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SEDIMENTS IN LOW-RELIEF COASTAL STREAMS AS EFFECTIVE FILTERS OF AGRICULTURAL NITRATE

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ABSTRACT: The Eastern Shore of Virginia comprises about 35-40% active cropland which receives extensive nitrogen fertilization to support corn, wheat, soybeans, tomatoes and cotton. The unconfined Columbia aquifer contains NO_3^- at concentrations of 8 to 25 mg N L^{-1} . The low relief streams that drain the peninsula into the seaside lagoons, and are fed from groundwater under both base flow and flood conditions contain a wide range of NO_3^- concentrations, 0.5 to 10 mg N L^{-1} , but many of the streams have a substantially lower NO_3^- concentration than the aquifers charging them. In Cobb Mill Creek the change in NO_3^- concentration is from 10-12 mg N L^{-1} in the groundwater to 1-2 mg N L^{-1} in the stream under base flow conditions. Piezometers inserted into this stream indicate the NO_3^- is removed from the water within a zone of 1 m below the stream sediment surface. Removal of these amounts of NO_3^- from the water over such a short distance suggests the mechanism for removal is bacterial denitrification. Enumeration of denitrifiers using molecular genetic methods, probing of the nitrous oxide reductase (*nos*) gene in a dilution-extinction mode, showed substantial numbers of the organisms coinciding with maximum nitrate removal. Acetylene-block measurements yielded the highest denitrification potential coincident with the nitrate disappearance. Organic matter (concentrations up to 20% w/w collocated with the zone of active denitrification) derived from riparian vegetation and buried in the sediments provides ample reducing agent for both NO_3^- reduction and the O_2 consumption that is prerequisite for denitrification. Stream sediments in low relief coastal streams may be a critical filter that removes nitrogen from discharging groundwater, provided there is an adequate input of organic matter from the surrounding riparian zone.

KEY TERMS: denitrification; nutrient flux; coastal stream

INTRODUCTION

Riparian zones surrounding streams are well known for their importance in removing contaminants, especially plant nutrients, from water discharging to local streams (Haycock and Burt, 1993, Hedin et al., 1998, Hill, 1996). Little has been reported on the ability of the stream sediments themselves to serve as an efficient filter for the removal of nutrients from discharging ground water. Removal of NO_3^- can be attributed to two processes, plant uptake, and bacterial denitrification.

Intensive use of nitrogenous fertilizers on agricultural land has led to extensive contamination of water supplies throughout the United States (Winter et al., 1998). Agricultural fields on upland soils fill the drainage waters with fertilizers flowing to streams and other receiving bodies. Nitrate contamination of groundwater and surface water is widespread since nitrate is very mobile in the environment. High concentrations of nitrate can lead to eutrophication, which contributes to the extensive growth of aquatic plants, depletion of oxygen, fish kills, and degradation of aquatic environments (Winter et al., 1998).

Agriculture is the dominant land-use on the small peninsula of the Eastern Shore of Virginia, as it accounts for approximately 50% of the land cover. Typical crops planted are soybeans, corn, and wheat or rye. In addition, tomatoes, cotton, and cucumbers are regionally grown. The hydrogeology of the Eastern Shore facilitates the infiltration of the nitrate from upgradient agricultural farms into the shallow groundwater. The shallow unconfined Columbia aquifer contains NO_3^- levels (10-20 mg N L^{-1}) (Chauhan and Mills, 2002) above USEPA drinking water limits (10 mg N L^{-1}). The flat land surface, dominated by well drained fine-sandy to sandy loam soils, facilitates groundwater contamination and the subsequent transport of nutrients to the streams, which ultimately contributes to nutrient enrichment of the Chesapeake bay and seaside lagoons. A small, low relief stream, Cobb Mill Creek, on the seaside of the Eastern Shore is fed exclusively from the surrounding sandy aquifer under base flow conditions. The NO_3^- -N concentrations of the stream are considerably lower (1-2 mg N L^{-1}) than the contributing groundwater (Chauhan and Mills, 2002, Mills et al., 2002). This discrepancy between high nitrate concentration in the discharging groundwater and low nitrate concentrations in stream water has prompted the examination of NO_3^- removal in the sediments of Cobb Mill Creek.

