An Ecosystem Experiment to Test Recovery of Nitrogen Assimilation Functions in Restored Freshwater Wetlands

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Abstract

We tested the influence of hydrology and nutrient loading on the development of wetland ecosystem processes that are important in improving water quality. In collaboration with researchers at Florida Gulf Coast University, we established and maintained a set of experimental mesocosms to execute four experimental treatments. Using a series of inflow and outflow pipes, we controlled the rate of water and nutrient loading to individual mesocosms, and the depth of standing water. Respiration and denitrification rates served as our quantifiable measures of wetland function and microbial activity. We assessed the diversity of microbes present to investigate the interactions between hydrology and microbial community development under different conditions. Mesocosms with high loading rates and deep standing water exhibited the highest rates of denitrification while mesocosms with low loading rates and shallow standing water had the lowest rates. Soil respiration was higher in mesocosms with shallow standing water. Like denitrification, nitrogen removal was higher in deep standing water. These findings were bolstered by the results of our microbial community analysis that showed higher microbial functional diversity in mesocosms with shallow standing water, an accepted indicator of soil health. The different trends between denitrification and respiration may be due to increased residence time of deep standing water. Our findings highlight the importance of soil microbial activity and hydrological conditions in establishing successful restored freshwater wetlands as a means of recovering ecosystem services.

Soil Collection and Field Work



Figure 3. **A)** One four inch deep soil core was taken from each mesocosm each week. These soil samples were refrigerated until they could be processed and used in a denitrification enzyme assay. Cores were taken in

<section-header>Denitrification and Respiration Denitrification Rate

Background





The Role of Wetlands in Nitrogen Transformation

Wetlands anaerobic soils and large soilwater interface facilitate the transformation
of nutrients. These transformations improve
water quality by removing nutrients from
the water and returning them to the soil,
atmosphere, and the floral communities in
usable forms. Anaerobic soil microbes are
responsible for these transformations as
they metabolize nitrate.

such a way as not to disturb vegetation. **B)** Respiration measurements were also taken each week in an effort to identify a possible relationship between soil respiration rates and denitrification rates as a function of treatment.

Enzyme Denitrification Assay

Helium → creates an anaerobic environment

Acetylene \rightarrow blocks the transformation of N₂O to N₂ because N₂O is measurable via gas chromatography and N₂ is not

Glucose + KNO₃ → ensure that energy and nitrate are not ratelimiting

Figure 4. Soil samples collected from the Buckeye Lake Mesocosms were



Figure 5. A) Denitrification rates varied among treatments. D treatments had the highest denitrification rates while S treatments had the lowest (p=0.0369) **B)** Soil respiration rates varied among treatments. S treatments had the highest respiration rates D treatments had the lowest (p=0.001).

Figure 1. Nitrogen sequestration and transformation in wetlands is a vital part of biogeochemical cycling.



used to preform a denitrification enzyme assay to determine the rate at which nitrogen is converted to N_2O . Gas samples are taken every 30 minutes and these samples are analyzed using a Gas Chromatograph to measure N_2O concentration.

Mesocosms with deep standing water had the highest percentages of nitrogen removal based on inflow concentration (figure not shown, p=0.088)

Conclusions

- Respiration rates are higher in soil that is not fully submerged
- Denitrification occurs at higher rates in conditions that are subjected to deeper standing water
- Not shown: microbial functional diversity is higher in soils with shallow standing water conditions
- The soil-water interface is a key indicator of the status of wetlands' biogeochemical capabilities and status
- Restoration projects should follow protocols that promote specific microbial diversity to tailor biogeochemical cycling on a per site basis

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Mesocosm Design







Figure 2. Mesocosm layout, individual design, and operation and maintenance. **A)** Mesocosms were setup in a 6x5 array. Water was sourced from Buckeye Lake and distributed via a series of tanks and valves. High water flow rates were maintained around 30 cm/week and low water flow rates were maintained around 10 cm/ week. Deep standing water was keep at 16 inches via standpipe and shallow standing water was kept at 12 inches via standpipe. **B)** The inflow pipe is shown on the left of the diagram, the standpipe is shown on the right of the diagram. Each mesocosm had equal amounts of soil, and gravel for drainage. Three *Juncus effusus* were planted in each mesocosm. **C)** Soil and water samples were collected once a week and water was delivered over a two day period.