**Final Report**

# The Biogeochemistry of Cove Point Marsh, Maryland: An Investigation of Carbon, Nitrogen, and Phosphorus Dynamics and the Role of the Microbial Community.

Karen L. Bushaw-Newton, Department of Biology, American University, 4400 Massachusetts Avenue, NW, Washington, DC 20016, bushaw@american.edu, 202-885-2203, 202-885-2182 (fax)

## Rationale

Identifying the mechanisms governing transport and the cycling of organic matter and nutrients in wetlands is of interest since "bottom-up" processes such as bacterial production often influence the rates of primary and secondary production in these systems. Cove Point Marsh represents a unique opportunity not only to further our understanding of carbon and nutrient (nitrogen and phosphorus) cycling in wetland systems but also, to enhance our understanding of the exchanges of nutrients between Cove Point Marsh and Chesapeake Bay.

## Objectives

- To quantify the concentrations of dissolved carbon, nitrogen, and phosphorus species in several locations within the marsh system and the adjacent Bay
- To determine potential exchanges of carbon and nutrients between the marsh system and the adjacent Bay
- To quantify relative microbial activities and community structures in locations throughout the marsh system

## Methods

Water and sediment were collected seasonally on September 10, 2004 (**Fa**ll), April 29 (**Sp**ring) and July 11, 2005 (**S**ummer) from six designated regions of Cove Point Marsh. Five regions were within the marsh and included an open water area (O), a forested wetland (F), a meadow area (M), an area dominated by sedges and cattails (C), an area dominated by *Phragmites australis* (P) and the sixth region was within the bay adjacent to the marsh.

***Water Studies***

*In situ* measurements of water parameters at all sites except the *P. australis* site and the Forest site in July (no standing water of depth, mainly dry) were made using the YSI 556 environmental multi-probe which measures temperature, pH, and oxygen concentrations. Salinity of the Bay was measured in September at 11 $^{o}/_{oo}$ using a refractometer.

At each site, water was collected in acid-washed 10 liter carboys (BOD filtered water studies, nutrients) and 2 liter containers (BOD whole water studies) and placed on ice during transport back to American University. Within 5 hours of collection, processing of samples was begun. Samples for chemical analyses were filtered through pre-combusted (450$^{o}$C for 4 h) Whatman glass fiber filters into acid-washed HDPE bottles for analyses of ammonium, nitrate and nitrite, soluble reactive phosphorus (SRP), and dissolved organic carbon (DOC)

*Chemical Analyses*--DOC concentrations were determined by high temperature catalytic oxidation using a Shimadzu TOC-5000 carbon analyzer (Academy of Natural Sciences, Philadelphia, PA). Sub-samples for nitrate, ammonium and soluble reactive phosphorus were stored in acid-washed HDPE bottles at –20$^{o}$C until analysis using standard spectrophotometric methods either through individual analyses (ammonium and SRP) or via an autoanalyzer (nitrate and nitrite and total dissolved nitrogen). Dissolved organic nitrogen was determined via subtraction of inorganic nitrogen species from total nitrogen components for the spring and summer samples.

*Biological Analyses-BOD Whole and Filtered Water Studies*--Bacterial respiration measured as loss of oxygen was monitored using the Winkler technique. Whole water from each site or water that had been pre-filtered through 3 µm nominal pore size filters (e.g., to assess the microbial component) was allowed to equilibrate to room temperature and pumped into replicate (3/time point) 60 ml BOD bottles. For both the spring and summer samples, oxygen concentrations were very low for the Forest and Cattail sites and rates of respiration were not able to be calculated. The bottles were incubated in the dark at room temperature for a period of 144 hours. Oxygen concentrations were measured at 0, 24, 48, and 144 hrs. In addition to the five sites, replicate

bottles were filled with distilled deionized water to incubate with the samples and account for any potential contamination or changes in room temperature. Oxygen consumption rates were calculated using a linear regression.

*Bacterial Enumeration*- Bacteria present in the whole water was quantified using Acridine Orange direct staining which fluorescently stains the cells. For the whole water 10 milliliter sub-samples were removed and preserved with 0.6 ml borate-buffered formalin.