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METHODS

Site Description

Cobb Mill Creek is a second-order, low-gradient (over a stream length of 2.9 km, the streambed elevation drops 10.7 m), groundwater-dominated creek in a 5.0 km² watershed on the Eastern Shore of Virginia (Figure 1). The study site (37°15.5' N, 75°55.75' W) is located along a non-tidal portion of the stream which drains to seaside lagoons. Cobb Mill Creek receives discharge from intensively cultivated fields on both sides, although there is an oak - maple - pine riparian forest with a width that exceeds 30 m in both directions at the study site. The Eastern Shore comprises extensive relict beach deposits, thus permeability is high in both the subsoil and substratum. The highly permeable soils are underlain by the shallow, unconfined Columbia aquifer, which consists of an eastward thickening wedge of sand and gravel unconsolidated sediments. The Columbia aquifer is underlain by the Yorktown- Eastover which lies below a confining unit at a depth ranging from 8-20 meters below ground elevation (Richardson, 1992).

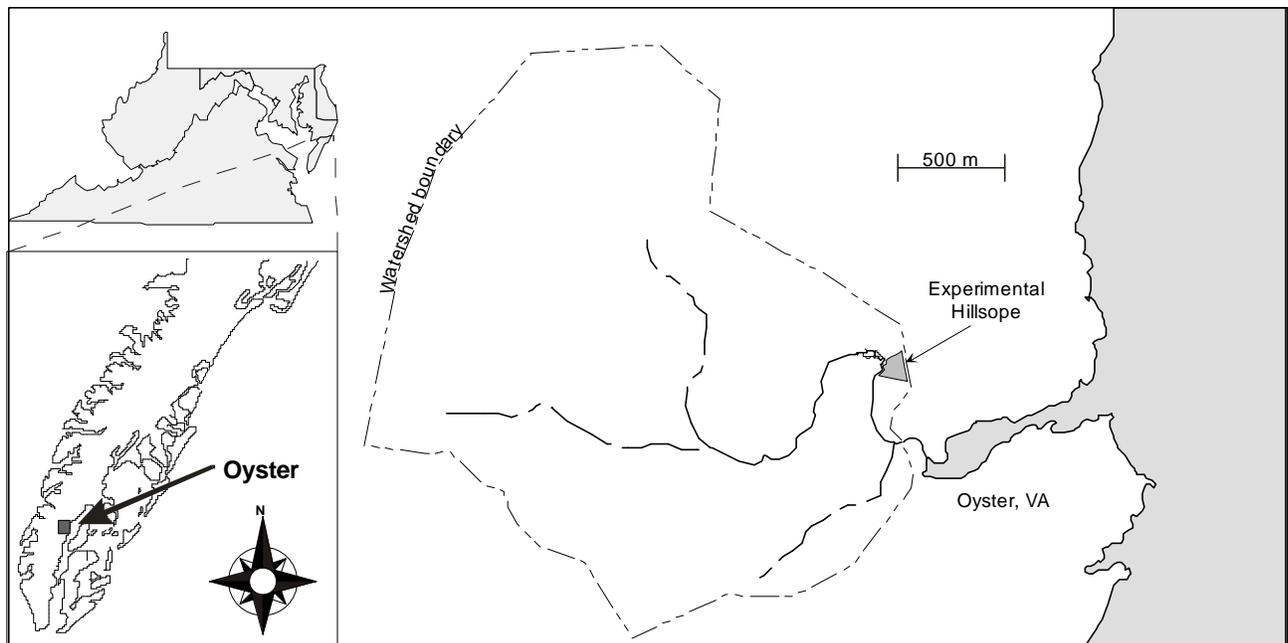


Figure 1. Location of the Cobb Mill Creek Field Site at the Anheuser-Busch Coastal Research Center

NO₃⁻ Profiles in Stream Sediment

Shallow piezometers were installed by driving 2.5-cm well casings with a solid point into the creek sediments. Openings in the casing consisted of several 4.8 mm holes around the circumference of the casing to enable accurate and precise placement of the openings at the desired depth. Permanent nests consisting of 3 piezometers were placed at two locations 10 meters apart in the stream. The openings ranged from about 20 cm to about 80 cm below the sediment surface. Depth profiles were also obtained by means of a portable mini-piezometer consisting of a series of 3-mm stainless steel tubes with slots cut near the pointed tips as described by Duff et al (1998).

Samples were withdrawn from a piezometer, filtered (0.2- μ m pore size), placed on ice, and returned to the laboratory for analysis. NO₃⁻ was analyzed by ion chromatography.

