AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
FUNDING CYCLE 2014 – 2016

TITLE: New Strategies to Reduce the Rate of Nitrification of Fertilizer N by a Combination of Selective Microbial Inhibition and Use of Organic and Slow Release N Fertilizers

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SUMMARY:

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 Oregon soils respond to organic N sources and enhanced efficiency forms of inorganic N at different seasonally relevant temperatures. In both organically managed and in fallowed agricultural soils, nitrification is totally dependent upon mineralization of organic N. We hypothesized that organic N might selectively stimulate AOA populations under NH_4^+ limiting conditions. As we have previously shown, AOA supported nitrification is generally slower than the rate supported by AOB, and reaches its maximum rate at lower levels of extractable NH_4^+ than does AOB-supported nitrification (Giguere et al 2015). Both of these attributes might be critically important criteria for efficient use of fertilizer N. With the expanding interest in utilizing organic N forms in agriculture, we want to determine how nitrification by AOA and AOB responds to applications of different types and levels of organic N of different C:N ratios. We hypothesize that the nitrification response to organic fertilizers may be different than that to slow release fertilizers (with urease inhibitors) or enhanced efficiency fertilizers (with nitrification inhibitors). Under these fertilizers with controlled NH_4^+ release AOA activity might be critical for promoting the availability of NO_3^- from late winter/early spring applications of NH_4^+ to winter wheat, and for stimulating successful establishment of fall planted crops. We believe that will provide enough data to develop useful correlates for a model to predict rates of nitrification based on AOA/AOB contributions (Booth et al 2005). We might also be able to develop a practical assay for soil testing purposes.

OBJECTIVES:

Objective 1: Use the octyne 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.

Objective 2: Use the octyne assay to determine the nitrification responses of AOA and AOB in cultivated soils in response to the addition of enhanced efficiency fertilizers (EFF).

PROCEDURES:

Experimental approach. Small portions of field soils will be incubated with either organic N, or a slow release form of N fertilizer (urea plus urease inhibitor), or enhanced efficiency fertilizer (EFF, urea plus nitrification inhibitor) at several NH_4^+ levels. Two sets of treatments will be imposed on each N type: with and without octyne (4 μM soil solution concentration, to discriminate between nitrification by AOA and AOB), and each treatment will be set up in triplicate. An acetylene control (6 μM soil solution concentration) will also be included for each N treatment to cause accumulation of NH_4^+ and allow us to measure the rate of N mineralization. All treatments will be incubated at temperatures relevant to soil conditions at the time of N applications in the field. Choice of organic-N or fertilizer-N will be influenced by the local sources of organic N or slow release N used in the specific region of the state. These experiments will be sampled over a period that will depend upon the speed with which N is nitrified.

SIGNIFICANT ACCOMPLISHMENTS:

Progress toward objectives.

Objective 1. Use the octyne 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.

Preliminary experiments were conducted to determine a suitable source of organic material for our experiments. We obtained red clover from cover cropped plots at Hyslop Farm which we separated into lower N (stems, S) and higher N (blooms and leaves, BL) fractions, and perennial grasses, minus the seed heads (G), from Long Term Fallow plots. Organic materials were dried at room temperature for 1 week, ground to a fine powder, and sent to the Central Analytical Laboratory for the determination of C and N contents. C:N ratios were 41.5, 26.6 and 12.8 in G, S and BL, respectively.

The organic materials were incubated with soils collected from Hyslop Farm winter wheat plots. We found that when the lower C:N ratio materials (G and S) were added as a soil amendment that there was no net nitrification, and in fact, the background levels of nitrate in the soil were reduced over time. This strongly suggested that the soils were C limited, and with the influx of organic C provided by the amendment, N was immobilized. This was backed up by observations in soil incubations which were treated with acetylene to prevent nitrification. In acetylene treated soils without G or S amendment NH_4^+ accumulated over the incubation period, whereas in acetylene treated soils with G or S amendment all background NH_4^+ was consumed and none accumulated over the incubation period.