***Sediment Studies***

Triplicate surface sediment samples (upper 5 cm) were removed using 2.54 cm diameter syringes (acid washed) with ends removed. Each sample was placed in a whirlpak bag and put on ice for transport back to American University. Within 5 hr of collection, sediment samples were processed. The upper 5 cm of each sediment sample was homogenized in a sterile 50 ml test tube with screw top. After homogenization, sub-samples were removed for elemental C & N analyses, bacterial enumeration, and genetic profiling studies.

*Chemical Analyses*—Percent organic matter based on ash free dry matter was measured. Oven-dried (60 $^{o}$C) sediment samples were weighed and placed in a muffle furnace and heated for 2 h at 500 $^{o}$C and then re-weighed. Elemental C and N content were measured on dried, crushed samples using a Carlo Erba Elemental Analyzer (Academy of Natural Sciences, Philadelphia, PA).

*Genetic Profiling*—For each sediment sample from each sampling period, DNA was extracted using the MoBIO Soil DNA extraction kits. The purified DNA samples were stored at -20$^{o}$C until amplification via polymerase chain reaction (PCR). The purified DNA was amplified via PCR using primer sequences specific for the 1070 F, 1392 R regions of a universal eubacterial 16S rRNA gene. After amplification, sub-samples of products were eletrophoresed on agarose gels to determine if the correct base size products were present. Amplified products from the sediment samples and water samples (extracted using MoBIO water DNA extraction kits) were then run using Denaturing Gradient Gel Electrophoresis (DGGE). DGGE analyses are useful to

determine the genetic diversity of a microbial community and compare microbe composition from different sites at the same time as subtle differences between species are visualized as different bands on the gel.

## *Results and Discussion*

### *Water Studies*

*In situ measurements*-Oxygen concentrations did not demonstrate a seasonal trend but there were clear differences between sites (Table 1). Oxygen concentrations were lowest in the Forest and Cattail sites for all seasons. In these two sites the flow of water is limited and organic matter is very concentrated which are potential contributing factors to low oxygen concentrations. Temperature followed a seasonal trend at all sites with lowest temperatures in the spring and highest temperatures in the summer. For all except the Cattail site, pH was variable with higher concentrations in the spring. Chesapeake Bay had the highest pH, which is expected due to the carbonate and ions in the estuarine water. The lack of variability at the Cattail site is potentially due to the high concentrations of organic acids, which may act as a buffer for this system.

**Table 1.** ***In situ*** **measurements of Oxygen concentration, temperature, and pH for the designated sites. (Fa=Fall, Sp=Spring, S=Summer) (ND=not determined)**

| Site | Dissolved Oxygen ($mg\ O_2\ L^{-1}$) | | | Temperature ($^oC$) | | | pH | | |
|---|---|---|---|---|---|---|---|---|---|
| | Fa | Sp | S | Fa | Sp | S | Fa | Sp | S |
| Open (O) | 5.0 | 7.8 | 7.5 | 25.1 | 15.8 | 30.6 | 7.0 | 8.4 | 7.7 |
| Forest (F) | 4.4 | 3.3 | ND | 21.5 | 12.7 | ND | 6.8 | 7.4 | ND |
| Meadow (M) | 7.3 | 6.0 | 7.4 | 26.0 | 14.6 | 27.8 | 6.6 | 8.5 | 7.4 |
| Cattail (C) | 3.8 | 1.7 | 2.3 | 24.0 | 14.4 | 26.6 | 6.8 | 6.8 | 6.5 |
| Chesapeake Bay (CB) | 5.0 | 10.2 | 7.4 | 25.1 | 13.9 | 27.8 | 7.0 | 8.4 | 8.3 |

