Project Title

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Keywords:

man-made pond; nutrient cycling; metabolism; terrestrial leaf litter; microbes

Abstract:

**The effect of terrestrial leaf litter on sediment organic matter processing and nutrient retention..**

The abundance of man-made ponds and lakes is increasing globally (Downing 2007) and in regions like Virginia, that lack natural ponds and lakes, man-made impoundments represent an important aquatic habitat. Despite the prevalence of man-made ponds in Virginia, there has been little research on how organic and inorganic nutrients cycle in these systems. Aquatic systems are an important component of the processing of terrestrially derived organic matter, and globally, natural aquatic systems process and decompose 70% of their total organic matter inputs (Tranvik et al. 2009). Terrestrial leaf litter supports microbial and animal production and supplies aquatic systems with organic and inorganic nutrients (Gessner et al. 1999). Furthermore ponds play an important role in watershed management due to their ability to retain nutrient run-off from the land (Johnston 1991, Hansson et al. 2005). This retention may be affected by terrestrial leaf litter, since as it decomposes in aquatic systems terrestrial leaf litter sequesters inorganic nutrients (Trant et al. 2013). Given that a significant portion of the Chesapeake Bay watershed drains Virginia, understanding how man-made ponds alter nutrient transport is vital to predicting appropriate nutrient management strategies.

Data collected by the Fortino lab to date indicates that man-made ponds near Farmville, Va, have an average of 82 mg of CPOM m-2 in the sediments, indicating significant inputs of terrestrial organic matter into these systems. Our proposed research project focuses on understanding the way in which man-made ponds contribute to the processing of watershed organic matter and nutrients via 2 research questions and 3 objectives:

**Research Question 1 -** *How does the input of terrestrial leaf litter affect sediment metabolism and nutrient retention in the sediments of man-made ponds?*

**Research Question 2 -** *How does the input of terrestrial leaf litter alter the sensitivity of pond metabolism to nutrient enrichment?*

**Objective 1 -** Quantify the effect of terrestrial leaf litter on the movement of nutrients across the sediment water interface.

We hypothesize that the presence of terrestrial leaf litter will increase the N and P fluxes into the sediments, due to the sequestration of N and P by the fungi on the decomposing leaves and increase the movement of dissolved organic nutrients out of the sediments due to the release of dissolved organic nutrients from the leaves (i.e., leaching).

**Objective 2 -** Quantify how terrestrial leaf litter alters microbial (bacteria and fungi) biomass and respiration.

We hypothesize that terrestrial leaf litter will increase microbial abundance and respiration, due to the release of dissolved organic matter from the decaying leaves, and growth on the leaf surface.

**Objective 3 -** Quantify how terrestrial leaf litter alters the response of sediment respiration to nutrient enrichment.

We hypothesize that terrestrial leaf litter will increase sediment respiration when the sediments are enriched with N and P, since litter associated fungi have been shown to be more nutrient limited that sediment microbial communities.

**Technical Approach and Outcomes**

To address our research questions and objectives we propose a single laboratory experiment that simultaneously manipulates the availability of terrestrial leaf litter and inorganic nutrients to the sediments of a man-made pond and measures changes in nutrient movement and microbial abundance. The experimental set-up will consist of 20, 300 ml septum topped glass jars filled with approximately 4 cm sediment collected from a local, man-made pond. The sediment samples will be run through a 250 μm mesh sieve to remove any terrestrial leaf litter particles and macroinvertebrates that may be present. The remaining volume in the jars will be filled with pond water samples collected at the time of sediment sample collection.

To create the terrestrial leaf litter treatments, 0.5 mg of senescent tulip poplar leaf disks will be added to the sediment surface of the treatment jars and the control jars (no leaf litter) will receive sufficient sieved pond sediment to equal the mass of organic matter added with the leaves.

The nutrient addition treatment will be created by adding NO3 and PO4 to raise the water concentration to twice the concentration measured in the original water samples from the pond, while control jars will be left unaugmented. The terrestrial leaf litter and nutrient treatments will be replicated 5 times and crossed in a complete factorial design (Table 1).

Table 1. Experimental design of the proposed complete factorial experiment. Rows indicate the levels of the terrestrial leaf litter treatment, columns indicate the levels of the nutrient addition treatment and the cells indicate the number of replicate jars in each treatment combination.

|  |  |  |
| --- | --- | --- |
|  | Ambient Nutrients | N + P Addition |
| No Terrestrial Litter | 5 | 5 |
| Terrestrial Litter Addition | 5 | 5 |

To evaluate the effect of terrestrial leaf litter on the nutrient and microbial dynamics of the system we will measure changes in five response variables

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1. inorganic nitrogen (NO3 and NH4) and soluble reactive phosphorus flux,
2. microbial respiration (measured as sediment oxygen demand),
3. dissolved organic matter flux (measured as near UV and visible light absorbance),
4. bacterial abundance, and
5. fungal biomass.

Sediment samples will be collected from a local, man-made pond with an Ekman dredge. Once back at the lab, the sediment samples will be run through a 250 μm sieve to remove any terrestrial leaf litter particles and macroinvertebrates that may be present. Next, 300 ml septum topped glass jars will be filled with four cm of the sieved sediment and the remaining volume in the jars will be filled using the pond water samples collected with a Van-Dorn sampler simultaneously with the sediment sample.

The treatments will be applied to the jars as described above and the sample jars will be incubated open at a constant temperature in darkness for 6 weeks. The jars will be sampled daily for the first week and weekly for the remaining 5 weeks. During each sampling event 25 ml of water will be removed from the jar and filtered through a 0.7 μm glass fiber filter, 1 ml of the water will be used to measure absorbance from 250 - 900 nm on a Nanopore Spectrophotometer. The remaining 24 ml will be frozen for nutrient analysis at Virginia Commonwealth University. A 5 ml sample will be filtered onto a black membrane filter (0.2 μm) and stained with a fluorescent nuclear stain (DAPI) for quantification of bacterial abundance. Bacteria will be counted on the stained filters using an epifluorescent microscope. In order to quantify sediment oxygen demand, we will refill the jars with pond water and then seal them without an airspace with a septum top lid. After 1, 3, and 6 hours of dark incubation, a 15 ml sample will be extracted from the jar using a glass syringe. Pond water will be simultaneously introduced to the jars with a second syringe while the samples are taken so that air will not be introduced into the jars during sample extraction. The oxygen content of each sample will be determined using the Winkler titration method (Carpenter ####) and the sediment oxygen demand will be calculated as the change in oxygen concentration of the water over time. At the conclusion of the 6 weeks, fungal biomass on the terrestrial leaf litter discs will be measured by extracting ergosterol (a fungi specific molecule) in methanol. The extracted ergosterol concentration will be measured by an outside laboratory.

**Timeline and Expected Results**

**Budget**

* Nutrient Analysis - $3600
* Ergosterol Samples - $1000
* Bacterial Abundance Quantification

1. DAPI Nuclear Stain for Bacterial Counts - $200
2. Membrane Filters - $100

**Total = $4900**