

The Role of Pond Sediments in the Removal of DOC and Tannin Compounds Through Abiotic and Bacterial Action

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Abstract

Pond sediment bioreactors taken from three Cape Cod area glacial kettle ponds demonstrate up to 98% removal of DOC that pass through them with a residence time of up to one week. Over 80% removal is observed in all core bioreactors after only one day's residence time. In combination with DOC analysis, a modified Prussian Blue Assay was developed to analyze the phenolic tannins and DOC present in the natural water samples. Phenolic tannins, forming a small portion of the TOC of the natural waters tested, are removed or increased in concentration after passing through bioreactors depending on type of core sediments, with sandy organic-poor sediments removing the highest concentrations of phenolics. Bulk water incubations from these same ponds demonstrated only 1-7% DOC and phenolic tannin removal, suggesting that sediment and biotic interactions are a strong factor in the degradation and sorption of these organic compounds.

Key Phrases: Modified Prussian Blue Assay, glacial kettle pond, coastal plain pond, water quality, phenolic tannins, humic acid, bulk incubation, wellpoint sampling, hydraulic conductivity

Key Words: Bioreactor, tannins, phenolic, DOC, groundwater, incubation, sediments, porosity, residence time, transit time

Introduction

Water is one of Cape Cod's greatest resources, and as the population increases with each year and fluctuates with massive tourist seasons, its groundwater is in growing demand. While water quality is greatly affected by agricultural runoff and pollutants, dissolved organic carbons in high concentrations are a common detriment to drinking water. In Hyannis, MA, high-volume pumping from the areas near tannin-rich lakes during the summer causes loss of water quality in the town (personal correspondence, Rich McHorney). Many previous studies have focused on the dangers of organic compounds such as tannins in drinking water sources as many of these compounds interact with other chemicals present in the environment or water treatment process to become carcinogenic, increasing the "bioaccumulation of these chemicals" (Maartens 1998). To protect our drinking water and the environment, a better understanding of groundwater and lake sediment interconnectivity is needed. Groundwater fed lakes on Cape Cod present a window into water-sediment interactions and water exchange going on beneath our feet. Given that many of these lakes remain clear year-round while others are brown with tannins and humic acids, individual lakes' sediments and groundwater interchange seem to greatly impact not only water color, but DOC and tannin content.

To look at these questions, sediment cores were collected from three Cape Cod area glacial kettle ponds situated on similar regions of mixed glacial till that received only ground and rainwater inputs. These cores were then flushed with water collected from Mary Dunn and Little Israel ponds, and the changes in concentration of phenolic tannins and DOC were monitored over a week. This study also presents a Modified Prussian Blue assay designed to quantitatively estimate the total phenolic tannin compounds found in natural waters and uses it to characterize sediment-affected water that passed through the experimental sediment bioreactors as well as ground and lake water. In looking at how tannins and DOC

are affected as groundwater empties into lakes and percolates through lake sediments to rejoin the water table, I hope to add to the understanding of one of our community's greatest assets.

Methods

Site Description

The three glacial kettle ponds (which could also be categorized as coastal plain ponds) maintain different levels of colored water throughout the year. Mary Dunn Pond in Hyannis is clear throughout the year, and is contrasted by the dark-brown Little Israel pond literally steps away. In Falmouth, the Punchbowl in Beebe Woods remains brown all year but its water is intermediately colored compared to the other two ponds. The DOC and phenolic tannin content as well as pH, ammonium, and phosphate concentrations in each lake's water are discussed in the results section (Tables 1 and 2). All ponds are situated on glacial till, but this provides a varied substrate across the Cape. While glacial outwash in some areas is well sorted and sand-sized, regions of silty clays and poorly sorted boulders are also present. Based on geological mapping of the area's glacial history (Masterson et al. 1997 and The Cape Cod Water Commission), both Little Israel and Mary Dunn ponds are located on well-sorted, sandy glacial till, while the Punchbowl in Falmouth is at the edge of a terminal moraine and has poorly-sorted substrate that prevents the use of wellpoints for groundwater collection. All ponds are surrounded by mixed deciduous forest containing predominantly black and white oak trees (*Quercus niger* and *Q. alba*), along with some beech (*Fagus grandifolia*), maple (*Acer saccharum*, and *A. saccharinum*) and pitch pine (*Pinus rigida*). Based on visual estimation, Little Israel pond is surrounded by a greater proportion of pitch pine than the other lakes. Historically, Little Israel has dried up completely in the dry periods of the summer, especially when the pump located on its shores is in operation; these episodes have also resulted in loss of water quality to the town of Hyannis, MA.

Bioreactors

My advisor Rich McHorney, collaborator Meghan Short, and I collected eight total sediment cores from the down-gradient sides of Mary Dunn (four cores), Little Israel (two cores), and Punchbowl (two cores) ponds on November 11, 2010 and November 17, 2010, respectively. After locating the down-gradient side of each pond (Cape Cod Water Commission), we used 5 cm acrylic tubing cut into meter-long sections to collect ~30 cm cores from each location. Size 10 rubber stoppers allowed us to form enough suction to pull up the cores without loss of sediment. We then placed a pre-cut, acid washed circle of 3M Scrub Pad on the base of the sediment to prevent later clogging of tubing, and plugged the other end of the cores. 20-L carboys filled with pond water from all three ponds were also collected on these days and used to flush the bioreactors later in the experiment.

In lab, we placed the cores and carboys immediately into a temperature controlled room set to 15° C, and wrapped them in black plastic to prevent light from reaching them and to mimic the temperature and light conditions of the bottom of the pond from which they were removed. We positioned each core upright and secured them to a shelving unit inside the fridge, then replaced the solid bottom stoppers with cored size 10 rubber stoppers through which we placed 5 cm lengths of 5 mm internal diameter glass tubing and linked these to 50 cm lengths of plastic tubing that emptied into 250 mL graduated

cylinders. For five days, the water originally collected with each core was allowed to percolate through the sediments and collect in the graduated cylinders. I collected this initial “through flow”, filtered it with a Swinex filter attachment and 25 mm GFF Whatman fiberglass filter, and froze it for preservation. To prevent uncontrolled organic materials from entering the cores from the organic matter and leaf litter that accumulated on top of the sediments in each pond, the top organic layers of each core were removed using a Geotech peristaltic pump, and the water was then replaced with water collected earlier from each pond. After porosity was calculated (please see “Determination of Porosity” section), excess water was removed from the top of each core using the Geotech peristaltic pump and was replaced with pond water from either Mary Dunn or Little Israel ponds. Mary Dunn cores 1 and 2 and Beebe Woods core 1 were filled with Mary Dunn water, while MD Cores 3 and 4, Little Israel cores 1 and 2 and Beebe Woods core 2 were filled with LI pond water. One seventh of the water contained in each core was removed each day with a acid-washed 60 mL syringe and filtered and frozen according to the methods described above. The longest residence time in bioreactors was a week for the t=7 timepoint. The addition of high-DOC and tannin-rich water to cores like those taken from Mary Dunn that are not normally accustomed to a high DOC concentration in the water is designed to “stress” these cores and their natural biota and gauge their reactions to this new chemistry. The same is true for the cores taken from Beebe Woods and Little Israel, that were taken from a higher-DOC environment and are now passing less concentrated water from Mary Dunn pond.

Determination of Porosity

In order to allow up to a week’s residence time of water in the core sediments, we needed to determine the actual water volume contained in the pore space of each core so that 1/7th of the volume could be removed each day. To determine the porosity, we first determined the flow rate of water through each sediment core while maintaining a constant head on each to keep the rate of water discharge stable. The remaining pond water was removed from each of the cores and a solution of 1g of NaCl to four liters of deionized water was added to each bioreactor. I maintained head levels throughout the procedure and measured the conductivity of samples that had passed through the cores at 10, 15 minute intervals. The excess liquid passed through each core was discarded and the collecting containers rinsed out with DI at these intervals to prevent the contamination of later samples. I collected samples at each of 10 intervals and graphed their conductivities to determine transit time for the water passing through the sediment column. As the first attempt at this porosity measurement failed, the following day we removed the extra NaCl solution from the cores and added freshwater from Mary Dunn Pond that had an initial conductivity of 82 μ S and flushed this freshwater through the sediments, again collecting and measuring conductivity every 15 minutes. The following equations detail the calculations used to determine the porosity.

Equation One:

$$V = \frac{q}{\phi}$$

Where V is the flow rate, q is the apparent velocity, and Ψ is the porosity. Variable q was determined with the following equations.

Equation 2:

$$Q = \frac{\text{Volume}}{\text{time}}$$

Where Q is the flow velocity of water through each core.

Equation 3:

$$q = \frac{Q}{A}$$

Where A is the area of the core in each tube.

Transit time for each core was determined by graphing the previously mentioned conductivity values measured at each of the 10 time intervals as the NaCl solution passed through the cores. The midpoint of the line on the salinity gradient connecting the points where the concentration of the solution moving through the core moves from low to high conductivity represents the true transit time. Transit time can then be used to calculate the velocity of flow with the following equation (Fig. 1).

Equation 4:

$$T = \frac{1}{V} \times H$$

Where T is transit time in hours, V is velocity of flow, and H is the height of a core in cm.

