Controls on the Distribution of Denitrification in Streambeds of the Eastern Shore of Virginia

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ABSTRACT

To examine controls on nitrate removal in groundwater discharging to streams on Virginia's Eastern Shore, organic matter, and nitrate (NO_3^-) concentrations, were examined in four different streams. Depth profiles of organic matter, chloride, and NO_3^- concentration were developed from intact sediment cores from the streams. Denitrification was then estimated as NO_3^- removal (ΔNO_3^-) from an additional set of cores in the lab, where *in situ* flow conditions were simulated. An artificial groundwater solution containing a known concentration of NO_3^- was pumped upward through the core, recovered at the top, and analyzed for NO_3^- with an ion chromatograph, and NO_3^- removal was then determined as the influx minus the efflux. Multilinear regression combined with principal component analysis examined the relative effect of each measured factor on ΔNO_3^- i.e., denitrification. Pumped columns were shown to remove up to 100% of added NO_3^-, though some columns did not show NO_3^- removal. In 3 of the 4 streams, denitrification *in situ* was primarily limited by NO_3^-. Regression analyses for NO_3^- concentration in porewater showed that NO_3^- is primarily influenced by Cl^- and depth below streambed. Organic matter (OM) was found to not be limiting in these streams, although it was the main driver for denitrification. Additionally, canopy cover had a significant effect on streambed organic matter content with forest-canopied streams having about twice as much OM as streams with open canopies. While this study shows the ability of certain streams to remove large amounts of NO_3^-, the large variability among streams does not support allowing more NO_3^- inputs to ESVA streams.

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INTRODUCTION

BACKGROUND

The low-lying, fertilized agricultural fields on the seaward side of Virginia's Eastern Shore are framed by, and interwoven with, low-relief streams that drain into the fragile, nutrient-sensitive coastal lagoons that line the peninsula. Nitrate-nitrogen (NO_3^--N) is applied as fertilizer to the fields directly or is formed from nitrification of applied ammonia (anhydrous NH_3 or NH_4NO_3). Nitrate in excess of plant needs reaches local streams, and can then be carried to the lagoons, where it contributes to their eutrophication (McGlathery et al. 2007). As the nutrient concentrations in receiving water bodies increase, excessive algal growth is often stimulated, and the eventual death and decay of the algae results in hypoxic conditions and death of organisms such as fish, crabs, clams, etc., that inhabit the lagoons (Howarth 2008, Howarth and Marino 2006, National Research Council 2000), including those of Virginia's Eastern Shore (Giordano et al. 2011). Understanding NO_3^- transport to, and through, these streams is especially pertinent to preventing eutrophication, as small streams are responsible for collecting most of the water and dissolved nutrients within a watershed (Peterson et al. 2001). Previous work has demonstrated that at least some of the streams draining to the seaside lagoons of the Eastern Shore can act as filters of nitrogen, and can remove substantial amounts of applied fertilizer N from groundwater discharging to the streams, largely through the process of denitrification (Flewelling et al. 2012, Gu et al. 2008, Gu et al. 2007, Mills et al. 2008).

In the environment, denitrification is the only way that reactive nitrogen (all forms of N except N_2) (Galloway and Cowling 2002, Galloway et al. 2002) can be removed completely from natural and engineered systems. Denitrification is the biological reduction of nitrogen in NO_3^- to N_2 through sequential steps (Figure 1). The process involves the use of NO_3^- as a terminal electron acceptor for the oxidation of organic carbon (primarily) or sulfur (less commonly) (Mills 2019). Nitrate is first reduced to NO_2^-, then, in order, to NO, N_2O, and finally N_2. In denitrifying microbes, each step in this process is carried out by a different enzyme (Philippot 2002). Many organisms (including green plants) are capable of reducing one or more of the nitroxy compounds in the denitrification sequence, often for assimilatory purposes, but true denitrifiers must contain nitrous oxide reductase (*nos*) which catalyzes the reduction of N_2O to N_2 (Averill and Tiedje 1982).

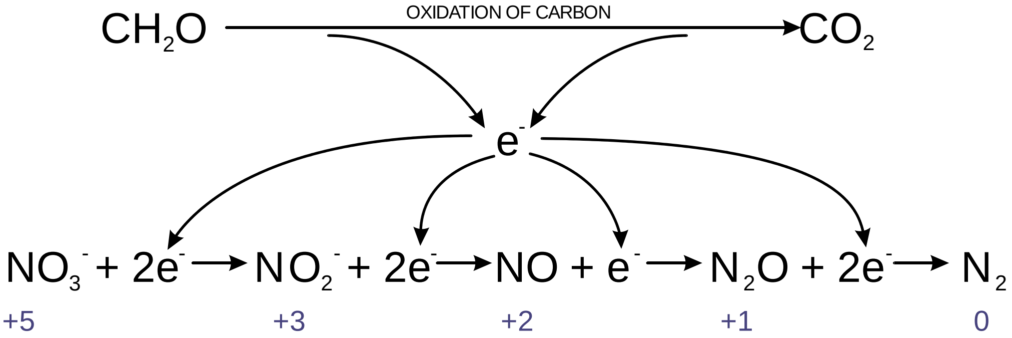

Figure 1. Denitrification is the process whereby electrons released during the respiration of organic carbon under anaerobic conditions are transferred to NO_3^- rather than O_2. Oxidation state of nitrogen in each species is shown below the relevant compound.

Denitrification requires four conditions to occur simultaneously: available organic matter (OM), low dissolved oxygen (DO) concentration, denitrifying bacteria, and nitrogen in an oxidized form, generally as NO_3^-. In this work, DO, OM, and NO_3^-, were examined both individually and in relation to each other in sediments of Eastern Shore streams. Regardless of the type of stream setting being examined (closed or open canopy, near or far from agricultural fields, and low or moderate topographic relief of the banks along the streambed), removal of NO_3^- by denitrification in the sediments requires that the water feeding the stream experience the same, denitrification-favorable conditions along its flow path. Note that evidence to date indicates that the removal of NO_3^- occurs in the sediment as the groundwater discharges to the stream. In streams examined for such, water-column processes do not significantly alter NO_3^- concentrations through the stream reaches as compared with the changes that occur in the sediments (A.L. Mills and M. Challand, unpublished data).

On the Eastern Shore, the streams are predominantly gaining streams, meaning that the major input of water to the stream is from groundwater entering the stream by traveling upward through the bed sediments, as opposed to overland flow (Mills et al. 2008). Water falls on the fertilized fields from precipitation or irrigation and picks up NO_3^- that has not been taken up by the plants as it percolates downward through the soil (Figure 2). From there, the water percolates further to the groundwater which is typically 2-3 m below ground surface in the agricultural areas (Accomack-Northampton Planning District Commission and Eastern Shore of Virginia Groundwater Committee 2013). Once in the groundwater, the NO_3^- travels laterally through the unconfined Columbia aquifer before rising vertically through the streambed sediments. Along this pathway, the NO_3^--rich water must encounter zones where denitrification conditions coincide for nitrate to be removed. These conditions are labile organic matter, low dissolved oxygen, the presence of denitrifying bacteria, the presence of NO_3^-, and sufficient residence time of nitrate-rich water in an area fit for denitrification (Burt et al. 1999). Most of the groundwater in the Columbia Aquifer contains oxygen at a level that should inhibit denitrification (Dunkel 2014), and organic matter is not generally abundant (Gu et al. 2007, Mills et al. 2008). Thus, the NO_3^- persists in the groundwater until high concentrations of organic matter are encountered in the streambed sediments through which the groundwater passes as it discharges to the local streams (Figure 2).

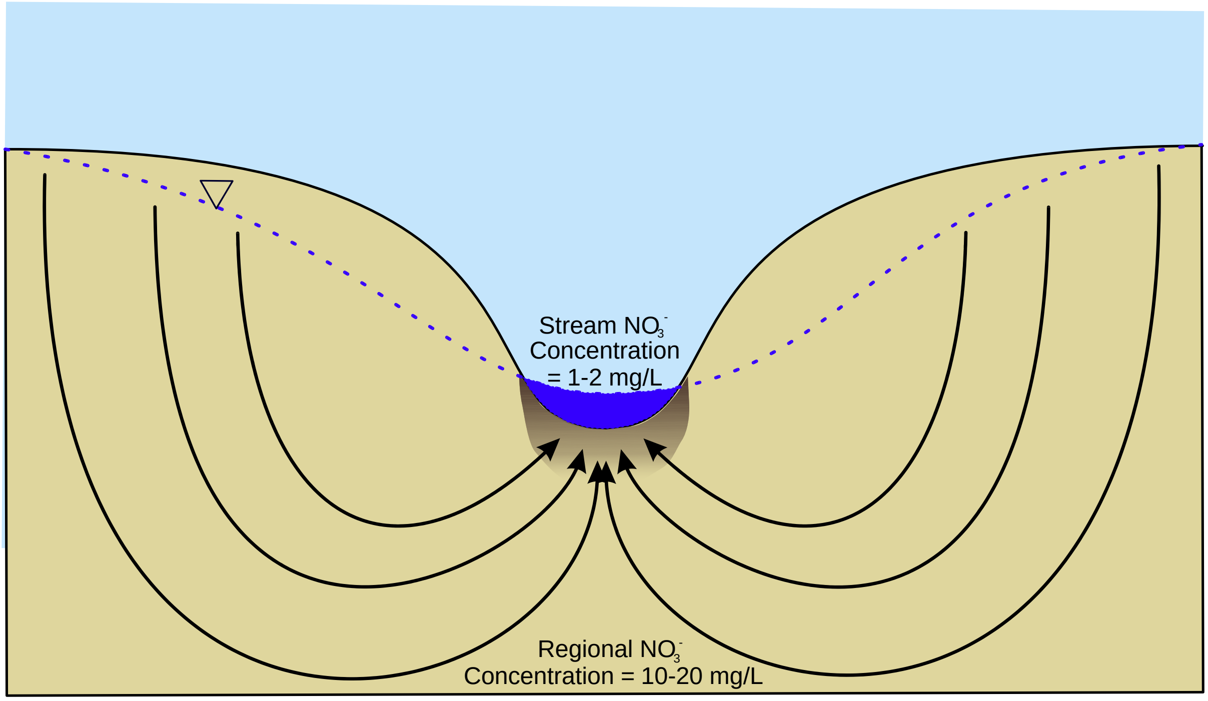

Figure 2. Typical water flow to streams from adjacent hillslopes. As NO_3^- -rich water passes through the stream sediments, O_2 is removed by microbial decomposers and NO_3^- is then removed by denitrifiers resulting in the removal of a large fraction (up to 90%) of the NO_3^- present. The unconfined aquifer represented here is the Columbia.

McFadden (2013) concluded that the lagoon-side Eastern Shore streams are “poised” for denitrification, although denitrification itself was not always observed for reasons that were never determined. Because some streams show strong denitrification while others seem to have none, understanding the controls on denitrification in the stream beds on the seaside of the Eastern Shore of Virginia is essential to understanding the nature of NO_3^- transport to the lagoons, and was, therefore, the topic of this research effort.

Organic Matter & Dissolved Oxygen.

Organic carbon molecules serve as energy sources for denitrifying bacteria (Figure 1) in stream beds. Labile carbon can stimulate denitrification directly by providing an energy source for bacterial growth, as well as indirectly, by fueling respiration that depletes dissolved oxygen (Gu et al. 2007, Piña-Ochoa and Alvarez-Cobelas 2006).

Because of the consumption of O_2, the biological activity powered by organic matter further leads to anoxic zones. Water that is not in frequent contact with the atmosphere can become oxygen depleted (hypoxic or anoxic) as the O_2 is used by aquatic life, including microbes in the decomposition of organic matter, to respire. Anoxic conditions are a necessary criterion for denitrification, and DO concentrations, while highly variable in water (Truesdale et al. 1955), can dictate areas where denitrification can and cannot occur.

Denitrifying Bacteria.

