MICROBIAL CHARACTERISTICS AND FATE OF STREAM BANK
LEGACY SEDIMENTS IN MID-ATLANTIC STREAMS

by

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ABSTRACT

The mobilization of anthropogenic sediments has become an increasing concern in Mid-Atlantic watersheds. A large portion of annual watershed sediment exports in Mid-Atlantic streams could be composed of legacy sediments. Despite this, we know very little about the microbial communities in legacy sediment and how they may affect nutrient processes in stream banks. There is additional uncertainty whether legacy sediments will act as a source or a sink once deposited into the water column. Legacy sediment stream banks were sampled across 15 different sites in northern Delaware, eastern Maryland, and southeastern Pennsylvania, USA. Multiple analyses were performed on the sediments to determine nutrient, heavy metal content, microbial community and functional gene abundance, and the sorption properties.

The focus of the first study was on the variation in microbial community structure and nitrogen transforming functional genes across four different land uses and by multiple stream bank depths. Results from the study indicated that variation in microbial community composition varied by land use and bank depth highlighting the complexity and spatial heterogeneity of each site. Nitrification genes were lower in abundance than the measured denitrification gene: nosZ which may influence nitrogen cycling within the stream banks. The interactions between microbes and the stream bank chemistry highlight the importance of looking at both aspects when concluding on the potential impacts legacy sediments have on water quality.

The second studies focused on if legacy sediments acted as a source or a sink for nutrients once deposited into streams. A variety of laboratory experiments were
conducted on coarse (>63µm) and fine (<63µm) fractions of legacy sediment collected from the fifteen sites. Sediments were incubated for 24 hours to determine the Phosphorus Sorption Index (PSI) and the Equilibrium Phosphorus Concentration (EPC₀). Sediments were also incubated in anoxic and oxic conditions to measure the amount of phosphorus release in both conditions. Additionally, legacy sediment was incubated in modified milk crates in two small tributaries for eight months along the Big Elk Creek. Results indicated that while the fine fraction sediment has a high sorption capacity, whether the sediment acts as a source or a sink will depend upon the environmental conditions of the stream water.

This thesis expands upon the current knowledge about legacy sediments and provides insight into microbial community variation in legacy sediment and their potential role in nutrient processing. Findings from this study also give insight into how legacy sediments may interact with the water column once deposited into streams. These findings will be of interest to natural resource managers, water quality scientists, TMDL planners, and the legacy sediment community.
Chapter 1
INTRODUCTION

The input of sediments into streams has become an increasing concern for scientists and managers in Mid-Atlantic watersheds such as the Chesapeake Bay (Chesapeake Bay Program 2018). Suspended sediments limit light penetration and reduce phytoplankton production (Hotzel and Croome, 1994; Henley et al., 2000). Increases in sedimentation can also reduce dissolved oxygen levels in the water column, reducing respiratory functions in fish (Goldes et al., 1988; Waters 1995). Another cause for concern are nutrients (e.g., nitrogen (N) and phosphorus (P)) that are bound to suspended sediments. Increased input of N or P into aquatic systems may lead to eutrophication, threatening aquatic species in both marine and freshwater environments (Smith 2003).

Legacy sediments are defined as post-settlement alluvium or colluvium abundant throughout valley bottoms and river floodplains in the Mid-Atlantic Region (James 2013). Historic millponds, along with widespread erosion following deforestation and agriculture resulted in the accumulation of sediments in streams and creeks throughout the Mid-Atlantic Region (Walter and Merritts 2008). The breaching of mill dams caused the water level to lower and additionally channel incision from the flowing water (James 2013). These incised channels, in combination with the accumulated sediment form vertical streambanks that can vary in size (Merritts et al., 2011, 2013; Walter and Merritts 2008). Legacy sediment streambanks are composed of predominately fine fraction sediment, making them susceptible to various erosive
processes (Merritts et al., 2011; Wegmann et al., 2013). Recent studies revealed that streambank erosion of legacy sediments might be a significant contributor to watershed sediment loads (Gellis et al., 2009; 2018; Cashman et al., 2018). As much as 50 to 100% of fine sediments exports in watersheds are attributed to stream bank sediment sources with legacy sediments being the predominant component (Banks et al., 2010; Gellis and Noe, 2013).

While legacy sediments have received increased attention within recent years, there is still substantial uncertainty regarding nutrient processing in legacy sediments and their impacts on aquatic ecosystems. Only a few studies have investigated concentrations of N and P for legacy sediments and how these concentrations change once deposited into streams. A recent report by the Chesapeake Bay Science and Technology committee (STAC, Miller et al., 2019) highlighted the major gaps in information regarding nutrients in legacy sediments. Additionally, little is known about microbial communities and their functionality within legacy sediments. Microorganisms are centrally involved in and have a direct effect on nitrogen transformations (Herrmann et al., 2011; Leininger et al., 2006; Zhang et al., 2016) by affecting processes such as nitrification, denitrification, mineralization, and immobilization (Johnson et al., 2005; Kirchman 2012). These communities are studied to understand and interpret how microbial populations respond to changes and how they may affect nutrient processing in the environment (Fierer and Jackson, 2006; Kim et al., 2016). To our knowledge, only one study exists that has studied microbial communities and processes in legacy sediments (Weitzman et al., 2014). Weitzman et al. (2014) studied the differences in N processing and the associated functional genes for both legacy sediments and the pre-colonial hydric soils buried beneath them. They
discovered that the buried hydric layer had low denitrification rates and microbial activity, which was contrary to what they expected (Weitzman et al., 2014). However, we do not know if the low microbial activity is seen across all legacy sediment stream banks, or if this was a site-dependent influence at the Big Spring Run location.

We aim to address these knowledge gaps by examining legacy sediment nutrient, and microbial characteristics across a variety of land use types within the Mid-Atlantic region. The first study analyzes microbial community structure and nitrogen transforming functional genes in legacy sediments. The second evaluates if legacy sediments are a source or a sink for nutrients once deposited in streams. The specific questions that were addressed were:

Question 1: How do the microbial communities and their functionality for legacy sediments vary with land use and stream bank depth? (Study 1)

Question 2: Are stream bank legacy sediments a source or sink for nutrients? (Study 2)

Question 3: What is the fate of legacy sediment nutrients under oxic/anoxic conditions? (Study 2)

This thesis is divided into several chapters, starting with the literature review. Chapter 3 is the manuscript associated with study 1 (Question 1). Chapter 4 is the second manuscript that addresses the fate of legacy nutrients in streams (Question 2 and 3). The last chapter, Chapter 5, draws conclusions on the two studies and provides insight into potential future research.
REFERENCES


Chapter 2

LITERATURE REVIEW

This literature review highlights major issues involving sediments and nutrients and then defines legacy sediments within streambanks. This review also explores the varying nutrient processes that occur within legacy sediments before ending on current microbial knowledge on legacy sediment.

2.1 Sediment and nutrient pollution and environmental consequences

An overabundance of sediment affects aquatic systems on multiple levels. The Chesapeake Bay Program ranks sediment pollution as the second most harmful pollution contributor to the overall health of the Bay. Sediment can reduce the amount of periphyton growing in streams (Steinman and McIntire, 1990). Suspended sediment can limit light penetration and reduce phytoplankton production (Hotzel and Croome, 1994); this affects the aquatic organisms throughout the food web by decreasing potential food sources, limiting production and growth in the process. Lloyd et al. (1987) found that if turbidity increased by 5 Nephelometric Turbidity Units (NTUs), primary production decreased by up to 13%. In a 25 NTU environment, primary production decreased by up to 50% (Lloyd et al., 1987).

Turbidity and sedimentation can also affect local fish populations. Depending on the species, certain fish may relocate to a new area. Otherwise, species that stay in a disturbed area may have adverse effects on their health. It has been shown that increased sedimentation and turbidity can reduce the dissolved oxygen levels in the water column and reduce respiratory function (Horkel and Pearson, 1976; Goldes et al., 1988; Waters, 1995). Sedimentation can also reduce spawning activity and
available spawning habitat (Ryan 1991). Overall, increased turbidity can cause a
trophic cascade that affects entire aquatic ecosystems (Henley et al., 2000).

While turbidity is an issue with sediment in suspension, nutrient pollution is
another issue associated with sediment. Sediment, especially composed of
predominately clay particles, once deposited in the water can release nutrients such as
ammonium and phosphorus (Fox et al., 2016; Wegmann et al., 2013). Phosphorus
binds to these clay particles that contain metal oxides such as iron (Fe), manganese
(Mn), and aluminum (Al) (McDowell and Sharpley, 2002). This sorption process can
occur at either baseflow or during storm events. However, nutrient bound sediment
when transported has the potential to carry these nutrients and negatively impact the
water quality of an aquatic ecosystem.

Higher concentrations of nutrients can be observed on a seasonal cycle. For
instance, higher concentrations of phosphorus have been observed during the summer
and autumn months, while lower concentrations are noted during the winter and spring
(Cooper et al., 2015). This increase could be attributed to multiple factors with a few
being: manure applications to croplands, the growth of P-rich biofilms that proliferate
in the summer, autochthonous P release from primary productivity during the growing
season, increased P-rich inputs from runoff and subsurface agricultural field drains,
and enhanced bed sediment P sorption under baseflow conditions (Hofmann and
Beaulieu, 2001; Cooper et al., 2015). The transport of soluble reactive phosphorus
from agricultural fertilizers can be another nonpoint source input into an aquatic
system. An increase in algal blooms and eventually eutrophication may occur due to
increased inputs of N or P into aquatic ecosystems. This may lead to a shift in nutrient-
limiting factors. In the case of freshwater lakes and streams, P is the limiting factor for growth in algae (Schindler 1977).

Eutrophication leads to many undesirable effects that are both costly for consumers on an economic level and are harmful to aquatic ecosystems (Carpenter et al., 1998). One-half of impaired lake areas and 60% of impaired river reaches in the US are from the results of eutrophication (USEPA 1996). Some, but not all adverse effects of eutrophication include depletion of deep-water oxygen, leading to increased release of P in oxic sediments (Kangro et al., 2007), threats to endangered aquatic species, reduction of water column transparency, and increased production of phytoplankton and suspended algae (Smith 2003). This applies to both marine and freshwater environments. The increase in nutrient pollution leads to the development and propagation of bloom-forming cyanobacteria, over other algal species. Cyanobacteria can lead to summer fish kills and impairment of drinking water quality (Kann and Smith, 1999; Cooke and Kennedy, 2001; Smith et al., 2003). For streams and rivers, the nutrient enrichment of flowing waters will have a great influence on the biomass and community structure of benthic and suspended algae (Smith 2003).

