Final Report 2011-2013

Contract Information

Title	The Constructed Wetland Dilemma: Nitrogen Removal at the Expense of Methane Generation?
Project Number	60030648
Start Date	6/1/2011
End Date	9/21/2013
Focus Category	Methanogenesis, Denitrification, Wetlands, Ecology
Keywords	Microbial Ecology, Biogeochemistry, Methanogenesis, Constructed Wetlands
Lead Institute	The Ohio State University
Principle Investigators	Paula Mouser and Gil Bohrer

Abstract

Fixed nitrogen (N) is required for the growth for all biological organisms, and agriculture is dependent upon nitrogen for fertilizer. When present at elevated levels in water resources, however, nitrate-N concentrations present human and environmental health risks, including contributing to the eutrophication of some water bodies and coastal zones. Ohio exports significant levels of nitrogen in its surface waters and groundwater to Lake Erie and the Mississippi Basin due to its geology and agricultural land management techniques. Constructed wetlands can be used for nitrogen removal through the biologically-mediated process of denitrification, where nitrite is reduced to nitrogen gas and released to the atmosphere. Unfortunately, denitrification in wetlands comes with the tradeoff of increased Green House Gas (GHG) production. Wetlands sequester large amounts of carbon (C) from the atmosphere, removing the most common GHG - CO_2 but produce another and more potent GHG – methane (CH_4). In order to allow development of wetlands as a solution for N removal without concerns of GHG emissions, it is critical to understand what factors control methane production, and how they relate to wetland aquatic conditions. The proposed work will examine the interaction between environmental factors (temperature, depth, and microsite) based on inocula-based laboratory experiments. Microcosms constructed from the sediments from distinct horizons within each microsite will serve as different sources of microbial communities and chemical substrates. Anaerobic incubation will determine maximum levels of methane and carbon dioxide production from biogeochemical processes. This will direct which factors should be incorporated as parameters in GHG emission models to predict carbon budgets at the ORWRP, and other wetlands.

Methodology

We used anaerobic microcosm experiments developed from wetland sediment and source water to measure the production of methane and carbon dioxide gases, while monitoring solution geochemistry. Two incubation temperatures were used on sediment collected from three distinct microsites across a 1-ha wetland and from two depth horizons. Microsite locations include: (1) an upland area, (2) an edge zone with emergent vegetation, and (3) an unvegetated, central basin. Headspace gas productions during a temporal incubation were used to develop linear gas production rates which were used to predict maximum gas flux. Statistical methods were used to identify the key factors related to maximum gas production.

Major Activities

We completed a series of experiments over a two-year period that tested the effect of environmental conditions on methane flux from sediment cores. Samples cores were taken in the dormant-season of vegetation growth (November) when water levels were low, and during the growing-season (July) when water levels were high. For the dormant-season experiment, composite 30-cm cores were sample within a 1-m^2 area for the upland, vegetated, and open-water sites (Figure 1). Cores were sampled again during the growing-season experiment at the vegetated and open-water microsites, but split into two 15-cm lengths (Figure 2). Samples were stored under an anaerobic atmosphere until bottles could be constructed using 25-g of sediment and 75-ml of intake water. Carbon amendment was made in a subset of bottles to test for carbon limitation of methanogenic activities. Bottles were placed under an atmosphere of pure N_2 gas and incubated for 77 days. Methane and carbon dioxide concentrations within the headspace were measured using gas chromatography to calculate linear rates of production. Based on sediment density, these rates were translated into equivalent maximum anaerobic flux on a surficial area basis. A linear fit model incorporating random effects to handle the pseudoreplication of samples was used to determine the significant factors acting on the maximum potential GHG fluxes. Concurrent microcosms were used to analyze the solution geochemistry through time.