Many bacteria denitrify. Some important denitrifying microbes in soils include *Alcaligenes faecalis* and *Pseudomonas fluorescens* (Knowles 1982). Almost all these bacteria possess all the enzymes necessary to reduce NO_3^- all the way to N_2. However, not all nitrate reducing bacteria can carry out complete denitrification as some organisms lack N_2O reductase and release N_2O as the final product rather than elemental nitrogen (Knowles 1982). Moreover, denitrifying bacteria are usually facultative anaerobes, meaning that they will use oxygen when it's available but can survive without it (Burford and Bremner 1975). Therefore, they will only reduce nitrate in hypoxic or anaerobic conditions. The fact that denitrifiers are facultative means that they are nearly always present in appreciable numbers in environments with fluctuating concentrations of oxygen (providing there is an adequate supply of NO_3^-).

Each step of the denitrification process is catalyzed by a different enzyme (Figure 3). In order, these enzymes are nitrate reductase, nitrite reductase, nitric oxide reductase, and nitrous oxide reductase (Philippot 2002). Nitrate reductase (coded for by the *nar* gene) reduces NO_3^- to NO_2^- . Nitrite reductase (*nir*) reduces NO_2^- to nitric oxide (NO). Nitric oxide reductase (*nor*) reduces NO to nitrous oxide (N_2O). Finally, nitrous oxide reductase (*nos*) reduces N_2O to dinitrogen gas (N_2). The gene that codes for the N_2O reductase is commonly used as the marker for denitrifying bacteria as it is the last in the chain and defines N_2 as the end product of the denitrification sequence. Furthermore, without N_2O reductase, the final denitrification product would be nitrous oxide, a powerful greenhouse gas (Lashof and Ahuja 1990).

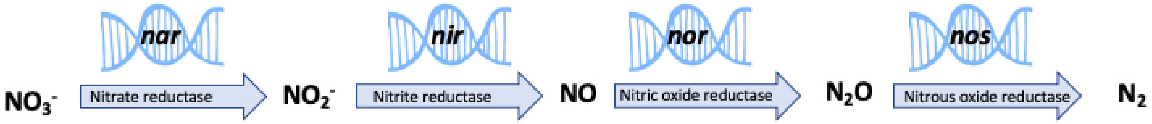

Figure 3. Genes and enzymes in the denitrification process.

NITRATE SOURCES AND DELIVERY.

Of course, denitrification cannot occur without (oxidized) reactive nitrogen in the system. Agricultural activity on the Eastern Shore provides many opportunities for nitrate to enter local groundwater. Reactive nitrogen as fertilizer is applied to agricultural fields primarily as anhydrous NH_3, which is usually nitrified to NO_3^-. Common agricultural practices work to limit denitrification in the field soils so that the nitrogen stays largely as NO_3^- , the favored form for crop uptake (Seitzinger et al. 2006). About half of the applied nitrogen goes into the crops, and the other half is lost to the environment, either the atmosphere or local water bodies (Galloway et al. 2004). An estimated 2.02×10^6 kg of reactive nitrogen is applied as fertilizer annually on the Eastern Shore as a whole (Johnson 2018).

While riparian buffers are instrumental in the remove of nutrients, particularly NO_3^-, from agricultural water (Hill 1996, Hill et al. 2000, Lowrance 1992, Machefert and Dise 2004, Pavel et al. 1996), this can only occur if the nutrient-rich water comes in contact with the active root zone of the plants. Alternatively, water can be forced into deeper flow paths, thereby completely bypassing the biologically active layer within the riparian zone that includes plant roots and active microorganisms (Gold et al. 2001, Vidon and Hill 2004). This second type of flow is more common on the Eastern Shore. This means that for the gaining streams of the Eastern Shore, the opportunities for denitrification must, largely, be realized in the sediments, before the water discharges to the stream, and the water must have sufficient residence time in the biologically active sediment to allow denitrification is to occur (Flewelling et al. 2012, Gu et al. 2008).

An area that provided ample organic matter but contained little to no DO would provide an effective environment for denitrification to occur. Furthermore, different investigators have found that characteristic differences in streams can have profound differences in inorganic N production and removal, making it ill-advised to generalize findings from one location to another (Alexander et al. 2000).

In Eastern Shore streams, NO_3^- concentrations can increase and decrease even within the same stream or along the same flow path (McFadden 2013). Therefore, individual streams may be subject to different controlling factors making it even more important that streams are studied individually and are not subjected to broad generalizations about denitrification patterns. Determining the factors controlling denitrification on the Eastern Shore an important next step in the effort to protect large bodies of water from eutrophication from nitrogen-fertilizer runoff.

RESEARCH QUESTION

What controls the distribution of denitrification in streambeds on the Eastern Shore of Virginia?

There are three factors known to control denitrification; these conditions are organic matter, denitrifying bacteria, and nitrate. The presence and distribution of organic matter naturally leads to another condition that can be included appropriately as a control: hypoxic or anoxic conditions. These controls must co-occur for a long enough period of time to observe measurable denitrification. It is reasonable to assume that some bacteria capable of denitrification are present in the sediments of each of these streams, and that they will be in abundance in proportion to the levels of the other controlling factors (Mills et al. 2008). Therefore, it is more important to look at the factors that will influence the activity (or lack of activity) of these bacteria, rather than the numbers of denitrifiers in any sample.

Previous research has shown that denitrification is not evenly distributed among streams (Dunkel 2014, McFadden 2013). To examine how these factors vary along the Eastern Shore, four streams were selected to represent the range of stream types: closed or open canopy and low or moderate topographic relief for the banks along the streambed. At each stream, organic matter and nitrate NO_3^- - in the sediments were quantified, and in each stream, nitrate removal by the sediments was determined in intact cores of sediment in the laboratory.

To quantify organic matter in the stream sediments, sediment cores were taken from each stream site. A full profile of organic matter by depth was created for each stream bed. Additionally, porewater was taken at the same depth intervals as samples for organic matter prior to the organic matter sampling. Composite profiles were then generated for each stream including organic matter, dissolved oxygen, and nitrate concentrations.

The effect of nitrate delivery was examined in a laboratory experiment designed to mimic and exaggerate field conditions to find both NO_3^- removal and potential NO_3^- removal. Artificial groundwater (AGW) (Bolster et al. 1999) with known NO_3^- concentrations was pumped through sediment cores from the streambeds. Nitrate loss from the solution after passage through the core represented denitrification as described by Dunkel (2014).

RESEARCH APPROACH

SITE DESCRIPTION.

The Eastern Shore of Virginia makes up the southern-most portion of the Delmarva Peninsula. The area is intensively cultivated, with 2038 crop fields on the seaward side accounting for 25.3% of the land that is in active crop production (Johnson 2018).

The underlying sediments are Pleistocene-aged, unconsolidated, and range from silty to gravely sands (Mixon 1985, Mixon et al. 1989, Richardson 1992). The unconfined Columbia aquifer underlies the unsaturated zone and carries nearly all the excess agricultural chemicals to nearby streams. Nitrate concentrations have been observed as high as 34 mg NO_3^--N /L (153 mg NO_3^- /L) (Hamilton et al. 1993). The waters of the aquifer are highly oxic and lacking in carbon, preventing nitrogen transformations (Mills et al. 2008). A ridge (the maximum elevation of which is about 13 m) runs down the Eastern Shore from north to south, dividing it into (Chesapeake) Bayside and Seaside portions (Sinnott and Tibbetts Jr. 1968). All four streams studied are on the seaside of this dividing line (Table 1, Figure 4).

Table 1. Location of the four streams

Stream	*Longitude (West)*	*Latitude (North)*	*Contributing Area[a] (Ha)*
Bundick Creek	75° 36’ 23.5’’	37° 45’ 57.7’’	31
Phillips Creek	75° 51’ 11.7’’	37° 27’ 27.7’’	482
Coal Kiln Creek	75° 46’ 12.5’’	37° 33’ 47.2’’	92
Cobb Mill Creek	75° 55’ 15.7’’	37° 17’ 28.9’’	627

[a] Contributing area is the area of a watershed upstream of the point in a stream where measurements are being made. Data are from Aaron Mills (unpublished).

Cobb Mill Creek (CMC) is a well-studied stream near Oyster, Virginia, in the southern part of the peninsula. Cobb Mill Creek drains into Oyster Marsh. It provides a contrast of different conditions all in the same stream. Although the overall watershed that feeds this stream is low-relief, CMC has a variety of bank slopes. Where the stream crosses the Mappsburg Scarp, there is a substantial hillslope leading from an agricultural field down to the stream. Additionally, the land cover dominating different reaches of the stream ranges from active agricultural fields, to forest, to marsh. In the area where sediment was collected for analysis and for NO_3^- removal experiments in the lab, the banks are densely covered with a mix of pines and hardwoods (Figure 5). The stream bed is mostly (>85%) fine and medium sands, with highly variable organic matter content along the stream (McFadden 2013). This site has previously been shown to actively denitrify and it may have the potential to remove more nitrogen than has been observed *in situ* (Dunkel 2014).

Phillips Creek is fed by a relatively small, 92-ha, watershed and drains to the Upper Phillips Creek Marsh. This stream is located near Nassawadox, VA. Its stream bed comprises mostly medium sand, with some silt and clay. Previous work by McFadden (2013) has shown that Phillips Creek has a lower hydraulic gradient than other Eastern Shore streams examined, and, as a result, some stream water may reenter the sediments in a hyporheic exchange, rather than groundwater strictly flowing upward without such exchange as observed in streams with a higher hydraulic gradient. Because organic matter was seen in substantial quantities at depth (60 cm) in the stream bed, McFadden (2013) hypothesized that denitrification occurs deeper in the sediments at Phillips Creek than has been examined previously. Sediment

Figure 5. Locations of Eastern Shore streams that were sampled.

Figure 4. Cobb Mill Creek at the sampling point. The image was taken in the fall and shows the large amount of organic material deposited in the stream at that time.

was collected at Phillips Creek, under a complete canopy similar to that of Cobb Mill Creek (Figure 6).

Figure 6. . Looking downstream at Phillips Creek. This reach is contained in a complete canopy.

<u>Coal Kiln Creek</u>, near Painter, VA, has banks of roughly two meters that drop steeply toward the water. The canopy is open, and the stream is exposed to the sky. However, low bushes and tall plants cover the banks, and the channel is largely filled with grass (Figure 6). There are several active agricultural fields upstream of the sampling site, although not immediately along the bank where sampling occurred. The streambed is soft. Under baseflow conditions, the stream is roughly 1.5 m wide at and around the sampling location.

Figure 7 The streambed of Coal Kiln Creek at the location where cores were collected.

<u>Bundick Creek</u> is the northern-most stream included in this study. Located in Metompkin,VA, it is an exceptionally shallow stream, effectively a ditch, nestled between two active agricultural fields. There is no grass or forest buffer between the stream and the fields; the lack of slope allows farm equipment to be used right along the edges of the stream (Figure 8). The adjacent agricultural fields leave an open canopy over the stream. The streambed is predominately sandy and often has a dense growth

of grasses and other plants filling the streambed. The stream is fed by a small (31 ha) watershed. While the stream is often less than a meter is width, storm events can quickly increase the size of the stream.

Figure 8. Bundick Creek at the point sampled showing the lack of a buffer and the proximity of the agricultural activity to the stream channel.

METHODS

FIELD METHODS.

A drive-point sampler was used to collect water from the streambed sediments and groundwater seasonally from Fall 2017 to Summer 2019. The drive point was a 1.5-m length of 2.54-cm (1-in) diameter steel electrical conduit that had been fashioned into a point at one end by flattening the conduit, removing part of the flattened area, and forming a sharp point with the remaining “flaps” (Figure 9). Four 3-mm holes were equally spaced around the conduit just above the point where the tubing had been collapsed. The drive point was driven into the streambed with a hammer so that at each sample point the small holes were open at the desired sampling depth below the streambed. Once a sampling depth was reached, the sampler was pumped dry two or three times to ensure the source of the sample was at the depth to which the holes in the wall had been driven. If the sampler could not be pumped clear, pumping continued until the water was free of the mud that entered during the driving process. Once a sample had been collected at a given depth, the sampler was driven deeper into the sediment and the sampling process and additional driving was repeated until all the samples had been collected for a given profile.