2.2 Legacy sediments – definition and origins

Before the pre-European settlement, streams in the Mid-Atlantic Region were categorized as wetland-like environments with narrow anabranching channels (Merritts et al., 2011; Walter and Merritts, 2008). These streams stored organic carbon and retained only trace amounts of fine sediment (Merritts et al., 2011; Walter and Merritts, 2008). During the late 17th to the 19th century, settlers dammed streams and constructed water power mills in order to meet industrial demand and produce goods such as paper, grains, and other products (James 2013; Walter and Merritts, 2008).
Throughout the Mid-Atlantic Region, over 65000 low-head mill dams were constructed across 1st to 3rd order stream valley bottoms (Merritts et al., 2011). Concurrently, sediment deposits were produced through intensive poor agriculture practices and rapid land clearance (Happ et al., 1940; Knox 1972,1977, 2006; Gellis et al., 2009; Merritts et al., 2011), and mining (Knox 1987; James, 1989; Marcus et al., 2001; Lecce et al., 2008; James 2013), which promoted widespread erosion and increased the sediment input to the water column (Walter and Merritts, 2008). This led to the accumulation of fine-grained sediment behind low-head mill dams within stream channels and millponds (Walter and Merritts, 2008). The post-settlement deposits are referred to as legacy sediments (James 2013; Walter and Merritts, 2008).

Legacy sediments are broadly defined as post-settlement alluvium or colluvium on river floodplains that are the result of anthropogenic practices generating sediment (James 2013). This sediment will continue to accumulate behind the mill-dam until it is removed, or naturally breaches. This results in the base-level lowering of the streams and channel incision (James 2013). After the breaching process, all that is left are atypically high legacy sediment stream banks (James 2013, Merritts et al., 2011), that are highly susceptible to fluvial and subaerial erosive processes (Fox et al., 2016; Gellis et al., 2017; Wolman 1959). As such, legacy sediments have been identified as substantial sources of sediment to stream channels during storm events (Gellis et al., 2013). These stream banks erode and can contribute sediment into streams for several decades post-dam-breaching (Merritts et al., 2013).

Legacy sediment stream banks could be comprised of four layers: pre-Holocene gravel, hydric soil, gleyed silt deposits, and post-contact legacy sediment (Merritts et al., 2011). The pre-Holocene layer (bottom) consists of poorly sorted cobbled
to boulder basal gravel that can be colluvial or periglacial in origin (Merritts et al., 2011). The hydric layer is a thick black organic carbon-rich layer that is remnants of past wetland-like environments. This sediment has been dated to be as young as 300 years BP to as much as 10500 years BP (Merritts et al., 2011). The gray silt deposits resting on top of the hydric layer is associated with early human activities like land clearing or burning, potentially occurring at the same time as major climatic events such as droughts and hurricanes (Merritts et al., 2011). The final layer (top) is referred to as post contact sediment that is a result of post-colonial mill dam construction and sediment erosion (Merritts et al., 2011).

2.3 Broader watershed/environmental challenges with legacy sediments

Legacy sediments are a critical source of sediment and impose challenges to water quality (Cashman et al., 2018; Gellis et al., 2009; 2016, 2018) to downstream aquatic systems. One study indicated that legacy sediment comprised 57% (± 25%) of the measured gross stream bank erosion which makes up a significant portion of stream bank erosion across the Baltimore County, Maryland drainage network (Donovan et al., 2015). Sediment exports are atypically high within the Piedmont region, given its low elevation and geology (Gellis et al., 2009). When legacy sediment streambanks erode, the coarse fraction deposition deposits itself along the channel bottom, while the fine fraction deposition is mobilized and transported downstream (Donovan et al., 2015). Large sediment inputs from early in the year can have a critical impact on the spring spawning season of biologically sensitive habitats within the Chesapeake Bay. The sediment can bury spawning beds, affect the growth of aquatic grasses, and ultimately harm the reproductive stages of fish spawns (Orth et al., 2010). Winter season storms, which are becoming more frequent in the Mid-
Atlantic region, have shown to have a greater negative impact on the aquatic ecosystems that make up the Chesapeake Bay (Dennison et al., 2012). An additional challenge that may impact legacy sediment contribution to the Bay is the increase of urbanization in the mid-Atlantic region. Streamflow variability is increased by urbanization. While legacy sediment is a critical source of sediment in mid-Atlantic waterbodies, there is still much uncertainty regarding the nutrient input from these sources (Miller et al., 2019).

**TMDL Issues**

The Chesapeake Bay Commission has struggled to meet Total Maximum Daily Load (TMDL) targets for nutrient and sediment loads within the Bay (USEPA, 2010). The Chesapeake Bay TMDL seeks to set limits of 185.9 million pounds of nitrogen, 12.5 million pounds of phosphorous, and 6.45 billion pounds of sediment inputs into the Bay watershed per year. The goal of these reductions (25% reduction in N; 24% reduction in P; and 20% reduction in sediment loads) is to ensure a complete restoration of the Bay and tidal rivers by 2025 (USEPA, 2010; Chesapeake Progress 2019).

Due to only recently being recognized as a tremendous concern for sediment input into the Chesapeake Bay, there is uncertainty regarding the overall impact legacy sediments may have on sensitive and vulnerable aquatic ecosystems downstream within the Bay (Fincham 2011). More attention has been given to legacy sediment in recent years, however, they are still not accounted for in sediment budgets and models. The most recent Chesapeake Bay Program Watershed Model (5.3.2) does not account for legacy sediment contributions into the Bay despite growing concerns (USEPA, 2010; Chesapeake Bay Program 2018). This may lead to a misallocation of resources.
and funds and may contribute to water management agencies not reaching TMDL goals for nutrient and sediment management (STAC, Chesapeake Bay Program 2017).

2.4 Processes influencing stream bank legacy sediment erosion, nutrient content, and fate in streams: Nitrification – Denitrification

Nitrification and denitrification are two important processes in N-cycling in soil and water. Nitrification is the process in which ammonium is converted first to nitrite and then oxidized to nitrate by aerobic microorganisms for energy (Brady and Weil, 2008; Johnson et al., 2005). It occurs mostly in aerobic conditions and when soil is warm. The denitrification process takes nitrate and converts it into the gaseous forms of N such as nitrogen gas (N₂) or nitrous oxide (N₂O). This process occurs in anoxic conditions by heterotrophic bacteria. This process removes N from the system and is an important ecosystem service performed in anaerobic areas like wetlands (Johnson et al., 2005).

Weitzman et al. (2014) compared N processing in upland soils and legacy sediments. Results from their study indicated that potential denitrification rates were low in the hydric layer of legacy sediments while they were found to be highest in the surface soils for both legacy sediments and upland soils. Despite the hydric layer being carbon-rich, the low rates were attributed to low microbial enzyme activity. The net nitrification rates for surficial layers in legacy sediment zones were 312 and 284% higher than the mid-layer and bottom layers (Weitzman et al., 2014). They concluded that legacy sediments might act as sources of NO₃ for waterways (Weitzman et al., 2014).
Mineralization – Immobilization

Mineralization is a process in which microbes convert organic N into inorganic forms. This process is temperature and moisture dependent. Immobilization is the opposite of mineralization in which microbes convert N from inorganic to organic forms (Johnson et al., 2005). The carbon to nitrogen ratio within soils can be used as a rough assessment of whether mineralization or immobilization is occurring. A C:N ratio of less than 25 to 1 can indicate that mineralization is occurring while a ratio with greater than 25 to 1 may indicate immobilization (Chen et al., 2014).

For forms of P, microbial transformations in soil solutions could convert inorganic into organic P. Organic P can be mineralized into inorganic P via the decomposition process (Holliday and Gartner, 2007). Ultimately, microbial activity, nutrient abundance, and organic matter lability will dictate the nutrient (N & P) mineralization rates in an ecosystem (Blinkley and Hart, 1989).

Only one study has quantified the C and N mineralization rates for legacy sediments. Potential C mineralization rates ranged from 223 to 1737 g m\(^{-2}\) year\(^{-1}\) in both upland and legacy sediment soils (Weitzman et al., 2014). These rates were found to be highest in the surficial layer compared to the middle and bottom depths. Weitzman et al. (2014) quantified N mineralization by determining potential net nitrification rates and potential net ammonification rates. Net nitrification rates ranged from 9.2 to 77.9 g m\(^{-2}\) year\(^{-1}\) across both landscape and depth. Potential net ammonification rates were negative and ranged from -5.5 to -47.8 g m\(^{-2}\) year\(^{-1}\) across both sites and depths (Weitzman et al., 2014).

Sorption - Desorption
Sediment has the properties to either adsorb or desorb P (Froelich 1988). Whether the sediment either adsorbs or desorbs P is based upon the relationship between the P concentration within a solution relative to the P concentration within the sediment particles. An isotherm is a relationship between the solution phase and the adsorbed phase of P concentrations where adsorption or desorption occur. Similar mechanisms have also been observed in the relationship between sediment and NH$_4^+$ (Wegmann et al., 2013). The extent to which adsorption or desorption occurs depends upon multiple factors such as charge properties of the sediment, pH, organic matter content, and mineral makeup (Brady and Weil, 2008).

There are three mechanisms for P adsorption in acidic soils. The first occurs when H$_2$PO$_4^{-}$ ions react with or adsorb to the surfaces of oxides containing metals like iron and aluminum (Fe$_2$O$_3$*3H$_2$O and Al$_2$O$_3$*3H$_2$O) (Brady and Weil, 2008). This allows dissolved P anions to attach to the positively charged oxides by electrostatic forces. If the P was not knocked off, over time, it could penetrate the mineral structure, thus making it more difficult to remove. This penetration reopens the adsorption site on the particle surface and creates a two-step adsorption process (Brady and Weil, 2008; Sparks 2003; Grundtner 2013). A second mechanism for acidic soils involves the chemical integration of P with the oxide surface. The binding is stronger and takes place predominately on clay particles. The P bound by this mechanism is low in solubility and desorption potential (Brady and Weil, 2008). The third mechanism occurs only when iron and aluminum hydrous oxides cover the bound P. This happens over time as the P becomes buried inside the oxide particle. Only extreme environmental changes will release this bound P (Brady and Weil, 2008).
For more basic or alkaline soils (pH = 8), multiple products are formed from the interaction between dissolved \(\text{PO}_4\) and Ca. The phosphate ions bind with calcium carbonate in soils and become chemically integrated like the second mechanism in acidic soils. The availability of P in these soils will be determined by the solubility of calcium bound P compounds (Grundtner 2013).

