* N. sinensis and *N. multiformis* respectively as described in Thion and Prosser (2014). The qPCR assayswereperformedwith realplex^{2}epGradient S Mastercycler(Eppendorf, Germany) and the quality of the qPCR amplicons was checkedbymelt curve analysis and 1% agarosegelelectrophoresis. qPCR efficiencies for amplification of AOA and AOB *amoA* genes were 87% and 90% respectively, with a r^2 value of 0.99. AOA and AOB growth were determined by subtracting the *amoA* abundance at time 0 day from that of day 28.

2.5**Statistical analysis**

All statistical analyses were performed using the program R 3.4.0 (http://www.rproject.org/). NO_3^- concentrations, nitrification rate, AOA and AOB growth in the soil experiment were analysed separately using a two-way ANOVA (Statistical Procedures for Agricultural Research package (agricolae)) with NH_4^+ concentration and inhibitor as fixed factors. Tukey HSD multiple post-hoc tests were used to assess the significance of the differences among the means.

III. Results

3.1**The effects of simvastatin on ammonia oxidiser growth**

The inhibitory effects of simvastatin on the ammonia oxidation activity of four AOA and three AOB representative isolates were determined by measuring nitrite production during incubation. Ammonia oxidation activity of *Ca.* N. sinensis and *Ca.* N.devanaterra (Fig. 1d and e) were inhibited by 8 µM simvastatin. *Ca.* N. franklandus, *N. viennensis* and AOB showed no inhibition at 8 and 16 µM (data not shown). The concentration of simvastatin therefore increased to 32 and 100 µM which subsequently lead to the increase in volume of DMSO used in one of the control experiments from 0.0098 to 0.17% v v^{-1}(volume of DMSO per total volume of culture). Ammonia oxidation activity in *Ca.* N. franklandus and *N. viennensis* was partially inhibited at 32 µM (data not shown). At 100 µM, ammonia oxidation activity was completely inhibited in C*a.* N. franklandus and *N. viennensis*(Fig. 1f and g), while AOB did not show inhibition at all concentrations of simvastatin tested in this study (i.e., 8, 16, 32 and 100 µM)(Fig. 1a - c). In addition, all AOA and AOB tested were not inhibited by 0.0098 to 0.17% v v^{-1} concentration of DMSO used as control (Fig. 1).