In contrast, when soils collected from Hyslop Farm winter wheat plots were incubated with the highest C:N material (BL), net nitrification occurred. In these incubations NO_3^- accumulation was stimulated to rates 3.5-fold higher than the no amendment control, and NH_4^+ accumulated in the acetylene treated controls. In future experiments BL amendments were used since they promoted measureable net nitrification rates.

We compared the response of AOA and AOB in Hyslop Farm wheat cropped soil incubated with 5 µmol/g soil organic N (BL), inorganic N (NH_4^+ added as NH_3 gas), or a no N amendment. In the absence of added N, all of the net nitrification was attributed to AOA and NO_3^- accumulated at a rate of 0.1 µmol/g soil/d. When soils were incubated with 5 µmol/g soil organic N the rate of net NO_3^- accumulation did not increase significantly over that of the no amendment control, and the AOA still contributed all of the net nitrification. However, when the soils were incubated with 5 µmol/g soil inorganic N, the overall rate of net NO_3^- accumulation increased to 0.32 µmol/g soil/d, and while the AOA rate did not increase, now AOB contributed 0.18 µmol/g soil/d. These observations suggests the following:

a) The AOA were not NH_4^+ limited in this cropped soil and were oxidizing NH_4^+ at their maximum rate regardless of N amendment. This agrees with previously published observations from our lab and in the literature (Giguere et al 2015).
b) The AOB were NH_4^+ limited but could not respond to the slow release of NH_4^+ from the organic N amendment.

Objective 2: Use the octyne assay to determine the nitrification responses of AOA and AOB in cultivated soils in response to the addition of enhanced efficiency fertilizers (EFF).

Experiments evaluated the response of wheat cropped soil from Hyslop farm to three forms of urea utilized in the Willamette valley: urea with no polymer coating, ESN which is a slow release urea that is polymer coated, and Agrotain which is a polymer coated urea containing the urease inhibitor N-(n-butyl) thiophosphoric triamide. Urea and ESN are commonly used by farmers in the Willamette valley while Agrotain is less commonly used. We found that in soil slurry assays incubated at 25ºC:

a) The polymer coating of the ESN form of urea did not have a significant effect on the accumulation of NH_4^+ in soil. In soil that was treated with acetylene to prevent nitrification 36.7 ± 7.4 µmol NH_4^+/g soil accumulated in urea treatments over ~200 h, while 26.9 ± 19.9 µmol NH_4^+/g soil accumulated in ESN soil treatments. The accumulation of NH_4^+ in soils treated with Agrotain plus urease inhibitor had a much slower rate of NH_4^+ accumulation (7.8 ± 1.6 µmol NH_4^+/g soil).
b) NO_2^- + NO_3^- accumulation due to soil nitrification was nearly the same in urea and ESN treated soil with 28.5 ± 5.4 and 29.6 ± 7.8 µmol NH_4^+/g soil accumulating in the two treatments, respectively; while NO_2^- + NO_3^- accumulation in Agrotain treated soil was 8.6 ± 2.0 µmol NH_4^+/g soil.
c) Generally, NO_2^- is not measured separately from NO_3^- in soil assays, but recent work in our lab (Giguere et al 2017)has shown that under some conditions NH_4^+ oxidation can become uncoupled from NO_2^- oxidation resulting in NO_2^- accumulation. We found that in urea and ESN treatments that all of the NO_2^- + NO_3^- was NO_2^-, suggesting that NO_2^- oxidation was not coupled to NH_4^+ oxidation under the assay conditions. However, in the Agrotain treatments NO_2^- was barely above the detection limit.
d) NO_2^- accumulation in soil has been implicated in the production of N_2O in agricultural soils. We found N_2O accumulation in the urea and ESN treated soils were significantly greater than in the Agrotain treated soils.