Stream bank sediment with high P concentrations can potentially erode and release P into the water column (Fox et al., 2016). Fox et al. (2016) found stream banks with concentrations exceeding 250mg kg\(^{-1}\) P should be considered a significant source of P to surface waters if erosion is occurring. This can be detrimental to aquatic ecosystems if fine sediments saturated with P travel downstream and reach anoxic waters which favor desorption. Anoxic conditions can potentially allow this saturated sediment to desorb more P into aquatic systems (Kleinman et al., 2007). The adsorption and desorption processes have been simulated via laboratory experiments in river bank sediment literature, (Grundtner et al., 2014), and are a crucial role in understanding legacy sediments impact on water quality.

**Sorption Isotherms**

There are three commonly used isotherms: linear, Freundlich, and Langmuir (Grundtner 2013). A linear isotherm shows a constant rate of P adsorption regardless of the solution P concentration. Freundlich has a slower rate a P adsorption as the solution P concentration increases. Langmuir shows an adsorption capacity that plateaus once a certain P solution concentration is reached. Several factors influence sorption isotherms including soil: water ratio, temperature, and exposure time (Grundtner 2013).
Giles et al. (1974) proposed to use four main shapes for modeling isotherms. These were “C,” “L,” “H,” and “S.” Linear shaped isotherms are modeled after “C” shapes. “L” and “H” are concave isotherms and are similar to each other. “H” isotherms are an extreme version of “L” isotherms (Limousin et al., 2007). These two shapes show a basic progression to saturation of the solid. Freundlich isotherms can take on these shapes. “S” isotherms have a point of inflection due to influence from two opposite mechanisms such as through the phenomenon “cooperative adsorption” (Hinz 2001). Non-polar organic compound usually forms this shape due to low affinity with clays (Limousin et al., 2007).
Figure 2.1: Four types of isotherms – each letter represents the shape of the isotherm. On the axes, C represents the remaining solute concentration for a compound, while Q is the concentration of the compound retained on solid particles (after Giles et al. 1974, figure from Limousin et al. 2007).

**Phosphorus Sorption Index (PSI)**

The Phosphorous Sorption Index (PSI) was a reference index developed by Bache and Williams (1971) that provided a less time-consuming option as opposed to using P sorption isotherms. It allowed for characterization of phosphate sorbing properties within soils that was comparable to the maximum sorption capacity of the soil ($P_{\text{max}}$ or $Q_{\text{max}}$). The PSI is the slope of the plot of sorption, $x$, divided by the log of equilibrium solution phosphate concentration, $C$, (Bache and Williams, 1971). It is expressed as and is considered a single-point isotherm:
\[ \text{PSI} (\text{L kg}^{-1}) = \frac{x}{\log C} \]

A study done by Wang et al. (2016), compared three different ways of expressing PSI in relation to \( P_{\text{max}} \). Wang et al. (2016) refer to PSI-b as L kg\(^{-1}\), similar to Bache and William (1971). A shortcoming of PSI-b is that it is difficult to explain its physical significance. To get around this, current literature uses the amount of P sorbed by the given soil (mg/kg) or \( x \) as the PSI value. This is expressed as PSI-a in Wang et al. (2016). Finally, they express PSI-c as the sum of PSI-a and soil test P (STP), which is commonly referred to as Olsen P (Wang et al., 2016). Each equation is expressed as follows:

- PSI-a (mg kg\(^{-1}\)) = \( \frac{(60-C) \cdot 0.025}{0.001} \)
- PSI-b (L kg\(^{-1}\)) = \( \frac{\text{PSI-a}}{\log C} \)
- PSI- c (mg kg\(^{-1}\)) = PSI-a + Olsen P

\( P_{\text{max}} \) – the maximum sorption capacity of soil or sediment, is a metric found throughout various studies (Table 2.1) (Hongthanat 2010; McDowell and Sharpley, 2002; Mozaffari and Sims, 1994). Wang et al. (2016), determined that PSI-a and PSI-b were linearly related to \( P_{\text{max}} \) and as such, can be used as a quick estimate of \( P_{\text{max}} \) in sediment and soil samples. Both measure the remaining P sorption capacity of the soil. For their Ontario soils, Wang et al. (2016) found that PSI-c was the most accurate estimate of \( P_{\text{max}} \). PSI-c could also be used for calculating the degree of phosphorus saturation (DPS) indices for soil P loss risk assessments (Pautler and Sims, 2000; Wang et al., 2016). Since PSI values can be directly compared to the P sorption maximum, there have been several papers that calculated the maximum sorption capacity for upland soils, including within the Delmarva region (Table 2.1).
Table 2.1: Phosphorus sorption index (PSI) found in the literature.

<table>
<thead>
<tr>
<th>Location</th>
<th>Soil Depth</th>
<th>Soil Type</th>
<th>PSI (mg/kg)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delaware Inland Bays Watershed</td>
<td>0-20 cm</td>
<td>Evesboro loamy-sand (Ag)</td>
<td>149</td>
<td>Mozaffari &amp; Sims 1994</td>
</tr>
<tr>
<td></td>
<td>20-40 cm</td>
<td>Evesboro loamy-sand (Ag)</td>
<td>136</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40-60 cm</td>
<td>Evesboro loamy-sand (Ag)</td>
<td>217</td>
<td></td>
</tr>
<tr>
<td>Delaware Inland Bays Watershed</td>
<td>60-80 cm</td>
<td>Evesboro loamy-sand (Ag)</td>
<td>263</td>
<td></td>
</tr>
<tr>
<td>Delaware Inland Bays Watershed</td>
<td>0-20 cm</td>
<td>Matawan sandy loam (Ag)</td>
<td>588</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20-40 cm</td>
<td>Matawan sandy loam (Ag)</td>
<td>2083</td>
<td></td>
</tr>
<tr>
<td>Delaware Inland Bays Watershed</td>
<td>40-60 cm</td>
<td>Matawan sandy loam (Ag)</td>
<td>2564</td>
<td></td>
</tr>
<tr>
<td>Delaware Inland Bays Watershed</td>
<td>60-80 cm</td>
<td>Matawan sandy loam (Ag)</td>
<td>1886</td>
<td></td>
</tr>
<tr>
<td>Delaware Inland Bays Watershed</td>
<td>0-20 cm</td>
<td>Matawan sandy loam (Field Border Area)</td>
<td>434</td>
<td></td>
</tr>
<tr>
<td>Delaware Inland Bays Watershed</td>
<td>20-40 cm</td>
<td>Matawan sandy loam (Field Border Area)</td>
<td>1562</td>
<td></td>
</tr>
<tr>
<td>Delaware Inland Bays Watershed</td>
<td>40-60 cm</td>
<td>Matawan sandy loam (Field Border Area)</td>
<td>2000</td>
<td></td>
</tr>
<tr>
<td>Delaware Inland Bays Watershed</td>
<td>60-80 cm</td>
<td>Matawan sandy loam (Field Border Area)</td>
<td>1923</td>
<td></td>
</tr>
<tr>
<td>Delaware Inland Bays Watershed</td>
<td>0-20 cm</td>
<td>Pocomoke sandy clay loam (Ag)</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td>Delaware Inland Bays Watershed</td>
<td>20-40 cm</td>
<td>Pocomoke sandy clay loam (Ag)</td>
<td>714</td>
<td></td>
</tr>
<tr>
<td>Delaware Inland Bays Watershed</td>
<td>40-60 cm</td>
<td>Pocomoke sandy clay loam (Ag)</td>
<td>212</td>
<td></td>
</tr>
<tr>
<td>Delaware Inland Bays Watershed</td>
<td>60-80 cm</td>
<td>Pocomoke sandy clay loam (Ag)</td>
<td>303</td>
<td></td>
</tr>
<tr>
<td>Delaware Inland Bays Watershed</td>
<td>80-100 cm</td>
<td>Pocomoke sandy clay loam (Ag)</td>
<td>564</td>
<td></td>
</tr>
<tr>
<td>Mahantango Creek Catchment (Central PA)</td>
<td></td>
<td>Agricultural catchment exposed stream bank</td>
<td>259</td>
<td>McDowell &amp; Sharpley 2002</td>
</tr>
<tr>
<td>Mahantango Creek Catchment (Central PA)</td>
<td></td>
<td>Agricultural catchment submerged bank sed</td>
<td>214</td>
<td></td>
</tr>
<tr>
<td>Rathbun Lake Watershed (Iowa)</td>
<td>0-20 cm</td>
<td>Olmitz Fine-loamy mix (Floodplain)</td>
<td>667</td>
<td>Hongthanat 2010</td>
</tr>
<tr>
<td>Rathbun Lake Watershed (Iowa)</td>
<td>20-40 cm</td>
<td>Olmitz Fine-loamy mix (Floodplain)</td>
<td>667</td>
<td></td>
</tr>
</tbody>
</table>

**Field Border areas separate crop fields from drainage ditches**
**Equilibrium P Concentration (EPC₀)**

The Equilibrium P Concentration (EPC₀) refers to the theoretical point at which no net sorption or desorption of P is occurring on the sediment (James et al., 2002; Froelich 1988). This value is compared with the solution P concentration to determine whether the sediment will act as a source or sink for P (Froelich 1988). If the solution concentrations are higher than the EPC₀, then P will generally be adsorbed onto the sediments. If the opposite is true and the EPC₀ value is higher, then P will be leached into the solution or desorbed from the sediment (House and Dennison, 2000; Webster et al., 2001). Table 2.2 provides EPC₀ values of various soils and sediment.

Studies have shown that there is a strong correlation between the EPC₀, soluble reactive phosphorus (SRP), and dissolved reactive phosphorus (DRP) in stream water (McDowell et al., 2018; Roberts and Cooper, 2018). The EPC₀ in sediments may change depending upon the SRP concentration in the sediment and stream water (Roberts and Cooper, 2018). Roberts and Cooper (2018), looked at the influence of sewage treatment works (STWs) on riverbed sediment to determine if it could act as a buffer for the increase in P concentrations. They found that regardless of STW influence, the riverbed sediments always acted as sinks in the water column. Despite higher concentrations of SRP in the rivers downstream of STWs, sediment near STWs had a higher capacity to absorb SRP (Roberts and Cooper, 2018).
Table 2.2: EPC$_0$ concentrations for various sediments and soils reported throughout literature.