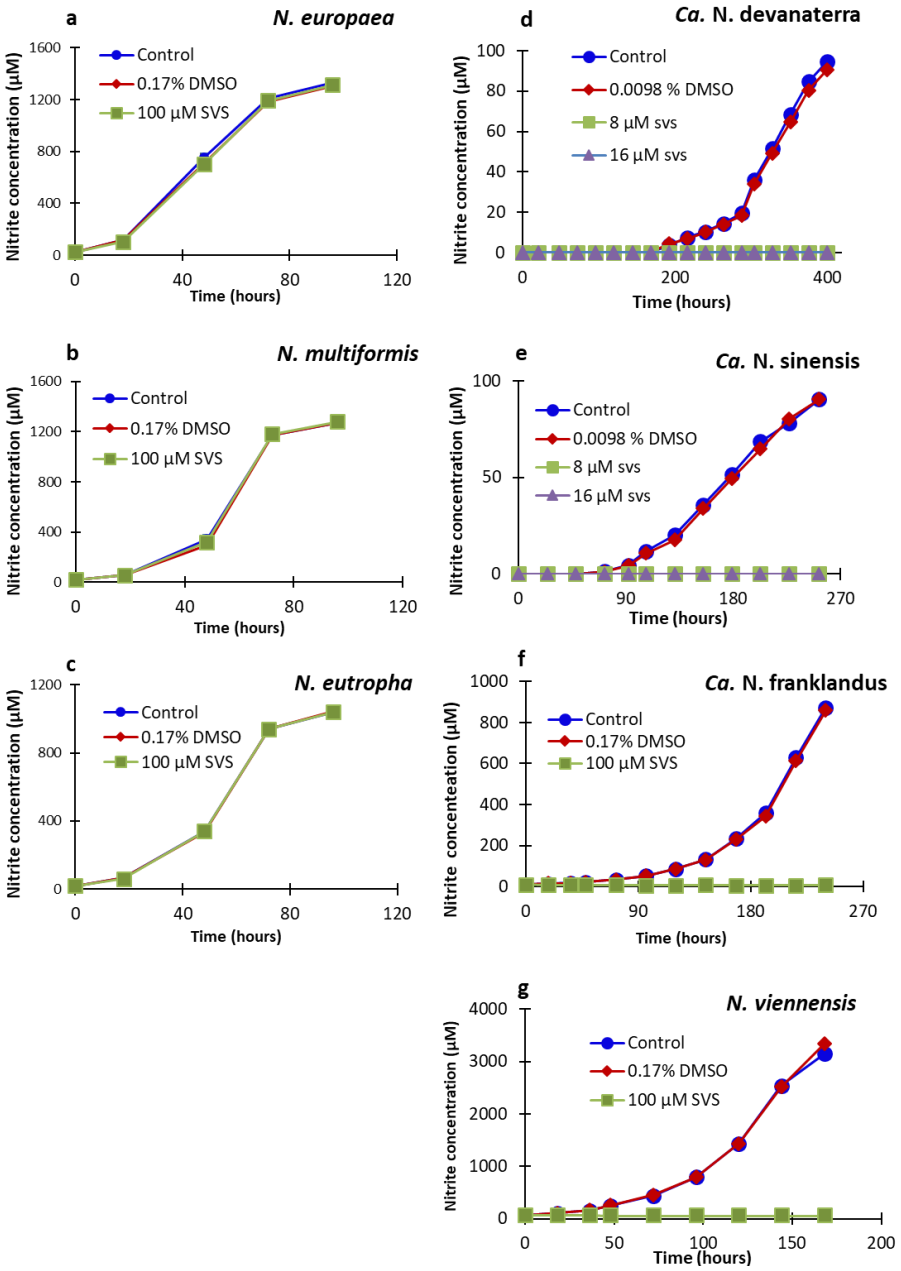

Figure 1: Effect of simvastatin concentrations on nitrite production by AOA (d - g) and AOB (a - c) in pure cultures. Control = cultures incubated without simvastatin and DMSO, svs = simvastatin, DMSO = dimethyl sulfoxide. Data are plotted as the mean and standard error of values from triplicate cultures.

3.2 NH_4^+/NH_3 oxidation and NO_3^- production in soil

In soil, NH_4^+ was not detected in control microcosms without inhibitor, with octyne and simvastatin at low NH_4^+ concentration after 28 days of incubation (Fig. 2a, b and c). In microcosms with high NH_4^+ concentration, the highest NH_4^+ oxidation was determined in the microcosms without inhibitor compared to octyne and simvastatin after 28 days of incubation, as more NH_4^+ were added to the control microcosm without inhibitor during incubation to maintain high NH_4^+ concentration than in microcosms incubated with octyne and simvastatin (Fig. 2a, b and c). As expected, acetylene completely inhibited NH_4^+ oxidation irrespective of NH_4^+ concentration. The increase in NH_4^+ concentration observed was due to NH_4^+ added through mineralisation activity in the soil during incubation (Fig. 2d).

However, in the control microcosms with DMSO only, NH_4^+ concentration was not detectable during incubation (Fig.2a). NO_3^- production increased significantly with time in microcosms with octyne, simvastatin, DMSO and control microcosms without inhibitor at low NH_4^+ concentration. There was no significant difference ($p > 0.05$) in the minimum nitrification rate in all treatments at low NH_4^+ concentration except in the acetylene treatment where inhibition of nitrification activity was complete (Fig. 2h). The nitrification rate (µg N g^{-1} day^{-1})

was significantly affected by NH_4^+ concentration and inhibitor. NO_3^- production and maximum nitrification rate in microcosms amended with high NH_4^+ concentration were significantly higher ($p< 0.001$) than minimum nitrification rate at low NH_4^+ concentration. Also, NO_3^- production and maximum nitrification rate in microcosms incubated with high NH_4^+ concentration were significantly higher in the control microcosms without inhibitor compared to octyne and simvastatin treatments (Fig. 2e, f, g and Fig. 3). At high NH_4^+ concentration, the sum of NO_3^- concentrations in microcosms incubated with octyne (AOA activity only)(194 ± 1.7 µg N g^{-1}) was higher than in microcosms incubated with simvastatin (AOB activity only)(145 ± 0.3 µg N g^{-1}), while the sum of NO_3^- concentrations in microcosms incubated with octyne and simvastatin (339 ± 2 µg N g^{-1}) is similar to NO_3^- concentrations in microcosms with no inhibitor (333 ± 21 µg N g^{-1})(Fig. 2e, f and g).