BENEFITS AND IMPACT:

Two articles have been published in respected journals.
A.E. Taylor, A. Giguere, C. Zoebelein, D.D. Myrold, P.J. Bottomley. 2016. Modeling of soil nitrification response to temperature reveals fundamental thermodynamic differences between ammonia oxidizing archaea and bacteria. *International Society for Microbial Ecology Journal*, advance online publication, December 20, 2016; doi:10.1038/ismej.2016.179.
Andrew T. Giguere, Anne E. Taylor, Yuichi Suwa, David D. Myrold, Peter J. Bottomley. 2017. Uncoupling of ammonia oxidation from nitrite oxidation: impact upon nitrous oxide production in non-cropped Oregon soils. *Soil Biology and Biochemistry*, 104:30 - 38.

Talks and posters have been presented at scientific meetings.
Giguere, AT., Taylor, A.E., Myrold, D.D., Bottomley, P. J. 2016. Uncoupling of ammonia oxidation from nitrite oxidation: impact upon nitrous oxide production in non-cropped Oregon soils. Abstracts of the Soil Science Society of America meeting, Phoenix, AZ.
A.E. Taylor, A. Giguere, A. Wagoner, G. Jones, D.D. Myrold, P.J. Bottomley 2016. Effects of Digested Dairy Manure on Soil Microbe Populations. EPA Region 10 Animal Feeding Operation/Confined Animal Feeding Operation Workshop. Oldfield Hall, Oregon State University, Corvallis OR.
A.E. Taylor, A. Giguere, C. Zoebelein, D.D. Myrold, P.J. Bottomley. 2016. Modeling of soil nitrification response to temperature reveals fundamental thermodynamic differences between ammonia oxidizing archaea and bacteria. International Society for Microbial Ecology Meeting, Montreal, Canada.
Giguere, AT., Taylor, A.E., Myrold, D.D., Bottomley, P. J. 2015. Uncoupling of ammonia oxidation from nitrite oxidation, and its impact upon nitrous oxide production in a grassland soil. Abstracts of the Soil Science Society of America meeting, Minneapolis MN.

Giguere, AT., Taylor, A.E., Myrold, D.D., Bottomley, P. J. 2015. Uncoupling of ammonia oxidation from nitrite oxidation, and its impact upon nitrous oxide production in a grassland soil. International Conference on Nitrification, Edmonton, Canada.
A.E. Taylor, A. Giguere, C. Zoebelein, D.D. Myrold, P.J. Bottomley. 2016. Modeling of soil nitrification response to temperature reveals fundamental thermodynamic differences between ammonia oxidizing archaea and bacteria. International Conference on Nitrification, Edmonton, Canada.

Two undergraduate students have been trained.
Mackenzie Frey. Microbiology student, graduated 2015.
Gavin Jones. Soil Science student, ready for great things and set to graduate 2017.

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM:

USDA-NIFA. The Impact of Archaeal and Bacterial Nitrifiers on the Fate of Digester N Applied to Fodder Crops

Oregon Dairy Farmers. The Impact of Nitrification on the Fate of Digester N Applied to Eastern Oregon Agricultural Soils

FUTURE FUNDING POSSIBILITIES:

Pending
Oregon Beef Council.
Current ARF Funding Cycle
Oregon Department of Agriculture Fertilizer Research Program Competitive Grant Program – 2017
In preparation
National Science Foundation Ecosystems (Due January 2017)
National Science Foundation Dimensions of Biodiversity (Due February 2017)

REFERENCES:
Booth MS, Stark JM, Rastetter E (2005). Controls on nitrogen cycling in terrestrial ecosystems: A synthetic analysis of literature data. *Ecol Monogr* 75: 139-157.

Giguere AT, Taylor AE, Myrold DD, Bottomley PJ (2015). Nitrification responses of soil ammonia-oxidizing archaea and bacteria to ammonium concentrations. *Soil Sci Soc Am J* 79: 1366-1374.

Giguere AT, Taylor AE, Suwa Y, Myrold DD, Bottomley P (2017). Uncoupling of ammonia oxidation from nitrite oxidation: Impact upon nitrous oxide production in non-cropped Oregon soils. *Soil Biology & Biochemistry* 104: 30 - 38.