<table>
<thead>
<tr>
<th>Location</th>
<th>EPC$_0$ Points (mg/L)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Redwood River Suspended Solids</td>
<td>0.074</td>
<td>James et al. (2002)</td>
</tr>
<tr>
<td>Minnesota River Suspended Solids</td>
<td>0.117</td>
<td>James and Larson (2008)</td>
</tr>
<tr>
<td>Lake Pepin Sediment</td>
<td>0.155</td>
<td>James and Barko (2004)</td>
</tr>
<tr>
<td>Minnesota River Basin Agricultural Soil</td>
<td>0.34</td>
<td>Fang et al (2002)</td>
</tr>
<tr>
<td>Eau Galle River</td>
<td>0.129</td>
<td>James and Barko (2005)</td>
</tr>
<tr>
<td>Colorado River</td>
<td>0.04</td>
<td>Mayer and Gloss (1980)</td>
</tr>
<tr>
<td>NY wooded streams</td>
<td>&lt;0.002</td>
<td>Klotz (1985)</td>
</tr>
<tr>
<td>Lower Mississippi River</td>
<td>0.108</td>
<td>Wauchope and McDowell (1984)</td>
</tr>
<tr>
<td>Lake Pepin Stream bank Sediment</td>
<td>&lt;0.1</td>
<td>Grundtner et al. (2014)</td>
</tr>
<tr>
<td>River Wensum Catchment (UK)</td>
<td>0.085</td>
<td>Roberts and Cooper (2018)</td>
</tr>
<tr>
<td>Mahantango Creek Catchment (Central PA)</td>
<td>0.02</td>
<td>McDowell &amp; Sharpley (2002)</td>
</tr>
<tr>
<td>Courthouse Creek Sediment VA</td>
<td>0.090</td>
<td>Sobotka (2011)</td>
</tr>
<tr>
<td>Kimages Creek Sediment VA (Legacy sediment)</td>
<td>0.010</td>
<td>Sobotka (2011)</td>
</tr>
<tr>
<td>Rathbun Lake Watershed (Iowa):</td>
<td></td>
<td>Hongthanat (2010)</td>
</tr>
<tr>
<td>Olmitz Fine-loamy mix (Floodplain)</td>
<td>0.185</td>
<td>Hongthanat (2010)</td>
</tr>
<tr>
<td>Olmitz Fine-loamy mix (Floodplain)</td>
<td>0.086</td>
<td>Hongthanat (2010)</td>
</tr>
<tr>
<td>Pre-Illinoian Till (Cut Bank)</td>
<td>0.013</td>
<td>Hongthanat (2010)</td>
</tr>
<tr>
<td>Nodaway Fine-Silty mix (Cut Bank)</td>
<td>0.12</td>
<td>Hongthanat (2010)</td>
</tr>
<tr>
<td>Colo Fine-silty mix (Floodplain)</td>
<td>0.232</td>
<td>Hongthanat (2010)</td>
</tr>
</tbody>
</table>

Processes described above for N and P are important for understanding how legacy sediments interact with the environment. These processes could be different depending on the location, water quality, and conditions of the channel.
Erosional Processes:

Streambank erosion can contribute 50 to 100% of sediment loads in Piedmont watersheds (Gellis and Noe, 2013). This erosion can be caused by a variety of mechanisms such as fluvial erosion generated by large storm flows (Gellis et al., 2017), freeze-thaw activity (Lawler 1993), or mass wasting (Fox et al., 2016). Gellis et al. (2017), found Tropical Storm Lee was an erosive event in which 82% of their measured streambanks eroded while 88% of streambanks that were aggrading before the storm became erosional. This highlights the potential that larger storms may switch erosive properties in stream banks. Sediment yields ranged from 161 to 376 Mg km\(^{-2}\) y\(^{-1}\) at their site in Upper Difficult Run, Virginia (Gellis et al., 2017).

Continuous freeze-thaw cycles or wetting and drying weaken streambanks and promote subaerial erosion (Merritts et al., 2013; Wolman 1959). Inamdar et al. (2018) found that freeze-thaw events followed by intense winter rainstorms exported high concentrations of suspended sediment, particulate organic carbon (POC) and particulate nitrogen (PN). At the 12 and 79 ha watershed within the mid-Atlantic region, suspended sediment levels were the highest recorded for February rainfall-runoff events over the past 10 years. Suspended sediment levels peaked at >5000mg L\(^{-1}\), POC was >250mg L\(^{-1}\), and PN was > 15 mg L\(^{-1}\) (Inamdar et al., 2018). This highlights the implications freeze-thaw cycles have on exports of sediment contributions.

Mass wasting occurs once the streambanks are weakened through fluvial or subaerial processes. Gravity causes the weakened streambank to collapse into the stream system (Lyons et al., 2015; Thorne 1982). This causes the sediment from the
streambanks to break off as “chunks,” if there is nothing holding the soil together. However, vegetation could reduce mass wasting due to roots reinforcing the soil (Fox et al., 2016). The erosive processes are key players in high concentrations of suspended sediment associated with storm events and legacy sediment stream banks (Inamdar et al., 2018).

2.5 Factors influencing stream bank legacy sediment erosion, nutrient content, and fate in streams

Particle Size

Particle size plays a key role in binding of P within soil. Total phosphorus (TP) in one study was found to have a direct association with the clay content in the soil (Day et al., 1987). It has also been noted that all forms of P are found to be highest in clay particles and lowest in sand particles (Hanley and Murphy, 1970). In the sand fraction, calcium bound P is the dominant form while for silts and clays it is iron bound P (Hanley and Murphy, 1970). The P found in sands is loosely bound compared to clay and silt, allowing for less sorption to occur (McDowell and Sharpley, 2002). Similar properties may be observed in legacy sediments as they are composed of mostly fine-grained sediment and on average consist of 64% silt/clay and 34% sand (Donovan et al., 2015).

Flow Type

Sediment and nutrient losses are also controlled by flow type (base flow vs. storm flow). Intense storms like hurricanes and tropical storms contribute a major proportion of annual sediment and nutrient exports from watersheds (Dhillon and
Inamdar 2013,2014; Gellis et al., 2017; Inamdar et al., 2015). Inputs of sediment and nutrients have also been found to be highest during storm events (Dhillon and Inamdar 2013; Gellis et al., 2017). Gellis et al. (2017) found that Tropical Storm Lee generated the highest suspended sediment load (5,560 Mg) compared to other months monitored during the 2 years and 10 months of study. This was 56% of the total suspended sediment loads during the study (Gellis et al., 2017).

Additionally, it has been found that up to 80% of annual TP loss from watersheds occurs during storm events while the remaining 20% is lost during base flow (Sharpley et al., 2008). Larger storms export larger amounts of sediment and soil containing additional TP. This is due to the increased volume of runoff that occurs as storm sizes increased. An increased amount of overland runoff and energy may erode particles enriched with TP (Sharpley et al., 2008.)

**Land Use**

Legacy sediment nutrient concentrations may be influenced by land use (Niemitz et al., 2013). Niemitz et al. (2013) collected and compared legacy sediment cores from both forested and agricultural watersheds at multiple depths. They analyzed the samples for metal and nutrient content and found that copper (Cu), lead (Pb), and P increased in the upper 100cm at the agricultural site, but not at the forested location. They then compared the chemical ratios for each sample (P/Al, Cu/Al, Pb/Al) to ratios of eastern United States soils and upper continental crust. These ratios exceeded natural conditions in the upper 100 cm of soil (Niemitz et al., 2013). However, below the upper 100 cm, both sites showed little variation, and the chemical ratios indicated natural conditions. They concluded that anthropogenic influences had
altered the chemistry of these surficial legacy sediments and that the remobilization of legacy sediment could act as an additional source of nutrients and trace elements to downstream ecosystems (Niemitz et al., 2013).

**Oxygen Levels**

Oxygen limitation greatly influences the iron and P interactions within the water column and may affect the redox potential of P fluxes (Coelho 2004). Under oxic conditions, iron and P bind together with iron being in the insoluble Ferric (III) form (Brady and Weil 2008). This allows the P to stay bound to the sediment. If dissolved oxygen levels drop or if the sediment is buried, creating anoxic conditions, then Fe$^{3+}$ is reduced to Fe$^{2+}$ or its Ferrous (II) form (Brady and Weil 2008). When this happens, the attached P is released into the water column and may act as a continuously recycled source of P over time (Brady and Weil 2008).

The continuously recycled P is a concern for aquatic ecosystems that are subjected to legacy sediment deposition (James et al., 1996). In a study done by Bischoff (2012), sediment was taken from 20 Minnesota lake sediments. Oxic P release varied by 0 to 0.8 mg/m per day while for anoxic conditions P release varied from 0 to 30mg/m$^2$ per day (Bischoff 2012). A recent study from Rahutomo et al. (2018), observed that for their incubations, the anaerobic conditions had low redox potential and increased pH. Organic labile P was found to increase in sediments subjected to low redox potential. As redox conditions changed over time, the younger sediments had a greater potential to contribute to elevated levels of P and may ultimately be released into aquatic systems (Rahutomo et al., 2018).
2.6 Variation in Microbial Characterization and Functionality

Microorganisms are the most abundant living organisms in terrestrial environments (Fierer 2006, 2017), and they are centrally involved in nutrient cycling within the environment (Fuhrman 2009). These communities composed of bacteria and archaea have a direct effect on nitrogen transformations (Herrmann et al., 2001; Leininger et al., 2006; Zhang et al., 2016) by affecting processes such as nitrification, denitrification, mineralization and immobilization (Johnson et al., 2005; Kirchman 2012). The communities may contain functional genes that encode enzymes such as ammonia monooxygenase (amoA) (Rotthauwe et al., 1997; Leininger et al., 2006) and nitrous oxide reductase (nosZ) (Throbäck et al., 2004; Zhang et al., 2016) that breakdown inorganic nitrogen. The functional genes and community structures are often analyzed to understand how microbial populations respond to environmental changes or disturbances (Fierer and Jackson, 2006; Kim et al., 2016). Despite numerous studies on microbial communities and their functionalities in soils and sediments (Griffiths et al., 2011; Hou et al., 2017; Kim et al., 2016; Li et al., 2016), there has only been one study that has employed microbial techniques on legacy sediments (Weitzman et al., 2014).

