**Nitrous Oxide Source Identification from a Fertilized Bioenergy Crop Soil**

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**Project Goals: Over 60% of global anthropogenic nitrous oxide ($N_2O$), a significant greenhouse gas, originates from fertilized agricultural soils. The major goals of this project were: 1) to resolve the relative contributions of ammonia-oxidizing microbes to nitrification and the production of $N_2O$ from a fertilized soil growing an important bioenergy crop, 2) to determine factors that stimulate $N_2O$ production, and 3) identify methods to suppress $N_2O$ production in such systems. This knowledge would allow for the implementation of more sustainable agricultural practices that would reduce negative environmental consequences currently facing large-scale bioenergy production.**

Nitrous oxide ($N_2O$) is a powerful greenhouse gas that is largely a consequence of the extensive use of synthetic nitrogen (N) fertilizer in agricultural practices. As the global population continues to grow, there will be an increasing demand for food, livestock, and bioenergy, requiring even further intensification of agricultural practices, N fertilizer use, and resulting $N_2O$ emissions. The processes responsible for most $N_2O$ production are microbially-controlled, and under aerobic conditions the controlling populations are ammonia-oxidizing archaea (AOA) and bacteria (AOB). However, the extent to which each group contributes to $N_2O$ production is not known, with published results often varying.

AOA and AOB population dynamics and activities in soils planted with the bioenergy crop switchgrass (*Panicum virgatum*) were characterized both in the laboratory and experimental field studies to resolve the influence of soils, soil chemistry, and management practices on $N_2O$ production. The relative contribution of each group was constrained by laboratory studies combining transcription, isotope fractionation, and selective inhibitor analyses. Real-world significance of the laboratory experiments was confirmed in a field study. The results, described below, provide knowledge that is central to mitigating agricultural greenhouse gas emissions.

Soil from switchgrass fields that previously received synthetic N amendments (fertilized) or no amendment (control) were used to establish a microcosm experiment, where new synthetic N was applied to the fertilized soil, and gas and soil samples were collected over the course of 10 days. The consumption of ammonia and balanced production of nitrate in the soils confirmed activity of ammonia-oxidizers during the experiment. Functional gene counts differed significantly between the two treatments for both ammonia oxidizers, with AOB gene counts increasing 45-fold after 10 days. Conversely, AOA gene counts increased only 1.5-fold after 10 days. Both AOA and AOB transcripts in the fertilized soil increased during the experiment, however AOB transcripts increased over 85-fold from day 0 to 10. Total $N_2O$ and total natural abundance $^{15}N$ ($\delta^{15}N^{bulk}$) from the gas samples were measured on an isotope ratio mass spectrometer (IRMS). Total $N_2O$ increased beginning on day 5, and reached a 75-fold difference

at day 7 from day 0 concentrations. Another 2.5-fold increase was observed from day 7 to 10. Relatively little $N_2O$ was produced in the unfertilized control soil microcosms, which retained a $\delta^{15}N^{bulk}$-$N_2O$ signature between -7 to +3 per mil. The $\delta^{15}N^{bulk}$-$N_2O$ from fertilized microcosms transitioned from values of -10 to -49 per mil during the course of incubation, indicating a shift in $N_2O$ production by AOA to AOB. Typical $\delta^{15}N^{bulk}$-$N_2O$ values from soil AOA range from -11 to -34 but can be as low as -68 per mil in cultures of AOB. Keeling plots indicated the major source of $N_2O$ had a $\delta^{15}N^{bulk}$ value of -45.5 per mil, representing a fractionation of -45 per mil relative to the $NH_4^+$ supplied. The distinct increase in AOB abundance and activity paired with rising $N_2O$ stemming from a single source within the fractionation range of AOB provided strong evidence that AOB dominated $N_2O$ production in these fertilized soils.

A second microcosm experiment was established to confirm the above results, as well as test the effectiveness of nitrification inhibitors on reducing $N_2O$ emissions from fertilized soils. Four different treatments were applied to the same soils types (control and fertilized) used in the first experiment: 1) acetylene to inhibit both AOA and AOB, 2) PTIO to selectively inhibit AOA, 3) 1-octyne to selectively inhibit only AOB, and 4) no inhibitor (positive control). Soil and gas samples were collected over a 10 day period. No decrease in soil ammonia or increase in nitrate or $N_2O$ in the acetylene-treated soil indicated that both ammonia oxidizers had been successfully inhibited. The 1-octyne treated soils produced nitrate and $N_2O$ at rates similar to those from the acetylene treatment, suggesting that AOA produced very little of these compounds. The no inhibitor control showed a balanced consumption of ammonia and production of nitrate, while $N_2O$ production was high. Importantly, the PTIO treatment showed slightly higher nitrate and $N_2O$ production than the no inhibitor control treatment. This suggested that inhibition of ammonia oxidation by the AOA served to direct more ammonia to the AOB and again indicated AOB dominated $N_2O$ production in these fertilized soils.

To evaluate the real-world significance of these findings, a field study was executed to measure AOA and AOB abundance in relation to $N_2O$ flux. Switchgrass plots receiving synthetic N fertilizer or no amendment (control) were sampled for soil and gas flux in April, July, and September 2013. The results were consistent with laboratory findings. The abundance of AOB correlated with ammonia application and seasonal $N_2O$ flux, implicating AOB as the major producers. There was no difference in AOA abundance between the two treatments. Other environmental factors, such as temperature, seemed to be a larger driver of their abundance in the field.

The anticipated increase in $N_2O$ emissions associated with agriculture is a great challenge for society. The results of our studies suggest that management practices that promote AOA and suppress AOB will help reduce microbial production of this atmospherically active gas.

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