When the drive point was driven to the desired depth, a water level recorder (Solinst Model 101 P2, Solinst Canada, Ltd., Georgetown, Ont. CA) was used to find the depth to water, and the length of the sampler (top to the openings) and the length of the sampler remaining above the sediment surface were then used to determine the depth of the openings below the sediment surface. Water samples were collected 30, 70, and 100 cm below streambed with a peristaltic pump (Masterflex Model W/S Portable Sampler, Cole-Parmer, Vernon Hills, IL) and Tygon® tubing inserted into the sampler. Each sample for laboratory analysis of anions was pumped through a flow-through analysis cup fitted on a sonde (YSI model XLR, Yellow Springs Instrument Co., Yellow Springs, OH) and was collected in acid washed and thrice-sample-rinsed

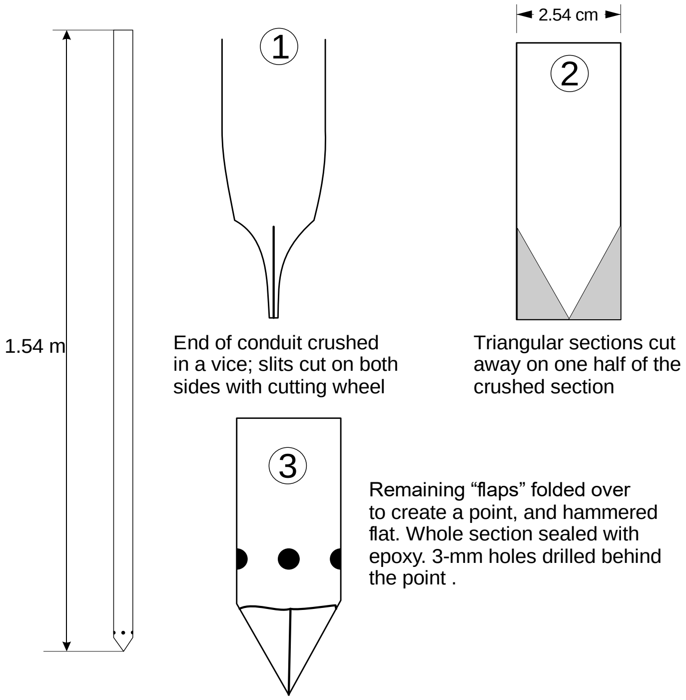

Figure 9. Drive point used to take samples of sediment pore water. Steps in the fabrication are depicted, along with the dimensions.

sampling bottles (Figure 10). Similar vertical sample profiles were taken along the north and south banks as well as the center of the stream at each site.

The sonde was used to record temperature, pH, and DO for the sample to establish *in situ* values. Sample bottles were filled to the brim to eliminate air, then capped and stored on ice until they could be returned to the lab for processing.

Profiles of organic matter in the stream beds were obtained from sediment cores taken from the center of each stream. Two cores were taken from adjacent locations at the center of each of four streams in early April 2019—one core to be used for OM analysis and the other for flow-through experiments. The cores were taken with 1.01-m long (40in), 7.62-cm diameter (3 in), Schedule 40 PVC pipes. Each pipe was sharpened on one end to make insertion into the streambed easier. The PVC was placed sharp-end-down at the desired location and the top end was covered with a wooden board. Then the wooden board was struck repeatedly with a 4.5-kg (10-lb) sledge hammer driving the pipe to the desired depth in the sediment. The depth to the sediment in the core tube and outside the core tube was measured and recorded to account for compression of the sediment, and the tube was filled with stream water. An expansion cap was placed in the top of the core and tightened to create suction so that a pipe wrench could be used to remove the core from the streambed while keeping the sediment in the core intact. A second expansion cap was put into the sharpened,

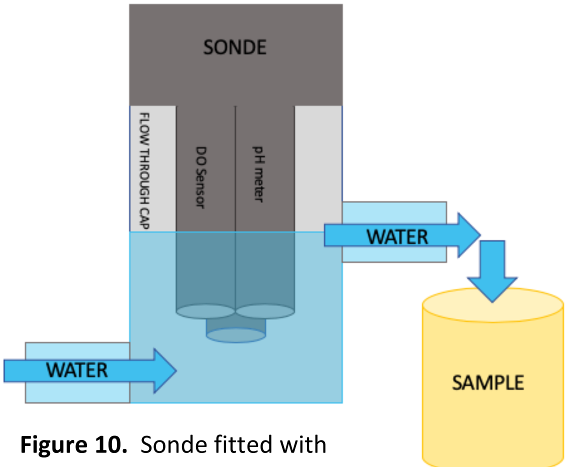

Figure 10. Sonde fitted with flow-through cap.

bottom end of the core. The capped cores were stood upright in a deep bucket of water to avoid overheating and transported back to the lab for analysis. Upon return to the lab, the cores were divided into two groups, each group containing one core from each of the four sites. The first set of cores were used for content (OM and NO_3^-) analyses and the second set was set aside to be used in a flow-through experiment.

ANALYTICAL METHODS.

Solute Analysis

Levels of NO_3^- in the stream and in pore waters of each stream were determined from the water samples collected in the field. Once the samples were collected, they were kept on ice or refrigerated at 4° C until analysis. Within 48 hr of return to the laboratory, the samples were filtered through 0.45-μm pore-size glass fiber filters. Concentrations of Cl^- and NO_3^- were determined with a Dionex ICS-2100 ion chromatograph (IC) and ICS Series AS-DV auto sampler, employing a CR-ATC Continuously Regenerated Anion Trap column, an AS-18 anion-exchange analytical column, and an ASRS 300 4mm RFIC self-regenerating suppressor. The AS-18 column uses KOH as the eluent for anion analysis. Before analyzing the samples, the IC was calibrated with a set of standards with known ion concentrations of 0, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 20.0, and 50.0 mg/L of each analyte ion. A full set of standards were run with the samples, both before and after the samples were analyzed, and some of the lower-concentration standards (0.1, and 1.0 mg / L) were also interspersed among the samples.

Organic Content Analysis.

Starting 5 cm from the top of the sediment in each core, a 3-mm hole was drilled through the PVC casing. A 10-mL syringe with a 40-mm, 16-gauge needle was used to obtain a water sample from the sediment at each depth. The pore water was filtered (0.45-µm pore diameter), placed in the refrigerator (4°C) until analysis, and processed in the IC with the other water samples. These samples were taken at 5-cm intervals for one core from each strea*m*.

Once the pore water samples had been collected for each depth, a hole saw was used to create 2.5-cm diameter holes over the existing small holes, which acted as pilot holes. Through these openings, a detipped, 10-mL syringe was used to take a sediment sample at each of the depths (Herlihy et al. 1988). These samples were weighed, then dried in a drying oven at 105°C for 24 hours. They were removed from the oven and weighed again to determine dry weight. The oven-dry samples were then ignited in a furnace at 500 °C for 24 hr , and the organic matter content was determined as the weight loss on ignition. This method of measuring organic matter provided a profile of organic matter content with depth in the stream sediments.

DENITRIFICATION IN SEDIMENT CORES.

One of the collected cores from each stream was used to examine denitrification in the sediments similar to studies done by Gu et al (2007) and by Dunkel (2014). To differentiate the cores used for lab studies from those dissected for solutes and organics, the cores used in the lab will be referred to as columns. For each column, the expansion cap was removed from the top, the water removed, and the PVC cut to within about a centimeter of the surface of the sediment. Glass wool was placed in the top space as a filter to keep sediment from moving during the applied flow. Two 3” (7.5 cm) test caps were prepared by drilling 3-mm holes through the center and inserting small nylon tubing-nipples through these holes. The nipples were glued in place and caulked with Goop® (Eclectic Products, Inc., Eugene OR). One test cap was fitted into the top of the core and secured with Goop®; the other was fitted into the bottom of the core, replacing the expansion cap, and caulked with Goop®. A rubber collar was secured to the bottom of each core using hose clamps and Goop®, providing extra support for the bottom cap. Starting 5 cm from the surface of the sediment, 3-mm holes were drilled into the core at 5-cm intervals until the top of the rubber collar at the bottom was reached. Needles with stopcocks were inserted into each of these holes and sealed with Goop® so that pore water could be sampled. (Figure 11). Once the Goop® had cured, each core was fastened to a wooden frame in an upright position using hose clamps. The nipple in the bottom cap served to allow inflow to the bottom core and the top nipple allowed outflow to be collected.

The pore volume of a core was determined by assuming a uniform porosity of $\Phi = 0.33$ for each core, a value that is similar to that used by others working in Eastern Shore stream sediments (e.g., Flewelling 2009, Gu 2007). The bed volume of each column was calculated from the interior diameter of the pipe and the length of the collected sediment in the pipe as measured once back at the lab. The pore volume was then estimated as 1/3 of the total volume. The average residence time for the cores was determined as the pore volume (L^3) divided by the discharge (L^3/T) (Equation 1), and it averaged 1360 minutes (23.7 hr) with an average discharge of 0.52 mL/min.

$$Retention\ time = \frac{Pore\ Volume\ [L^3]}{Flow\ rate\ [L^3/T]} \qquad \text{Equation 1}$$

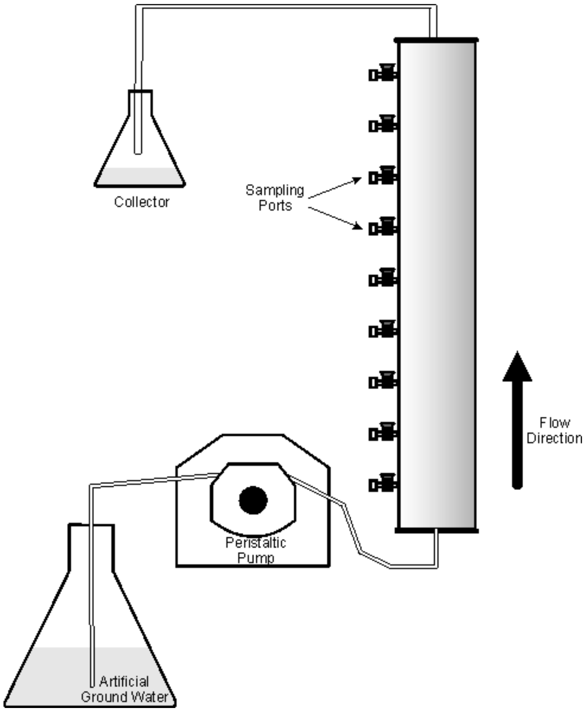

Figure 11. Schematic view of sediment columns showing arrangement of sampling ports, 5cm apart.

An artificial groundwater solution (AGW) was pumped upward through the upright column at the lowest pump setting (1.6).The formulation of the AGW solution was that of Bolster et al (1999) with the amount of NO_3^- adjusted to a predetermined concentration (63 mg/L NO_3^- on the IC). The AGW contained per liter of deionized water, 60 mg $MgSO_4 \cdot 7H_2O$, 36 mg $NaHCO_3$, 36 mg $CaCl_2$, 20 mg KNO_3, and 25 mg $CaSO_4 \cdot 2H_2O$. Columns were pumped for a minimum of three days without any leaks before being sampled (enough time for three full pore volumes to pass the column) to ensure the column reached a steady state. Once the column had reached steady state, sampling was conducted. Small samples (< 5 mL) were withdrawn using a clean syringe from each of the stopcocks (sampled top to bottom) to obtain values of NO_3^- for generation of NO_3^- profiles and calculations of NO_3^- removal (ΔNO_3^-) in the columns. Samples from the outflow flask were also collected from the Cobb Mill Creek column for NO_3^- analysis.

Data Analysis.