Weitzman et al. (2014), studied the differences in N processing and microbial activity in legacy sediment and the hydric layer. They found that the surface soils had a significantly greater nitrifier population index when compared to the mid-layer and relict hydric soils along the stream bank. However, the buried hydric layer was found to be low in denitrification and microbial activity (Weitzman et al., 2014). Additionally, their study also looked at the enzymatic activity in legacy sediments. They found that enzymatic activity was significantly higher in surface soils compared to mid layer and bottom soils. However, overall, there was low enzymatic activity
across the stream banks (Weitzman et al., 2014). This study suggests that overall microbial activity may be low in legacy sediments. However, another study looking at non-legacy sediment had different results. Kim et al. (2016) looked at the distribution and activities of microbial populations involved with N – cycling in riparian and stream sediments and found that the riparian sediment had higher rates of nitrification and denitrification occurring compared to the stream sediment. The riparian sediment was rich in organic matter and extracellular enzyme activities.

Functional gene abundance represents the functional role microbial communities have in regards to nutrient processing (Kan 2018). The nitrifier functional gene amoA is composed of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) which aid in ammonia oxidation (Herrmann et al., 2001; Leininger et al., 2006). There have been several studies that look at the effects of nutrients on the abundance and activity of amoA genes, but have concluded with conflicting results (Liu et al., 2014; Wang et al., 2014; Zhao et al., 2013). For instance, Liu et al. (2014) found that AOA abundance for their lake sediment negatively correlated with NH$_4$-N and positively correlated with NO$_3$-N and TP, while there were no significant correlations for AOB. On the other hand, Wang et al. (2014) found that AOB diversity for agricultural and reservoir sediment had significant positive correlations with pH and total nitrogen while AOA diversity was potentially negatively affected by NO$_3$-N and NH$_4$-N. While this is only a contrast between two studies, this highlights the complexity of microbial communities and functional genes. Environmental factors may influence the community structure, abundance, and functionality of microorganisms (Kirchman, 2012).
A variety of factors may influence microbial community signatures including, temperature, pH, soil type, soil chemistry, contemporary changes in the environment, and oxygen exposure (Kirchman 2012). Microbial community variation in legacy sediment stream banks may be affected by these factors, especially oxygen exposure. Locations with mill dams present will have a higher water level behind the dam (Merritts et al., 2011, 2013), creating saturated and anoxic conditions for the stream banks. These communities may change once mill dams are removed – creating a more aerobic environment. Phylum level shifts have been observed when floodplain sediments became hydrologically connected (Arigroff et al., 2017). Arigroff et al. (2017) found that Firmicutes increased in relative abundance, while Actinobacteria and Acidobacteria decreased as the sediment became more saturated and anoxic. These phyla are prominent in soils and sediments (Griffiths et al., 2011; Janssen, 2006; Kielak et al., 2016) and may be present in legacy sediment. The kinds of phyla that reside in legacy sediment is currently unknown. However, various microbial techniques can be used to determine the microbial communities and their functionality in sediments.

**Microbial Techniques for Characterization**

Environmental bulk samples, from soil or sediment, can contain thousands of microbial communities which can be identified through next-generation sequencing (NGS) technology (Shokralla et al., 2012). Taxa present within a sample can have their sequences matched with a standard reference library of known sequences (Shokralla et al., 2012). NGS technology can match these organisms with high confidence (Shokralla et al., 2012). By using this technology, one can observe slight
changes in community structures when there are fluctuations by natural or anthropogenic means (Leininger et al., 2006). Other analyses have looked at soil bacterial biodiversity by looking at 16S rRNA amplicons. In the instance of Roesch et al. (2007), they found that agricultural management of soil may significantly influence the diversity of bacteria and archaea.

Two molecular techniques that are being used for this study are Quantitative Polymerase Chain Reaction (qPCR) and High Throughput Sequencing (Klindworth et al., 2013). qPCR allows us to analyze the functional genes of microbial populations and determine the abundance of that gene within sediments (Kan 2018). By measuring multiple genes, we can determine if specific genes are more prevalent than others (e.g., nitrifiers vs. denitrifiers). High throughput Illumina sequencing characterizes the microbial populations allowing one to discern the potential variations such as between land usages.

An example of the usage of qPCR techniques was demonstrated in a paper by Thompson et al., (2016). They identified the impact of a major pesticide spillage in the River Kennet and how this influenced microbial functional genes and the ecosystem of the river. They used qPCR techniques on the microbial communities, both upstream and downstream of the river and found the pesticides caused both direct and indirect effects. One of the genes analyzed, the ammonia monooxygenase gene (amoA), was thirty times more abundant than previous measurements. It was concluded that the increase in decaying invertebrates caused a rise in amoA abundance (Thompson et al., 2016).

High throughput sequencing uses a sequencing methodology called Illumina sequencing (Degnan and Ochman, 2012). In 2012, the Illumina methodology was
1/100 of the cost per reading than the 454 pyrosequencing – another notable methodology that is used for sequencing (Degnan and Ochman, 2012). Illumina generally reads much shorter sequence lengths compared to 454 pyrosequencing, but the length of the sequence can be increased by merging paired-end reads from the same amplicon (Gloor et al., 2010). Between the 454 pyrosequencing and Illumina sequencing, data must be separated between biological variation and amplicon sequencing errors. This has prompted amplicon-specific error-correction methods, most of which were made for 454 pyrosequencing and not Illumina sequencing (Reeder and Knight, 2010). Quality filtering the construction of Operational Taxonomic Units (OTUs) is used to reduce the number of errors in Illumina sequencing (Schloss et al., 2009). DADA2 (Divisive Amplicon Denoising Algorithm), an open-source R package (Callahan et al., 2016), is used to disentangle biological variation from sequencing errors. The program infers sample composition by dividing amplicon reads into partitions consistent with the error model within the program. The algorithm for denoising is built on a model of errors in Illumina-sequenced amplicon reads. This provides a clean output of the sequences from the sample, allowing someone to determine the potential communities within a sample (Callahan et al., 2016). Both qPCR and high throughput sequencing techniques allow us to compare microbial communities and their functionality between varying depths, land use, and environmental conditions.
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Chapter 3

MICROBIAL COMMUNITY COMPOSITION AND NITROGEN TRANSFORMATION GENES OF LEGACY SEDIMENTS IN THE MID-ATLANTIC REGION, USA

Abstract

Stream banks with legacy sediments may contribute substantial amounts to sediment exports from Mid-Atlantic watersheds. However, there is considerable uncertainty regarding nutrient content and nutrient export from legacy sediment. Additionally, there is uncertainty regarding the role of microbial communities in influencing nutrient processing, and implications of these processes for receiving waters. In this study, we sampled 15 different stream banks at multiple depths throughout four watersheds in the Mid-Atlantic Region. High throughput sequencing of 16S ribosomal RNA genes indicated that microbial communities from stream bank sites varied by land use, depth, and on a site-specific basis. In general, the most abundant microbial taxa in legacy sediment were Acidobacteria (25-45%), Proteobacteria (15-40%, mainly alpha, beta, gamma, and delta subclasses), Chloroflexi (1-5%), Actinobacteria (1-10%), Nitrospirae (2-10%), Firmicutes (1-10%), Verrucomicrobia (1-5%), Gemmatimonadetes (1-5%), AD3 (1-5%), Planctomycetes (1-5%), Bacteroidetes (1-5%), and OD1 (1-3%). NMDS plots used to display variations among sites and samples showed distinct separations between agriculture and urban groups (p = 0.001) as well as suburban and urban groups (p = 0.001). Site-specific separation of two stream banks from urban locations: Cooch’s
Bridge and Brandywine Zoo were also present. The two urban sites: Cooch’s Bridge and Brandywine Zoo, drove the separation of urban locations. There was also significant variation in microbial community composition between the bottom and top layer sediment. Only relative abundances of *Acidobacteria* and *Proteobacteria* differed between depths (both \( p < 0.007 \)). The total *Proteobacteria* taxa were significantly different between urban and suburban land use (\( p = 0.028 \)). Several weak but significant positive correlations were observed between the stream bank chemistry (metals and nutrients) and microbial phyla. Real-time PCR analysis on nitrogen transformation genes suggested that denitrifying microbes (*nosZ* genes) dominated in lower saturated sediment layers, while nitrifiers (*amoA* genes) were more abundant in near-surface sediment layers, however, *nosZ* was more abundant than *amoA* across all sites and depths. Compared to ammonia-oxidizing bacteria (AOB), nitrifying archaea (AOA) were more predominant in legacy sediments. This study provides new insights into the microbial communities and their N-cycling functionality in legacy sediments within freshwater ecosystems.

3.1 Introduction:

Suspended sediment is the second leading cause of water quality impairment following excess nutrients in the Chesapeake Bay (Chesapeake Bay Program 2018). Excess sediment can limit light penetration in aquatic systems and thus reduces the primary production of phytoplankton and aquatic vegetation (Hotzel and Croome, 1994; Henley et al., 2000). Sediment can also carry nutrients such as nitrogen (N) and phosphorus (P) in water bodies (Yang et al., 2008). Inputs of nutrients may lead to
eutrophication and algal growth, which subsequently create anoxic or hypoxic conditions in downstream bays and estuaries (Smith 2003).

Anthropogenic, or legacy sediment, has been identified as a potentially important sediment source to aquatic systems, particularly in the mid-Atlantic US (James 2013; Miller et al., 2019). Widespread erosion following deforestation and poor agricultural practices post-European settlement led to the accumulation of large amounts of sediment behind low-head mill dams (Walter and Merritts, 2008). Throughout the Mid-Atlantic region, over 65000 low-head mill dams were constructed across 1st to 3rd order stream valley bottoms (Merritts et al., 2011).

Many of the milldams have since been removed or have breached resulting in channel incision due to base level lowering of the streams. Not surprisingly, this has resulted in stream banks that are more susceptible to fluvial and subaerial erosive processes (Fox et al., 2016; Gellis et al., 2017; Wolman 1959). Due to stream bank erosion, legacy sediments have been identified as major contributors to annual sediment exports in the Chesapeake Bay watershed (Cashman et al., 2018; Gellis et al., 2009, 2018) and may account for 50 to 100% of suspended sediment loads in some systems (Banks et al., 2010; Gellis and Noe, 2013). While our understanding of legacy sediment inputs continues to improve, there is a significant lack of understanding of how legacy sediments influence the inputs and export of nutrients in fluvial networks. We also know little about how incised stream banks and associated hydrologic and biogeochemical conditions influence microbial communities in legacy sediments and their potential effects on nutrient cycling.

