The Effect of Agricultural Management Regimes on the Soil Microbial Denitrification Community Structure and its Impact on Nitrous Oxide Emissions

Summary:

Production agriculture to provide food for an ever-growing world population has resulted in the large scale use of inorganic fertilizers (Erisman, Sutton et al. 2008). The resulting increase of nitrogen in soil has far-reaching consequences (Canfield, Glazer et al. 2010). Nitrogen availability is regulated by microbial transformations in the nitrogen cycle, and agricultural inputs can disrupt the natural cycle (Galloway, Dentener et al. 2004). Important microbial transformations of the nitrogen cycle are nitrification and denitrification. Nitrification converts ammonium to nitrate, which presents pollution risks for groundwater and aquatic ecosystems. Denitrification can remove reactive N from terrestrial ecosystems through conversion of nitrate to molecular nitrogen via a stepwise process ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$) but can also produce nitric oxide/nitrous oxide during incomplete denitrification (Zumft 1997). Nitrous oxide (N_2O) is an extremely potent greenhouse gas (Canfield, Glazer et al. 2010). A range of organisms are capable of denitrification including archaea, bacteria and fungi. The efficiency of denitrification (and production of GHG through incomplete denitrification) is influenced by the composition of the microbial community. Denitrification in soil also depends on oxygen availability, available carbon, redox conditions, pH, nitrate, and temperature, but most importantly on soil moisture, which plays a determining role in the other factors (Butterbach-Bahl, Baggs et al. 2013). The presence of more carbon and nitrogen substrates increases the rates of denitrification, which in turn increases the rate of incomplete denitrification and its byproduct of nitrous oxide. These factors can also influence the composition of soil microbial communities. These drivers of microbial communities and their denitrification processes are influenced by land use and management practices, thus investigating how microbial community structure and function differ among types of land use and also their response to specific ecological drivers can lead to better management of nitrous oxide emissions in intensively managed agricultural landscapes.

Methodology:

Soil samples will be obtained from fields that are managed with the following practices: chisel plow and ridge till, with and without cover cropping. We will also obtain soil samples from agricultural wetlands that treat agricultural runoff, and from denitrifying bioreactors that treat nitrate-laden tile drain effluent. The agricultural plots are located at the University of Illinois South Farm. The plots to be used are planted in a maize/soybean rotation and are fertilized with a surface liquid urea ammonium nitrate (UAN) broadcast application just prior to planting. The two denitrifying bioreactors planned for this study are also located at the University of Illinois South Farm. They consist of lined trenches filled with wood chips and receive effluent from a tile drain system from the study field. PVC sampling ports allow access for collecting woodchips from the depths of the bioreactor. The wetland sites are located on the Franklin Research and Demonstration Farm in McLean County, and these wetlands receive tile drain effluent for treatment. We will carry out sample collection over six sampling dates per management regime per year; the dates will be split between times expected to have relatively high N_2O emissions, such as following fertilizer application or incorporation of cover crop residues or during periods of high flow for the bioreactors, as well as during periods expected to have relatively low N_2O

emissions. A standard suite of soil chemical and physical parameters will be measured at the time of each sampling, including soil moisture, pH, SOM, total N and total C, and ammonium and nitrate concentrations.

Standard denitrification studies of soil or woodchip samples will be modified using several known methods to distinguish between contributions of bacteria and fungi. A modified acetylene inhibition method allows the measurement of the nitrous oxide produced by specific blockage of the last step of denitrification (Tiedje, Simkins et al. 1989, Royer, Tank et al. 2004). N_2O concentrations will be determined by use of gas chromatography using an electron capture detector.

A variety of microbial functional groups are involved in nitrogen cycle activities including archaeal and bacterial and fungal denitrifiers, and nitrous oxide consuming bacteria which are not denitrifiers (Butterbach-Bahl, Baggs et al. 2013). We can assess the abundance of these groups using quantitative PCR (qPCR) of functional genes specific to them. We will use Illumina MiSeq amplicon sequencing of diagnostic functional genes to compare community composition of all abundant functional groups across management practices.

We will relate specific microbial populations or specific microbial assemblages to production of nitrous oxide by multivariate analysis of the sequencing and qPCR data. The analysis will also identify ecological drivers or management practices that influence microbial community structure and function.

Objectives and Expected Results

Objectives

The methods described above will allow us to analyze the soil and woodchip samples from a variety of agricultural management practices (and runoff mitigation strategies) for denitrification potential and nitrous oxide emissions. The molecular microbial ecology methods and ecological analyses will produce detailed information about size and composition of the microbial population present in those samples, and the ecological drivers that shape the communities and their functions. Our 4 major objectives for this work are listed below:

Objective 1: Measure N_2O emissions for different agricultural and nutrient management practices (ridge till and chisel plow with and without cover cropping; wetlands; and denitrifying bioreactors) for at least 6 sample dates over the course of the season.

Objective 2: Characterize the microbial community associated with production and consumption of N_2O in each management regime using functional gene markers for nitrifiers, denitrifiers, and N_2O-consuming microorganisms (including fungi). Characterize seasonal variations in the microbial community.

Objective 3: Determine the potential denitrification and incomplete denitrification rates for denitrifiers in soil or bioreactor woodchip samples from each management regime, and also determine the contributions of bacterial and fungal denitrifiers.

Objective 4: Determine the management factors and specific ecological drivers that have the greatest influence on microbial community composition, denitrification, and N2O production in each management practice.

Expected Results

- We will be able to compare denitrifying activity in agricultural production to that of wetlands and bioreactors.
- Fungal denitrifiers will be important in wetlands and bioreactors since they are more abundant in less physically disturbed environments.
- The expectation for the nitrous oxide emissions is that soil in the agricultural fields will have a higher output than wetlands or bioreactors due to fertilization. Among agricultural management practices fertilized fields should have higher nitrous oxide emissions due to increased available nutrients.
- Seasonal patterns will be evident when soils are flooded and more denitrification can take place in the anoxic conditions. However, seasonal trends in agricultural fields will correspond more highly to nutrient inputs. It has also been noted that fungal populations increase over time following planting which may mean N_2O production will increase correspondingly.
- Understanding the abundance of and diversity of the microbes responsible for the production and consumption of nitrous oxide will be important in determining the best management regime.
- High nitrous oxide production will be correlated to low abundance of *nosZ*.

Conclusions

The relationship between the soil microbial community, agricultural nitrogen inputs, and management practices to the reactive nitrogen pollution produced from farming needs to be addressed. While some strategies have been developed to mitigate the runoff of nitrate into the aquatic environment (for example, denitrifying bioreactors and agricultural wetlands), their potential to generate nitrous oxide is a concern which needs to be more fully investigated. We propose that studying the microbial community composition in soil samples from a variety of agricultural management regimes along with analysis of denitrification potential and N_2O emissions will help us determine meaningful relationships of the community to GHG emissions. Combining these findings with results from other expert teams at the University of Illinois will allow an overarching model to be developed that can identify management practices for farmers which minimize soil, air, and water reactive nitrogen pollution while retaining good crop yields and revenue potential. The synergy from a multidisciplinary analysis of the problem by groups that have not traditionally worked together should advance our understanding of how to abate pollution caused by reactive nitrogen from agriculture.

Participants

The work will be carried out by graduate student Natalie Stevenson under the supervision of Professor Angela Kent. Two undergraduate students will participate in this project in Summer 2015.

References

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