Removal of NO_3^- in cores and columns was taken as a direct indicator of denitrification (Dunkel 2014, Gu et al. 2008). Removal of nitrate (termed ΔNO_3^-) was calculated for each core as the difference between the inflow (known) concentration and the outflow (measured) concentration using Equation 2.

$$\Delta NO_3^- (Nitrate\ Removed) = (NO_3^-)_{inflow} - (NO_3^-)_{outflow} \qquad \text{Equation 2}$$

Denitrification in the columns was determined as the loss of nitrate as the AGW passed through the column, i.e., ΔNO_3^- for the entire column.

Statistical analyses, including regression, tests of differences of means, and principal components analysis were performed in R and/or SPSS.

RESULTS

STREAM SEDIMENTS & CORES

Organic Matter in the Sediments

Organic matter in cores collected from each of the four streams varied among the streams and with depth in the sediment (Figure 12). Bundick Creek had the least organic matter of the four streams and also the least variable distribution (Figure 12). Organic matter ranged from 0-6% with a mean of 1.9% dry weight (Table 2). As might be anticipated, OM content was greatest at the surface. Coal Kiln Creek had more organic matter than did Bundick Creek and the OM content was more variable. OM varied from 0-21% with a mean of 6.9% of the dry

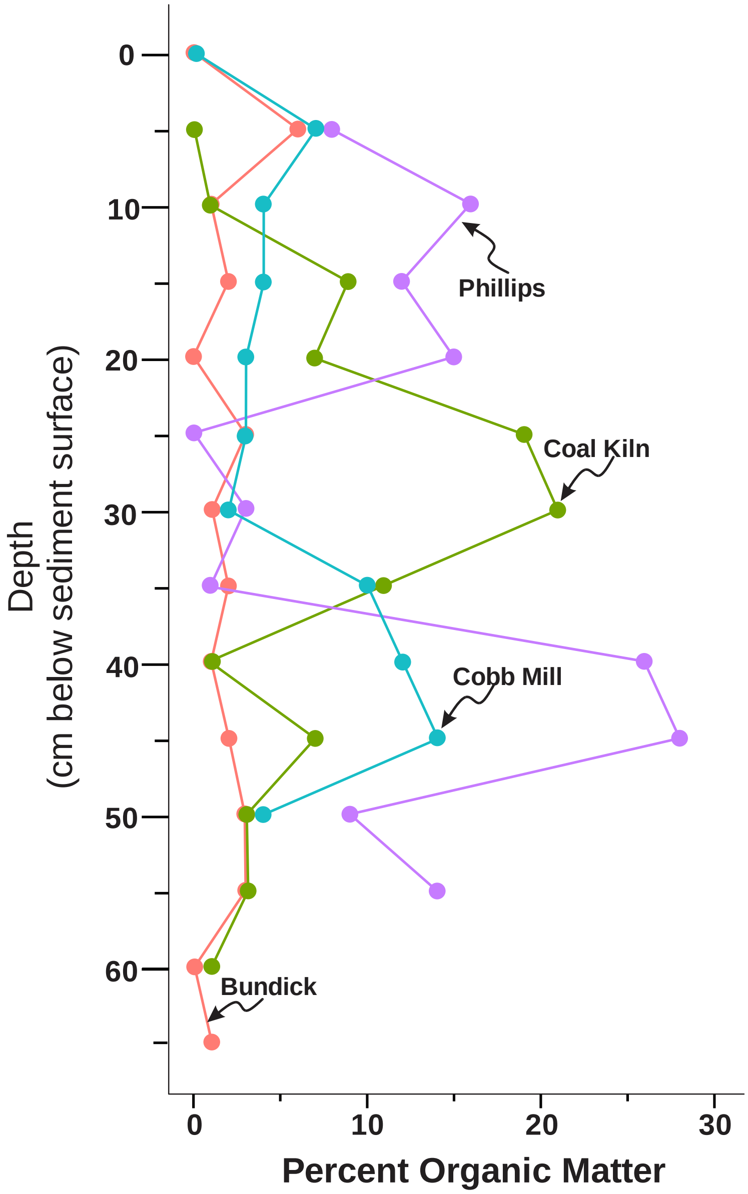

Figure 12**.** Organic matter as percent of dry weight. Samples were taken every 5 cm through a core from each of the four streams.

weight (Table 2). OM increased gradually from the surface downward to a maximum of 21% at 30 cm below the streambed and then decreased with depth below 30 cm (Figure 12).

Cobb Mill Creek sediments had organic matter concentrations similar to those of Coal Kiln Creek. The mean was also 6.9%, although there was less variability, with a range of 2-14% of dry weight (Table 2). Organic matter was greatest at 45cm below the streambed. From there, OM decreased to 30 cm then increased again towards the surface (Figure 12).

Phillips Creek sediments contained the highest concentration of organic matter of the four streams examined. OM content ranged from 0-28% with a mean of 12% of dry weight (Table 2). OM in these sediments was greatest at depth and generally decreased towards the surface with the maximum OM content occurring 45 cm below the streambed surface (Figure 12).

Table 2. Organic matter (expressed as percent weight of dry weight) in sediment cores. Mean values are the average of organic matter content determined at 5-cm intervals in the core.

	Bundick Creek	*Coal Kiln Creek*	*Cobb Mill Creek*	*Phillips Creek*
Mean	1.9	6.9	6.9	12
Standard Deviation	1.5	6.8	4.6	8.8
Max	6	21	14	2.8
Min	0	0	2	0

Effect of Canopy Cover on OM in the sediments

Bundicks and Coal Kiln both have open canopies and Cobb Mill and Phillips both have closed canopies over the sampling area (Figures 5-8). The mean OM content for Bundicks and Coal Kiln Creeks was 1.9% and 6.9%, respectively, and for Cobb Mill and Phillips Creeks, OM as percent of dry weight was 6.9% and 12% respectively.

The average OM for the open-canopy streams was 4.3% and for that of the closed-canopy streams was 9.8%. A t-test that compared OM content between the open-canopy and closed-canopy streams showed that the difference between the two groups (for OM content over the entire core length) was statistically significant ($p = 0.012$)

Dissolved Oxygen in the Sediments

Oxygen content was difficult to measure reliably using the sonde connected to the drive-point sampler, and many of the results were completely unrealistic. Several values obtained at

Phillips Creek and Cobb Mill Creek were extremely high (e.g. 14 to 15 mg O_2 /L) and seem useless. Because results from those streams were so unreliable, DO data are only reported for drive-point porewater samples from two streams (Table 3). The numbers obtained for these streams suggest that the oxygen concentration in the sediment at or below 30 cm was around saturation. The water temperature in the groundwater was about 15°C which corresponds to a saturation value of 10.07 mg O_2 /L.

Table 3. Dissolved O_2 (mg O_2/L) in stream and sediment pore water taken in the field using a peristaltic pump, drive point, and sonde.

	Bundick Creek	*Coal Kiln Creek*
Stream (0cm)	5.5	8.65
Shallow (30cm)	10.6	8.95
Medium (70 cm)	10.6	9.54
Deep (100cm)	9.71	8.07

Nitrate and Chloride in Pore Water Extracted with the Drive-Point Sampler

Pore-water sampling was conducted with the drive-point device near each bank (denoted as North and South) and in the center of the channel to establish *in situ* concentrations and to assist in selecting streams from which to collect cores (Table 4). Data were not obtained for Phillips or Cobb Mill Creek due to the inability to insert the drive point into the sediment or to withdraw water through the drive point. Bundick Creek showed a relatively stable NO_3^- concentration through the sediment. Nitrate in Coal Kiln Creek was low in concentration throughout the profile in the north-bank and stream center samples, but NO_3^- concentrations were higher in the south-bank profile than in the other two profiles. The surface water concentration of NO_3^- exceeded the concentration in the sediments of the north-bank and stream-center profiles, whereas concentrations obtained at 30 and 70 cm below the sediment surface in the south-bank profile exceeded the stream water concentration. Chloride concentrations in Coal Kiln Creek sediments were also largely uniform from a depth of 1 m to the sediment surface. The stream water concentration of Cl^- was slightly higher than was observed in the pore water.

Table 4. Porewater samples were collected using a drive point at Bundick and Coal Kiln and analyzed for NO_3^- and Cl^- concentrations. All ion concentrations are reported in mg/L of the named ion. 0-cm depth refers to stream water.

Depth (cm)	*Bundick Creek*		*Coal Kiln Creek*	
North Bank	NO_3^-	Cl^-	NO_3^-	Cl^-
0	8.76	38.1	10.19	17.9
30	7.07	28.5	2.58	14.0
70	7.42	29.9	0.36	11.9
100	9.86	30.6	0.38	13.4
Center of Stream	NO_3^-	Cl^-	NO_3^-	Cl^-
0	8.76	38.1	10.19	17.9
30	9.94	30.0	1.8	15.7
70	9.6	30.9	0.22	15.3
100	8.84	30.9	0.13	14.8
South Bank	NO_3^-	Cl^-	NO_3^-	Cl^-
0	8.76	38.1	10.19	17.9
30	8.18	29.8	11.6	18.5
70	5.9	29.6	16.7	11.5
100	9.86	25.5	0.97	15.1

In sediments of Bundick Creek, the NO_3^- concentrations were higher than those of Coal Kiln Creek (although the surface-water concentrations were quite similar). The concentrations observed were generally uniform throughout the profiles with higher concentration seen in the stream water than in the pore-water. The three profiles were similar for all the sampling locations in the stream. Chloride in Bundick Creek was substantially higher than observed in Coal Kiln Creek, and the difference between the NO_3^- concentration and Cl^- concentration in Bundick Creek was much greater than seen in Coal Kiln Creek.

Nitrate in Sediment Cores used for Organic Matter Analysis

Nitrate concentrations were determined in samples of pore water withdrawn from the same sediment cores as were used for organic matter analysis. In some cases, particularly Cobb

Mill Creek, water could not be obtained from the sediment with a syringe and needle, and so data from locations where water could not be withdrawn are missing. Bundick Creek had a mean concentration of 4.4 mg NO_3^-/L with a range of 1.1 to 18.4 mg /L. The highest concentration of NO_3^-/L, was at 65 cm below the streambed, the deepest point sampled. The concentration declined sharply to 3.1 mg NO_3^-/L at 55 cm and was thereafter relatively constant to the surface (**Error! Reference source not found.**).

Coal Kiln Creek had a mean concentration of NO_3^- of 1.1 mg/L with a maximum of 6.0 mg/L and a minimum of 0.1 mg/L. Through the length of the core, NO_3^- was nearly constant at less than 1 mg NO_3^- /L except for the 40-cm depth, where a concentration of 6.0 mg NO_3^-/L was observed.

The Cobb Mill Creek core was difficult to extract water from, and only two samples could be obtained. In both samples (20 cm and 5 cm depth), the NO_3^- concentration was 1.0 mg/L.

Samples could not be withdrawn from the Phillips Creek core below the 25-cm interval. In the samples that were obtained, the pore water had a mean of concentration of 3.4 mg NO_3^- /L with a minimum of 0.6 mg/L and a maximum of 6.7 mg/L seen at 15 cm below the streambed surface. The concentration of NO_3^- was relatively consistent throughout the core (**Error! Reference source not found.**).

Dependence of NO_3^- Concentration on Other Measured Variables in Cores

Multivariate regressions were performed for NO_3^- concentration against OM, Cl^-, and depth for all the streams (Table 4), and for the core from each stream (except for Cobb Mill Creek and Phillips where not enough data points were available for the analysis) (Table 5). All The data reported include an R^2 for the regression equation and significance of the equation and the individual coefficients.