Organic Matter and Platinum Electrode Potential

Total organic matter was determined on core samples collected from Cobb Mill Creek sediments. Cores were collected by driving 7.5-cm diameter lengths of PVC pipe with a sharpened cutting edge into the sediment to depths ranging from 50 to 100 cm. Extracted cores were sealed at both ends and maintained in an upright position during their return to the laboratory.

Cores were refrigerated (4°C) until processing. Beginning at the sediment surface, small holes were drilled in the side of the core at 10 cm intervals. The holes were immediately filled with silicone sealer to prevent loss of water from the core. Platinum electrode potential measurements were made by inserting a Pt wire through the silicone into the sediment. The wire was connected by a length of copper wire to a millivolt meter. The circuit was completed by placing a reference electrode in the water covering the core and connecting the electrode to the other terminal on the meter.

To sample the cores for organic matter after taking Pt electrode measurements, holes were cut in the side of the core tube with a 16-mm diameter hole saw using the previously drilled holes as pilot holes. Sediment was removed from the opening with a spatula, placed in tared weighing dishes, dried at 100°C for 24 hr, weighed, and ignited at 500°C for 24 hr. Organic matter was determined as the loss of weight from the oven dried sample upon ignition.

Denitrification Potential

The potential for denitrification activity in the sediments was determined over depths from 2 cm below the sediment surface to 70 cm below the surface in samples collected from cores using the acetylene block technique (Galavotti, 2004). Cores were taken from the sediment as described above. The PVC liner was marked at 10-cm intervals starting at the sediment surface and 16-mm diameter holes were bored at each depth. As each hole was cut, sediment was removed by using a de-tipped, 10-mL syringe. The sediments samples were placed in 60-mL serum bottles with 15 mL of artificial groundwater and capped with a septum and crimped shut with a metal cap. Artificial groundwater consisted of the following salts dissolved in 1 L of deionized water: 60 mg $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 36 mg NaHCO_3 , 36 mg CaCl_2 , 25 mg $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$. Nitrate was not added to the sediment slurries. In situ pore water in the sample was the only source of nitrate. The sediment suspension was made anaerobic by bubbling with N_2 gas for 5 minutes. 5% acetylene was injected into the sediment suspension. The bottles were shaken on a rotary shaker during the incubation (ambient temperature), and headspace samples were collected over time and assayed for N_2O by gas chromatography with an electron capture detector. Rates were determined using the linear portion of the N_2O -production curve.

Abundance of Denitrifying Bacteria

Denitrifying bacteria were quantified by estimating the abundance of the nitrous oxide reductase gene, *nosZ* using the primer set developed by Scala and Kerkhof (1998). Because probing for a gene can only indicate presence or absence of the gene, quantification was carried out by application of most-probable-number (MPN) methodology using approaches developed for other organisms (Feray et al., 1999). Briefly, serial dilutions were made from samples and multiple portions were taken from the dilutions and amplified by PCR using the *nosZ* primer set. The presence or absence of the gene was determined by observation of the presence or absence of the amplified product after electrophoretic separation in an agarose gel. The number of positive responses in each dilution were then used to calculate the most-probable-number of denitrifiers using an MPN calculator available on the web (<http://www.i2workout.com/mcuriale/mpn/>).

RESULTS

NO_3^- concentrations in samples from various depths in the sediments of Cobb Mill Creek declined from levels ranging from 6 to 13 mg $\text{NO}_3^- \text{-N L}^{-1}$ at a depth of 50-60 cm to stream concentrations that ranged from 1.5 to 2.5 mg $\text{NO}_3^- \text{-N L}^{-1}$ (Figure 2). The greatest change in the NO_3^- concentration was observed in the upper 20 cm of the sediment. Coincident with the greatest loss of NO_3^- was the highest concentration of organic matter (Figure 3). Although the maximum average concentration was about 3%, the concentration at that level was as high as 20% in a few cores. Core samples from that depth were often a dark brown color indicative of high organic content, whereas the sand itself is usually an off white color reflecting its origin as an old beach deposit.

Platinum-electrode potentials were always just above 300 mV except for the depths associated with elevated organic matter content (Figure 4). While these potentials are only indicative of the general redox status, the fact that they fall below 300 mV in the region containing the maximum organic matter suggests that oxygen is likely absent, or at least mostly depleted in that portion of the sediment.