With these equations, we calculated the porosity of each core, and as their values were all around 0.25 (+/- 0.05), we decided to use this porosity for all of the cores and the volume removed each day reflects this assumption.

Wellpoint Sampling and Bulk Decomposition

In addition to the bioreactor setup, five liter water samples were collected in replicate from each of the three ponds and allowed to incubate under the same dark, 15° C conditions as the cores. These samples were collected at the same time as the carboys used to refill the cores with water, and were sampled at their collection and at 5 following intervals to determine phenolic tannin and DOC content. These samples were collected every two to three days, and were filtered and frozen in the same manner as the samples that passed through the bioreactor sediments described above. Additionally, the initial pH, phosphate, and ammonium concentrations of each pond's water were tested. The pH was analyzed with the Fisher Scientific Accumet pH/conductivity meter model 20, the ammonium was analyzed with the Cary 50 Scan UV-Visible Spectrophotometer, and phosphate concentrations were analyzed with the Shimadzu UV-Visible Spectrophotometer 1601.

We took groundwater samples on the down-gradient sides of Mary Dunn and Little Israel ponds near where the cores were taken using a wellpoint sampler and handpump. In Mary Dunn and Little Israel ponds, the down-gradient side is on the east/southeast side (Cape Cod Water Commission). In both

locations, we took two transects of wellpoint samples spaced about two meters apart. The Mary Dunn transects began on shore about a meter from the water's edge and moved into shallow, 0.5 m water over three total points on each transect. The Little Israel transects also began on shore about a meter from the water's edge, but contained four points each with the fourth point located in about a meter of water. More pressure was required to create a filter pack and pull up groundwater from the sediments of this pond, and in some cases, we were not able to withdraw any groundwater from these silty, mucky sediments (Table 1). These groundwater samples were filtered and frozen in the same manner as the other water samples to preserve their concentrations of DOC and phenolic tannins for later measurement.

Determination of Dissolved Organic Carbon Concentrations

We analyzed the concentrations of DOC in all water samples with the O-I-Analytical Aurora Model 1030 with Autosampler 1088. Groundwater and bulk decomposition samples were thawed and analyzed without dilution, but 2.5 mL of the bioreactor sediment-flow samples were all diluted with 10 mL of deionized water. Each of the four runs conducted with my samples began with a series of standard solutions of potassium hydrogen phalate (KHP) in 1, 5, 10, 20, 50, and 100 ppm concentrations. These solutions were made on December 6, 2010, and kept in the fridge until use.

Development of a Modified Prussian Blue Assay

The original Prussian Blue assay described by Hagermann in her *Tannin Handbook*, was devised by Price and Butler in 1977, and instead of using ferric chloride, uses ferric ammonium sulfate as the first reagent. When I first ran the assay with the recommended hefty dilution of 50 mL of added DI water to only 6.01 mL of reagent and sample combined, the concentrations of my samples proved to be too low in tannins to react enough for spectrophotometric measurement. The reaction becomes viable for natural water samples containing low concentrations of phenolic tannins when reagent one, 0.10 M $\text{FeNH}_4(\text{SO}_4)_2$ in 0.10 M HCl, and reagent two, 0.008 M $\text{KFe}(\text{CN})_6$ are used in higher concentrations with a greater volume of sample.

Maintaining the rest of the procedure described in Hagermann 2006, I added 5 mL of natural water sample to a 15 mL falcon tube and added 1 mL of reagent one and 1 mL of reagent two. Exactly 10 minutes after the addition of reagent two, I used the Shimadzu UV-Visible Spectrophotometer to measure the absorbance of the sample at 720 nm. The assay is standardized against 1, 2.5, 5, 10 and 25 μM solutions of tannic acid. As tannic acid is difficult to dissolve, gentle heating (below 30° C) of the solutions while stirring for an hour does not appear to damage the solution and allows for total dissolution. Standard curves for a representative tannic acid standard showed linearity and were repeatable between the two days I used the Modified Prussian Blue assay to analyze samples (Fig. 2).

All solutions were made from dry chemical provided by Sigma-Aldrich, with the exceptions of the concentrated HCl which was provided by Fisher Scientific and the ammonium iron (III) dodecahydrate p.a. provided by Acros Organics.

Results

Bulk Incubation Degradation of DOC and Tannins

The water samples taken in replicate from Little Israel, Mary Dunn, and Punchbowl ponds were sampled for DOC and tannin content six total times during the study from when they were collected on November 11, 2010 through December 6, 2010. On November 12, 2010, I analyzed the water samples for pH and concentrations of ammonium and phosphate to better characterize the water chemistry from which the cores used in the experiment originated (Tables 1 and 2). We analyzed the samples collected at each time point for DOC and phenolic tannin concentrations the week of December 6, 2010, and found minimal degradation of both compounds in the bulk incubations (Fig. 3). Although some degradation of organic compounds did occur in all of the bulk incubations, usually in the 1-6% range (Table 3), the amounts were dwarfed in comparison to the degradation measured when water from the same ponds were allowed to flow through the bioreactors (Table 4).

Bioreactor Degradation of DOC and Tannins

Although the initial concentrations of DOC and phenolic tannins in the organic-rich and clear pond water added to each of the eight bioreactors varied substantially from 1225 μM to 9600 μM DOC and 0 to 1.1 μM tannin content (Table 1), samples collected from each of the cores showed between 85 to over 98% DOC removal over a week's residence time in the core sediments (Table 4). Graphical analysis of DOC removal in each of the cores showed very little continuing takeup of DOC after the first time point representing one day's residence time for water in the cores, and nearly complete removal of the DOC present in the water initially added (Fig. 4). Regardless of the core's original environment, sediment characteristics, or the type of water (clear or organic-rich) that flowed through each bioreactor, all cores removed over 85% of the DOC originally present in the water, with a mean DOC removal of 96% (Table 4). In addition, the cores that processed organic-rich water removed a higher percentage of DOC than the cores with clear water added. Mary Dunn cores 1 and 2, from a clear pond with clear water added, removed only about 85.5 and 91.5%, respectively, while their replicate cores 3 and 4 removed over 98% of the DOC present in the more organic-rich water (Table 4). A similar relationship can be seen with the two bioreactors taken from the Punchbowl in Beebe Woods; although these cores' original pond environment contains an intermediate level of DOC and tannins in its water compared to the other ponds (Table 1), the bioreactors showed similar amounts of DOC removal from both types of water as the cores from the clear Mary Dunn pond. The Beebe Woods bioreactor with clear water added removed about 82% of DOC, while the core with organic-rich water added removed about 98% of DOC.

The removal of phenolic tannins measured with the Modified Prussian Blue assay is less uniform across the bioreactors. While we measured 75-80% removal of phenolics from the two Mary Dunn cores with organic-rich water added (Fig. 5.A), only about 16% of phenolics were removed from the Beebe Woods Core with organic-rich water added and the standard deviation for a week's values for this sample is nearly 15% (Table 4). All of the cores that showed tannin removal and not *addition* are composed of predominantly sandy sediments and originated from ponds containing low to intermediate levels of phenolic tannins (0-0.5 μM , respectively). Cores taken from Little Israel pond showed gains of

tannin ranging from 0.25-1.2 μM , the latter of which is higher than the original phenolic concentration of the organic-rich water added to the cores at 1.1 μM (Table 4). Mary Dunn core 2 and Beebe Woods core 1 to which we added clear, tannin-free water showed varying amounts of tannin gain ranging from 0.082-1.42 μM (Table 4). All cores, with the exception of Mary Dunn cores 3 and 4 to which we added organic-rich water, showed greater fluctuations in phenolic tannin concentrations over the course of the week, with nearly half of data points containing phenolic concentrations smaller than the water initially added to each reactor. Overall, however, the gains in phenolic tannin concentration for these cores were large enough to override any small losses earlier in the experiment (Fig. 5. B-D).

Well Point DOC and Tannin Concentrations

The groundwater samples taken from the two transects at Mary Dunn and Little Israel ponds showed large changes in DOC concentration between points on each transect, with the highest values for both tannins and DOC occurring in the groundwater taken from the farthest down-gradient sample point (Fig. 8). In the Mary Dunn transects (Fig. 6), DOC concentrations down-gradient (onshore) are about 2400 μM and are only about 1600 μM directly beneath pond sediments. Compared to the DOC concentration measured in the pond water, 1225 μM , there is an accumulation of DOC in groundwater in the groundwater compared to the pond water, but an overall decrease compared to the surrounding groundwater (Fig. 6). The concentration of phenolic tannins is similarly decreased directly below the pond sediments in Mary Dunn Pond. Although we found no measurable tannins in the water from the pond, the groundwater contained between 0.08 and 0.05 μM of phenolic tannins (Transects 1 and 2, respectively) at the points sampled farthest down-gradient, and no phenolic tannins in the groundwater collected from the points directly below the pond sediments (Fig. 6).

Little Israel groundwater transects showed higher DOC concentrations in all samples than the Mary Dunn groundwater, but showed the same decreased concentrations of both tannins and DOC in groundwater taken from directly below lake sediments (Fig. 7). The farthest up-gradient groundwater had a DOC concentration of about 6800 μM , and this value decreased to about 2000 μM in groundwater from below lake sediments. Phenolic tannin concentrations decreased in groundwater taken from below lake sediments as well, with a value of about 0.1 μM compared to 0.75 μM in shoreline groundwater.