Microorganisms are the most abundant and widely distributed living organisms in terrestrial environments (Fierer 2006; 2017 and citations therein). They maintain
ecosystem function and integrity through energy and nutrient cycling (Strickland et al., 2009). For instance, microbial communities are comprised of bacteria and archaea which have a direct effect on nitrogen transformations (Herrmann et al., 2011; Leininger et al., 2006; Zhang et al., 2016) by affecting processes such as nitrification, denitrification, mineralization, and immobilization (Johnson et al., 2005; Kirchman 2012). These communities may contain a variety of functional genes (Rotthauwe et al., 1997; Leininger et al., 2006; Throbäck et al., 2004; Zhang et al., 2016) that breakdown different forms of inorganic N to varying extents. Microbial community structures are analyzed and documented to better understand and interpret how populations respond to environmental changes or disturbances (Fierer and Jackson, 2006; Kim et al., 2016), but little is known about environmental controls that influence community and functional gene abundance in legacy sediments. While there have been many studies that analyzed microbial community and functional gene abundance in soils and river sediments (Griffiths et al., 2011; Hou et al., 2017; Kim et al., 2016; Li et al., 2016), to our knowledge only one study has investigated microbial communities in legacy sediments, (Weitzman et al., 2014). Weitzman et al. (2014) studied the differences in N processing and associated genes for legacy sediments and the pre-colonial hydric soils buried below the legacy sediments. Contrary to their expectations, they found low denitrification and microbial activity for the buried hydric layer (Weitzman et al., 2014). Nevertheless, these results underscore the complexities and need to study microbial communities and their functionality within legacy sediment to better understand their role in nutrient cycling and how environmental drivers regulate microbial diversity.
The objective of this study was to determine the composition and diversity of microbial communities in stream bank legacy sediments and their potential implications for nitrogen processing and cycling. Specific questions we are addressing include:

1. What are the compositions and relative abundances of microbial communities in legacy sediments?
2. How do microbial communities vary across land use, bank depth, and environmental gradients and what are the key drivers?
3. What does the nitrogen functional gene distribution tell us about nitrogen processing?

This research is the first comprehensive study analyzing the microbial community structure within legacy sediment and may pave the way for future studies within the field. We address these knowledge gaps by examining legacy sediments for 15 different stream bank sites located in the Chesapeake and Delaware Bay drainage basins in Maryland, Delaware, and Pennsylvania, USA. Legacy sediment sites covered four different land uses including forest, suburban, urban, and agriculture. Sediment microbial communities were determined using high throughput sequencing, and nitrification and denitrification functional gene abundance were measured through quantitative polymerase chain reactions (qPCR).
3.2 Site Description and Methods:

Legacy sediments were collected from 15 sites over five streams – Big Elk Creek, White Clay Creek, Christiana River, Gramies Run and Brandywine Creek in northern Delaware, southeastern Pennsylvania, and northeastern Maryland (Table 3.1, Figure 3.1). Sampling sites were selected based on contemporary land use, safe access, sampling permits, locations of milldams (existing and breached/removed) from historical maps, and visual identification of legacy sediments in the stream banks. Contemporary land use categories were determined considering two approaches: the stream water NO$_3$-N concentrations at baseflow and the National Land Cover Database (2011). Stream water grab samples were collected on the same day as the stream banks were sampled and analyzed for NO$_3$-N concentrations. If locations where the NLCD classifications were unclear, the stream water NO$_3$-N concentrations were used to help determine the land use type. Sites were classified as “forested” locations if NO$_3$-N concentrations were less than 2 mg*L$^{-1}$; “urban” and “suburban” locations were between 2-3 mg*L$^{-1}$ NO$_3$-N, and “agricultural” sites if concentrations exceeded 5 mg*L$^{-1}$ NO$_3$-N. These concentrations reflect the values of headwaters within the surrounding area.
Figure 3.1. Map showing 15 sampling sites for legacy sediments and their associated land uses forested, suburban, urban, and agriculture.
Table 3.1: Legacy sediment sampling sites with coordinates, land use, bank heights, sample numbers and depth sampled and stream water depth at the time of sampling.

<table>
<thead>
<tr>
<th>Site Name</th>
<th>Name Abbreviation</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Land Use</th>
<th>Bank Height (cm)</th>
<th>No. of depths sampled</th>
<th>Water Depth at Bank (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gramies Run</td>
<td>GMT</td>
<td>39.6856</td>
<td>-75.8503</td>
<td>Forested</td>
<td>168</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Middle Run</td>
<td>MR</td>
<td>39.7214</td>
<td>-75.7304</td>
<td>Forested</td>
<td>168</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>Cider Mill</td>
<td>CDM</td>
<td>39.6923</td>
<td>-75.7553</td>
<td>Suburban</td>
<td>274</td>
<td>4</td>
<td>28</td>
</tr>
<tr>
<td>Casho Mill</td>
<td>CM</td>
<td>39.6829</td>
<td>-75.7799</td>
<td>Suburban</td>
<td>244</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>Cottage Mill</td>
<td>RH</td>
<td>39.6567</td>
<td>-75.761</td>
<td>Suburban</td>
<td>152</td>
<td>4</td>
<td>n/a</td>
</tr>
<tr>
<td>Byrnes Mill</td>
<td>BYR</td>
<td>39.698</td>
<td>-75.6652</td>
<td>Urban</td>
<td>244</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>Brandywine Creek</td>
<td>BZ</td>
<td>39.7587</td>
<td>-75.5549</td>
<td>Urban</td>
<td>152</td>
<td>4</td>
<td>n/a</td>
</tr>
<tr>
<td>Cooch’s Bridge</td>
<td>COB</td>
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<td>-75.7423</td>
<td>Urban</td>
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<td>61</td>
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<tr>
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<td>-75.7493</td>
<td>Urban</td>
<td>122</td>
<td>4</td>
<td>10</td>
</tr>
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<td>Big Elk Bridge</td>
<td>BEB</td>
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<td>Agriculture</td>
<td>259</td>
<td>4</td>
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</tr>
<tr>
<td>Camp Bonsul Road</td>
<td>CB</td>
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<td>-75.8828</td>
<td>Agriculture</td>
<td>411</td>
<td>6</td>
<td>23</td>
</tr>
<tr>
<td>Nature Center Beach</td>
<td>NCB</td>
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<td>-75.663</td>
<td>Agriculture</td>
<td>259</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Scott’s Mill 2</td>
<td>SM2</td>
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<td>Agriculture</td>
<td>274</td>
<td>4</td>
<td>2.5</td>
</tr>
<tr>
<td>Scott’s Mill 3</td>
<td>SM3</td>
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<td>Agriculture</td>
<td>305</td>
<td>4</td>
<td>2.5</td>
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<tr>
<td>Tweed’s Mill</td>
<td>TM</td>
<td>39.7261</td>
<td>-75.7675</td>
<td>Agriculture</td>
<td>137</td>
<td>4</td>
<td>n/a</td>
</tr>
</tbody>
</table>
3.2.1 Field Sampling and Collection:

At each sampling location, stream bank height, GPS coordinates, number of depths sampled, and the depth of water at the bank were recorded (Table 3.1). Stream water grab samples were collected for nutrient analyses and photos were taken of each stream bank (Appendix A2). Sampling depths were determined based on sediment color, bank height, and texture. Samples were labeled based on a site-code (Table 3.1) and the depth (cm) at which they were collected.

When stream water levels were low and safe access was possible, samples were collected by standing in the stream and collecting samples laterally from the bank. The surficial one inch of the bank was scraped off before sample collection. Four to five samples were collected at each depth to account for spatial heterogeneity and composited into one sample. Samples were collected using a clean trowel or an auger. When stream water levels were too high to stand in the stream, or it was unsafe, bank samples were collected by augering down into the bank.

Following collection, samples were placed in sterile Ziploc bags and put on ice until they were brought back from the lab for further analyses. For each site and depth, a 20g microbial subsample was taken from the homogenized samples and was placed into a sterile Whirlpak bag and frozen at -80°C until further analysis. On average, four to five samples were collected at each stream bank site and for different depths. However, depending on stream bank height, some locations had as little as three samples while others had as many as seven samples. This resulted in a total of 67 sediment samples across the 15 stream bank sites.
3.2.1.1 Chemical Analysis:

This microbial study was performed with a companion study investigating nutrient and metal concentrations in legacy sediments (Lutgen 2019). Nutrient and metal analyses are described in detail in Lutgen (2019). A subset of each homogenized sample (~100g) was air dried underneath a fume hood at ambient temperature for 48 hours. Dried samples were pre-sieved using a 2mm mesh to remove large debris from the sample. The dried subsample was then ground and sieved into two different particle-size classes, coarse (>63µm) and fine (63µm) fractions using an RX-29 RoTap® sieve shaker. Once sieved, samples were frozen and stored at -20°C for testing.

Mehlich-3 elements (STPM105-01), nutrient analysis for nitrate (NO₃-N) and ammonium (NH₄-N) via KCl extractions (STPM111-01), and microwave digestion (EPA_3051) were done by the University of Delaware Soil Testing Labs. Additional subsamples of each size fraction were sent to the Central Appalachians Stable Isotope Facility for %C and %N by combustion and carbon (δ¹³C) and nitrogen (δ¹⁵N) isotopes (CASIF; Frostburg, MD). Analyses were done for the following nutrient and metal characterizations: Mehlich 3 extractable elements (Mehlich, 1984; Sims et al., 2002) which included: Fe, Al, P, K, Ca, Mg, Mn, Zn, Cu, B, S, Si. Additionally measured characterizations included: NH₄-N, NO₃-N, As, Cd, Co, Cr, Cu, Ni TP, Pb, Si, Zn; %TC, %TN, δ¹³C, δ¹⁵N. Particle size was also determined for each sample using a Beckman Coulter LS 13 320 Particle Size Analyzer (%coarse, %fine, %sand, %silt, %clay) (Indianapolis, IN).

