

Partitioning Nitrous Oxide (N₂O) Emissions from Ammonia Oxidizing Bacteria (AOB) and Ammonia Oxidizing Archaea (AOA) in Corn and Switchgrass Ecosystems

Di Liang^{1,2,3*} (liangdi@msu.edu) and **G Philip Robertson**^{1,2,3*}

¹Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, MI; ²W.K. Kellogg Biological Station, Michigan State University, Hickory Corners, MI; ³Great Lakes Bioenergy Research Center, Michigan State University, East Lansing, MI

<https://www.glbrc.org/research/sustainability>

Project Goals:

Our goal in this project is to understand the relative importance of ammonia oxidizing bacteria (AOB) and ammonia oxidizing archaea (AOA) as sources of nitrate and nitrifier-derived nitrous oxide (N₂O) in potential bioenergy cropping systems. In particular we aim to understand how AOA and AOB respond to nitrogen fertilizer in switchgrass vs. corn cropping systems. We use 1-octyne, a recently developed and tested chemical inhibitor of AOB (Taylor *et al.*, 2013, Taylor *et al.*, 2015) to distinguish activities and emissions from AOA vs. AOB.

Abstract:

Nitrous oxide (N₂O) is a potent greenhouse gas with a global warming potential 300 times higher than CO₂. The atmospheric N₂O abundance has increased from a preindustrial baseline of 270 to 329 ppb_v as of 2016 (<http://cdiac.ornl.gov>, accessed on December 20, 2016), largely due to human activities. N₂O also reacts with electronically excited oxygen atoms O (¹D) in the stratosphere to form nitric oxide (NO), which catalyzes ozone depletion (Portmann *et al.*, 2012). Gross anthropogenic N₂O emissions of 6.2 Tg N₂O-N yr⁻¹ are dominated by agriculture (Davidson and Kanter, 2014). As agricultural soils contribute N₂O more than any other anthropogenic components, understanding sources of N₂O from agricultural soils is critically important for developing N₂O mitigation practices (Paustian *et al.*, 2016).

Soil N₂O emissions mainly result from two microbial processes including denitrification and nitrification. Nitrification converts ammonia (NH₃) to nitrite (NO₂⁻) and nitrate (NO₃⁻), with N₂O as a byproduct (Robertson and Groffman, 2015). Nitrification is performed mainly by two taxa: ammonia oxidizing bacteria (AOB) and ammonia oxidizing archaea (AOA). Recent studies have confirmed that both AOA and AOB are able to produce N₂O (Stieglmeier *et al.*, 2014). However, the relative contribution of AOA and AOB to N₂O is still unclear. Our understanding of the mechanisms regulating N₂O production from AOA and AOB is hindered mainly by the difficulties of isolating pure cultured ammonia oxidizers. For example, there is currently only one pure culture of soil AOA (*Nitrososphaera viennensis* EN76) available for physiological studies.

In this study, we examined the seasonal responses of AOA and AOB following nitrogen fertilizer application to annual (corn) and perennial (switchgrass) cropping systems. We took soil samples before and 1, 5, 13, 21, and 42 days after fertilization in late fall of 2015 and spring and summer of 2016. Using 1-octyne as a selective inhibitor targeting AOB in short-term (24h) incubations, we were able to separate the N_2O and NO_3^- contributed by AOA and AOB, respectively.

Our results show that: 1) In both corn and switchgrass ecosystems, AOB were the main contributors to nitrate accumulation in spring and summer (60-75% of accumulated nitrate) while in late fall AOA and AOB contributed equally; 2) AOB dominated N_2O emissions (> 80%) in spring and summer in both corn and switchgrass ecosystems, but in late fall AOA and AOB contributed equally to N_2O in corn while AOB were the major N_2O contributors (60%) in switchgrass ecosystems; 3) AOB but not AOA produced more nitrate in response to fertilization in both corn and switchgrass; the AOB response lasted 42 days; and 4) both AOA and AOB produced more N_2O in response to fertilization in corn but not in switchgrass.

We conclude that AOA and AOB have different responses to nitrogen fertilization in different cropping systems. In most seasons more nitrate and N_2O was produced by AOB than AOA, and AOB also tended to be more responsive to fertilizer additions.

References

1. Ravishankara AR, Daniel JS, Portmann RW. Nitrous oxide (N_2O): the dominant ozone-depleting substance emitted in the 21st century. *Science*, 2009, 326(5949):123-5, doi: 10.1126/science.1176985.
2. Portmann RW, Daniel JS, Ravishankara AR. Stratospheric ozone depletion due to nitrous oxide: influences of other gases. *Philos Trans R Soc Lond B Biol Sci*, 2012, 367(1593):1256-64, doi: 10.1098/rstb.2011.0377.
3. Davidson EA and Kanter D. Inventories and scenarios of nitrous oxide emissions. *Environ. Res. Lett*, 2014, 9:105012, doi:10.1088/1748-9326/9/10/105012.
4. Paustian K, Lehmann J, Ogle S, Reay D, Robertson GP, Smith P. Climate-smart soils. *Nature*, 2016, 532(7597):49-57, doi: 10.1038/nature17174.
5. Robertson, GP and Groffman PM. Nitrogen transformations, 2015, Pages 421-446 in E. A. Paul, editor. Soil microbiology, ecology and biochemistry. Fourth edition. Academic Press, Burlington, Massachusetts, USA.
6. Stieglmeier M, Mooshammer M, Kitzler B, Wanek W, Zechmeister-Boltenstern S, Richter A, Schleper C. Aerobic nitrous oxide production through N-nitrosatin hybrid formation in ammonia-oxidizing archaea. *ISME J*, 2014, 8(5):1135-46, doi: 10.1038/ismej.2013.220.
7. Taylor AE, Vajjala N, Giguere AT, Gitelman AI, Arp DJ, Myrold DD, Sayavedra-Soto L, Bottomley PJ. Use of aliphatic n-alkynes to discriminate soil nitrification activities of ammonia-oxidizing thaumarchaea and bacteria. *Appl Environ Microbiol*, 2013, 79(21):6544-51, doi: 10.1128/AEM.01928-13.
8. Taylor AE, Taylor K, Tennigkeit B, Palatinszky M, Stieglmeier M, Myrold DD, Schleper C, Wagner M, Bottomley PJ. Inhibitory effects of C2 to C10 1-alkynes on ammonia oxidation in two Nitrososphaera species. *Appl Environ Microbiol*, 2015, 81(6):1942-8, doi: 10.1128/AEM.03688-14.

This work was funded in part by the DOE Great Lakes Bioenergy Research Center (DOE BER Office of Science DE-FC02-07ER64494) and DOE OBP Office of Energy Efficiency and Renewable Energy (DE-AC05-76RL01830).