*Nutrient Concentrations*-Cove Point Marsh is dominated by organic carbon and nitrogen and not inorganic nutrients (Table 2). Dissolved organic nitrogen dominated the nitrogen pool and dissolved organic carbon was high for all sites with lowest concentrations in the Bay site and highest concentrations in the Cattail and Forest sites. The Meadow site, which had lower concentrations of DON and DOC compared with the other marsh sites, was dominated by macrophytes and the sediment base has more clay and less sand than the other sites which may explain the lower concentrations. DOC tends to adsorb onto clay particles, which decreases

availability. Concentrations of ammonium and nitrate are low in this system and do not indicate anthropogenic control (e.g., nitrate concentrations > 1 mg $NO_3$-N $L^{-1}$) though levels were highest in the Chesapeake Bay site adjacent to the marsh. The marsh is not a source of nitrate to the Bay but the Bay is potentially a source of nitrate to the marsh during storm events. Ammonium concentrations were highest in the Forest and Cattail sites most likely due to the low oxygen concentrations. Ammonium production occurs at low to zero oxygen concentrations. For the open water site, ammonium concentrations were high for the fall sample and one plausible explanation is photo-ammonification. My previous research demonstrated that humic waters (e.g., waters high in organic acids) have the potential to produce ammonium after exposure to sunlight due to the breakdown of bonds within the humic matrix. It should be noted that all these concentrations are considered low and while they are useful for fueling food webs these concentrations are not considered toxic. Soluble reactive phosphate was low in all systems indicating potential limitation.

**Table 2. Concentrations of Nutrients in Cove Point Marsh (All values are averages, n=3) (ND = Not determined)**

| Site | Ammonium ($\mu$g $NH_4$-N $L^{-1}$) | | | Nitrate ($\mu$g $NO_3$-N $L^{-1}$) | | | SRP ($\mu$g $PO_4$-P $L^{-1}$) | | | DON (mg N $L^{-1}$) | | | DOC (mg C $L^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Fa** | **Sp** | **S** | **Fa** | **Sp** | **S** | **Fa** | **Sp** | **S** | **Fa** | **Sp** | **S** | **Fa** | **Sp** | **S** |
| O | 257 | 15 | 8 | 10 | 16 | 3 | 22 | 20 | 27 | ND | 1180 | 1515 | 22 | 12 | 16 |
| F | 250 | 129 | ND | 17 | 2 | ND | 36 | 19 | ND | ND | 1976 | ND | 8 | 39 | ND |
| M | 2 | 7 | 13 | 2 | 0.7 | 6 | 17 | 10 | 21 | ND | 495 | 539 | 5 | 6 | 6 |
| C | 8 | 115 | 10 | 4 | 19 | 3 | 180 | 31 | 77 | ND | 1328 | 1966 | 28 | 52 | 20 |
| CB | 65 | 56 | 16 | 129 | 520 | 4 | 40 | 10 | 27 | ND | 419 | 584 | 4 | 2.7 | 2.5 |

*Biological Oxygen Demand*-Rates of respiration were highest in fall and summer compared with spring (Table 3). Whole water rates demonstrated that all sites had biologically active water columns. The microbial component contributed from 28 to 38% of the oxygen consumption in the fall samples to 12 to 39% of the oxygen consumption in summer samples. These results indicate that bottom-up processes (e.g., microbially controlled) are an important component of these systems.

| Table 3. Respiration Rates for Whole Water and Microbial Communities of Sites of Cove Point Marsh (ND = not determined) | | | |
|---|---|---|---|
| **Whole** | **Fall (mg $O_2$ $L^{-1}$ $hr^{-1}$)** | **Spring (mg $O_2$ $L^{-1}$ $hr^{-1}$)** | **Summer (mg $O_2$ $L^{-1}$ $hr^{-1}$)** |
| Open | 0.0308 | 0.0275 | 0.0607 |
| Forest | 0.0264 | anoxic | anoxic |
| Meadow | 0.0319 | 0.0166 | 0.0246 |
| Cattail | 0.056 | anoxic | anoxic |
| Chesapeake Bay | 0.0237 | 0.0117 | 0.0434 |
| | | | |
| **Microbial (0.3 $\mu$m filtered)** | | | |
| Open | 0.0117 | ND | 0.0086 |
| Forest | 0.0101 | ND | anoxic |
| Meadow | 0.0104 | ND | .0097 |
| Cattail | 0.0157 | ND | anoxic |
| Chesapeake Bay | 0.0091 | ND | .0053 |

*Bacterial Enumeration*-For all sites other than the Cattail site, bacterial concentrations were highest in the summer compared with fall (Figure 1). Bacteria concentrations were highest in the Meadow waters during summer. These results are not conclusive (only one replicate per site) and are too preliminary to make any conclusions. However, these results do show that there is a strong bacterial population at all sites.