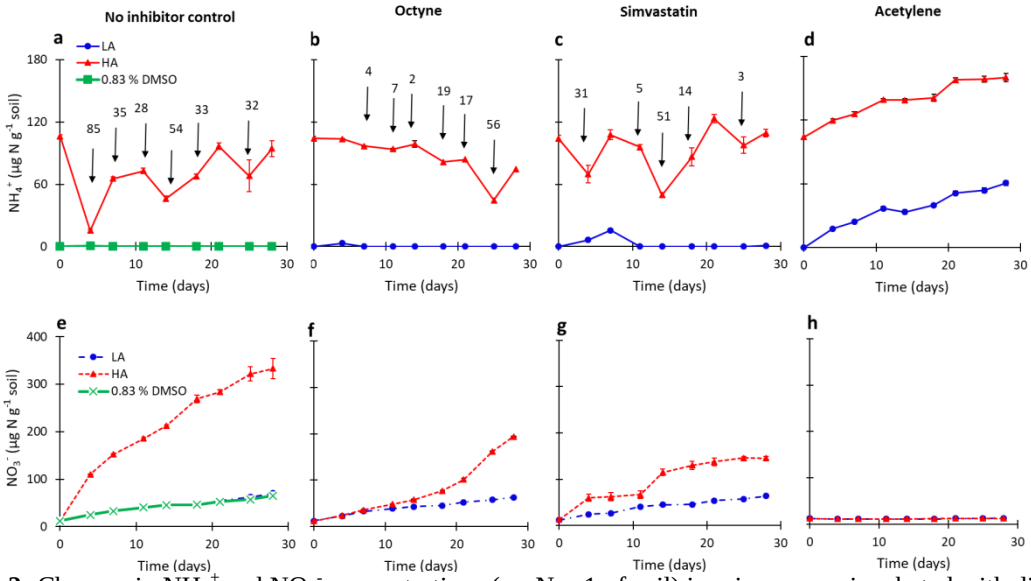

Figure 2: Changes in NH_4^+ and NO_3^- concentrations (µg N g-1 of soil) in microcosms incubated with different nitrification inhibitors and different NH_4^+ concentrations. LA = low $NH4^+$(blue); HA = high NH_4^+(red); DMSO (0.83% v v^{-1}) = green, the values and arrows are the concentrations of NH_4^+(µg N g-1 of soil) and times of addition, respectively. Data are plotted as the mean and standard error of values from triplicate microcosms.

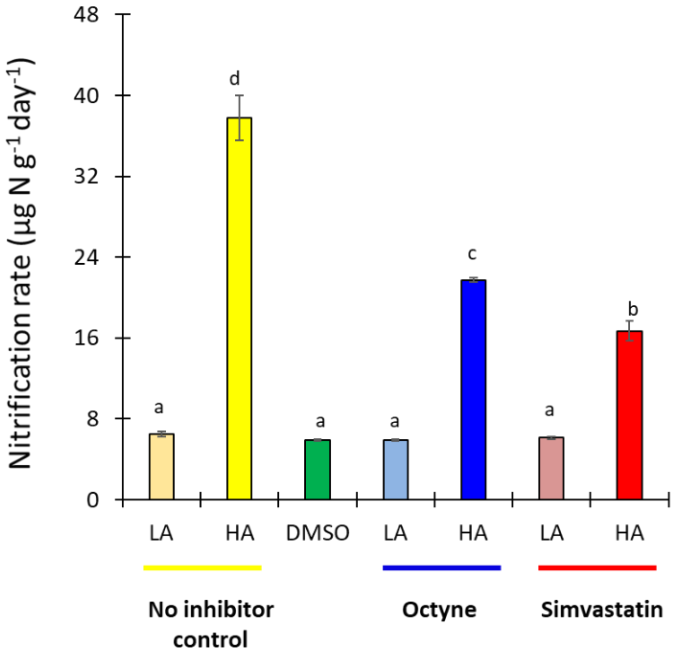

Figure 3: Minimum and maximum nitrification rates (µg N g^{-1} day^{-1}) in microcosms incubated with different nitrification inhibitors and different NH_4^+ concentrations. LA = low NH_4^+; HA = high NH_4^+ concentration. Data are plotted as the mean and standard error of values from triplicate microcosms. Different letters indicate significant difference.

