Abundance of Ammonia-Oxidizing Bacteria and Archaea Under Long-Term Maize Cropping Systems

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Introduction

- The first step in nitrification is often rate limiting (conversion of ammonia to nitrite) and until recently was thought to solely be facilitated by ammonia oxidizing bacteria (AOB). It is now known that ammonia oxidizing archaea (AOA) also possess the ammonia monooxygenase gene (amoA), the functional gene necessary for the oxidation of ammonia to nitrite (Venter et al. 2004; Treusch et al. 2005), but the extent of its role in soils is unknown.
- Much of the literature from cultivated soils has found that AOA abundance greatly outnumbers AOB abundance and that AOB abundance is less resilient to changes in soil conditions.
- Xu et al (2012) found that in acidic soils the abundance of AOB decreased while AOA abundance was unaffected. Another study on the effect of various fertilizer types found that AOB abundance was also affected while AOA abundance was not (Shen et al. 2008).
- Currently more studies are looking into the specialized roles of AOB and AOA in nitrification.
- Our study is a long-term (25 yr+) fertilized, monoculture maize field with no-tilled and disk-tilled plots. The average field pH is around 5.6 and acidity is postulated to be one of the drivers of AOA abundance over AOB.
- This long-term study allows for unique look into the habitat drivers of AOB and AOA abundance. Herein we report on 1) the long term abundance versus the change in abundance after planting of maize 2) the effect of habitat modification (no tillage versus disk tillage) and 3) the impact of long-term N fertilization rate on AOB and AOA abundance.

Materials and Methods

Field Site:
Two sampling dates: Pre-Plant and 4 days after planting (post-plant)
Disk Tillage versus No Tillage
Continuous Corn
0, 40, 80, 120, 160 kg N ha^{-1} yr^{-1}

DNA Extractions:
500mg of Freeze-Dried Soil
MoBio Ultra Clean® soil DNA isolation kits
Tubes were held in the MoBio Vortex Adapter tube holder.
Modifications included: Tubes were incubated at 70^{0}C for 10 mins in a water bath after solution S1 was added.
Followed by the 'Alternative Protocol for maximum yields' according to manufacturer's specifications.

Standard Curve:
Cells derived from Dr. Kim Cook's lab (USDA-ARS, Bowling Green, KY) were grown in LB broth and purified using Wizard Plus Minipreps DNA purification system.
Concentrations were calculated using NanoDrop
10-fold dilutions between 10^8-10^2 were used
AOB efficiencies between 94% and 115% % R^2 values of 0.981-0.999
AOA efficiencies between 91% and 99% with R^2 values of 0.990-0.998

Quantitative Real-Time PCR:
AOB primers: AmoA 1F/2R
AOA primers: AmoA 19F/643R
Samples were carried out in triplicate 20µL reactions using Sybr Green I dye
Single PCR products were validated using Melt Curve Analysis and Gel Electrophoresis

References / Acknowledgements

Venter, J.C., et al. (2004). Environmental genome shotgun sequencing of the Sargasso Sea. *Science* **304(66).**

Treusch, A.H., et al. (2005). Novel genes for nitrite reductase and Amo-related proteins indicate a role of uncultivated mesophilic crenarchaeota in nitrogen cycle. *Environmental Microbiology* **7(12).**

Xu, Y., Yu, W., Qiang, M., Zhou, H. (2012). Responses of bacterial and archaeal ammonia oxidisers of an acidic luvisols soil to different nitrogen fertilization rates after 9 years. *Biol Fertil Soils* **48(7).**

Shen, J., Zhang, L., Zhu, Y., Zhang, J., He, J. (2008). Abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea communities of an alkaline sandy loam. *Environmental Microbiology* **10(6).**