Measurements of denitrification potential yielded profiles with a maximum generally coincident with the organic matter maximum / Pt electrode potential minimum (Figure 5). As opposed to the other measurements, which achieved maxima (or a minimum in the case of Pt electrode potential) around 20 cm depth, denitrification potential increased starting around 40 cm below the surface and continued to increase to the surface. This is probably because large numbers of denitrifiers are

facultative anaerobes that use O_2 as the terminal electron acceptor when it is present, but will respire using NO_3^- , if available, in the absence of O_2 .

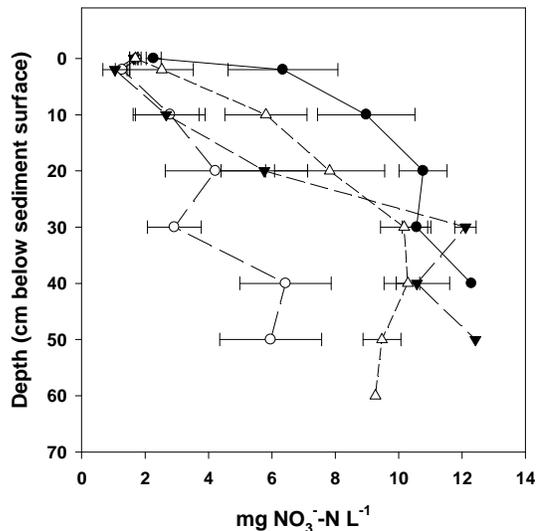


Figure 2. Profiles of NO_3^- concentration in Cobb Mill Creek sediments. Results are from a series of samples collected with the mini-piezometer at several locations at different times during the summer and early fall.

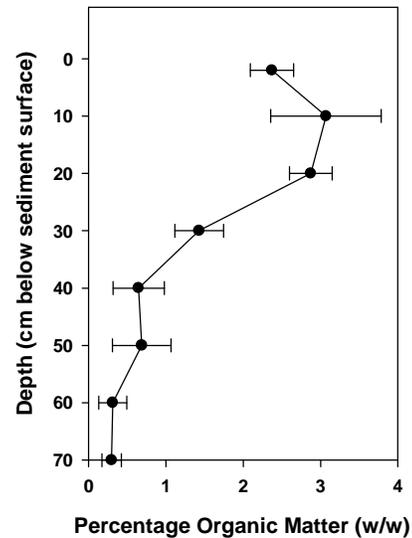


Figure 3. Organic matter content in cores from Cobb Mill Creek. Results are averages of 5 cores, and error bars represent the standard error of the mean.

Measurements of denitrification potential yielded profiles with a maximum generally coincident with the organic matter maximum / Pt electrode potential minimum (Figure 5). As opposed to the other measurements, which achieved maxima (or a minimum in the case of Pt electrode potential) around 20 cm depth, denitrification potential increased starting around 40 cm below the surface and continued to increase to the surface. This is probably because large numbers of denitrifiers are facultative anaerobes that use O_2 as the terminal electron acceptor when it is present, but will respire using NO_3^- , if available, in the absence of O_2 .

The number of denitrifiers, as measured by the combined PCR-MPN approach, increased beginning at around 50 cm below the surface and were a maximum between 30 cm below the sediment surface and the surface (Figure 6). Large errors associated with both the denitrification potential measurements and the numbers of denitrifiers make difficult a conclusion that these profiles are dissimilar.

DISCUSSION

Although riparian buffers contribute to the removal of nutrients entering surface waters in a variety of situations, the importance of the stream sediments as filters of NO_3^- in low relief coastal streams may be equal to or greater than the removal by processes such as plant uptake and denitrification in riparian forests or grass buffer strips. The water discharging more or less vertically to the stream channel represents water that has traveled the furthest distance from the stream, i.e. from the fertilized agricultural fields as opposed to the shallow, horizontal flow expected from the riparian zone itself. Indeed, at Cobb Mill Creek, profiles of NO_3^- taken from wells and piezometers in the adjacent riparian forest show a surface region in the saturated zone, that is significantly lower than that in the deeper groundwater which is, presumably, flowing to the stream from the agricultural fields beneath the riparian zone (Galavotti, 2004, Gu, 2007).