3.3**AOA and AOB growth in soil**

There was detectable growth of AOA in microcosms incubated with no inhibitorirrespective of NH_4^+ concentration. NH_4^+ concentration had no significant effect on the growth of AOA in microcosms incubated with no inhibitor ($p > 0.05$). In microcosms incubated with octyne, there was detectable growth of AOA at low and high NH_4^+ concentration, with highest growth in microcosms incubated with high NH_4^+ concentration. However, there was no detectable growth of AOA in microcosms incubated with simvastatin irrespective of NH_4^+ concentration (Fig. 4A). There was no detectable growth of AOB in microcosms incubated with no inhibitor at low NH_4^+ concentration, but AOB growth was highest in microcosms incubated with high NH_4^+ concentration without inhibitor. There was no detectable growth of AOB in microcosms incubated with octyne, irrespective of NH_4^+ concentration. In microcosms incubated with simvastatin, there was a detectable growth of AOB at low and high NH_4^+ concentration, with greatest growth obtained in microcosms incubated with high NH_4^+ concentration (Fig. 4B). Both inhibitors, and NH_4^+ concentration had a significant effect on AOA and AOB growth after 28 days of incubation, ($p > 0.001$, $p > 0.001$ (AOA); $p = 0.001$ and $p > 0.001$ (AOB)) for inhibitors and NH_4^+ concentration, respectively.

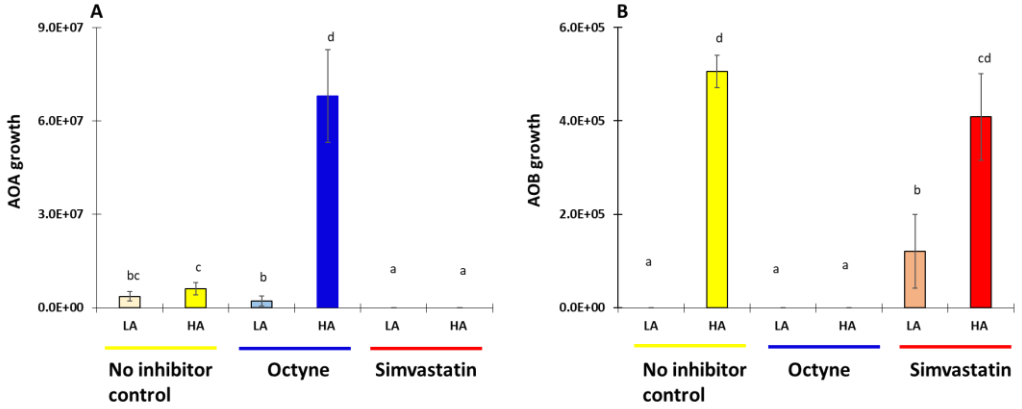

Figure 4: AOA (A) and AOB (B)growth in soil with different nitrification inhibitors at different NH_4^+ concentrations. LA = low NH_4^+, HA = high NH_4^+. Data are plotted as the mean and standard error of values from calculated from triplicate microcosms, different letters indicate significant differences among the treatments.

IV. Discussion

It has been observed that simvastatin inhibits nitrification activity in all AOA pure cultures tested at varying concentrations with no inhibition of AOB. These results support previous studies of the inhibitory effect of simvastatin on several archaea and heterotrophic bacteria [4,18,25,49,35,50,15]. The lowest inhibitory concentration of simvastatin (8 µM) obtained in the present study was higher than the inhibitory concentration obtained in previous studies of the non-ammonia oxidising archaea *Thermococcus kodakaraensis* and*Haloferaxvolcanii*, where inhibitory concentrations were 5 and 0.3 µM, respectively [24,49]. The highest inhibitory concentration of simvastatin (100 µM) obtained was higher than the inhibitory concentration obtained for *Sulfolobusislandicus*(16 µM)[50]. However, the reason(s) for the differences in the inhibitory concentration of simvastatin in different AOA strains is/are not well understood. The inhibitory activity of simvastatin in the present study is linked to pH, as the results indicate that acidophilic strains of AOA (*Ca.* N. sinensis and *Ca.* N.devanaterra) are inhibited by lower concentration of simvastatin than neutrophilic AOA strains (*Ca.* N. franklandus and *N. viennensis*). The present study is the first to investigate the inhibitory effect of simvastatin on AOA and AOB.