While neither groundwater tannin nor DOC concentrations in Mary Dunn groundwater were higher than in the pond's water, and both tannin and DOC concentrations were less than pond water concentrations in the groundwater of Little Israel, the difference in amount of organic compounds found in these groundwater samples are large. Groundwater beneath Mary Dunn pond sediments contain 67% less DOC than are present in the down-gradient water and almost no tannins (Fig. 6). In Little Israel groundwater, samples from beneath pond sediments showed about 30% less DOC and about 13% less tannins than found in the groundwater from down-gradient samples (Fig. 7).

Discussion

Bulk Incubations:

Small amounts of DOC and phenolic tannin degradation indicate a slow rate of bacterial or abiotic activity, but determining the proportions of biotic and abiotic degradation would require further study. As the bulk water samples were not filtered before placement in the dark, temperature controlled room, biotic factors may play a more significant role than abiotic degradation. Although photodegradation of DOC and other complex organic compounds can be considerable in natural waters, and may even increase bacterial abundance 35-55% with irradiation of samples (Tranvik 1999), my samples were kept in the dark from the time of their collection, so these effects are unlikely. Kaplan tested abiotic degradation of DOC through sterile sediments and found an average of 3% drop in DOC concentrations due to abiotic degradation and sorption onto sediments (Kaplan 1995). The bulk incubations conducted in this experiment did not have contact with sediments, so sorption is also less of a possibility as there was no particulate noticeable in the incubations. Both of these studies suggest that the amount of DOC and tannin degradation observed are consistent with previous research.

Bioreactors:

As all of the bioreactors removed substantial amounts of DOC, it seems that sedimentology of the cores themselves and the original chemistry of the pond is less important than the biota that populate each reactor and the chemistry of the water flowing through them. Bioreactors, regardless of origin, removed higher percentages of DOC when flushed with organic-rich water, while cores with clear water were less efficient at DOC removal (Table 4). Again, it seems that biological factors exert the most influence on DOC removal. This is supported by a Finnish study that found 81% of TOC was removed in the first 0.6m of the larger (18 m in length with a 0.5m diameter) pond sediment bioreactor (Kolehmainen et al. 2009). Kaplan also determined that the biology of his reactors greatly enhanced DOC removal (Kaplan 1995). Both of these studies, however, allowed months-long residence times for water flowing through bioreactors, and the initial lake water put through the cores was measured at 3400 and 4000 μM DOC, respectively. Due to these differences, it is difficult to make a direct comparison between this experiment and these other studies.

While phenolic tannins can be broken down and even metabolized by some bacteria, a large portion of the change in tannin concentration can likely be attributed to sorption and desorption from organic material present in the sediments. Some components of sediments, especially clay minerals, iron oxides, and organic matter, readily attach to phenolic compounds such as the tannins found in the Little Israel and Beebe Woods Pond waters (Moore and Ramamoorthy 1984). Clay minerals, however, readily release phenolics in moist environments (Moore and Ramamoorthy 1984). The clayey, organic-filled sediments of Little Israel likely absorbed tannins and other phenolic compounds in situ as lake water rich in phenolics flowed through them. When these cores were then flushed with water, it's likely that clay minerals in the sediments released some of their attached phenolics, increasing the concentration found in the water that had flowed through these bioreactors (Fig. 5). Conversely, the sandy, organic-poor sediments from Mary Dunn pond removed tannins that were present in the water added to the column, suggesting that biotic removal of tannins is dominant in these cores. There must, however, be some phenolics that were present in the column before the start of the experiment, as the Mary Dunn cores with added clear, tannin-free water saw small increases in tannin concentration in the water that flowed through them (Fig. 5). The overall degradation of tannins that passed through sandy cores suggests that

sediment type, and not the chemical composition of the water itself is the most important factor in phenolic tannin concentration.

Groundwater DOC and Tannin Content

Although the bioreactor experiments showed about 98% DOC removal, the groundwater data collected from the transects at Mary Dunn and Little Israel ponds show less of a difference in DOC and tannin concentrations between groundwater sampled from the shore and from beneath the pond sediments. As transects were conducted moving from on shore towards the pond against the flow of groundwater, variations in sediment size were observed. Closest to the shore, the coarse pebbles and gravel allows for higher hydraulic conductivity as it is more porous, so there is a shorter residence time in sediments nearer to the shore (Fig. 8). Groundwater samples taken from directly beneath the pond where sediment was finer-grained and more organic-rich had a lower hydraulic conductivity and longer residence times, maintaining longer sediment contact and opportunities for (a)biotic degradation (Fig. 8). In both Mary Dunn and Little Israel ponds, concentrations of both tannins and DOC decreased in groundwater that was sampled up-gradient, or towards the pond (Fig. 6 and 7). As discussed above, the sediments beneath the pond are finest and it will take water a longer time to flow through them, thereby allowing more microbial removal and sorption of DOC and tannins. The groundwater passing through the coarser sediments has less time for biotic or abiotic chemical changes to occur, and thus contains higher concentrations of DOC and tannins.

Less removal of DOC and tannins measured in groundwater taken from beneath in situ sediments suggests that the bioreactors' residence times of a week may be longer than they would be in the natural environment. The greater amounts of removal seen in the bioreactors may be due to increased microbial and abiotic activity on water in contact with sediments for longer periods than in situ. Although we deliberately removed one seventh of the core water from the reactors each day, it is possible that the groundwater exchange is higher in the ponds. More hydrological examination of the groundwater interactions would be illuminating in this case.

Summary

Although preliminary characterization of pond sediment DOC and tannin removal seems promising for water quality management in the region, further determination of the hydrological and biological interactions of groundwater with glacial kettle ponds and sediments is needed. As a follow-up study, characterizations of bacterial abundance and productivity along with determinations of the amount of sorption and desorption in bioreactors, bulk incubations, and groundwater would be logical and enlightening. In this study, however, the main conclusions are as follows:

- Whole water incubations only removed 3% of DOCs and 7% of tannins. These values are consistent with previous research (Kaplan 1995).
- Between 85 and 98% of DOC in bioreactors are removed after a week, depending on the initial DOC concentration of the water added. Mean DOC removal is 96%.
- Tannin concentrations in water passing through bioreactors depend primarily on sediment type

- Groundwater concentrations of DOC and phenolic tannins suggest that residence times in situ are lower than experimentally tested.

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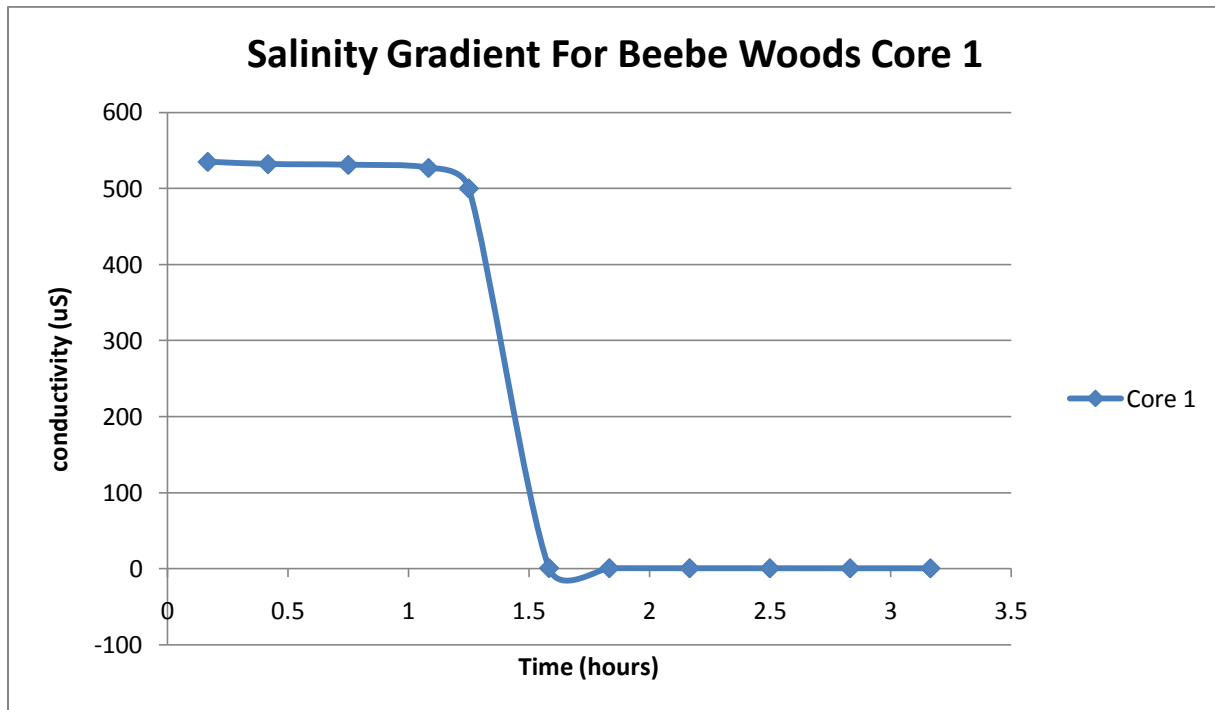


Figure 1: Example of a salinity gradient measured to calculate the porosity of Beebe Woods Core 1. The midpoint of the steep gradient that develops where the wave of fresh water “breaks through” the core’s pore spaces and reaches the bottom is the actual transit time.