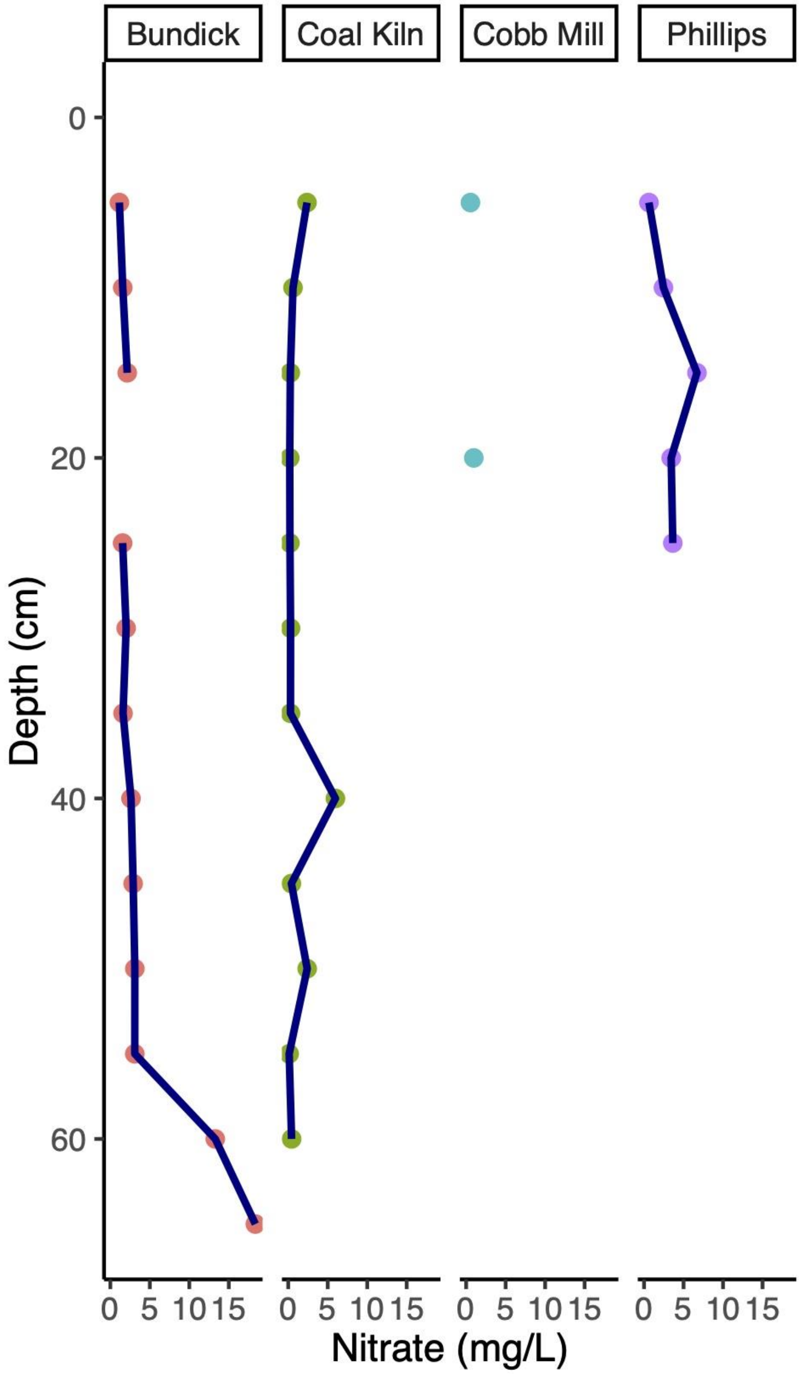

Figure 13. Nitrate concentrations from sampled pore water in collected cores. Pore water was sampled every 5cm from a core taken from each of the four streams.

Because a predictive model was not sought, the regression equations are not given in these results; however, the equations with their coefficients are given in tables included in Appendix A. Reported here are the standardized coefficients. Standardization is done by expressing the coefficients in Z space. That is, the units of the coefficients are standard deviations, rather than the native units. Doing this standardization removes the effect of units of different scale and magnitude so that direct comparison of the coefficients may be made. Such an approach is useful for comparing the coefficients to determine the relative importance of the variable for each coefficient to the dependent variable.

Table 5. Regression data and significance for regression models of sample cores. Significant relationships ($p < 0.1$) are bolded. Coefficient refers to standardized coefficients used to compare across factors with different units.

Component	*Standardized Coefficient*	*Significance*
Bundick Creek		
Full Model ($R^2 = 0.905$)		**0.000**
Cl^-	0.685	**0.000**
Depth	0.816	**0.000**
OM	-0.299	**0.041**
Coal Kiln Creek		
Full Model ($R^2 = 0.594$)		**0.055**
Cl^-	-0.795	**0.023**
Depth	-0.351	0.212
OM	-0.147	0.575

In Bundick Creek the regression coefficients suggest there was a significant positive relationship between NO_3^- concentration and depth, and a significant negative relationship between NO_3^- and OM. The value of this regression is also seen in that all of the coefficients in the equation were statistically significant for Bundick.

The full model results for Coal Kiln regression were not as strong as those of Bundick Creek; R^2 values were 0.905 and 0.594 for Coal Kiln and Bundick Creeks, respectively. The coefficients for Cl^- and depth were oppositely signed from their Bundick Creek counterparts, and the Cl^-

coefficient was the only one that was significant. However, **Error! Reference source not found.** shows that the NO_3^- concentration changed very little with depth in the core, such that strong relationships among the variables might not be expected.

With NO_3^- as the dependent variable using all the data from all four of the cores produced understandable results (Table 6). Aggregating the data allowed for the inclusion of values from Phillips and Cobb Mill Creeks. The regression had a modest R^2, but result was significant. The coefficients obtained for both Cl^- and depth were also significant. The coefficient for organic matter was very small and not significant.

Table 6. Regression data and significance for regression model of data from all cores. Significant relationships ($p<0.1$) are bolded. Coefficient refers to standardized coefficients used to compare across factors with different units.

Component	*Coefficient*	*Significance*
Full Model (R^2 = 0.33)		**0.004**
Cl^-	0.447	**0.012**
Depth	0.516	**0.004**
OM	-0.037	0.828

Dependence of NO_3^- Removal (Denitrification) on Other Measured Variables in Cores

Principal Component Analysis (PCA) was performed to attempt to understand how these factors combine to influence denitrification. As with the last regression for the aggregated data above (Table 6), PCA analysis included core data for all the streams. The analysis applied to all the cores from all sites generated two components that together explained 73% of the variance in the data (Table 7). The first principal component (PC), PC1, comprised NO_3^- which had a positive component loading of 0.855 (Table 8), Cl^- at a loading of +0.612, and OM with a negative loading of -0.595. Component 2 mostly comprised Depth with a coefficient of 0.850.

Chloride also loaded somewhat strongly at a value of -0.598. Once again, the relationship between organic matter and NO_3^- concentration was negative, like the regressions that were run on these same data.

Table 7. Variance explained by the extracted principal components.

Component	*% of Variance Explained*	*Cumulative % of variance explained*
1	40.5	40.5
2	32.6	73.1
3	18.8	91.8
4	8.1	100

Table 8. Factor loadings on the first two extracted principal components.

	PC	
	PC1	*PC2*
Depth	0.402	0.850
NO_3^-	0.855	0.290
Cl^-	0.612	-0.598
OM (avg)	-0.595	0.374

EXPERIMENTAL DETERMINATION OF DENITRIFICATION (NO_3^- REMOVAL) IN EASTERN SHORE STREAM SEDIMENTS

Overall, NO_3^- concentrations were greater in the pumped columns than in the cores (Figure 14) due to the concentration of NO_3^- that was present in the AGW (63mg NO_3^- /L). In the Bundick Creek column, NO_3^- concentration generally decreased towards the surface (although there were intervals in which NO_3^- seemed to increase substantially), with NO_3^- levels being below detection at 5 and 10 cm.

In the Coal Kiln Creek column, NO_3^- concentration was greatest at the lowest port with 89.6 mg/L NO_3^- and decreased towards the surface with the minimum concentration of 35.8mg/L observed at 15 cm.

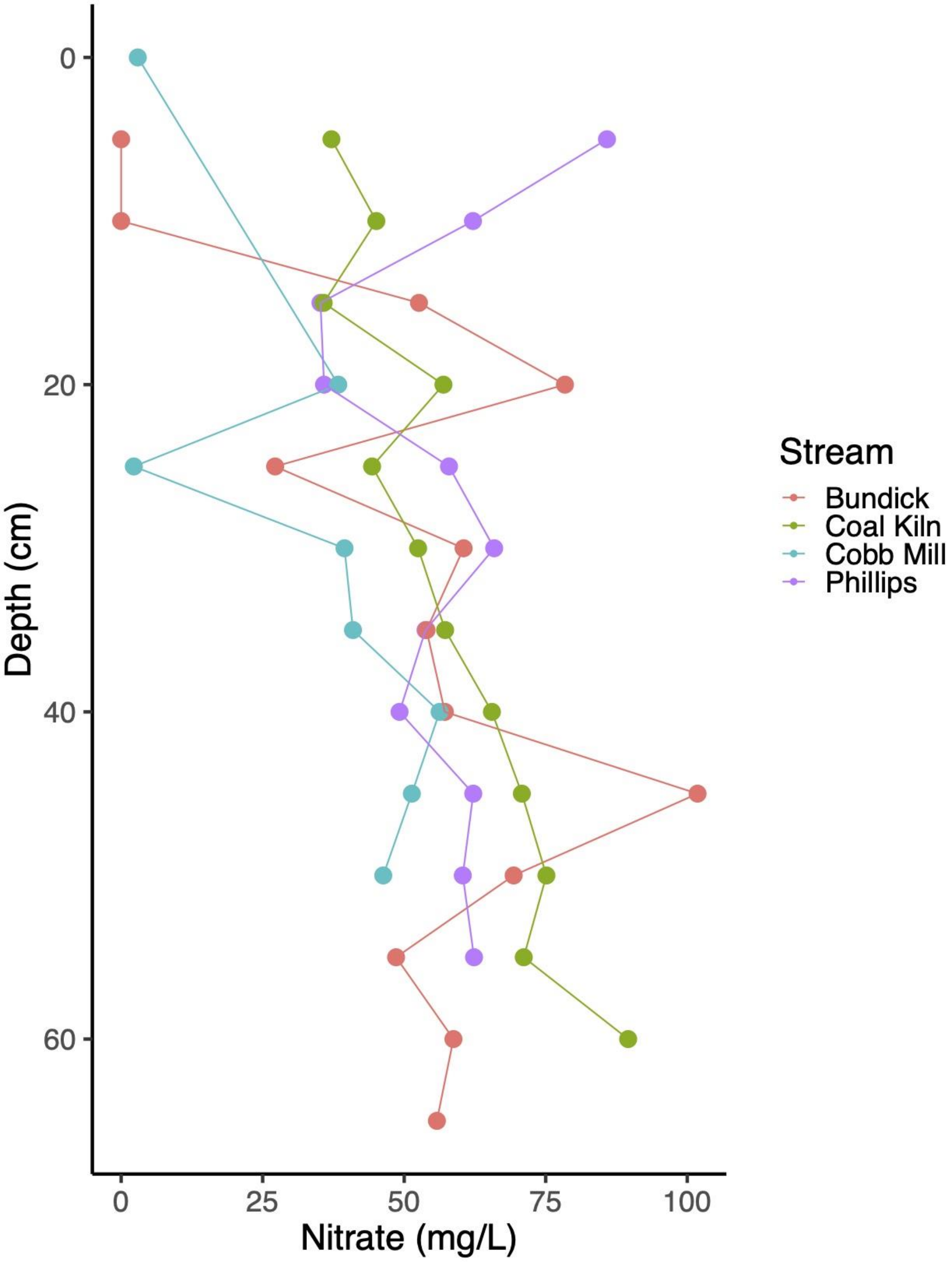

Figure 14. Nitrate concentrations from pore water pulled from cores used for flowthrough experiments. AGW added to bottom of each column had 63 mg NO_3^-/L.

The Cobb Mill Creek column had an overall decrease in NO_3^- mg/L from the base to the outflow. The highest concentration of 56.3 mg NO_3^-/L was observed at 40 cm below streambed and the lowest concentration of 2.2 mg/L was observed in the outflow.

The Phillips Creek column yielded an overall increase in NO_3^- from the bottom to the surface with the maximum concentration of 163.5 mg/L in the outflow; however, NO_3^- decreased from 62.3mg/L at the lowest port to a minimum of 35.2 mg/L at 15 cm. The subsequent increase in concentration in the top 15 cm of the column as the AGW moved toward the outlet cannot be explained.