The aim of the microcosms study is to investigate whether AOA or AOB are more active when competition is relieved by use of selective inhibitors. The detection of NH_4^+ oxidation activity with NO_3^- production in microcosms incubated with octyne irrespective of NH_4^+ concentration (Fig. 2b and f) coupled with the detectable growth of AOA irrespective of NH_4^+ concentration without inhibitor and a significant growth of AOA at high NH_4^+ concentration in soil incubated with octyne (Fig. 4A) are evidence that ammonia oxidation activity and growth of AOA in soil is not inhibited by high NH_4^+ concentration ($\leq$100 µg N g^{-1}). These results support previous physiological and ecological studies of high NH_4^+ tolerance by AOA[19,16,36,12,13] but disagree with Di *et al.*(2009; 2010) that AOA only grow under the supply of low NH_4^+ concentration in soil. Therefore, this provides evidence that tolerance to high concentrations of NH_4^+ is not a niche differentiation factor between AOA and AOB in the soil. Nevertheless, AOB grow better than AOA at high NH_4^+ concentration in the absence of inhibitors, but AOA dominate NH_4^+ oxidation activity at low NH_4^+ concentration (Fig. 4A), without

detectable growth of AOB at low NH_4^+ concentration in microcosms without inhibitors (Fig. 4B). These results indicate that AOB are better competitors at high NH_4^+ concentration than AOA, while AOA are better competitors than AOB at low NH_4^+ concentration. These results support previous studies by Hink *et al.*(2017) who detected growth of AOA in soil amended with high NH_4^+ concentration and therefore suggested that the differences in ability of AOA and AOB to compete for NH_4^+ at different NH_4^+ concentration was a niche differentiation factor between AOA and AOB rather than tolerance to high NH_4^+ concentration. Inhibition of AOB growth in microcosms with octyne, irrespective of NH_4^+ concentration, was in agreement with previous studies of octyne as a potent AOB inhibitor [44,13]. The present study also demonstrates the use of acetylene as a potent nitrification inhibitor which supports previous studies by Offre *et al.*(2009) and Gubry-Rangin *et al.*(2010).

The inhibition of AOA, detection of NH_4^+ oxidation activity and growth of AOB in soil incubated with simvastatin (Fig. 2,and 4), irrespective of NH_4^+ concentration, support the results of culture experiments and confirm simvastatin as a potent AOA inhibitor. The detection of AOB growth at low NH_4^+ concentration in the presence of simvastatin indicates that despite the preference of AOB for high NH_4^+ concentration in soil, NH_4^+ oxidation and growth of AOB in soil is not limited by low NH_4^+ concentration and thus, confirms that AOB are only outcompeted by AOA for NH_4^+ in soil at low NH_4^+ concentration when AOA and AOB are both growing without inhibition. Also, the detection of ammonia oxidation activity and growth of AOB when AOA are inhibited at low NH_4^+ concentration compared to AOB growth when no inhibitor is applied at low NH_4^+ concentration, coupled with higher growth of AOA in the presence of octyne at high NH_4^+ concentration is an indication that both AOA and AOB grow better in soil when competition is relieved using selective inhibitors. Although it was expected that AOB growth in microcosms incubated with high NH_4^+ concentration in the presence of simvastatin would be higher than AOB growth in microcosms with no inhibitor due to lack of competition. But AOB growth was higher in the control microcosms, even though there was not significant difference between them ($p > 0.05$). The reasons for no significant difference in AOB growth between the two treatments are not well understood.

V. Conclusion

Both AOA and AOB could oxidise ammonia in soil, irrespective of ammonia concentration. The differences in the ability of AOA and AOB to oxidise ammonia at different concentration rather than ammonia concentration is therefore suggested as a niche differentiation factor between AOA and AOB in soil.