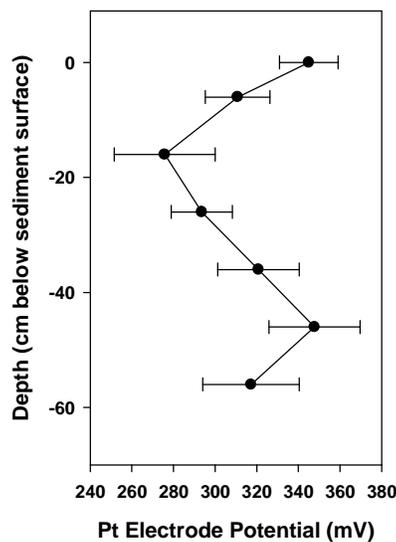


Figure 4. Redox potential at various depths in Cobb Mill Creek sediments measured as Pt-electrode potential. Error bars are one standard error of the mean.

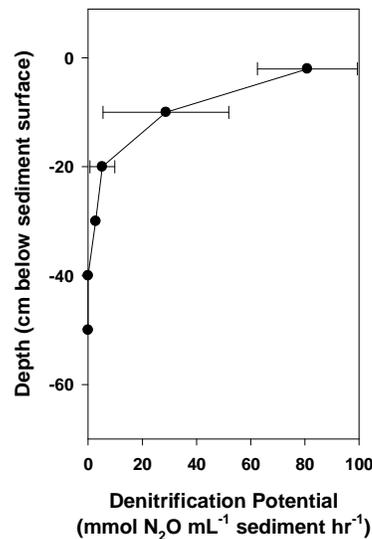


Figure 5. Denitrification potential as measured with the acetylene block technique. Data points are the mean of 9 cores and the error bars represent 1 standard error of the mean.

Given that there are no plants in the sediments in the area under study, plant uptake in the stream cannot contribute to the removal of NO_3^- in the vertically discharging water. The good correspondence of conditions conducive to denitrification, elevated levels of denitrification potential and numbers of denitrifiers to the region in the profile where NO_3^- most rapidly disappears supports our assertion that denitrification is completely responsible for NO_3^- removal. Experiments in our laboratory using intact cores through which NO_3^- -laden groundwater was passed showed that the sediments, in particular the top 20-30 cm of the sediments could remove all of the NO_3^- from the discharging water at flow rates similar to those seen *in situ* (Gu et al., 2007).

Although the sediments in Cobb Mill Creek are capable of removing all the NO_3^- from the discharging groundwater, the riparian forest plays a critical role. Organic inputs from litter fall that are worked into the sediments during storm reworking of the sediments drive the NO_3^- -removal process. There is little organic matter in the groundwater in most of the Columbia aquifer, at least not enough to cause anaerobiosis or denitrification. Thus, aerobic groundwater proceeds, with its NO_3^- load intact, toward the stream. As the water moves vertically toward the stream it encounters the elevated organic levels in the upper portion of the sediments. Aerobic microbes there oxidize the organics with O_2 , thereby depleting the concentration to the point where denitrification is possible. At that point, the microbes respire the organics using NO_3^- as the electron acceptor. Thus organic inputs from the riparian zone control the entire process in the stream sediment.

Note that that stream sediment removal in coastal streams is not a universal process. For example, in the upper part of the DelMarVa peninsula, Denver et al (2003) reported that NO_3^- concentrations in streams were not different from those in the underlying groundwater, and inferred that denitrification was, therefore, not an important process there. Furthermore, heterogeneity in organic matter distribution and in distribution of hydraulic conductivity, allow NO_3^- to leak through in some parts of the stream while it is completely removed in others (Flewelling et al., 2007). That heterogeneity is a partial explanation for the existence of NO_3^- in the stream water at the observed levels.

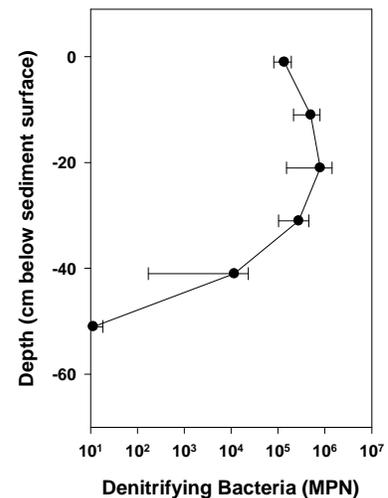


Figure 6. Most probable number of denitrifying bacteria with depth in Cobb Mill Creek sediments. Data points are the mean obtained from 8 different cores. Error bars are 1 standard error of the mean.

Clearly, denitrification in stream sediments can reduce the stream loading of NO_3^- from upland watersheds to the coastal estuaries, but the extent of the effect of the process on total NO_3^- removal, and what are the ultimate controls on the process that determine its distribution are questions that remain to be addressed.

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