Project Title

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Project Description:

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. Globally, natural aquatic systems process and decompose 70% of their total organic matter inputs (Tranvik et al. 2009). Terrestrially derived organic matter supports the production of microbes and invertebrates and supplies aquatic systems with inorganic nutrients (e.g, nitrogen and phosophorus), and dissolved organic carbon (Gessner et al. 1999). Despite the recognized importance of terrestrial organic matter in natural aquatic systems, its role in man-made ponds is still largely unexplored. 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). As terrestrial leaf litter decomposes in aquatic systems it sequesters inorganic nutrients (Trant et al. 2013), suggesting that the presence of leaf litter in the sediments may augment the retention of nutrients by ponds. 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, suggesting significant inputs of terrestrial organic matter into these system. 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 principle research questions:

**Research Question 1 -** *How does the input of terrestrial leaf litter affect sediment metabolism and nutrient retention or release from 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?*

To answer these questions, we developed three objectives and hypotheses.

**Objective 1 -** Quantify the effect of CPOM on the flux 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 leavesincrease the DOC flux out of the sediments, and increase the SOD.

**Objective 2 -** Quantify how terrestrial leaf litter alters microbial biomass.

We hypothesize that terrestrial leaf litter will increase water column bacterial abundance, increase sediment bacterial abundance due to the release of dissolved organic matter from the decaying leaves, and increase fungal biomass on the leaves themselves.

**Objective 3 -** Quantify how CPOM alters the response of sediment metabolism to nutrient enrichment.

We hypothesize that terrestrial leaf litter will increase SOD when the sediments are enriched with N and P, since litter associated fungi have been shown to be N and P limited in streams.

**Technical Approach and Outcomes**

We propose a single laboratory experiment to address the research questions and objectives. The experimental set-up will consist of 300 ml septum topped glass jars will be filled with approximately 4 cm sediment collected from a local, man-made pond. The sediment samples will be run through a 250 **μ**m mesh sieve to remove any terrestrial leaf litter particles and macroinvertebrates that may be present. The remaining volume in the jars will be filled using the 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. The control jars will receive sufficient sieved pond sediment to equal the mass or organic matter added with the leaves.

The nutrient addition treatment will be created by adding NO3 and PO4 at twice the concentration seen 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. 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 |

The proposed experiment will evaluate changes in five response variables:

1. inorganic nitrogen (NO3 and NH4) and soluble reactive phosphorus flux,
2. sediment oxygen demand,
3. dissolved organic matter flux (measured as absorbance),
4. sediment and water column bacterial abundance, and
5. fungal biomass.

Water and sediment samples will be collected from a local, man-made pond. Once back at the lab, the sediment samples will be run through a 250 micro mesh strainer to remove any CPOM particles and macroinvertebrates that may be present. By running the sediments through the strainer, we should be left with only SOM, which will be a necessary component later in the experiment. Next, 300 ml septum topped glass jars will be filled with approximately four cm of the recently-sifted SOM. The remaining volume in the jars will be filled using the pond water samples collected at the time of sediment sample collection. To create the CPOM samples, 0.5 mg of tulip poplar leaves will be added to the sediment surface. Prior to being weighed, the leaves used will undergo senescence, before being air dried and then conditioned for 24 hours. The final two treatments will be created by adding NO3 and PO4 at 2x the concentration seen in the original water samples from the pond to some of the jars without CPOM and some of the jars with CPO

The sample jars will be incubated 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. Each sampling event will consist of 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), stained with DAPI for quantification of bacterial abundance. Bacteria will be counted on the stained filters using an epifluorescent microscope. In order to quantify SOD, we will refill the jars with pond water and then seal them without an airspace with a septum top lid. A15 ml sample will be extracted from the jar after 1, 3, and 6 hours of dark incubation 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 ####). At the conclusion of the 6 weeks, fungal biomass on the terrestrial leaf litter discs will be measured using an egersterol extraction.

**Budget**

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

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

**Total = $4900**