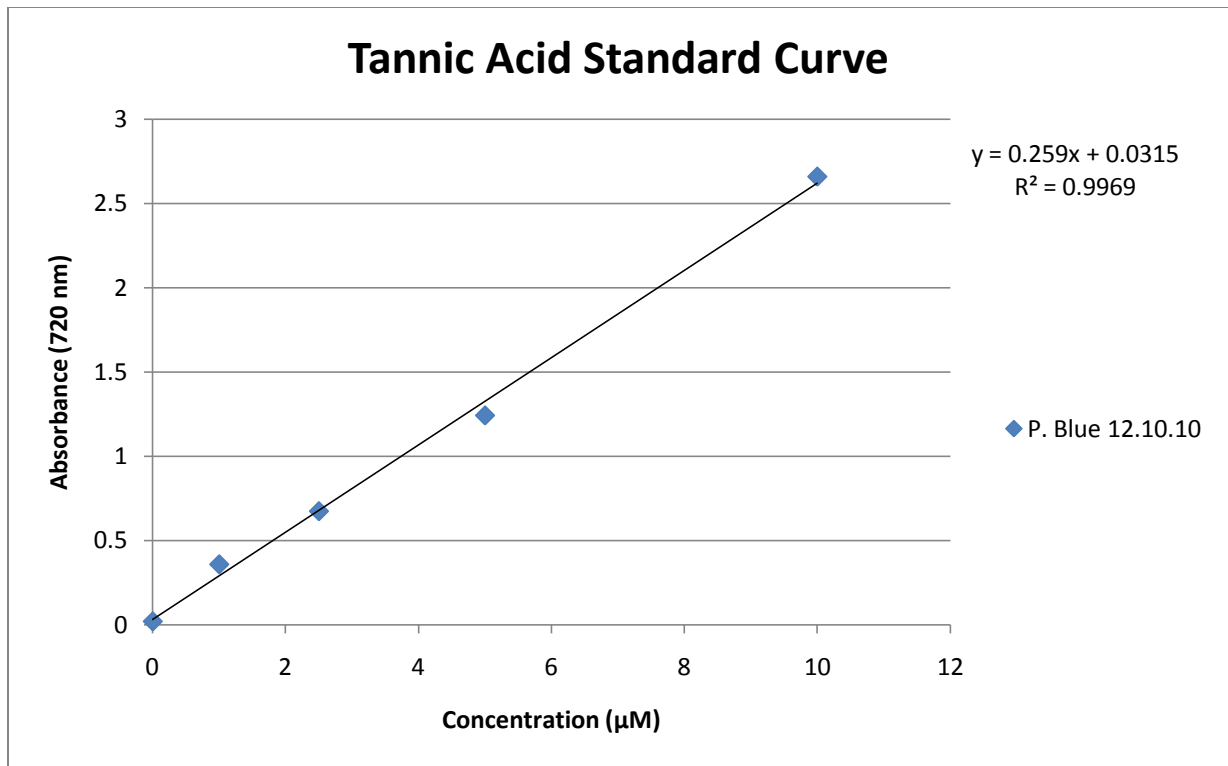


Figure 2: Representative standard curve for the modified Prussian Blue assay for phenolic tannins standardizing against tannic acid.

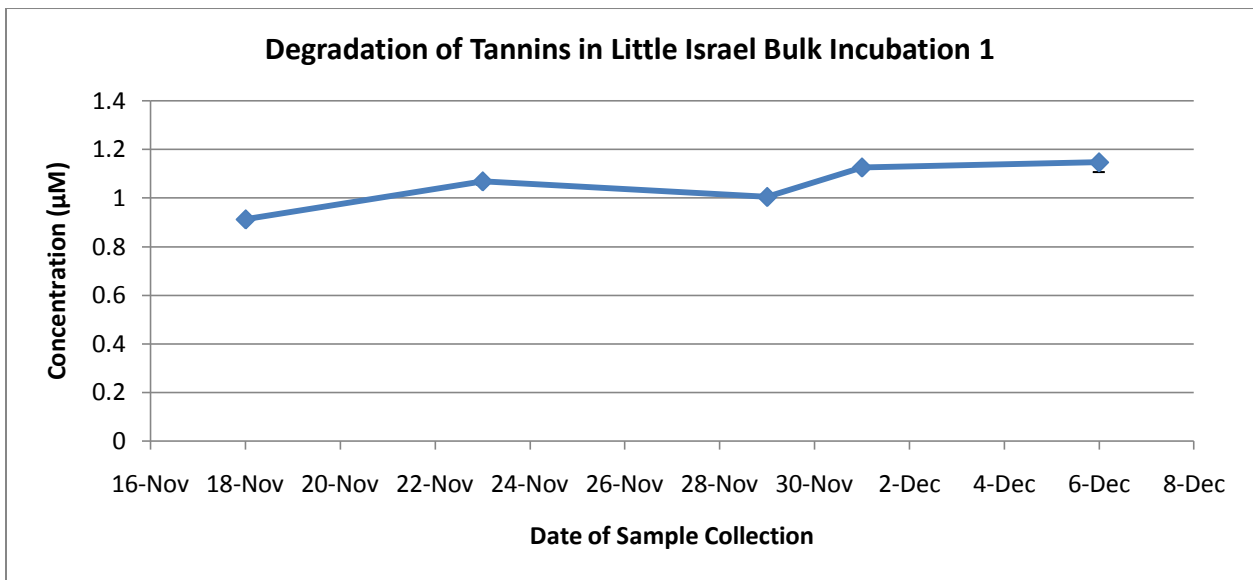
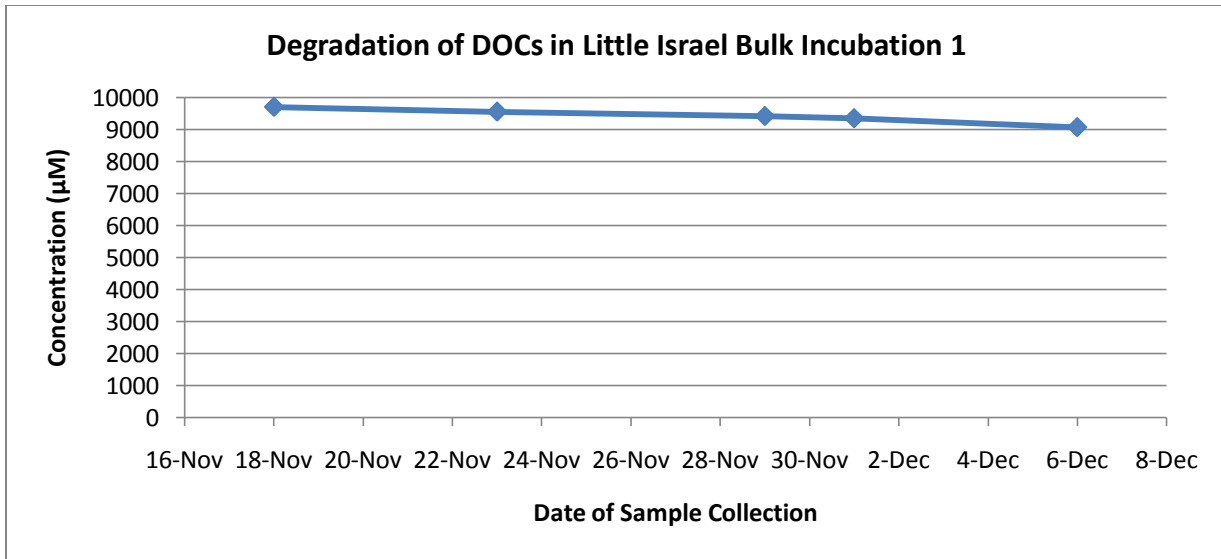
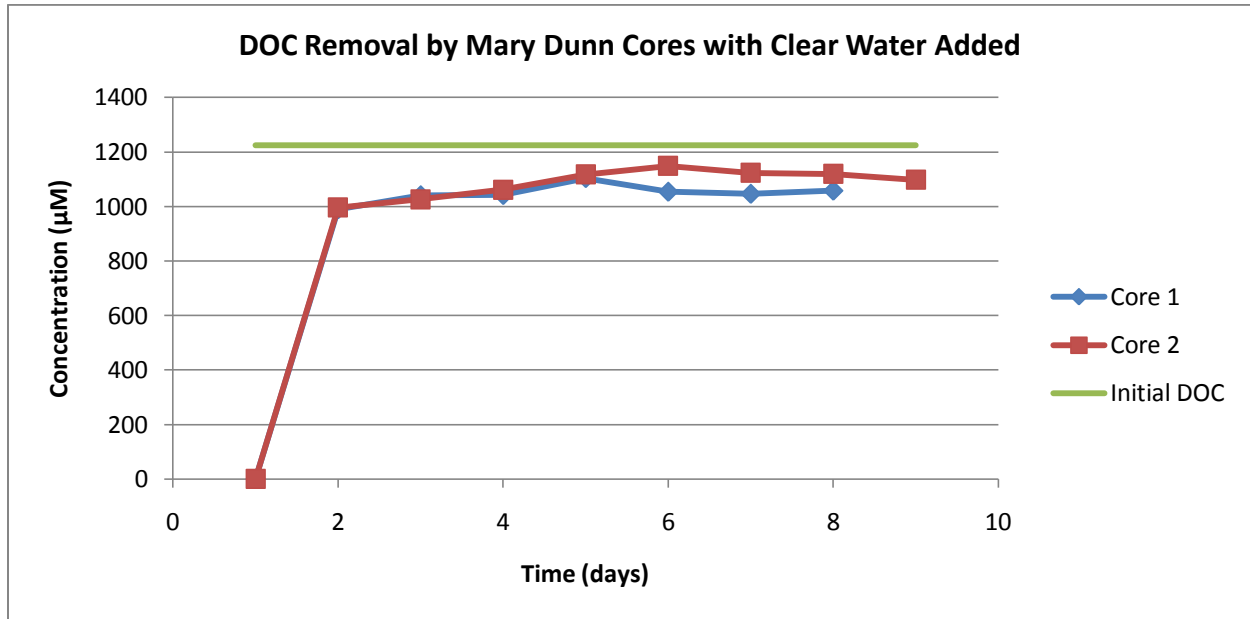
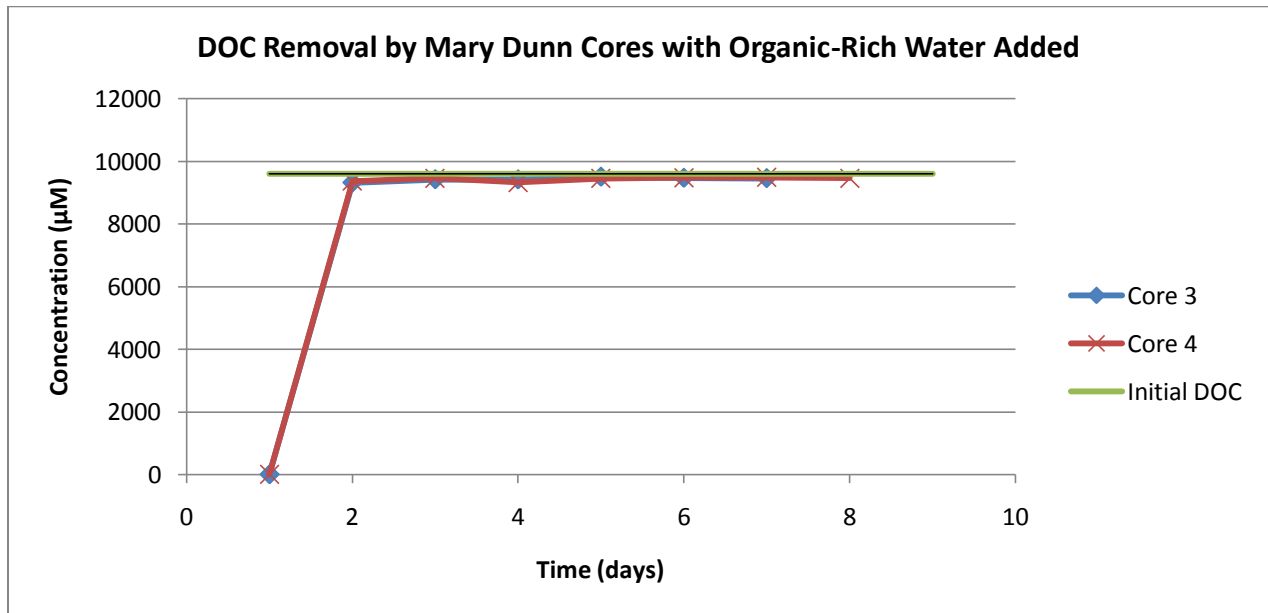