Acknowledgements

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References

[1]. Bartram, A. K., Jiang, X., Lynch, M. D. J., Masella, A. P., Nicol, G. W.,Dushoff, J. and Neufeld, J. D. Exploring links between pH and bacterial communitycomposition in soils from the Craibstone ExperimentalFarm. FEMS MicrobiolEcol 2014; (87): 403 – 415.
[2]. Bello, M.O. The effect of major environmental factors on archaeal and bacterial ammonia oxidisers in soil. PhD dissertation, University of Aberdeen Scotland UK, 2018, 75 – 94.
[3]. Brown, J. R. and Doolittle, W. F. Archaea and the prokaryote-to-eukaryote transition. Microbiol MolBiol Rev1997;(61): 456 – 502.
[4]. Cabrera, J. A., Bolds, J., Shields, P. E., Havel, C. M. and Watson, J. A. Isoprenoid synthesis in *Halobacterium halobium*. Modulation of 3-hydroxy-3-methylglutaryl coenzyme A concentration in response to mevalonate availability. J Biol Chem 1986; (261): 3578 – 3583.
[5]. Catão, E. C. P., Lopes, F. A. C., Rubini, M. R., Nardoto, G. B., Prosser, J. I. and Krüger, R. H. Short-term impact of soybean management on ammonia oxidizers in a Brazilian savanna under restoration as revealed by coupling different techniques. Biol Fert Soils2016; (52): 401 – 412.
[6]. Delmotte, P. and Delmotte-Plaquee, J. A new antifungal substance of fungal origin. Nature 1953; (171): 344 – 345.
[7]. Di, H. J., Cameron, K. C., Shen, J. P., Winefield, C. S., O'Callaghan, M., Bowatte, S. and He, J. Z. Ammonia oxidising bacteria and archaea grow under contrasting soil nitrogen conditions. FEMS MicrobiolEcol2010; (72): 386 – 394.
[8]. Di, H. J., Cameron, K. C., Shen, J. P., Winefield, C. S., Ocallaghan, M., Bowatte, S. and He, J. Z. Nitrification driven by bacteria and not archaea in nitrogen-rich grassland soils. Nature Geosci2009; (2): 621 – 624.
[9]. Goldstein, J. L. and Brown, M. S. Regulation of the mevalonate pathway. Nature 1990; (343): 425 – 430.
[10]. Gubry-Rangin, C., Nicol, G. W. and Prosser, J. I. Archaea rather than bacteria control nitrification in two agricultural acidic soils. FEMS MicrobiolEcol2010; (74): 566 – 574.
[11]. Hayatsu, M., Tago, K., Uchiyama, I., Toyoda, A., Wang, Y., Shimomura, Y., Okubo, T., Kurisu, F., Hirono, Y., Nonaka, K., Akiyama, H., Itoh, T. and Takami, H. An acid-tolerant ammonia-oxidising γ-proteobacterium from soil. ISME J 2017; (11): 1130 – 1141.
[12]. Hink, L., Gubry-Rangin, C., Nicol, G. W. and Prosser, J. I. 'The consequences of niche and physiological differentiation of archaeal and bacterial ammonia oxidisers for nitrous oxide emissions'. ISME J 2017;1751 – 7362.
[13]. Hink, L., Nicol, G.W. and Prosser, J.I. Archaea produce lower yields of N_2O than bacteria during aerobic ammonia oxidation in soil. Environ Microbiol2016; (00): 00-00.
[14]. Hofferle, S., Nicol, G. W., Pal, L., Hacin, J., Prosser, J. I. and Mandic-Mulec, I. Ammonium supply rate influences archaeal and bacterial ammonia oxidizers in a wetland soil vertical profile. FEMS MicrobiolEcol2010; (74): 302 – 315.
[15]. Jain, S., Caforio, A. and Driessen, A. J. M. Biosynthesis of archaeal membrane ether lipids. Microbial PhysiolMetabol2014;(5): 1 – 16.

[16]. Jung, M-Y., Kim, J-G., SinninghеDamsté, J. S., Rijpstra, W. I. C., Madsen, E. L. and Kim, S-J. A hydrophobic ammonia-oxidising archaeon of the *Nitrosocosmicus* clade isolated from coal tar-contaminated sediment. Environ Microbiol Rep2016; (8): 983 – 992.

[17]. Kemp, J. S., Paterson, E., Gammack, S. M., Cresser, M. S. and Killham, K. Leaching of genetically modified *Pseudomonas fluorescens* through organic soils: influence of temperature, soil pH, and roots.Biol Fert Soils 1992;(13): 218– 224.

[18]. Lam, W. L. and Doolittle, W. F. Mevinolin-resistant Mutations Identify aPromoter and the Gene for a Eukaryote-like 3-Hydroxy-3 methylglutaryl-coenzymeA Reductase in the Archaebacterium. *Haloferaxuolcanii.* JBiol Chem 1992;(267): 5829 – 5834.