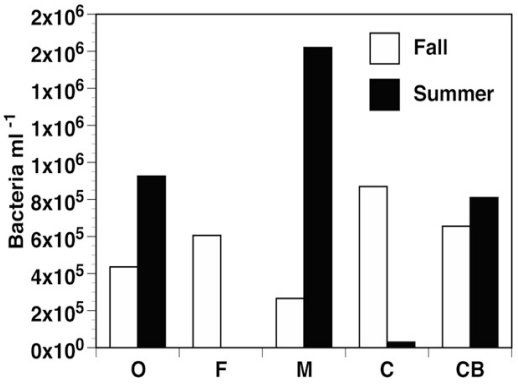

Figure 1. Bacterial concentrations for the water column samples

***Sediment Studies***

*Chemical Analyses*-Total organic matter content was variable between sites and changed over the season. The Open water site had the lowest concentration of organic matter in the sediment. The sediments for these samples were dominated by sand which has little to no organic matter. Similarly, the Chesapeake Bay samples were also lower reflecting higher sand content. The Forest and Cattail sites had higher concentrations of organic matter reflecting the higher concentrations of organic matter and followed the pattern of the dissolved organics. For fall and

spring, higher concentrations of total organic matter were found in the *Phragmites australis* site but very low concentrations were found in the summer sample. Previously, *P. australis* sediments have been shown to accrete organic matter and these results are not typical and indicate further investigation.

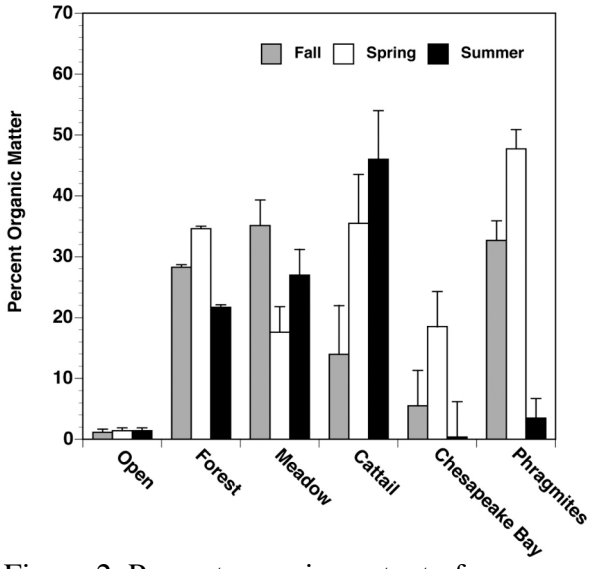


Figure 2. Percent organic content of sediments (n=3)

*Percent Carbon and Nitrogen*-Results of the elemental analyses of the sediments were similar to those of the percent organic matter content. The sediments of the Cattail and *Phragmites australis* sites had the highest carbon and nitrogen concentrations. The plants that dominate these sites tend to accrete organic matter.