Figure 3: Graphs showing degradation of DOC and tannins in a representative bulk water incubation from Little Israel pond. Note the small changes in concentration over the period of measurement.

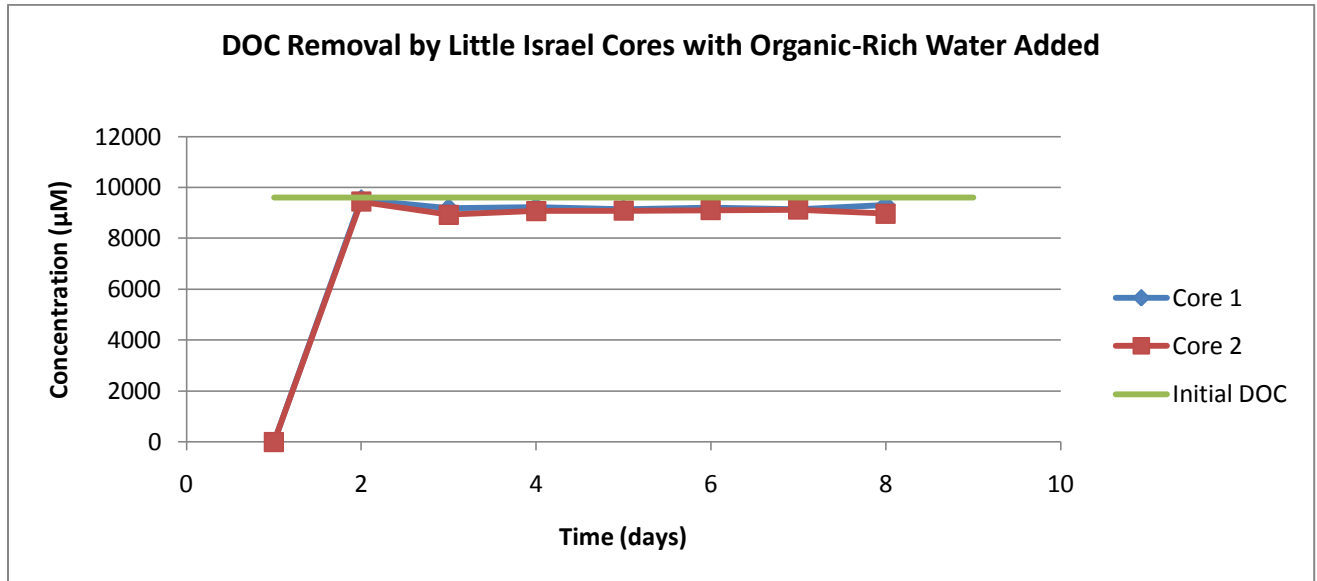
A.



B.



C.



D.