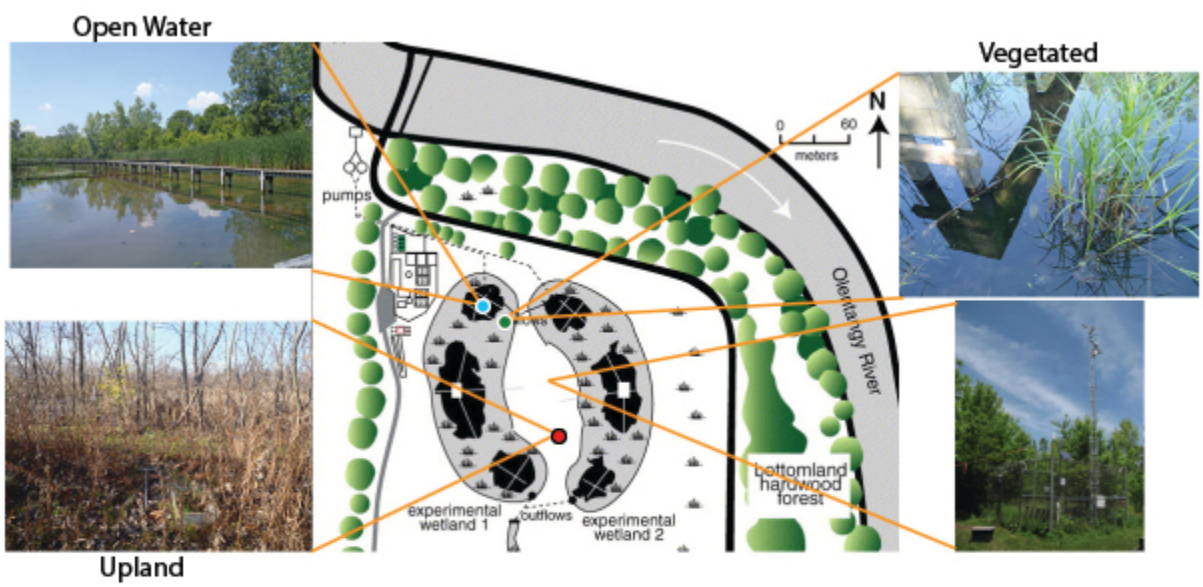

Figure 1. Samping locations used for collection of cores at the ORWRP wetland site. Images show the areas from which cores were taken through the course of both experiments. The location of the eddy flux meteorological tower used for flux comparisons is also shown.

In addition to the above described experiments, we characterized the microbial communities initially present within the four sediments collected during the growing-season (open-water/vegetated microsites at the shallow/deep horizons). High-throughput 454-pyrotagged sequencing was used to obtain >1000 sequence reads of the V4 region of 16S rRNA gene for the prokaryotic community. These DNA primers target both *Bacteria* and *Archaeal* members in order to identify the most prevalent operational taxonomic units (OTUs). The virtual QIIME pipeline was used to align sequences to the Greengenes database for a taxonomic analysis. A directed approach was taken to analyze the microbial community members that, based on sequence alignment to genus-level OTUs, have been identified within the literature as belonging to a functional guild critical to methanogenesis and GHG flux.

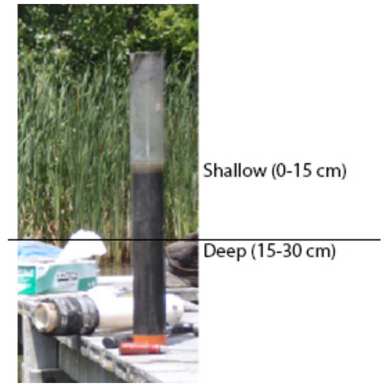

Figure 2. Open-water microsite core from the ORWRP. The shallow and deep horizon depths are indicated by the black line.