Additionally, radiocarbon dating using C¹⁴ isotopes was performed for two precolonial hydric soil layers - 127 cm depth at GMT and the precolonial leaf layer.
near the 396 cm sample at SM3 (Table 3.1). The mean ages were determined to be 950 and 220 years for GMT and SM3 soil samples, respectively.

3.2.1.2 Microbial Analysis

Genomic DNA was extracted from composite subsamples (not partitioned in fine and coarse) using a PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Inc.) following the manufacturer’s instructions. DNA concentrations and purity were measured by using an ND-2000 NanoDrop spectrometer (Thermo Fisher), and frozen at -80°C until future analyses. High Throughput Sequencing was performed to analyze detailed microbial community structures in legacy sediments. Nitrification and denitrification genes were quantified with Quantitative Polymerase Chain Reactions (qPCR) (QuantStudio™ 3 System) among legacy sediment from different land uses and bank depths.

High throughput sequencing and data processing

A total of 67 samples (15 sites with varying depths) were sequenced using the Illumina MiSeq platform at the University of Maryland Center for Environmental Science core facility (http://www.umces.edu/baslab). Partial 16SrRNA (V3-V4 hypervariable regions) gene fragments were amplified (Klindworth et al., 2013) and sequenced following 16S Metagenomic Sequencing Library Preparation protocol from Illumina (https://support.illumina.com/) previously described (Kan 2018). Raw illumine sequences were cleaned and analyzed using the DADA2 R package (Version 13.8, Callahan 2016). Briefly, low-quality sequences and chimeras were removed, and only high-quality data with at least 250bp were maintained in downstream analysis. Rarefaction curves were generated within the DADA2 script, and amplicon sequence
variants (ASVs) were normalized based on 99% coverage with a cutoff at 50,000 sequences per sample (Appendix A6).

**qPCR analysis**

Nitrogen transformation genes for nitrifiers and denitrifiers were used to determine functional gene abundance within the legacy sediments – ammonia monooxygenase (*amoA*) genes for ammonia oxidizing bacteria (AOB) and archaea (AOA) and nitrous-oxide reductase (*nosZ*) for denitrifying microorganisms.

The Sybr Green qPCR method (QuantiTect SYBR® Green PCR Kit (Qiagen)), was used to quantify these genes on a QuantStudio™ 3 System (Thermo Fisher). PCR reactions were set up and contained 1μL of DNA and: 10μL Sybr Green mix, 0.4 μL Primer 1, 0.4μL Primer 2, 0.4μL Bovine Serum Albumin (BSA), and 7.8μL PCR grade H₂O. The AOA primers used were *amoA*-1F (Francis et al., 2005) and *amoA*-1R, AOB Primers used were *amoA*-1F, and *amoA*-2R (Rotthauwe et al., 1997), and *nosZ* used nosF (Kloos et al., 2001) and nosZR¹⁶²² (Throbäck et al., 2004). For each functional gene: AOA, AOB, and *nosZ*, samples were exposed to the following thermal program: 1 cycle (50°C for 2 mins), 1 cycle of initial denaturing (95°C for 2 mins), 50 cycles of denaturing (15s at 95°C), annealing, (15s at 58°C for AOA and AOB; 56°C for *nosZ*), and extension (1 min at 72°C). Finally, a melting curve analysis was done for 15s at 95°C with dissociation from 60°C – 95°C. Each sample was run in triplicates or quadruplicates. For each functional gene, tenfold dilutions series were generated from frozen plasmids. Five points were used to generate a standard curve. For the AOA plasmid concentrations ranged 2.1 x 10¹ to 2.1 x 10⁵ ng, AOB’s concentrations ranged from 1.2 x 10¹ to 1.2 x 10⁵ ng, and *nosZ* ranged 1.1 x 10³ to 1.1
x 10^7 ng respectively. Standard curves were only accepted when the R² value was greater than 0.96. Mean amplification efficiency values for each gene were: AOA (111.37), AOB (75.48), and nosZ (69.52) The copy number per gram of wet soil was then calculated for each qPCR result by the following equation:

\[
(1) \quad CN_g = CN_{qPCR} \times (E / S_g)
\]

where CN_g is the copy number per grams of soil (Copy # * g⁻¹); CN_{qPCR} is the quantity of copy numbers in the qPCR reaction; E is the elution volume; S_g is the amount sediment used for DNA extraction in grams.

3.2.2 Statistical Analyses

Aggregated and normalized amplicon sequence variants (ASV) tables were used to calculate Chao1 richness (Chao 1984), Shannon, and Simpson diversity indices in DADA2. The Bray-Curtis similarity/dissimilarity (Bray and Curtis 1957) between the relative abundance of all identified taxa (at genus level) and Non-metric Multidimensional Scaling (NMDS) was conducted with employing the MDS procedure in SAS/STAT (v9.4, SAS Institute Inc., Cary, NC, USA). The scree plot (goodness-of-fit criterion or stress plotted against a number of dimensions) suggested that the first two dimensions were sufficient in defining the overall dimensionality of the input data matrix. Stress values close to or less than 0.1 indicated a good ordination with little risk of misinterpretation of the distribution pattern (Clarke 1993). Permutational multivariate analysis of variance (PERMANOVA) based on Bray-Curtis dissimilarities was performed to test significant differences between groups (land use, depth, and sites) (Anderson 2001, 2008) with a Bonferroni correction.
PERMANOVA functions were implemented in the R-package vegan (Oksanen et al., 2018) using the adonis function and the number of permutations was set to 999.

Environmental parameters were correlated with microbial parameters using SAS (Version 9.4, 2013). Environmental parameters included streambank chemistry: nutrient and metal content within the stream banks, bank depth, particle size, and stream water NO$_3$-N concentrations. Bulk sample values for nutrients and metals were determined using the following equation:

$$ (2) \ BV = (C \times %C) + (F \times %F) $$

where BV is the bulk sample value for nutrients and metals; C is the concentration for the coarse fraction; %C is the percentage of the sediment that is coarse; F is the concentration for the fine fraction; %F is the percentage of sediment that is fine. Depth was categorized into four groups based on percent depth to facilitate comparisons by sites. These layers were: 0% - 25% (Top Layer), 26% - 50% (Upper Middle Layer), 51% - 75% (Lower Middle Layer), and 76% - 100% (Bottom Layer). Spearman’s ranked correlation coefficients were determined between these environmental parameters and NMDS results.

Additionally, correlations (Pearson) were determined between environmental parameters, (metals, nutrient concentrations, depth, land use, particle size) and microbial functional gene abundance in JMP (version 14.0.0, 2018). Significant differences in microbial phyla and select genera, functional genes, Chao1 richness, Shannon and Simpson indices with depth and land use were determined using ANOVA and Tukey-Kramer HSD (p = 0.05). At the genus level, ANOVAs were
performed for *Candidatus Solibactor usitatus, Geobacter,* and *Nitrospira* populations to see if there were any statistically significant variations across depth classifications.

### 3.3 Results:

#### 3.3.1 Microbial Community Composition and Diversity:

Legacy sediments across the 15 sites predominantly contained the phylum *Acidobacteria* which comprised 25-45% of the overall microbial community relative abundance (Figure 3.2). This was followed by the phylum *Proteobacteria* representing between 15-40%. *Alphaproteobacteria* was the dominant class of *Proteobacteria* within legacy sediment (10-15%). Other prominent phyla were *Chloroflexi* (1-5%), *Actinobacteria* (1-10%), *Nitrospira* (2-10%), *Firmicutes* (1-10%), *Verrucomicrobia* (1-5%), *Gemmatimonadetes* (1-5%), *AD3* (1-5%), *Planctomycetes* (1-5%), *Bacteroidetes* (1-5%), and *OD1* (1-3%) in terms of relative abundance (Figure 3.2). The following genera were observed in the sediment based on high relative abundances and known functionality: *Candidatus Solibactor usitatus, Nitrospira,* and *Geobacter.* The relative abundance for each of the genera were 0-4%, 0-1%, and 0-4% respectively.
Figure 3.2. Relative abundance (%) of major bacterial phyla across 15 sites. *Proteobacteria* was further divided into alpha, beta, gamma, delta, and other.

Shannon diversity index ranged from 5.92 to 7.46 (mean: 6.63 ± 0.36) while the Simpson diversity index ranged from 0.996 to 0.999 (mean: 0.998 ± 0.0007). Inverse Shannon ranged from 254 to 1331 (mean: 585 ± 241). Chao1 richness ranged from 745 to 2924 (mean: 1405 ± 511). TM_132 indicated the highest species richness between samples (2924). The sample BZ_76 contained the highest Shannon and Simpson diversity (7.46 and 0.999). There was no significant difference between Chaol richness and Shannon and Simpson diversity indices when compared across land use or depth (p > 0.05).
3.3.2 Variation in composition with bank depth and land use:

Bacterial community structure differed between types of land use, with sample depth, and at specific sites (Figure 3.3). There was an overall difference in bacterial communities by contemporary land use classifications (Figure 3.3a; Table 3.2, p = 0.001) that was driven by communities which were distinct between agriculture and urban sites (F = 1.685, p = 0.024) and urban and suburban sites (F = 1.751, p = 0.006) (Figure 3.3a and Table 3.2). There was a statistically significant difference between the bacterial communities collected across different depths (Figure 3.3b; p = 0.001, Table 3.2). For the depth categories, there were significant difference between the Top and Bottom layers (F = 2.43, p = 0.006) and the Upper Middle and Bottom layers (F = 1.77; p = 0.006) (Figure 3.3b and Table 3.2). Microbial community structure also varied on a site by site basis with a distinct separation observed at two sites: Brandywine Zoo and Cooch’s Bridge; each had separate community structures compared to other sites F = 2.24, p = 0.003; and F = 1.74, p = 0.003) (Figure 3.3c and Table 3.2).
Figure 3.3. NMDS plots showing bacterial community structures in legacy sediments. Samples classified by a) land use between samples; b) depth; c) site-specific separation.
Table 3.2. Overall and pair-wise comparisons of microbial community composition analyzed with PERMANOVA using Bray-Curtis distance with Bonferroni adjustment

<table>
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<th>Comparison</th>
<th>d.f</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>$R^2$</th>
<th>p value</th>
<th>p adjusted</th>
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<td><strong>Overall Land use</strong></td>
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<td>Ag vs. Urban*</td>
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<td>1.47</td>
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<tr>
<td>Bottom vs. Top*</td>
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<td>Site</td>
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<td>1.97</td>
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<td>Residuals</td>
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<td>26.33</td>
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<td><strong>Contrasts</strong></td>
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<td>N vs. BZ*</td>
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<td>2.244</td>
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<td>0.001</td>
<td>0.003</td>
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<td>N vs. COB*</td>
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<td>1.736</td>
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<td>0.003</td>
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<tr>
<td>BZ vs. COB</td>
<td></td>
<td>1.543</td>
<td>0.236</td>
<td>0.031</td>
<td>0.093</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: MS, mean sum of squares; N, all 15 sites; BZ, Brandywine Zoo; COB, Cooch’s Bridge; * highlights significance
Candidatus Solibactor usitatus relative abundance differed with depth (Appendix A11) (One-way ANOVA p = 0.004) with significant differences between the Top Layer and Bottom Layer (p = 0.011) and Upper Middle and Bottom Layer (0.006) (all Tukey-Kramer-HSD). However, the relative abundance of Geobacter and Nitrospira did not vary by legacy sediment depth or with land use (p > 0.05).