Because the NO_3^- concentrations in the outflow of most of the columns were unrealistic compared to the concentration of NO_3^- added to the base of the column. The data from the outflow has been ignored. Additionally, it is not clear why several of the columns had initial concentrations of NO_3^- that were well above the input concentration, nor why large increases in NO_3^- were occasionally seen in the columns. Because of these anomalous observations, the ΔNO_3^- for the entire core was calculated on the basis of the value obtained at the 5-cm sampling port, except for Phillips Creek, and there the 10-cm value was used, and the input value was taken to be the concentration at the first sampling port after the inlet. Using that approach, the NO_3^- concentration in Cobb Mill Creek decreased by 93.22%, Coal Kiln lost 58.53%, Phillips lost 0.25%, and 100% of the NO_3^- added to the Bundick Creek column was removed as the AGW passed through the column. It is possible that the very high concentrations of NO_3^- observed for some columns at the outlet, e.g., Bundick Creek, arose because the receiving flask for the outflow allowed for evaporation of the water, thus increasing the concentration of ions in that flask. It may also be that some ammonium was released during the decay of the organics in the sediments, and nitrification of the ammonium in the receiving flask added nitrate above the original concentration. It is unknown, however, if either of these processes were active in any of the sediment column systems.

Dependence of NO_3^- Concentration on Other Measured Variables in Columns

Multilinear regressions for NO_3^- (the dependent variable) *vs.* OM, depth, and Cl^- had moderate to high values of R^2, which indicated that the regression explained much of the variance in the data (i.e., the equation was a good "fit"). Although the majority of the regressions using aggregated data from all the streams were significant, Individual variables within those regressions (*viz.*, OM, depth, Cl^-) often were not (Table 9).

In Phillips Creek, the regression had a generally poor fit. Chloride had the greatest impact on NO_3^-, followed by OM, then depth. However, none of these relationships were significant. Chloride showed a positive correlation, OM had a negative correlation, and depth had a correlation nearly equal to zero.

Bundick Creek had a moderately good regression fit. Chloride had the strongest positive relationship with NO_3^-, followed by depth. OM had the weakest relationship with NO_3^- , and this was, again, a negative relationship. None of the coefficients were statistically significant.

The fit to the Cobb Mill Creek was especially strong (R^2=0.985). However, this model was primarily driven by a very strong positive response with Cl^-. Depth had a moderate positive response with NO_3^-, and the coefficient indicating the dependence on OM was very small and not significant, but once again the response was negative.

A regression performed on the variables from columns of all four streams yielded a reasonable (and significant) fit that was predominately driven by Cl^- and depth (Table 10).

Table 9. Regression data and significance for regression models for NO_3^- of flowthrough columns. Significant relationships ($p<0.1$) are bolded. Coefficient refers to standardized coefficients used to compare across factors with different units.

Component	*Coefficient*	*Significance*
Bundick Creek		
Full Model (R^2=0.487)		**0.098**
Cl^-	0.354	0.232
Depth	0.299	0.311
OM	-0.258	0.339
Coal Kiln Creek		
Full Model (R^2=0.916)		**0.000**
Cl^-	0.036	0.750
Depth	0.907	**0.000**
OM	-0.191	0.119
Cobb Mill Creek		
Full Model (R^2=0.985)		**0.003**
Cl^-	0.856	**0.002**
Depth	0.257	**0.060**
OM	-0.006	0.950
Phillips Creek		
Full Model (R^2=0.102)		0.849
Cl^-	0.273	0.149
Depth	-0.034	0.930
OM	-0.104	0.795

Table 10. Regression data and significance for regression model of all four laboratory columns. Significant relationships ($p<0.1$) are bolded. Coefficient refers to standardized coefficients used to compare across factors with different units.

Component	*Coefficient*	*Significance*
Full Model (R^2 = .417)		**0.000**
Cl^-	0.449	**0.001**
Depth	0.413	**0.002**
OM	-0.057	0.643

DISCUSSION

The objective of the research reported here was to examine factors that control denitrification in streambed sediments of Virginia's Eastern Shore to understand better how denitrification might be distributed among the many streams found there. As pointed out by McFadden (2013), many of the streams on the Eastern Shore are poised for substantial denitrification, but not all of them seem to be denitrifying. In the present study, removal of NO_3^- was examined along with sampling for the distribution of NO_3^-, organic matter, Cl^- and dissolved oxygen in sediment pore water both in the field with the drive point, and in the lab in water extracted from intact cores. Additionally, laboratory experiments, in which an artificial groundwater solution of standard composition was passed through intact cores, was used to investigate the potential for the sediments to denitrify whether denitrification was indicated in the field or not. Finally, data collected from these exercises were analyzed using multi-linear regression and principal component analysis for comparison of the contribution of each of the variables examined to the distribution of NO_3^- and to the removal of NO_3^- in the sediments.

Multivariate regression analysis can be used to generate models that predict a variable based on the expression of other variables in a system. The approach can also be used to examine the relative importance of driving variables on an outcome (dependent variable). In this work, the relative dependence of NO_3^- and Δ NO_3^- on OM, depth, and Cl^- was sought with less interest in generation of a predictive model.

Two coefficients are generated for each independent variable in a regression. The first, Beta (β), is the coefficient one would use in generating a predictive equation. Differences in scale for units of independent variables may greatly skew these coefficients so they are not useful for direct comparisons (although they are appropriate for determination of the contribution of the variable to the overall regression slope). Standardized Beta on the other hand accounts for the varying magnitudes of units for each of the independent variables (these values are created such that they all have the same units, i.e., standard deviations, so they are directly comparable one with another). Standardized Betas are, therefore, useful for comparisons of influence on the dependent variable among independent variables.

PCA was only done for the cores, not the columns, as the columns were artificially pumped and would not allow for truly independent components to be generated. The two major components generated by the PCA for sampling cores explain a good deal (73%) of the variance in the concentration of NO_3^-. PC1 shows the direct relationship between NO_3^- and Cl^- and inverse relationship between NO_3^- and OM. Both of these are expected. PC2 shows the direct relationship between NO_3^- and depth, meaning that NO_3^- concentrations generally increase with distance from the sediment surface.

The change in nitrate concentration (representing the amount of N denitrification) was regressed against each of these two PCs individually. Neither regression had a good fit ($R^2 < .4$), indicating that neither of the two components satisfactorily explained the variance in ΔNO_3^-. While the PCA regressions have no statistical significance, they are consistent with the overall patterns of denitrification (ΔNO_3^-) observed in both cores and columns. Finally, a multilinear regression of ΔNO_3^- against both PCs produced no useful results.

In some flowthrough cases, NO_3^- concentrations were greater than that in the AGW. Although nitrogen might be added to the discharging AGW through organic matter decomposition, the amount of N needed to produce the increases in NO_3^- seen could not account for the levels of NO_3^- that were observed in some of the columns. Furthermore, the product of organic decay is NH_4^+ which would need to be nitrified to NO_3^- to account for the observed increases. Given that there was some denitrification observed in the columns, the sediment in the columns were, by necessity, anaerobic, conditions that would preclude the necessary nitrification.

STREAM OVERVIEWS

How cores and columns align across streams

Overall, Bundick had the least OM of any of the four streams. While the field core indicated that denitrification (i.e., NO_3^- loss) was greatest between 65 and 55 cm, the pumped column showed the greatest loss of NO_3^- (denitrification) from 20 to 10 cm. Neither of these coincided with a depth that was richest in OM. Field measurements from Bundicks and Coal Kiln both had DO concentrations that were above saturation conditions (>10.07 mg/L). It is safe to consider the groundwater and deep pore water (i.e., below about a meter) at these streams to be oxic (Gu et al. 2007), however the exact values reported here are not reliable.

In both the core and column from Coal Kiln, denitrification was observed. The core showed denitrification from 40-35 cm that corresponds with the low DO concentrations of 1.23-4.62 mg/L. However, this is slightly deeper than the layer of abundant OM between 20-35 cm. Previously, a moderately negative relationship between Cl^- and NO_3^- was observed at Coal Kiln (McFadden 2013).Consistent with McFadden's findings (McFadden 2013), nitrate concentrations increased and then decreased along the depth of both the column (Figure 14) and the core (Figure 13) .

Overall, NO_3^- concentrations in Phillips Creek sediments were similar throughout the length of the core and column (Figure 13, 14). There was little evidence of meaningful denitrification in the column experiment, and there was only a small decrease in NO_3^- in the core from 15-5 cm. These observations are consistent with those of McFadden (2013) for Phillips Creek; he also observed that while conditions for denitrification existed, little activity was seen. The uniform NO_3^- profile in these sediments may be because a lens of fine-textured impermeable material in the Phillips Creek streambed precludes water movement upward through the sediment. This assertion is supported by the observation that the upward head gradient in these sediments is roughly equal to 0 (McFadden 2013) , and it may, therefore, be insufficient to overcome the low permeability, especially given the low slope seen for the banks at Phillips. .

Significant NO_3^- loss was observed in the column of sediment from Cobb Mill Creek. Most of this loss occurred from 20 cm to the surface. Unfortunately, no porewater samples could be obtained during that interval, so finer detail for NO_3^- could not be acquired. Cobb Mill Creek remains the most studied stream of the four considered here, and the data presented here are consistent with earlier findings (e.g., Galavotti 2004, Gu et al. 2008, Gu et al. 2007, Mills et al. 2008)

CORE REGRESSIONS

Of the two streams that had enough data points to perform regressions on their cores, only one model had very good fit, (Bundick, R2 = .905). Coal Kiln had goof fit (R2=. 594) but with only one statistically significant coefficient. The significant relationship was between NO_3^- and Cl^- in the Coal Kiln Creek sample core.

Compared to the model for the Bundick column, which had moderately good fit (R^2=.487) but no significant relationships, the model for NO_3^- concentration in the core from Bundick Creek was predominately driven by depth below the streambed surface (Table 5). In both core and column, depth had the largest impact on NO_3^- concentration, though the coefficient was not significant in the model for the column. In tandem, these models suggest the importance of anoxic conditions in denitrification in Bundick sediments. Field measurements show that the groundwater that feeds Bundick Creek is oxic as it enters the OM-rich sediments. This pattern of oxygenated groundwater seems to hold across the Eastern Shore (ESVA) based on field observations collected as background research to select the four sites studied here (Table A-5). However, robust DO data are not available for the four streams here to confirm this for the four sites in question.

The significant relationship between Cl^- and NO_3^- in the cores is interesting, because Cl^- was not added to the cores as a tracer, but still appears to act as one. Depth had a positive relationship with NO_3^-. The positive coefficient indicates NO_3^- decreases to the surface, suggesting *in situ* denitrification in the sediments. This is consistent with preliminary field measurements taken of pore water.

In general, water input, as determined by depth and Cl^- concentration, seems to have more of an effect on NO_3^- concentration than does OM. This suggests that OM was present in large enough quantities to sustain the denitrifying microbes on the time scale the cores were transported and stored.

COLUMN REGRESSIONS

In the columns, the amount of Cl^- in samples showed a relationship to NO_3^- concentration. Cl^- had a significant positive association with NO_3^- in Cobb Mill Creek and small positive relationship with NO_3^- in Bundick and Phillips Creeks. Depth also was an important factor in the model's prediction of NO_3^-. It was highly significant, and large, in magnitude as a variable in the model for Coal Kiln Creek, but the magnitude was substantially lower in Cobb Mill Creek and Bundick Creek, although the coefficients were significant in both of those streams. In all of the streams, the coefficient was negative, suggesting that NO_3^- decreased as OM increased. This is expected and suggests a slight limiting effect of OM on NO_3^- removal though not significant. In other words, these negative coefficients suggest that the more OM that is available, the less NO_3^- observed and likely more NO_3^- removed through microbial denitrification. With respect to Cobb Mill Creek, Gu (2007) demonstrated that once the organic-rich layer was reached, there was, effectively, an infinite supply of (dissolved) organics. Gu was able to maintain actively denitrifying columns of Cobb Mill Creek sediment in the laboratory for (at least) several months, and the columns removed NO_3^- at the same rate throughout the experiment until they were dismantled. If OM concentration is well above that which limits the rate or extent of denitrification, a regression model would yield a coefficient near 0, as seen here for Cobb Mill Creek. Column studies such as conducted here have not been previously attempted at the other streams, so a similar interpretation cannot be made for those streams.