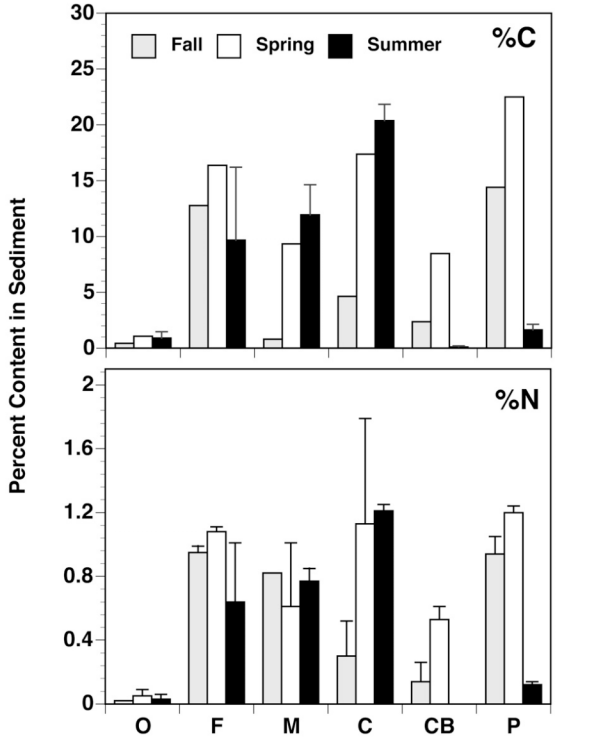


Figure 3. Percent carbon and nitrogen in sediments from all sites for three seasons (n=3)

*Genetic Profiling*-The preliminary results of the DGGE analyses showed that genetically there are some species that are similar between sites (bands at the same level are indicative of species that are the same) as well as species that are unique to certain sites. For example, the replicate samples from the Meadow site show the same banding pattern and those of the forest site show the same banding pattern but there are differences between the banding patterns of the two sites. Interestingly, there are also seasonal differences between the water samples for spring and summer indicating changes in diversity with seasons. For example, in the

CB-Sp sample compared with the CB-Su sample, there are six clear bands seen in the summer sample compared with only five bands in the spring sample. Concentrations of bacteria are quantified by the intensity of the band. For the CB-Sp sample there are higher concentrations of certain species compared with the same species found in the summer sample. DGGE analyses are ongoing and will resume in the spring 2006.

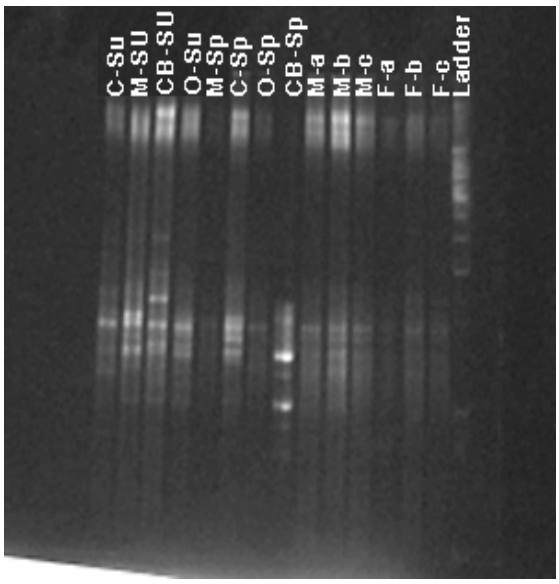

Figure 4. DGGE analysis of select Cove Point water and sediment samples

***Preliminary Conclusions***

Cove Point Marsh is a freshwater marsh with areas of concentrated organic matter. Bacterial processes are an important component of the marsh transforming nitrogen compounds (e.g., production of ammonium) as well as processing organic matter both dissolved and particulate. The marsh is not a source of nitrate to the Bay but may be a potential sink though further investigations are required to determine this linkage. The sediment composition of the marsh plays a role in organic matter accretion with sediments consisting mainly of sand (i.e., the Open site and Chesapeake Bay) having lower concentrations of organic matter compared with sites with little or no visible sand content (e.g., Cattail, Forest, Meadow, and *Phragmites australis*). The various sites of the marsh are rich in microbial diversity with species that appear unique to different sites. The genetic profiling studies suggest the need for further investigation to discern why the communities demonstrate both temporal and spatial differences.

***Antibiotic Resistance Studies (See attached report)***

Though not part of the initial study, Christine Whitehead used sediment samples from Cove Point Marsh for her honor's thesis project. Christine Whitehead studied natural antibiotic resistance in bacteria. She isolated several species of bacteria from the marsh sediment that are resistant to different antibiotics and conducted tests to determine each bacterial species.