Findings

In dormant season experiments, average potential methane flux rate for open-water samples was 3- to 5-fold greater than the VEG and UP wetland microsites (Figure 3). Rates for bottles held at 30ºC were about 3-fold higher than methane and carbon dioxide fluxes from bottles kept at 20ºC. A positive linear correlation was observed between potential carbon dioxide and methane fluxes within microcosms (r^2 = 0.28, p = 0.025, Pearson correlation analysis). Average potential methane flux in growing-season treatments was 70% higher than the dormant-season, while average potential carbon dioxide flux, on the other hand, was 20% lower in growing-season treatments than the dormant-season (Figure 2C-D, respectively). Microsite, temperature, and depth had no significant independent effects on mean potential methane flux during the growing season. However, the interaction between depth and temperature was significant, indicating the increased potential methane flux from shallow microsites was caused by temperature. Mean rates at 30ºC were approximately double those at 20ºC for both potential methane and carbon dioxide flux. Sodium acetate amendment to deep open-water sediments significantly increased production of methane and carbon dioxide compared to ambient samples (Figure 3). Methane flux estimate measured in microcosms were found to be comparable to upper range measurements previously recorded by chambers and eddy flux covariance at the site.

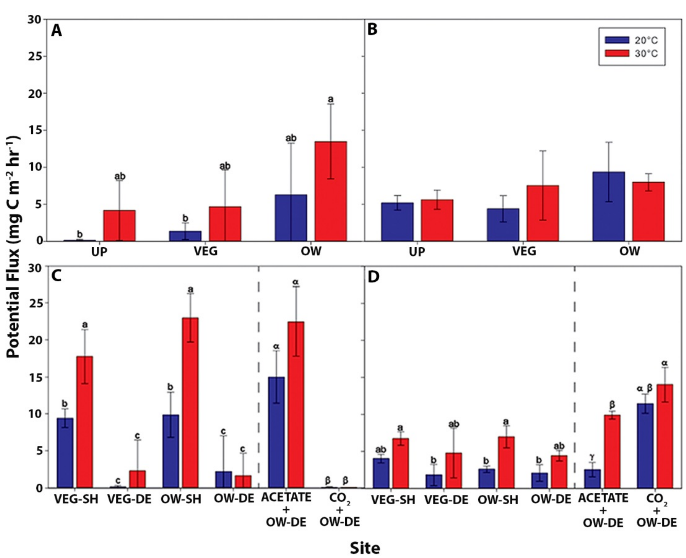

Figure 3. Methane and carbon dioxide flux for dormant season (A and B, n=3) and growing season (C and D, n=5) experimental treatments, respectively. Bars show the standard deviations upland = UP; vegetate = VEG; open-water = OW) microsites at shallow (SH) or deep (DE) horizons. Lower case letters represent significant difference in mean fluxes between treatments. Dashed line indicates the bottles containing acetate or carbon dioxide amendments, with Greek letters indicating significant differences in mean flux.

A comparison of the microbial community members at the phylum hierarchal classification level revealed differences in dominant taxa between the four sediments (Figure 4). Higher richness was observed in the shallow vegetated sediments while lower richness was seen in open-water, deep sediments. The *Proteobacteria,* primarily the α- and δ-*proteobacteria,* were the most dominant group in site sediments. The *Euryarchaeota* (containing methanogens) were of greatest abundance at the open-water microsite. The vegetated microsite contained a greater number of *Nitrospirae* relative to the open-water microsite. Other dominant taxa include: *Bacteroidetes*, *Chloroflexi*, *Verrucomicrobia*, *Acidobacteria*, and *Actinobacteria*.