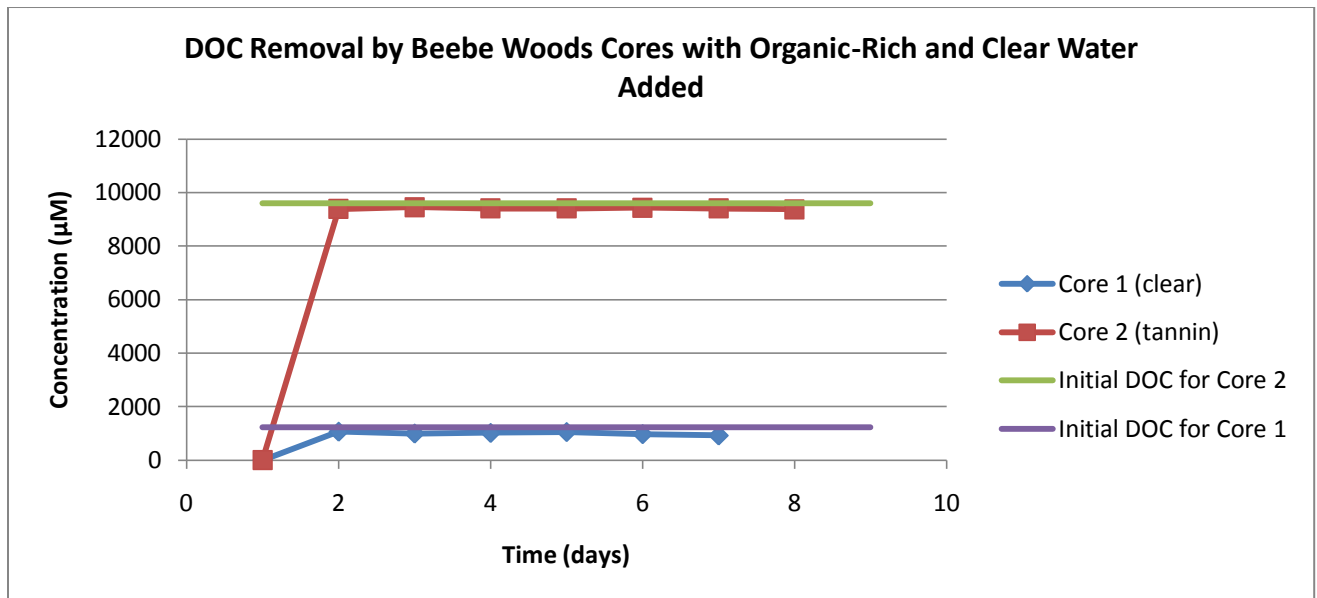
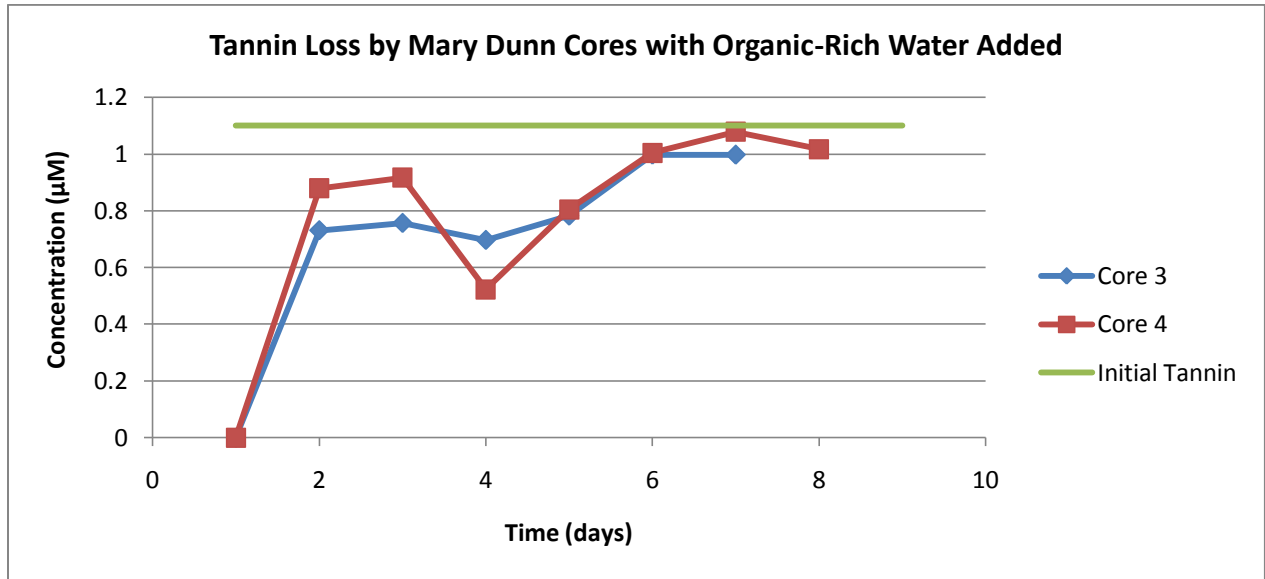
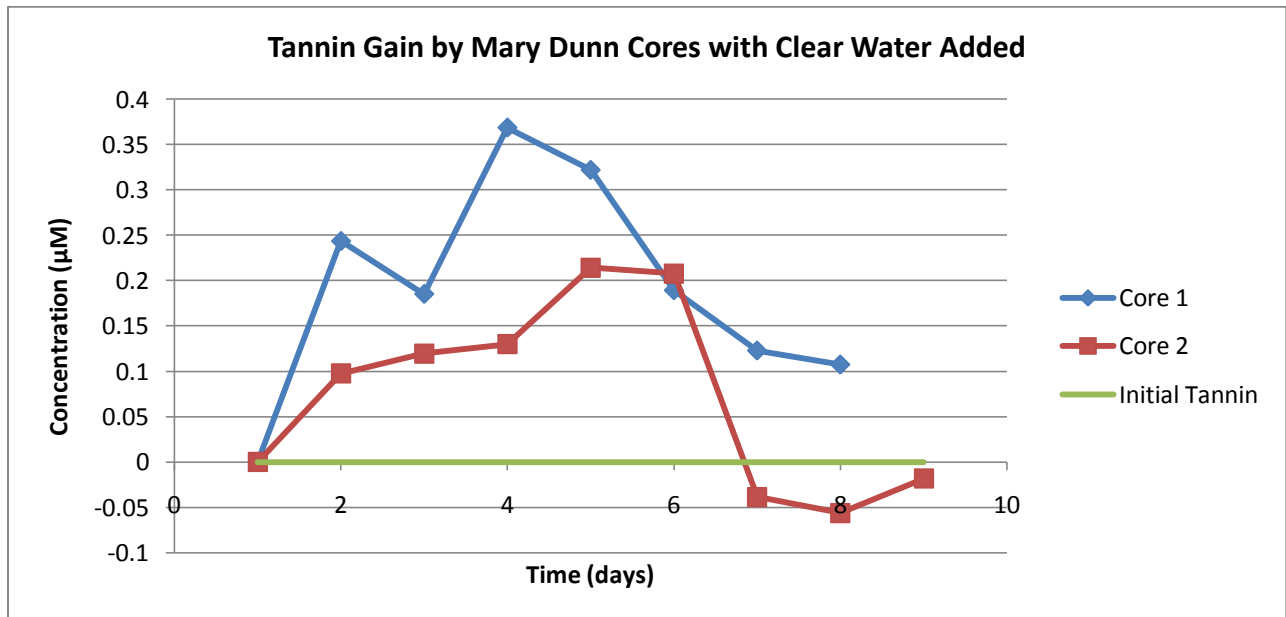


Figure 4: Graphs showing the amount of DOC removed (μM) over the course of a week during which water from either the organic-rich or clear ponds flowed through the eight bioreactor cores. Part A shows DOC removal by Mary Dunn cores 1 and 2 to which had clear water added. Part B shows DOC removal by Mary Dunn cores 3 and 4 to which organic-rich water was added. Part C shows the two cores taken from Little Israel pond and their DOC removal from the organic-rich water from the same pond. Part D shows DOC removal by the two cores from the Punchbowl in Beebe Woods; one core received clear water, the other organic-rich. The linear trends on each of the graphs show the initial DOC concentrations of the water that was added to each of the cores.

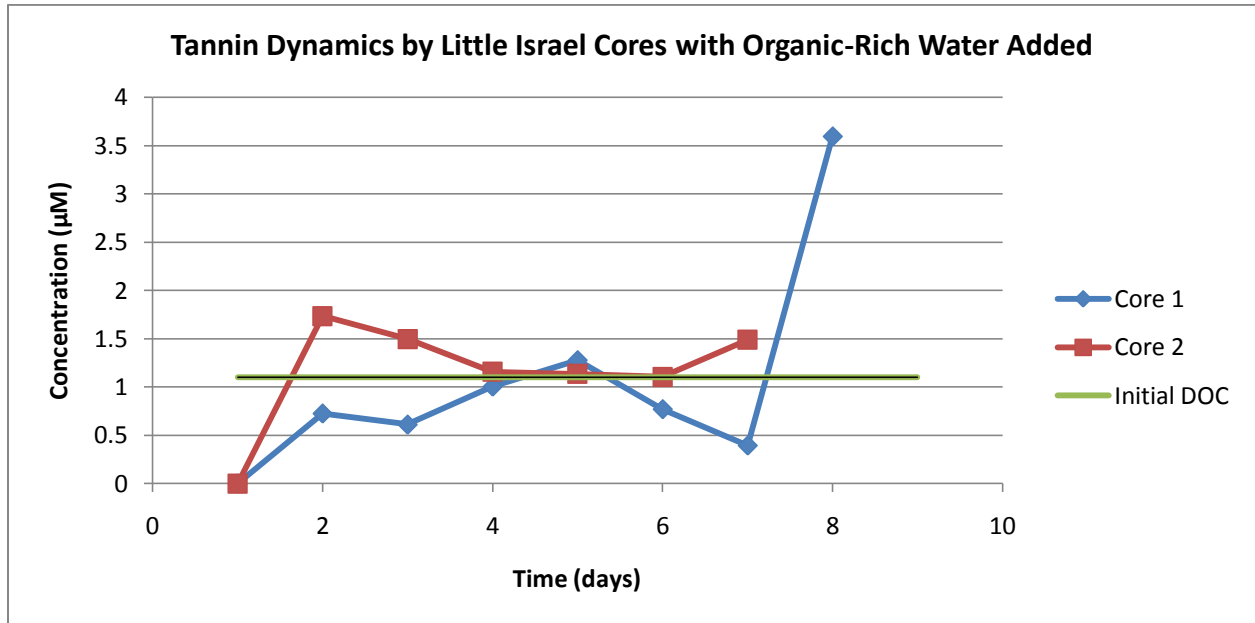
A.



B.



C.



D.