A positive relationship of NO_3^- with Cl^- (i.e., a coefficient of high magnitude) suggests that the concentration of NO_3^- depends on flow of groundwater through the column. The AGW was made with standardized concentrations of Cl^- and NO_3^- with the objective that Cl^- could act as a conservative tracer and indicate AGW distribution through the column. Cl^- and NO_3^- are both small, monovalent anions, though NO_3^- is subject to microbial activity and Cl^- is not. In the case of denitrification, Cl^- concentrations should remain relatively constant through the column, while NO_3^- concentrations should decrease along the same flow path. The positive relationship between Cl^- and NO_3^- indicates that residual NO_3^- in the column (NO_3^- that is not removed in a given step), moves along with Cl^- up the column.

A positive relationship of NO_3^- with depth was also observed in three of the four columns. As depth is measured as the distance below streambed, the positive relationship indicates that NO_3^- concentration is greatest where AGW is pumped into the bottom of the column and generally decreases along the flow path to the surface. This is consistent with previous work on the sediments in Cobb Mill Creek and other Eastern Shore streams (Dunkel 2014, Gu et al. 2007, McFadden 2013). The AGW is fully oxygenated when it enters the base of the column. As the solution spends more time in contact with respiring microbes in the sediment, DO is consumed. Once DO is sufficiently diminished, denitrifying bacteria will use NO_3^- as an electron receptor for respiration thereby decreasing the concentration of NO_3^- in the pore water (Gu et al. 2008, Gu et al. 2007).

The profiles of NO_3^- in Figure 14, show that the concentration of NO_3^- remains relatively constant throughout the lower portions of the columns, and then decreases substantially in the top ~1/3 of the columns.

COMPARISON OF NO_3^- DISTRIBUTION IN COLUMNS AND CORES

Because the AGW formulation was higher in NO_3^- concentration than the *in situ* groundwater, the laboratory columns had greater concentrations of NO_3^- than their corresponding cores. For example, (Cosans 2015) found that the groundwater (100 and 150 cm below the streambed surface) at Bundick Creek did not exceed 13.5 mg/L NO_3^- during any season during her study period. This starting concentration is small enough that the sediments of Bundick are not saturated with respect to NO_3^- and suggests that in the field, denitrification in Bundick sediments is limited by NO_3^-.

The greater starting concentration of NO_3^- in columns compared to cores also naturally affected the predominant pattern of NO_3^- in each core-column pair. While most of the cores had NO_3^- concentrations that started low and remained low, NO_3^--enriched columns showed greater NO_3^- loss and variation over their length.

In some cases in the flowthrough columns, the sampled pore water had a greater NO_3^- concentration than the AGW. In most cases, the difference is small (<10%) and could easily be due to some preferential flow paths through the columns or minor sampling error or IC analytical difference. The notable exceptions are Bundick 20 cm, Bundick 45 cm, Cobb Mill Creek 40 cm, and Cobb Mill 45 cm. In Bundick Creek, these NO_3^- increases were not accompanied by corresponding increases in Cl^- concentrations which would be expected if there were concentration of AGW or an analysis error. Theoretically, NO_3^- could be generated by nitrification, but the increase is so great that sampling error is more likely to be to blame for these anomalies. In Cobb Mill Creek there is an increase in Cl^- at 40 cm and 45 cm, corresponding with the elevated NO_3^-. This means that concentration of AGW by preferential flow path is a likely explanation. At 25 cm in Cobb Mill Creek, there is a sharp decrease in NO_3^-. Here, there is also a sharp decrease in Cl^-. Concentrations of NO_3^- and Cl^- at 20 cm and 30 cm are consistent with each other. The small NO_3^- concentration at 25 cm in Cobb Mill Creek is likely a sampling or analytical error and should be disregarded and not considered to be denitrification.

ORGANIC MATTER

The best explanation for the lack of significant relationships between NO_3^- and OM is not that OM is not important in denitrification in these settings. Rather, it is more likely that OM is not limiting in the core and columns examined here, at least between the time that they were collected, transported, and then pumped in the laboratory. Columns with continuous flow of AGW for up to eight weeks showed NO_3^- removal at that time. OM measurements were taken from un-pumped cores, giving an estimate of a starting value for OM. While no OM measurements were taken after pumping, it is unlikely that OM, initially measurable in units of per cent would be depleted in the “spent” columns. Indeed, the model developed by Gu et al. (2007) included organic matter as a term in the multiple Monod formulation. He assumed that the total organic matter present in the sediments represented an infinite pool for driving denitrification, and he controlled concentration of “labile” dissolved organic matter by assuming a that a constant fraction of the organic matter was made available for O_2 consumption and denitrification through microbial action.

Despite the lack of abundant significant relationships between NO_3^- concentration and OM concentration, in every case where such a relationship was developed, the sign on the standardized coefficient was negative. That outcome is not surprising given the important role of organics in microbial respiration that depletes, in turn both O_2 and NO_3^-. The lack of significance for many of the OM/NO_3^- relationships signified by the standardized coefficients does not allow for a quantitative assessment of the role of organic matter, but the unvarying direction of the relationships (always negative) provides clear evidence that organic matter is a primary driver of NO_3^- removal in the sediment of these streams. Indeed, the only explanation for anything other than a strong quantitative relationship is that when a high concentration of NO_3^- contacts a high concentration of OM, both are high. However, in such an instance, it is usually seen, here and in previous studies (Dunkel 2014, Gu et al. 2007), that the NO_3^- disappears quickly when OM is present, and dissolved O_2 has been removed by respiration.

If OM were limiting, stronger, more frequently significant inverse relationships between OM and NO_3^- concentrations would be expected. While standardized coefficients in cores and columns were small in magnitude, the uniformity in direction cannot be overlooked, especially as compared to depth or Cl^-. Coefficients for these variables were often larger than those obtained for organic matter, but the variability in direction suggests that no universal conclusions may be drawn from them that would allow generalization to many other streams. These findings suggest that streams on the ESVA have ample OM to deplete the DO from oxic groundwater and subsequently denitrify even at elevated concentrations of NO_3^-.

CANOPY COVER AND OM

The two open canopy streams, Bundicks and Coal Kiln, had greater OM regression coefficients compared to the closed canopy streams, Cobb Mill and Phillips. The open canopy streams also had smaller P values for the relationship between OM and NO_3^-, though still not significant. This supports the idea that OM did not have significant relationships with NO_3^- because it is not limiting. The open canopy streams had smaller average OM contents, possibly making OM more limiting in those streams compared to closed canopy streams.

It was hypothesized that canopied streams would have greater OM concentrations, especially near the surface, from leaf litter and debris collecting in the stream. It was further hypothesized that the distribution of organic matter in the sediments would be different for the streams with closed canopies as compared with those having open canopies. The open-canopied streams had abundant vegetation growing in the stream channel, at least during the warmer months, and the roots growing in the sediment might be expected to create a deeper organic-rich layer, that when the roots die, would contribute substantially to sub-surface organic matter in addition to that contributed by the parts of the plants above the sediment. Figure 12 shows the organic matter profiles for the four streams, and while the two canopied streams did have the two greatest OM at 5cm of all the streams, the expected pattern of deeper, of high OM content was not observed. Both canopied streams had their greatest OM content between 45and 35 cm. Open-canopied streams have OM higher in the sediments, closer to the surface. On the whole, however, streams under a full canopy have a significantly higher OM content than streams sediments in streams with an open canopy. While the open-canopied streams receive enough sunlight to support vegetation in and around the streambed, the amount of vegetative growth, and thus the infusion of organic matter to the sediment, is much less than in streams where a forest canopy deposits a very large amount of organic material as leaves in the fall. An interesting comparison yet to be made is the difference between deciduous and evergreen canopies. Given that deciduous forests drop leaves largely in the fall, whereas evergreens shed needles throughout the year, a different pattern of organic matter accumulation in sediments might occur. No examination of that assertion was done in this study, as the riparian buffer of both streams comprise a mixture of the two types such that differentiation would be impossible.

ASSUMPTIONS & LIMITATIONS

The column experiment described here assumes a strong vertical flow in the upward direction. Also, flowthrough columns assume that the force of advection be much greater than that of dispersion, meaning that NO_3^- would move upwards rather than laterally through the core. Piston flow, as seen in columns, distributes solutes across the diameter of the column. The only place where uniform distribution of solute would not be observed is in a situation with heterogeneity (cracks, gaps or other preferred flow paths) in any dimension. For example, Phillips Creek column likely had two different flow systems. While some porewater moved slowly upward through the core and had contact time with organic matter for denitrification, other porewater flowed preferentially through macropores where there would be little NO_3^- removal. The sampling method used here would homogenize porewater samples across a given depth, in effect averaging the NO_3^- concentrations from these two systems. While no macropores were directly observed, this idea is supported by observations from content analysis sampling of the core.

In other words, the laboratory conditions created in the flowthrough columns mimic wet, high-NO_3^- conditions that may be observed seasonally on ESVA but would not prevail year-round. The assumption of perfect piston flow is not necessarily met in the streams. This means that water in the streams could enter at shallower depths and move predominately in a lateral direction through the stream beds. Such, alternative groundwater flow paths could change the denitrification potential of streams because nitrate-rich water would have less contact-time with, or completely by-pass, deep OM, which would limit oxygen removal from the groundwater, thereby limiting denitrification.

As both *in situ* groundwater and AGW are oxic, the flowrate must be slow enough for microbial respiration to deplete the DO enough for measurable denitrification to take place (Flewelling et al. 2012, Gu et al. 2007). Past studies (Dunkel 2014, Gu 2007) indicate the importance of pore water velocity on NO_3^- removal as it affects the amount of contact time between denitrifying bacteria and NO_3^- in ESVA streams (Flewelling et al. 2012, Gu et al. 2007). Large amounts of OM at depth make depletion of DO more likely and therefore denitrification subsequently more likely. Longer residence time can also lead to increased depletion of DO through microbial respiration. When there is less DO available, microbes will use NO_3^- , a less favorable electron receptor, for respiration.

The method of core collection allowed for minimal disturbance to replicate *in situ* conditions as closely as possible. Specifically, the core extraction method preserved location and stratification of organic matter and microbial populations through the sediment.

The flow rate used in this study was a specific discharge of 1.56 cm/hr. Gu et al. (2007) reported that denitrification decreases as groundwater flow rates increase from 0.5 to 1.5 cm/hr, but that both of these groundwater-flow rates support greater denitrification than a groundwater-flow rate of 3 cm/hr. This means that the specific discharge of the flowthrough cores in this experiment in the realm for moderate nitrate removal.

IMPLICATIONS, APPLICATIONS, AND GOING FORWARD

These cores show abundant OM deeper than previously seen in ESVA streams (Galavotti 2004, Gu et al. 2007). This study supports McFadden’s (2013) initial findings that OM is present at significant levels well below the top 20 cm of the streambed in Coal Kiln and Phillips Creeks. Additionally, OM is greatest at 45 cm below the streambed in Cobb Mill Creek, indicating a deeper biologically active zone than seen by Gu et al. (2007), although the general pattern of OM distribution and NO_3^- removal is similar in the two studies.

McFadden had hypothesized that some denitrification may have occurred deeper in Phillips than the depth to which he collected cores. While the cores collected for this study were not significantly deeper, the column experiments injected nitrate-spiked AGW between 65 and 50 cm below the streambed. The flowthrough experiments do not rule out a deeper reactive zone in Phillips but do suggest a lower groundwater NO_3^- concentration. This small groundwater NO_3^- concentration may also contribute to the lack of observed denitrification both in the core and column. Similar to McFadden's research, the NO_3^- concentrations in Phillips Creek sediments remained relatively constant with depth in both the column and core.

