Soil Processes in Restored Wetlands

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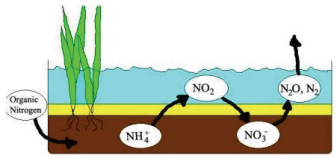
Introduction

Denitrification is a process through which nitrate is reduced to nitrite or nitrogen. Denitrification Enzyme Assays (DEAs or simply denitrification) are used to determine the amount of N2O being produced by the denitrifying bacteria in the soil. Soils with higher amounts of denitrifying bacteria present would be expected to have higher DEAs because they have more bacteria producing N2O than soils with fewer denitrifying bacteria.



Wetland Nitrogen Cycle (Gooselink,2001)

Background

This project is a collaborative effort between OSU (Dept. of Crop and Soil Science and Dept. of Geosciences), the federally-funded Natural Resources Conservation Service (NRCS) and Portland Metro. Its goal is to evaluate the ability of restored wetland sites along the Tualatin River Basin to provide ecosystem services. These services include nitrogen removal (looking specifically at plant uptake of nitrogen, the evolution of N2O, and denitrification) and conservation of diversity.

Sources

•Drury, C.F., et al. "Denitrification Techniques for Soils". Soil Sampling and Methods of Analysis. 2007. P. 471 – 494

•Rich, J.J. and D.D. Myrold. "Community composition and activities of denitrifying bacteria from adjacent agricultural soil, riparian soil, and creek sediment in Oregon, USA". Soil Biology and Biochemistry. 2004. **36:** 1431 -1441.

•Groffman, P.M. et al. "Denitrification". Standard Soil Methods for Long-Term Ecological Research. 1999. P. 272-288

•Henry, S., et al. "Quantification of denitrifying bacteria in soils by NirK gene targeted real-time PCR". Journal of Microbiological Methods. 2004. **59**: 327 – 335.

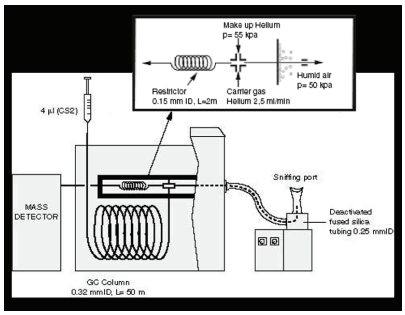
•Henry, S., et al. "Quantitative detection of the NosZ gene, encoding nitrous oxide reductase, and comparison of the abundance of 16S rRNA, narG, nirK, and nosZ genes in soils". American Society for Microbiology. 2006. **72**: 5181 – 5189.

•Initial proposal:
Santelmann, M. and D. D. Myrold. "Vegetation and Soil Processes in Restored Wetlands". 2009.

Measuring Denitrification Enzyme Assays (DEAs)

Soil samples are measured for their percent moisture content. Once this is known, 20 grams of soil are placed into an 125 ml Erlenmeyer flask with 25 ml of a glucose-nitrate solution added to create a soil slurry. The flasks are capped with rubber stoppers. The flasks are then attached to a vacuum pump/Argon tank via a needle and repeatedly have the headspace air removed and replaced by Argon (an inert gas). After several repetitions, the flask is attached solely to the Argon tank and gas is allowed to flow into the flask and out through an "exit needle" for about 1 minute to insure that only Argon gas is in the headspace.

After flushing each flask with Argon, 10 ml of acetylene gas is added to each flask to catalyze the production of N2O gas. The flasks are then placed on an orbit shaker during the duration of the Gas Chromatograph (GC) measurements.



The GC measurements are taken by removing 500 ul of headspace volume from each flask and injecting into the GC (illustrated above). The gas sample passes through a column that separates the gases, which then pass through a detector, and the resulting signal is converted by an integrator that calculates the area of each peak. The N2O peak appears roughly 3-3.5 minutes after injection of the sample. Assuming this is the case for every sample, we take the peak area given by the integrator at roughly this time and use it to calculate nanograms of N2O-Nitrogen present in each soil sample.

$$PV = nRT$$

Nanomoles of N2O gas is calculated using the Ideal Gas Law, PV = nRT, where n is the variable of interest. R is a constant with units of L*atm/K*mol. T is room temperature in Kelvin. V is 500 ul). Pressure is assumed to be the partial pressure of the flask, calculated by finding the total volume of the flask and subtracting out the volume of soil slurry.

Rates of ng of N2O-N per g of soil per minute (ng N2O-N/g soil/minute) is a linear relationship.