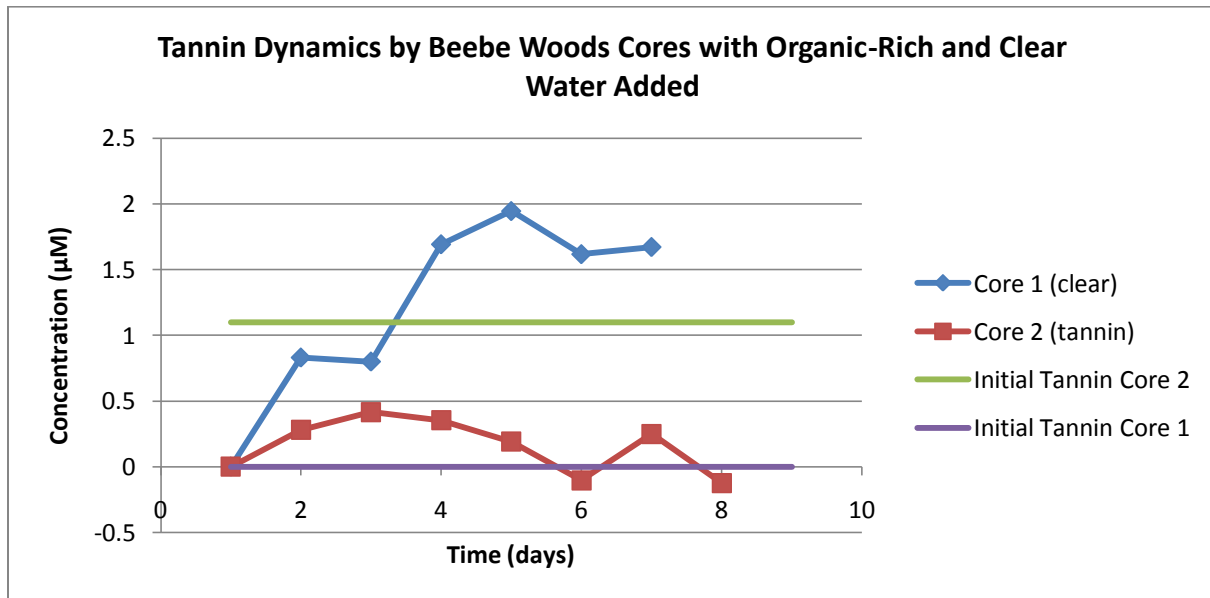
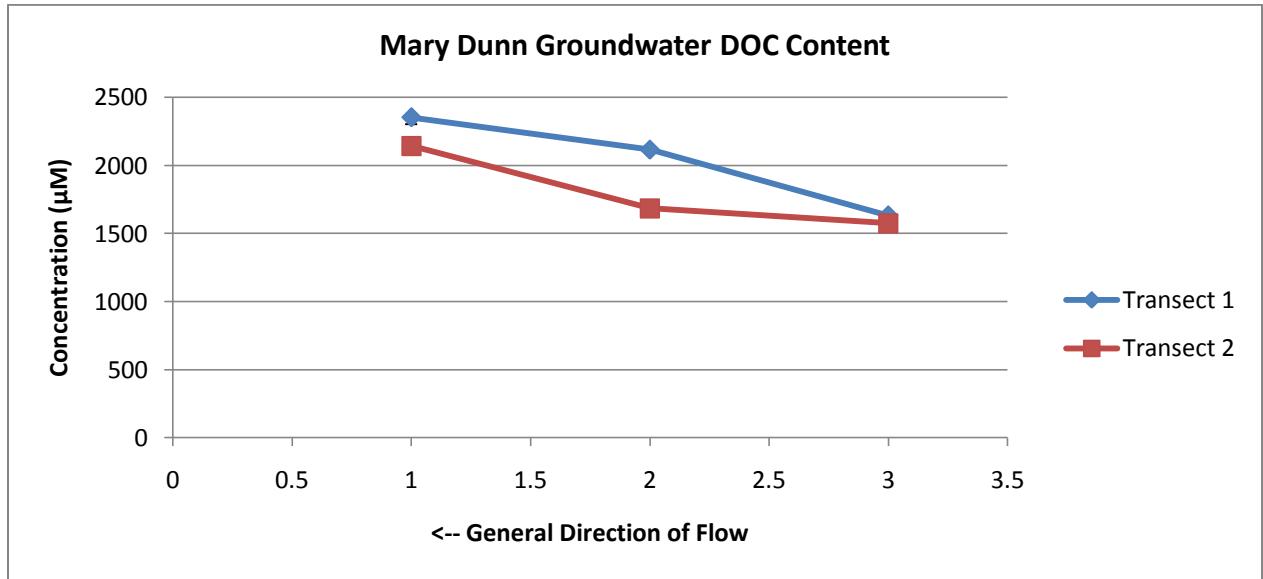


Figure 5: Graphs showing changes in concentration of phenolic tannins over a one-week experimental period. Part A shows loss of phenolic tannins in Mary Dunn Cores 2 and 3 with addition of organic-rich water. Part B shows substantial gain in tannins by Mary Dunn cores 1 and 2 with clear water added that did not contain measurable phenolic tannins. Part C details tannin dynamics in Little Israel cores 1 and 2 with organic-rich water added, with overall gain in tannin concentration from that of the original water added. Part D shows tannin dynamics for Beebe Woods cores 1 and 2 to which we added clear and organic-rich water, with overall tannin gain for both bioreactors. Linear portions marked "Initial Tannin" show tannin concentrations measured in the clear or organic-rich water added to the bioreactors.

A.



B.

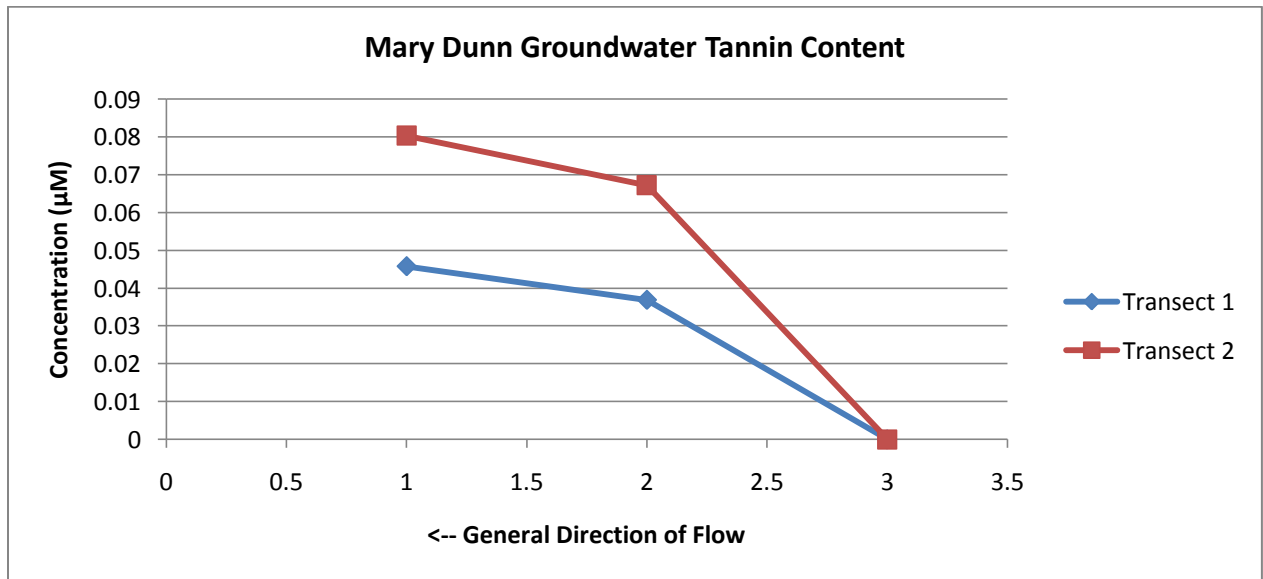
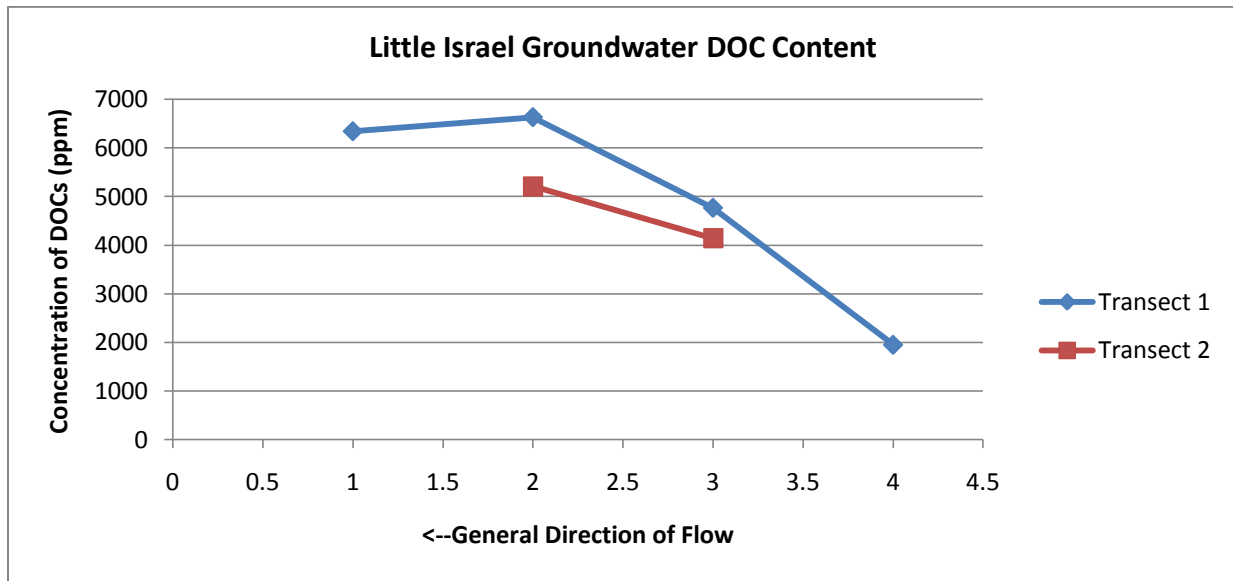


Figure 6: Groundwater concentrations (μM) of DOC and phenolic tannins for two transects in Mary Dunn Pond. X-axis corresponds to the wellpoint sampler's position in relation to the downgradient flow of groundwater; point 1 is on shore, point 3 is sampled from sediments directly below the pond.