Each small section of streams examined was not intended to characterize an individual stream's denitrification pattern along its length to the coastal lagoons. Rather, the four stream sections studied, with their differences in topography and canopy cover, are meant to illustrate the range of existing conditions and possibilities for denitrification of streams on the seaside of ESVA. In fact, stream reaches for core collection were chosen to represent open and closed canopy as well as flat and sloped local topography. These factors (canopy and topography) do not have clear implications on OM distribution, as evidenced by OM profiles, on the scale examined.

Conclusions

Not all of the streams examined in this studied exhibited denitrification. Denitrification was observed in the flowthrough columns of three streams and the cores three streams. Denitrification in columns ranged from 100% nitrate removed in Bundick Creek to no NO_3^- removed over the column length of Phillips Creek. This pattern in Phillips Creek was also observed by McFadden (2013) who noted that the stream did not demonstrate meaningful NO_3^- removal despite being "poised for denitrification" with sufficient stream sediment OM and abundant NO_3^- input from groundwater.

The columns that did experience denitrification showed the potential for greater nitrate removal in the flow-through column setting than was observed in the field. This finding is consistent with work by Dunkel (2014) who showed that Cobb Mill Creek microbial denitrification rates were limited by NO_3^- concentration.

The consistently negative regression coefficients associated with OM make a strong case for its importance with respect to denitrification. In each core and column examined, increased OM corresponded with decreased NO_3^- concentrations. While these relationships were not necessarily statistically significant, this pattern was the most consistent relationship observed. Similarly, the PCA analysis showed OM with a moderate, negative contribution to PC1. Again, this demonstrates the inverse relationship observed between NO_3^- concentration and OM content in the cores.

In the laboratory experiment, NO_3^- was added in excess of field background concentrations. While NO_3^- seems to be the limiting factor for denitrification *in situ*, AGW this high in NO_3^- allowed for the influence of other factors on denitrification to be examined. In this case, it makes sense that OM would be the limiting, and therefore a decisive factor in denitrification, particularly because of the effect of OM on removing DO. More available OM leads to depletion of DO in sediment pore water, creating hypoxic or anoxic zones capable of denitrification. The OM-rich sediments also would provide the fuel for bacteria to carry out denitrification. Especially, as NO_3^- input concentration and flow rate were standardized across columns, it is understandable, and expected, that OM would be a prominent controlling factor.

Of course, all of this depends not only on OM being present, but also biologically available. Gu et al. (Gu et al. 2007) made the assumption that a uniform fraction of the total OM present was made available to the denitrifying bacteria over time, and his regression models that incorporated that assumption predicted the distribution of NO_3^- in laboratory columns of sediment from Cobb Mill Creek extremely well. Further work should examine the quality of OM present in sediments to determine if OM from different vegetation (e.g., grass vs. deciduous trees vs. evergreens) contributes to differences in denitrification among ESVA streambeds. As demonstrated here, OM content is significantly different among streams based on canopy cover. Streams under closed canopies had significantly more OM than those with open canopies. This is important because denitrification was not demonstrated to be proportional to OM content in any quantitative sense (such as a regression).

The step-wise nature of the denitrification process is evident in the flowthrough column experiments. Near the bottom of the column, where pore water is still oxic from AGW, no NO_3^- removal is observed. Mechanistically, NO_3^- removal is primarily determined by anoxic conditions, which are generated by microbial decomposition of OM.

More reliable DO measurements are required to confirm the exact locations, and DO concentrations, that allow denitrification to begin in these columns. These measurements would be especially important at the lesser-studied streams in this study: Bundicks, Coal Kiln, and Phillips Creeks.

Finally, the observation of denitrification in these streams supports the assertion that these ESVA streams can be effective filters of agricultural NO_3^- as it is currently applied. However, there is wide variability in denitrification among the streams that wee the subjects of this study, and it is most likely that the scale of variability is no less in the large number of streams not examined. Not all streams will support similar rates or quantities of nitrate removal as others. Given this critical limitation, these results should not be used to justify allowing increased NO_3^- input to ESVA soil and water in order to allow the streams to continue to protect the seaside lagoons from further nutrient enrichment from agriculture.

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APPENDIX – data collected or calculated that were not presented in the body of the thesis

Table A- 1 Equations produced from regressions for NO_3^- on OM, Cl^-, and depth in core pore water. The “All Core” model was developed from aggregated data from all four streams’ cores. Each coefficient shows the contribution of that factor; however, the coefficients are in the native units of each factor and are therefore not able to be directly compared to one another.

CORE REGRESSION EQUATIONS	
Bundick Creek	R^2= 0.905
$Bundick\ Nitrate = -33.4 + (0.306 \cdot Cl^-) + (0.225 \cdot Depth) - (104 \cdot OM)$	
Coal Kiln Creek	R^2= 0.594
$Coal\ Kiln\ \ Nitrate = 16.425 - (0.247 \cdot Cl^-) - (0.034 \cdot Depth) - (3.589 \cdot OM)$	
All Cores	R^2= 0.33
$All\ Core\ NO_3^- = \ -6.501 + (0.07 \cdot Cl^-) + (0.113 \cdot Depth) - (2.583 \cdot OM)$	

Table A- 2 Equations produced from regressions for NO_3^- on OM, Cl^-, and depth in the column experiment. The “All Column” model was developed from aggregated data from all four columns. Each coefficient shows the contribution of that factor; however, the coefficients are in the native units of each factor and are therefore not able to be directly compared to one another.

COLUMN REGRESSION EQUATIONS	
Bundick	R^2= 0.487
$Bundicks\ Nitrate = \ -22.710 + (1.031 \cdot Cl^-) + (0.436 \cdot Depth - (456.919 \cdot OM)$	
Coal Kiln	R^2= 0.916
$Coal\ Kiln\ Nitrate = 23.548 + (0.141 \cdot Cl^-) + (0.830 \cdot Depth) - (44.667 \cdot OM)$	
Cobb Mill	R^2= 0.985
$Cobb\ Mill\ Nitrate = \ -9.256 + (0\ .578 \cdot Cl^-) + (0.418 \cdot Depth) + (2.223 \cdot OM)$	
Phillips	R^2= 0.102
$Phillips\ Nitrate = 47.302 + (1.68 \cdot Cl^-) - (0\ .029 \cdot Depth) - (15.892 \cdot OM)$	
All Columns	R^2= 0.417
$Column\ Nitrate = \ -.862 + (0.537 \cdot Cl^-) + (0.517 \cdot Depth) - (16.604 \cdot OM)$	

Table A- 3 Values of analytes in cores collected to characterize sediment and pore-water analyte concentrations.

Stream	*Depth (cm)*	*Chloride (mg/L)*	*Nitrate (mg/L)*	*OM (%)*
Phillips	5	0.6175	123.983	8
	10			16
	15	2.4556	96.0696	12
	20	6.6978	91.3114	15
	25	3.4319	101.124	0
	30	3.6397	100.124	3
				1
				26
				28
				9
				14
Bundick	5	132.3839	1.147	6
	10	112.5776	1.5735	1
	15	108.8464	2.1386	2
	20			0
	25	94.9146	1.5571	3
	30	100.7062	2.0023	1
	35	94.7869	1.6082	2
	40	94.2254	2.6176	1
	45	93.9309	2.8884	2
	50	93.8334	3.1011	3
	55	95.4446	3.0957	3
	60	112.782	13.2853	0
	65	114.6777	18.3636	1
Coal Kiln	5	60.6906	2.3756	0
	10	59.085	0.6027	1
	15	60.673	0.2607	9
	20	57.533	0.1908	7
	25	64.6874	0.2145	19
	30	59.6394	0.2918	21
	35	54.7808	0.2811	11
	40	43.4731	5.9744	1
	45	50.6617	0.3976	7
	50	53.873	2.4001	3
	55	59.0465	0.1151	3
	60	54.955	0.4239	1
Cobb Mill	5	99.2936	0.5414	0
	10	104.5484		3
	15	100.3432		3
	20	98.8881	0.967	2
	25			10
	30			12
	35			14
	40			4

Table A- 4 Values of analytes in cores used as laboratory columns for examining denitrification (NO_3^- removal) under controlled condition. Note that the percentage of organic matter presented here were actually determined in the cores collected for sediment characterization and are, therefore, identical to the values that appear in **Table A- 3**

Stream	Depth (cm)	Chloride (mg/L)	Nitrate (mg/L)	OM (%)
Phillips	0	178.40	163.52	-
	5	55.8739	85.8214	8
	10	98.7703	62.1498	16
	15	59.0319	35.2134	12
	20	52.3601	35.8424	15
	25	77.9209	57.9181	0
	30	132.4533	65.8967	3
	35	69.5247	53.7696	1
	40	66.2269	49.1815	26
	45	73.8368	62.2006	28
	50	77.3902	60.3518	9
	55	67.6387	62.3374	14
Bundick	0	62.56	221.44	
	5	45.1883	0	6
	10	57.5786	0	1
	15	66.2662	52.6138	2
	20	66.6472	78.3991	0
	25	63.6326	27.1986	3
	30	72.5886	60.4954	1
	35	64.0725	53.9514	2
	40	62.6209	57.18	1
	45	63.6038	101.8216	2
	50	89.5615	69.337	3
	55	64.9581	48.5688	3
	60	63.0792	58.7001	0
	65	67.9473	55.7771	1
Coal Kiln	0	NA	NA	-
	5	85.2413	37.147	0
	10	81.3271	45.0768	1
	15	69.904	35.7699	9
	20	72.0879	56.9327	7
	25	74.8572	44.3276	19
	30	81.1301	52.4587	21
	35	76.1843	57.2338	11
	40	78.5401	65.494	1
	45	77.8391	70.7854	7

Table A-4 cont.

Stream	Depth (cm)	Chloride (mg/L)	\itrate (mg/L)	OM (%)
	50	79.5773	75.1223	3
	55	78.4797	71.1194	3
	60	79.8276	89.5658	1
Cobb Mill	0	55.3781	2.9398	0
	20	71.3069	38.3502	3
	25	4.2033	2.2417	3
	30	56.9051	39.4549	2
	35	55.8944	40.9535	10
	40	85.5423	56.2566	12
	45	73.1547	51.352	14
	50	61.6743	46.3007	4

Table A-5 Values of analytes collected *in situ* to characterize sediment and pore-water analyte concentrations of streams on the Eastern Shore used only for background information in this study.

Depth	**North Bank**		**Center**		**South Bank**	
Tommy's	NO_3^-	Cl^-	NO_3^-	Cl^-	NO_3^-	Cl^-
0	26.56	166.98	26.56	166.98	26.56	166.98
30	7.43	148.75	15.28	160.22	19.49	178.13
70	37.52	134.35	38.15	137.4198	13.45	147.98
100	48.03	134.23	20.10	135.83	7.55	146.18
Frogstool	NO_3^-	Cl^-	NO_3^-	Cl^-	NO_3^-	Cl^-
0	2.10	81.15	2.10	81.15	2.10	81.15
30	.90	107.28	2.90	68.65	2.09	76.70
70	1.19	89.56	0.59	76.96	21.19	45.15
100	0.71	92.51	1.06	80.22	26.23	44.77
Pungo	NO_3^-	Cl^-	NO_3^-	Cl^-	NO_3^-	Cl^-
0	3.76	73.02	3.76	73.02	3.76	73.02
30	0.95	80.81	3.75	70.52	3.54	74.29
70	0.68	86.09	0.75	52.88	2.02	47.40
100	0.79	67.87	0.83	51.97	0.56	43.57
Holt	NO_3^-	Cl^-	NO_3^-	Cl^-	NO_3^-	Cl^-
0	15.61	64.25	15.61	64.25	15.61	64.25
30	8.71	62.30	1.87	56.15	1.46	133.28
70	3.84	61.42	3.11	54.16	18.08	56.23
100	24.53	67.17	5.33	61.40	20.00	58.73