Factors Affecting Nitrous Oxide Production from Ammonia Oxidizers and Possible Mitigation Options

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Project Goals: Biofuels produced from perennial crops, like switchgrass (*Panicum virgatum*), are a potential alternative to fossil fuels. Traditional cultivation practices often require the addition of synthetic nitrogen. However, much of the applied nitrogen is lost to the leaching of nitrate produced by nitrifying microorganisms. In addition, those organisms contribute directly and indirectly to the production of the greenhouse gas nitrous oxide (N_2O). Over 60% of anthropogenic N_2O production originates from fertilized agricultural soils. The goals of this project were: 1) to evaluate the environmental and land management factors that affect N_2O production from cultivated switchgrass, 2) to resolve the relative contributions of ammonia-oxidizing microorganisms to nitrification and the production of N_2O, and 3) to identify methods to reduce or suppress N_2O production in such systems.

Cellulosic ethanol produced from perennial feedstock crops, such as switchgrass (*Panicum virgatum*), is a potential replacement for fossil fuels. However, high biomass yields require the addition of nitrogen (N) fertilizers that often has negative environmental impacts, including nitrate leaching and N_2O production. N_2O has a global warming potential ~300X greater than carbon dioxide, and emissions are expected to continually increase due to higher demand for crops and livestock from a growing world population. The processes responsible for most N_2O production are microbially controlled, and under oxic conditions the contributing populations are ammonia-oxidizing archaea (AOA) and bacteria (AOB). However, the extent to which each group contributes to ammonia oxidation and N_2O production, and the environmental and land management factors that affect their populations, are not known, with published findings differing.

The contributions of these two groups to ammonia oxidation and N_2O production were investigated in the field and laboratory. Soil from fields of switchgrass located at two sites, Prosser and Paterson, WA, differing in soil texture (silt loam vs. loamy sand, respectively), were examined for ammonia oxidizer community changes with N source and agricultural practices, as well as for correlation with N_2O production. Complementary lab studies using field soils constrained the relative contribution of each group using transcription, isotope fractionation, and selective inhibitor analyses.

During two field seasons, N_2O fluxes were measured monthly from April through October from fields of switchgrass receiving no N (control), 224 kg/y of inorganic N (agronomic rate), and N derived from intercropped leguminous alfalfa (*Medicago sativa*). Simultaneously, soil samples were collected and assayed for AOA and AOB abundance via quantification of the gene encoding one subunit of the ammonia monooxygenase (*amoA*). N_2O fluxes from fertilized plots were up to 16X higher immediately after N application and irrigation than from unfertilized control plots. Importantly, intercropping reduced N_2O flux to one-third of that from fertilized treatments. However, the 2-year average biomass yield from intercropped plots (16.8 ± 1.1

Mg/ha/yr) was intermediate between fertilized (24.5 ± 1.2 Mg/ha/yr) and control (10.5 ± 1.1 Mg/ha/yr) plots. AOA were the dominant in all treatments and were more abundant in the intercropped treatment than the control and fertilized treatments. Only AOB abundance was positively correlated with N_2O emissions.

Terminal restriction fragment (TRF) analysis of *amoA* revealed a significant impact of management on AOA populations. Communities in native soils were similar, despite sampling sites being some distance apart. In contrast, populations in managed fields were comprised of different genotypes of both AOA and AOB. Those changes in population structure were correlated with soil pH and texture (i.e., particle size).

The same field soils were used to establish a series of microcosms receiving, or not receiving, N fertilizer. Temporal fluctuations in N_2O emissions and isotopic composition were associated with N amendment and changes of AOA and AOB genes and transcripts. AOB *amoA* gene counts increased 45-fold after 10 days, whereas AOA increased only 1.5-fold following N addition. Although *amoA* transcripts of both AOA and AOB increased during the experiment relative to no-N controls, AOB transcripts increased over 85-fold from day 0 to 10, indicating a much greater response of AOB to N addition. Control soils retained a $\delta^{15}N^{bulk}$-N_2O signature between -7 to +3 per mil, indicative of AOA fractionation, while the $\delta^{15}N^{bulk}$-N_2O from fertilized microcosms reached values of -49 per mil, which are within the fractionation range of AOB.

Selective inhibitors of nitrification were used to further document the relative contributions of AOA and AOB to ammonia oxidation and associated N_2O emissions in response to N addition. Four inhibitor treatments were applied to control and fertilized soils: 1) acetylene to inhibit both AOA and AOB, 2) PTIO to selectively inhibit AOA, 3) 1-octyne to selectively inhibit AOB, and 4) no inhibitor (positive control). After 10 days, the acetylene-treated soil showed no decrease in ammonia or increase in nitrate or N_2O, indicating both ammonia oxidizers had been inhibited. The 1-octyne-treated soils showed a lack of nitrate and N_2O accumulation similar to the acetylene treatment, suggesting 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. In the PTIO treatment, nitrate and N_2O production were similar to the no inhibitor control treatment, suggesting AOB as the major N_2O producers. A titration of N additions with selective inhibitors indicated that, even when small amounts of synthetic N were supplied, AOB were stimulated and produced N_2O in this soil.

Our results provide evidence that agricultural practices, as well as soil properties, can affect ammonia-oxidizing communities and N_2O emissions. Synthetic N fertilizer, even in small amounts, led to N_2O production by AOB. Intercropping switchgrass with alfalfa does enhance biomass yields (relative to control plots) and reduces N_2O emissions from AOB (relative to fertilized plots). Selective inhibitors that promote AOA and suppress AOB could be a useful strategy to reduce microbial production of N_2O in bioenergy feedstock croplands.

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