[19]. Lehtovirta-Morley, L. E., Ross, J., Hink, L., Weber, E. B., Gubry-Rangin, C., Thion, C. E., Prosser, J. I. and Nicol, G. W. Isolation of *"Candidatus Nitrososocosmicus franklandus"*, a novel ureolytic soil archaeal ammonia oxidiser with tolerance to high ammonia concentration. FEMS Microbiol. Ecol.2016;(057).

[20]. Lehtovirta-Morley, L. E., Stoecker, K., Vilcinskas, A., Prosser, J. I. and Nicol, G. W. Cultivation of an obligate acidophilic ammonia oxidiser from a nitrifying acid soil. PNAS USA 2011; (108): 8420 – 8425.

[21]. Levičnik- Höfferle, Š., Nicol, G. W., Ausec, L., Mandić-Mulec, I. and Prosser, J. I. Stimulation of thaumarchaeal ammonia oxidation by ammonia derived from organic nitrogen, but not added inorganic nitrogen. FEMS MicrobiolEcol 2012;(80): 114 – 123.

[22]. Li, Y., Ding. K., Wen, X., Zhang, B., Shen, B., Yang, Y. A novel ammonia-oxidising archaeon from wastewater treatment plant: Its enrichment, physiological and genomic characteristics. Sci Rep 2016; (6): 23747 – 23758.

[23]. Martens-Habbena, W., Qin, W., Horak, R. E., Urakawa, H., Schauer, A. J., Moffett, J. W., Armbrust, E. V., Ingalls, A. E., Devol, A. H. and Stahl, D. A. The production of nitric oxide by marine ammonia oxidising archaea and inhibition of archaeal ammonia oxidation by a nitric oxide scavenger. Environ Microbiol2015; (17): 2261 – 2274.

[24]. Matsumi, R., Manabe, K., Fukui, T., Atomi, H. and Imanaka, T. Disruption of a sugar transporter gene cluster in a hyperthermophilic archaeon using a host-marker system based on antibiotic resistance. J Bacteriol2007; (189): 2683 – 2691.

[25]. Miller, T. L. and Wolin, M. J. Inhibition of growth of methane-producing bacteria of the ruminant forestomach by hydroxymethylglutaryl-CoA reductase inhibitors. J Dairy Sci 2001; (84): 1445 – 1448.

[26]. Nicol, G. W., Leininger, S. and Schleper, C. Distribution and activity of ammonia oxidising archaea in natural environments. *In: Nitrification*. ASM: Washington, DC 2011; 157 – 178.

[27]. Nicol, G. W., Tscherko, D., Embley, T. M. and Prosser, J. I. Primary succession of soil Crenarchaeota across a receding glacier foreland. Environ Microbiol2005; (7): 337 – 347.

[28]. Offre, P., Prosser, J. I. and Nicol, G. W. Growth of ammonia oxidising archaea in soil microcosms is inhibited by acetylene. FEMS MicrobiolEcol 2009; (70): 99 – 108.

[29]. Onodera, Y., Nakagawa, T., Takahashi, R. and Tokuyama, T. Seasonal change in vertical distribution of ammonia oxidising archaea and bacteria and their nitrification in temperate forest soil. Environ Microbiol 2010;(25): 28 – 35.

[30]. Oremland, R. S. and Capone, D. G. Use of specific inhibitors in biogeochemistry and microbial ecology. AdvMicrobial Ecol 1988;(10):285 – 383.

[31]. Prosser, J. I. Soil Nitrifiers and Nitrification. *In: Nitrification.ASM*:Washington, DC 2011; 347 – 383.

[32]. Prosser, J. I. and Nicol, G. W. Archaeal and bacterial ammonia oxidisers in soil: the quest for niche specialisation and differentiation.Trend Microbiol2012; (20): 524 – 531.

[33]. Rotthauwe, J. H., Witzel, K. P. and Liesack, W. The ammonia monooxygenase structural gene *amoA* as a functional marker: molecular fine-scale analysis of natural ammonia oxidising populations.Appl Environ Microbiol 1997;(63): 4704 – 4712.

[34]. Santangelo, T. J., Cubonova, L., James, C. L. and Reeve, J. N. TFB1 or TFB2 is sufficient for *Thermococcus kodakaraensis* viability and for basal transcription in vitro. J Mol Biol2007; (367): 344 – 357.