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Results

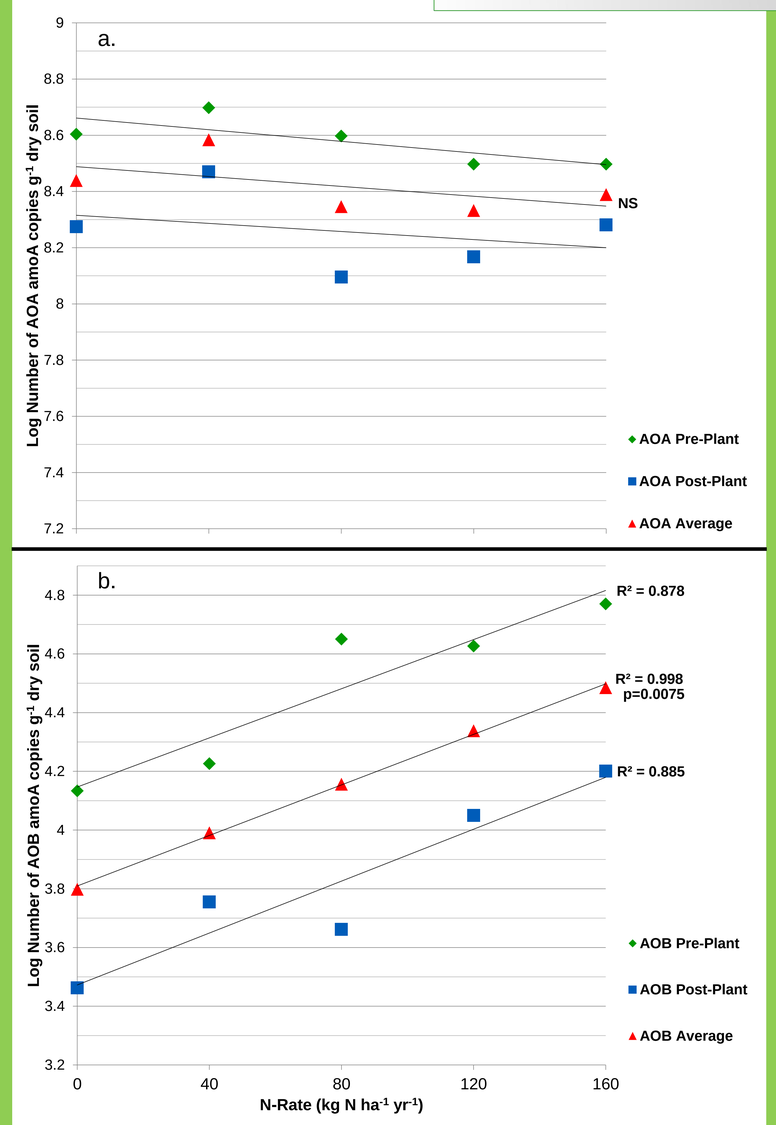

Figure 1: Nitrifying (a) archaeal (AOA) and (b) bacterial (AOB) amoA abundance by N rate for pre- and post-plant soil samples

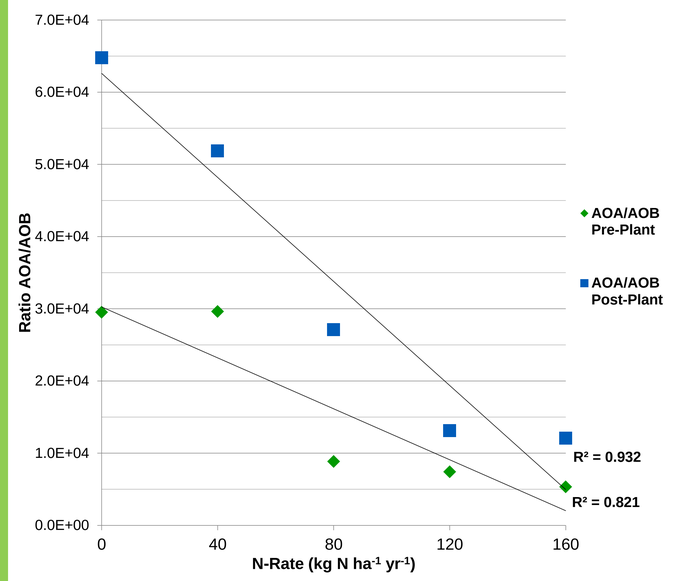

Figure 2: Ratio of AOA to AOB abundance over a N rate gradient for pre- and post-plant soil samples

Figure 3: AOB and AOA abundance by tillage practice for pre- and post-plant soil samples

Discussion

- AOA abundance greatly outnumbered AOB abundance (Fig. 1a,b) in this long-term continuous maize system, and may reflect acidic conditions created by ammonia-based fertilizer addition.
- AOB, but not AOA abundance, increased with increasing N rate (Fig. 1a,b).
- Immediately after planting AOB and AOA abundance decreased up to 10 fold, possibly due to soil disturbance during planting and to a lesser extent N fertilizer addition (Fig. 1a,b).
- The ratio of AOA/AOB declined with increasing N rate and was largely due to increased AOB abundance (Fig. 2). The decline in the ratio was greater post-planting in response to increased AOB abundance and a slight decline (non significant) in AOA abundance with N rate (Fig. 2).
- AOB abundance, and pre-plant AOA abundance, was greater under no tillage compared to disk tillage (Fig. 3).
- Overall, AOB abundance was more responsive to N-rate, tillage and harvest time, compared to AOA suggesting AOA to be more resilient to soil management and environmental conditions.

Conclusions

- Tillage has a greater influence on the abundance of AOB than AOA.
- To a similar extent, N-rate also has an effect on AOB abundance, but not on AOA.
- AOB responds to soil management to a much greater extent than AOA, suggesting greater resilience of AOA to environmental perturbation.
- We hypothesize that this may stem from AOB and AOA occupying distinct microhabitats in the soil of varying physicochemical composition, stability and turnover.
- Further investigation into habitat driven niches of AOB and AOA is currently being explored.