AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
FUNDING CYCLE 2013 – 2015

TITLE: Influence of ammonia oxidizing Thaumarchaea on N fertilizer use efficiency (8196A)

RESEARCH LEADER: Peter J. Bottomley

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SUMMARY: In Oregon agricultural soils, N fertilizers such as urea and ammonium (NH_4^+) are rapidly transformed/oxidized to nitrate (NO_3^-) by microbes in a process referred to as nitrification. NO_3^- is more water soluble than NH_4^+ and is easily taken up by plant roots, but also has potential to be leached from the soil. We have been unable to control the microbial transformation of fertilizer N to NO_3^- and it has been difficult to balance economically optimum N fertilization rates and minimize N losses. Until 2005, ammonia (NH_3) oxidation in soils was thought to be carried out by uniquely specialized NH_3-oxidizing bacteria (AOB). Soil AOB have been studied for over 100 y and attempts have been made to slow down their activity using nitrification inhibitors, slow release N fertilizers, and by careful timing of N fertilizer applications. However, in 2005 the nitrification paradigm was changed by the discovery that a completely different type of soil microbe called thaumarchaea can perform NH_3 oxidation. Only a few strains of thaumarchaeal NH_3-oxidizers have been isolated and proven to grow by oxidizing NH_4^+. They are given the acronym AOA to distinguish them from the AOB. AOA are widely distributed in soils throughout the world, as are AOB. However, because AOA are usually more numerous in soil than AOB, this has led to speculation that AOA are responsible for most soil nitrification..

With the assistance of a previous ARF award, we developed a cheap, fast, and technically simple assay to discriminate between the activities of AOA and AOB in soil. This assay takes advantage of differences in the sensitivity of AOA and AOB to acetylenic compounds (alkynes). We have discovered that commercially available alkynes of carbon chain lengths C6-C9 can completely stop NH_3 oxidation by AOB, but have no effect on NH_3 oxidation by AOA. This assay (**C8**) can be used to discriminate between AOA and AOB mediated NH_3 oxidation in soils in a 24-48h incubation.

In this ARF grant we proposed to use a laboratory-based experimental approach to assess how overall nitrification rates and the relative contributions of AOA and AOB in agriculturally important soils from across Oregon respond to organic and inorganic N sources at different seasonally relevant temperatures. In both organically managed and in fallowed agricultural soils, nitrification is totally dependent upon mineralization of organic N.

OBJECTIVES: Objective 1: Use the C8 assay to determine the nitrification response of different cultivated soils to increasing NH_3 levels over a range of temperatures encompassing early spring through fall conditions. Evaluate the relative contributions of AOA and AOB to nitrification at each temperature and N level.

Objective 2: Use the C8 assay to determine the nitrification responses of AOA and AOB in cultivated soils in response to the addition of organic N or other slow release forms of N. Determine if different groups of AOA respond to inorganic versus organic N sources.

SIGNIFICANT ACCOMPLISHMENTS:

In 2013 an invitation was extended to A.E. Taylor to speak at the **3rd International Conference on Nitrification, in Tokyo, Japan**. This exposure led to collaboration with laboratories in Vienna, Austria and a publication.

Two articles have been published in a highly respected journal.
A.E. Taylor, Kc Taylor, B. Tennigkeit, M. Palatinszky, M. Stieglmeier, D.D Myrold, C. Schleper, M. Wagner, and P.J. Bottomley. 2015. Inhibitory Effects of C_2 to C_{10} 1-Alkynes on Ammonia Oxidation in Two *Nitrososphaera* Species. Applied and Environmental Microbiology, 81:6.
A. E. Taylor, N. Vajrala, A.T. Giguere, A.I Gitelman, D. D. Myrold, L. Sayavedra-Soto and P. J. Bottomley. 2013. Use of aliphatic n-alkynes to discriminate soil nitrification activities of ammonia-oxidizing thaumarchaea and bacteria. Applied and Environmental Microbiology, 79:21:6544-6551.

Two manuscripts are in preparation.
A.E. Taylor, A. Giguere, B. Tennigkeit, D.D. Myrold, P.J. Bottomley. (2015). Temperature dependency of the relative contributions to soil nitrification by ammonia oxidizing archaea and bacteria.
A. Giguere, A.E. Taylor, B. Tennigkeit, D.D. Myrold, P.J. Bottomley. (2015). Nitrification responses of soil ammonia-oxidizing archaea and bacteria to ammonia additions.

BENEFITS & IMPACT:
1) The octyne assay was shown to be effective over a broad range of soils from Oregon. Octyne can be useful in short-term assays to discriminate AOA and AOB contributions to soil nitrification (Taylor et al 2013).
2) We now have work with pure cultures to back up our observations in soils. Octyne resistance was confirmed in three strains of AOA and octyne sensitivity in two strains of AOB. In addition we have observations related to the physiology of AOA that will be interesting to other soil nitrification workers. We showed that higher concentrations of octyne partially inhibit AOA activity in a manner that is: (a) time independent, (b) fully reversible, (c) not competitive with NH_4^+, and (d) without effect on the competitive interaction between NH_4^+ and acetylene, and raises the possibility that kinetic and catalytic properties of AMO from AOA and AOB might differ (Taylor et al 2013 and 2015).
3) We have demonstrated that AOA and AOB activities in soil occur at different temperatures. AOB are active at temperatures $\leq 23^oC$ while AOA activity occurs at temperatures $\geq 23^oC$ (Taylor et al, in preparation). This strongly suggests that AOB are primarily responsible for oxidation of N fertilizer applied when the soil is ~10°C and could be targeted for inhibition, whereas AOA are likely responsible for most nitrification in warm soils under early fall conditions.
4) We have demonstrated that AOA are responsible for the bulk of nitrification that occurs in response to mineralized N, and that they are also responsible for nitrification when very low additions of NH_4^+ are added (14 – 28 µg NH_4^+/g soil). The maximum rate of AOA activity is lower than the corresponding rates for AOB. This suggests that AOA are the nitrifiers to oxidize NH_4^+ when slow release fertilizers are utilized.

ADDITIONAL FUNDING RECEIVED: NIFA: foundation Grant. Identifying the drivers and consequences of archaeal and bacterial contributions to soil nitrification. P.J. Bottomley, D.D. Myrold, and A.E. Taylor co-PIs.

FUTURE FUNDING: NIFA grant pending. Impacts of Archaeal and Bacterial Nitrification on Cycling and Fate of N in Agroecosystems.