[35]. Santangelo, T. J. and Reeve, J. N. Genetic tools and manipulations of the hyperthermophilic heterotrophic archaeon *Thermococcus kodakarensis. Koki Horikoshi(ed.),* Extremophiles Handbook 2011; 431 – 538.

[36]. Sauder, L. A., Albertsen, M., Engel, K., Schwarz, J., Nielsen, P. H., Wagner, M. and Neufeld, J.D. Cultivation and characterization of *Candidatus Nitrosocosmicus exaquare*, an ammonia-oxidising archaeon from a municipal wastewater treatment system.ISME J 2017;(11): 1142 – 1157.

[37]. Sauder, L. A., Ashley, A., Ross, A.A. and Neufeld, J. D. Nitric oxide scavengers differentially inhibit ammonia oxidation in ammonia oxidising archaea and bacteria. FEMS Microbiol Lett 2016; (052).

[38]. Schleper, C. and Nicol, G. W. Ammonia Oxidising Archaea Physiology, Ecology and Evolution. Adv Microbial Physiol2010; (57):1 – 41.

[39]. Singh, S. N. and Verma, A. The potential of nitrification inhibitors to manage the pollution effect of nitrogen fertilisers in agricultural and other soils. a review Environ Microbiol 2007;(9): 226 – 279.

[40]. Sirtori, C.R. The pharmacology of statins.Pharmacological Research2014; (88): 3 – 11.

[41]. Skinner, F.A. and Walker, N. Growth of *Nitrosomonas europaea* in batch and continuous culture. Arch Microbiol 1961;(38): 339 – 349.

[42]. Stopnisek, N., Gubry-Rangin, C., Hofferle, S., Nicol, G. W., Mandicˇ-Mulec, I. and Prosser, J. I. Thaumarchaeal ammonia oxidation in an acidic forest peat soil is not influenced by ammonium amendment.Appl Environ Microbiol2010; (76): 7626 – 7634.

[43]. Subbarao, G. V., Ito, O., Sahrawat, K., Berry, W. L., Nakahara, K., Ishikawa, T., Watanabe, T., Suenaga, K., Randon, M. and Rao, I. O. Scope and strategies for regulation of nitrification in agricultural systems-challenges and opportunities. Cri Rev Plant Sci 2006;(25): 303 – 335.

[44]. Taylor, A. E., Vajrala, N., Giguere, A. T., Gitelman, A. I., Arp, D. J., Myrold, D. D., Sayavedra-Soto, L. and Bottomley, P. J. Use of Aliphatic n-Alkynes to discriminate soil nitrification activities of Ammonia-Oxidising Thaumarchaea and Bacteria. Appl Environ Microbiol2013; (79): 6544 – 6551.

[45]. Thion, C. and Prosser, J. I. Differential response of non-adapted ammonia-oxidising archaea and bacteria to drying-re-wetting stress. FEMS MicrobiolEcol2014; (90): 380 – 389.

[46]. Tourna, M., Stieglmeier, M., Spang, A., Könneke, M., Schintlmeister, A., Urich, T., Engel, M., Schloter, M., Wagner, M., Richter, A. and Schleper, C. *Nitrososphaera viennensis*, an ammonia oxidising archaeon from soil. PNAS USA 2011; (108): 8420 – 8425.

[47]. Tourna, M., Freitag, T. E., Nicol, G. W. and Prosser, J. I. Growth, activity and temperature responses of ammonia oxidising archaea and bacteria in soil microcosms.Environ Microbiol2008; (10): 1357 – 1364.

[48]. Verhamme, D. T., Prosser, J. I. and Nicol, G. W. Ammonia concentration determines the differential growth of ammonia-oxidising archaea and bacteria in soil microcosms. ISME J2011; (5): 1067 – 1071.

[49]. Wendoloski, D., Ferrer, C. and Dyall-Smith, M. L. A new simvastatin (mevinolin)-resistance marker from*Haloarculahispanica*and a new*Haloferaxvolcanii*strain cured of plasmid pHV_2. Microbiol2001; (147): 959 – 964.

[50]. Zheng, T., Huang, Q., Zhang, C., Ni, J., She, Q. and Shena, Y. Development of a Simvastatin Selection Marker for aHyperthermophilic Acidophile,*Sulfolobusislandicus.* ASM 2012; (78): 568 – 574.