A.



B.

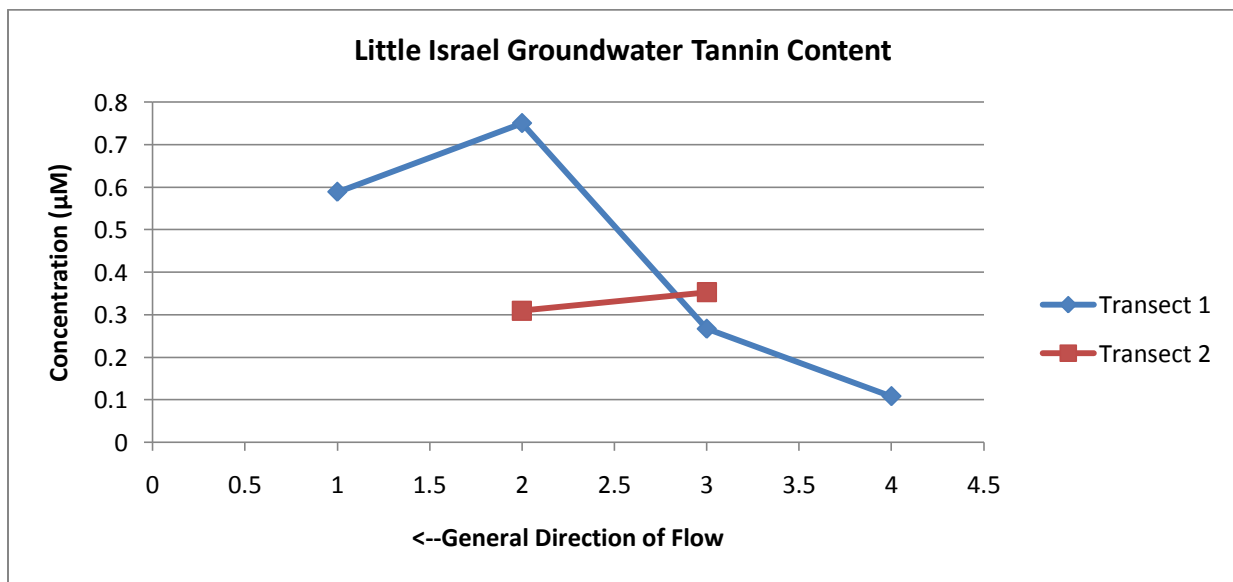


Figure 7: Groundwater concentrations (μM) of DOC and phenolic tannins for two transects in Little Israel Pond. X-axis corresponds to the wellpoint sampler's position in relation to the downgradient flow of groundwater; point 1 is on shore, point 4 is sampled from sediments directly below the pond. We were unable to collect groundwater from points 1 and 4 on Transect 2 due to clayey, mucky sediments.

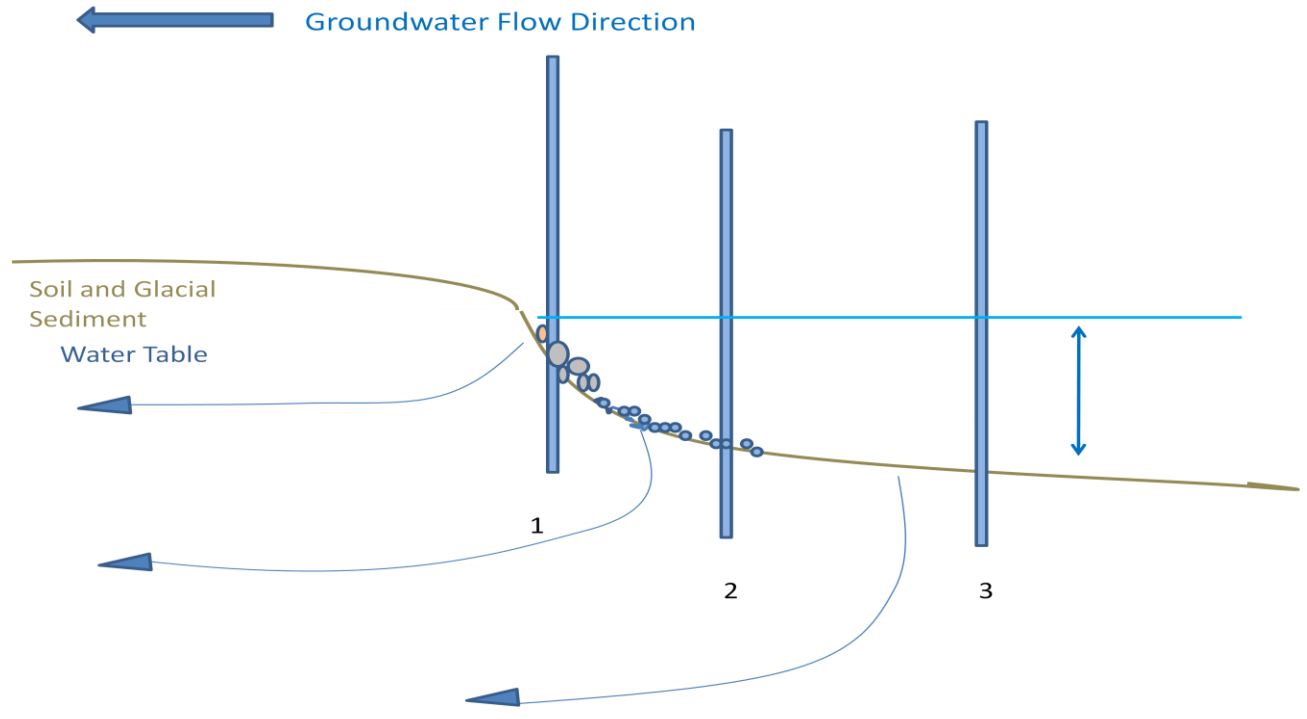


Figure 8: Schematic showing groundwater sampling transects at Mary Dunn and Little Israel ponds. Water contours show direction of groundwater flow. Note differences in sediment size as the transect moves into the pond and finer sediments.

Table 1: Initial chemical and sedimentary conditions of each pond used in the study.

Pond Location	Initial DOC (μM)	Initial Tannin (μM)	Sediment Description
Mary Dunn, Hyannis MA	1225	0	Sandy, coarser grained (0.5-1 mm), occasional dark bands of organic material occur at 10-15 cm
Beebe Woods, Falmouth MA	3075	0.05	Sandy, rounded grains, dark organic material in 1 cm layers begin at 10 cm depth
Little Israel, Hyannis MA	9600	1.1	Silty, black-dark brown with organic matter throughout, leaf litter layer 5-10 cm

Table 2: Initial water chemistry for the samples taken from all three sites. The samples were allowed to degrade without sediment contact in a temperature controlled room at 15°C.

Sample Origin	Replicate	PO ₄ ³⁻ (μM)	NH ₄ ⁺ (μM)	pH
Beebe Woods	1	0.04	2.16	5.01
Little Israel	1	0.31	8.48	4.95
	2	0.15	7.72	4.73
Mary Dunn	1	0.02	1.51	5.82
	2	0.05	1.52	5.77

Table 3: Degradation of DOC and phenolic tannin compounds described as percentages of the original content for each of the bulk incubation samples.

Bulk Water Sample	Replicate	DOC Degradation (%)	Tannin Degradation (%)
Beebe Woods	1	4.2	6.9
Little Israel	1	6.5	6.9
	2	1.3	6.7
Mary Dunn	1	4.4	--
	2	1.2	--

Table 4: Summary table of each core's initial environment and water chemistry compared to the total percentages of DOC removed and phenolic tannins removed or gained.

	Initial Conditions		Core	DOC removed (%)	Tannins Removed (%)	Tannins Gained (uM)
	DOC (uM)	Tannins (uM)				
Cores with Mary Dunn (clear) Water Added	1225	0	Mary Dunn Core 1	85.5 +/- 2.5		0.22 +/- 0.097
			Mary Dunn Core 2	91.5 +/- 4.1		0.082 +/- 0.10
			Beebe Woods Core 1	82.1 +/- 4.3		1.42 +/- 0.48
Cores with Little Israel (Organic-rich) Water Added	9600	1.1	Mary Dunn Core 3	98.2 +/- 0.6	75.1 +/- 11.2	
			Mary Dunn Core 4	98.2 +/- 0.65	80.8 +/- 16.9	
			Little Israel Core 1	96.2 +/- 1.4		1.2 +/- 1.01
			Little Israel Core 2	94.8 +/- 1.6		0.25 +/- 0.23
			Beebe Woods Core 2	98 +/- 0.23	16.4 +/- 17.9	