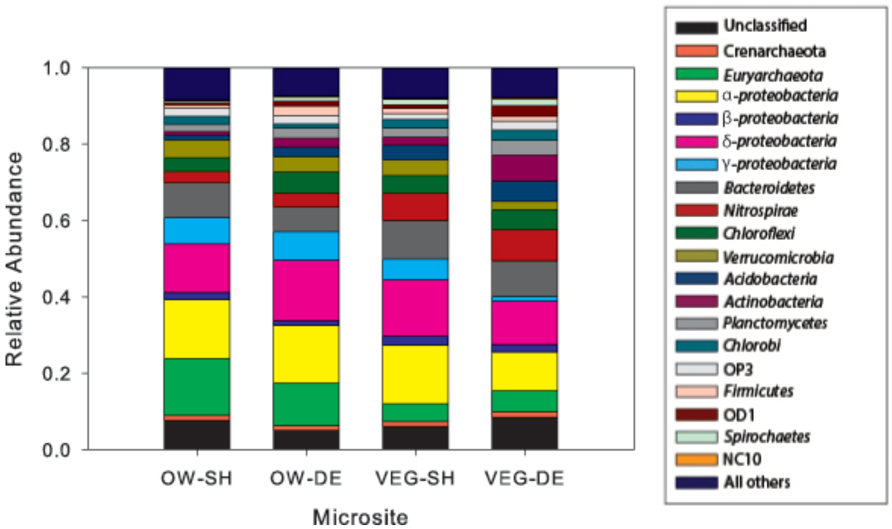

Figure 4. Dominant phyla (with *Proteobacteria* divided into class) for the four sediments sampled detected for the 16S rRNA gene using 454-pyrosequencing. Samples include the vegetated (VEG) and open-water (OW) microsites at the shallow (SH) or deep (DE) horizon. "All Others" represents phyla which were present at <1% across the site.

Significance

A microcosm approach was useful to show that temperature caused an increase in methane flux from temperate freshwater wetlands. Fold changes measured in the laboratory were consistent with those measured *in situ* by chambers and eddy flux across a similar temperature range. Results suggest that although deep sediments contain microbial members associated with methanogenesis, they have weak methanogenic capabilities as a result of substrate availability and competition by bacteria utilizing other electron acceptors. Open water and vegetative microsites had similar potential methane flux from shallow sediments during the growing season, and as hypothesized, fell along the upper range of fluxes measured in the field. If methanogens are respiring at similar rates across microsites, this suggests that larger-scale processes (e.g. redox boundaries, vegetation piping) greatly alters overall emission rates across these wetland microsites.

Publications/Conferences (since 6/2011)

(*Denotes the student working on the project)

<u>Publications</u>

*Brooker, M.R., Bohrer, G., Mouser, P.J. (2014) Variations in Potential CH_4 Flux and CO_2 Respiration from Freshwater Wetland Sediments that Differ by Microsite Location, Depth, and Temperature. *Ecological Engineering* (in review).

Conference Presentations

*Brooker, M.R., Bohrer, G., Mitsch, W.J., Mouser, P.J. Factors Influencing Methane Flux Potential from Wetland Sediments. Oral presentation *Ohio River Basin Consortium for Research and Education 2012 Scientific Symposium*, July 18-20, 2012, Athens, OH.

Mouser, PJ, *Brooker, M., Mitsch, W., Bohrer, G. Factors Influencing Microbial Gas Production Rates in a Constructed Wetland Ecosystem. Oral presentation *American Meteorological Society, First Conference on Atmospheric Biogeosciences,* May 29-June 1, 2012, Boston, MA.

Mouser, PJ, *Brooker, M., and Bohrer, G. Factors Influencing Microbial Gas Production Rates in Wetland Sediments. Oral presentation *4th International EcoSummit on Ecological Sustainability: Restoring the Planet's Ecosystem Services,* Sept 30-Oct 5, 2012, Columbus, OH.

*Brooker, M.R.; Mouser, P.J. and Bohrer, G. Quantifying *in situ* rates of methanogenesis and denitrification in wetland sediments. Poster, *4th International EcoSummit on Ecological Sustainability: Restoring the Planet's Ecosystem Services,* Sept 30-Oct 5, 2012, Columbus, OH.

Students Supported By Project 6/2011--9/2013

One student worked on the project: Michael Brooker – who completed a MS degree through Environmental Sciences Graduate Program in 9/2013.

Awards or Achievements

1. Department of the Interior, USDA, "An integrated approach to foster science-based management of agricultural drainage channels in the Western Lake Erie basin", 2013-2016. PI Jon Witter, co-PI Paula Mouser. $659,979.

Professional Placement of Graduates

Michael Brooker has graduated and continued on to the Ph.D. track of the Environmental Sciences Graduate Program at The Ohio State University.