3.3.3 Interaction with environmental gradients:

To further explore the drivers of differences between microbial communities in legacy sediments at urban and suburban sites, and specifically the two urban sites, Cooch’s Bridge and Brandywine Zoo, we examined how this subset of sites related to environmental gradients (Figure 3.4). Microbial community structure was correlated with stream bank chemical parameters and microbial phyla (Figure 3.4; Spearman ranked correlations, p ≤ 0.01). The microbial community was also correlated with particle size composition of the stream banks (Spearman ρ ranged from -0.23 to 0.25). There were no significant correlations between particle size and community structure across all sites (p > 0.05). The two locations with distinct community structures, Brandywine Zoo, and Cooch’s Bridge were driving the relationships between community composition, metals, and nutrients (Figure 3.4a and Figure 3.4b). We observed additional positive correlations between M3-Al and M3-Fe in Bottom Layer samples. Following this, Geobacter was correlated with M3-Fe to explore this relationship (Appendix A10) further. There was a weak but statistically significant positive correlation between Geobacter and M3-Fe ($R^2 = 0.08$, $p = 0.019$). No significant correlations were observed between NMDS coordinates and stream water NO$_3$-N concentrations (p > 0.05).
Figure 3.4. Correlations of stream bank chemistry with NMDS separation of microbial community structure. Only significant a) chemical parameters ($p \leq 0.01$) and b) microbial phyla are shown with vectors.
3.3.4 Nitrogen Transformation Genes:

Nitrous-oxide reductase gene, *nosZ* was more abundant than *amoA* abundance of Bacteria and Archaea at the 15 streambank locations (Figure 3.5). The highest abundance of *nosZ* functional genes occurred in the bottom layer (SM3_396) of the Scotts Mill 3 site (2.1 E+07 *nosZ* copies g\(^{-1}\) soil, Figure 3.5). Stream banks contained a greater abundance of AOA than AOB (Appendix A7). AOB genes were detected in only 16 of the 67 samples. The highest *amoA* gene abundance was observed at the top layer of the Brandywine Zoo stream bank (1.1 E+07 nitrifier copies g\(^{-1}\) soil, Figure 3.5). Overall, there were no significant differences between the log-transformed functional genes when classified by depth (p > 0.05). Abundances for three N-cycling genes were the highest in sites classified under urban and agricultural land use sites, and lowest for forested sites (Appendix A3, A4, A5). There was a significant difference in the log-transformed nitrifier functional gene abundance between forested and urban sites (p = 0.006).
Nitrogen functional gene abundances were weakly correlated with several stream bank chemistry parameters and genera (Table 3.3). Nitrifier gene abundance positively correlated most strongly with M3-Ca and M3-Cu ($r = 0.54; \ p < 0.001$ and $r = 0.56; \ p <0.001$, respectively). Denitrifier nosZ gene abundance positively correlated most strongly with %TC and %TN ($r = 0.49; \ p < 0.001$ and $r = 0.40; \ p = 0.001$, respectively). The abundance of one bacterium genus known to be a nitrifier, *Nitrospira*, had a significant positive correlation with log transformed AOA gene abundance ($r^2 = 0.40; \ p < 0.001$) (Appendix, Figure A12).
The pattern of site-specific variation in functional genes and community compositions led us to highlight a focal set of sites to show specific depth trends in N-cycling gene abundance at sites (Figure 3.6a-d). Gramies Run legacy sediment, contained a high abundance of \textit{nosZ} genes at 127cm depth (sample GMT_127) while \textit{amoA} abundance at this depth was below detection. The \textit{Acidobacteria} in the pre-colonial layers buried beneath the legacy sediment (GMT_127 and GMT_152) was lower in relative abundance than the legacy sediments sampled above them (20% and 15% respectively) (Figure 3.6a). The precolonial layer organic horizon was most pronounced for this location (dark horizon with elevated %C), compared to the other sampled locations. Brandywine Zoo had an overall high abundance of nitrogen transformation genes compared to the other sampling locations. For Brandywine Zoo, \textit{Acidobacteria} relative abundance decreased at the lowest depth (mean: 25.3% ± 0.09% to 16.7%) while relative abundances of \textit{Proteobacteria} (mean: 33.7% ± 0.77% to 47%) increased at the lowest depth (Figure 3.6b). Legacy sediment at Scotts Mill 3 increased in \textit{nosZ} gene abundance with depth. A relative increase in \textit{Proteobacteria} (mean: 29.5% ± 2.5% to 37.0%) and \textit{Chloroflexi} occurred in the lowest depth sampled (396 cm) while \textit{Acidobacteria} decreased (mean: 35.0% ± 2.0% to 23.8%) (Figure 3.6c). Nature Center Beach sediments varied in functional gene abundances across depths, with no specific pattern observed.

Phyla relative abundances varied arbitrarily with between depth. \textit{Acidobacteria} relative abundance increased moving vertically down the stream bank profile while \textit{Actinobacteria} and \textit{Firmicutes} decreased at the lowest depths (Figure 3.6d).
Table 3.3. Pearson Correlations of nitrogen functional genes (amoA and nosZ) and stream bank chemistry. Significant correlations (p<0.01) are highlighted in bold.

<table>
<thead>
<tr>
<th>Nitrogen transformation genes</th>
<th>Stream bank Chemistry</th>
<th>r</th>
<th>p-value</th>
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<tr>
<td>amoA (AOA + AOB) (nitrifiers)</td>
<td></td>
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<td></td>
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<tr>
<td>M3-Al</td>
<td>-0.3079</td>
<td>0.0112</td>
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<tr>
<td>M3-P</td>
<td>0.4633</td>
<td>p &lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>M3-Ca</td>
<td>0.5429</td>
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<td></td>
</tr>
<tr>
<td>M3-Zn</td>
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<td>0.0031</td>
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<tr>
<td>M3-Cu</td>
<td>0.5615</td>
<td>p &lt; 0.0001</td>
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<tr>
<td>M3-B</td>
<td>0.4105</td>
<td>0.0006</td>
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<tr>
<td>M3-Si</td>
<td>0.2931</td>
<td>0.0161</td>
<td></td>
</tr>
<tr>
<td>amoA (AOA + AOB) (nitrifiers)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td>0.3668</td>
<td>0.0023</td>
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<tr>
<td>Cr</td>
<td>0.3277</td>
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<tr>
<td>%TN</td>
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<tr>
<td>nosZ (denitrifiers)</td>
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<td>M3-P</td>
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<tr>
<td>M3-Fe</td>
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<tr>
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<tr>
<td>%TC</td>
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<tr>
<td>%TN</td>
<td>0.404</td>
<td>0.0007</td>
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Discussion:

The results from this study highlight the spatial heterogeneity and variability of microbial communities in stream bank legacy sediments. The variation between bacterial communities was correlated with land use, bank depth, and site-specific variance and may be driven by the local legacy sediment chemistry. Nitrogen transformation genes, *amoA*, and *nosZ* were also found to be highly variable across sites. This variability is not surprising given the diversity of environmental gradients and history of the stream banks.
3.4.1 Variation in microbial community composition of legacy sediments

Twelve different phyla had greater than 1% relative abundance across all sites indicating potentially dynamic interactions between microbial processes in legacy sediment. One of these phyla, *Acidobacteria*, was the most dominant phylum observed in legacy sediments and is recognized as one of the most diverse phyla within soils (Dunbar et al., 2002; Fierer, 2017; Kielak et al., 2016). Our observations indicate that 25-45% of stream bank microbial communities consisted of *Acidobacteria* which is within the range 20-52% of microbial communities in soil habitats (Dunbar et al., 2002; Janssen, 2006). *Acidobacteria* and *Proteobacteria* were the only phyla that showed statistically significant differences in relative abundance with depth (Appendix A8, A9), while only *Proteobacteria* showed a statistically significant difference between land use types. The two were the most abundant phyla found in legacy sediment making up the combined total of about 55 – 65% of the total abundance across each stream bank.

Microbial communities varied in composition and abundance across the 15 different sites. Samples from sites that were classified under agriculture and urban contemporary land use were significantly different from each other. Additionally, suburban and urban sites were also significantly different from each other (Figure 3.3, Table 3.2). This was not surprising to us as we expected to see differences due to the nutrient and metal concentrations within the stream banks. NMDS plots indicated grouping between two sites with distinct bacterial communities (Brandywine Zoo and Cooch’s Bridge) driving urban site separation. These sites received high influences of anthropogenic input and were also significantly different from the rest of the sampled sites (Table 3.2). The Brandywine River has been exposed to industry along its banks for the past 200 years (Maynard 2015) while Cooch’s Bridge potentially receives
highway runoff since it is adjacent to a major highway. The two sites had high levels of Mehlich 3 Fe, Ca, K, Mg, and Cu, among other metals and nutrients compared to the other sampled sites (Lutgen 2019). The elevation of metal and nutrients possibly due to urbanization is postulated to be the reason why these sites are separated from the other samples from other sites.

Our findings indicate that microbial communities within legacy sediments have a strong relationship with local stream bank chemistry. Correlation analyses between stream bank chemistry parameters driving NMDS patterns further demonstrate the bacterial community separation of samples from Cooch’s Bridge and Brandywine Zoo (Figure 3.4a). Combining this with the correlation analyses of phyla groups provides robust statistical relationships. Iron was one of the driving factors for microbial communities at Cooch’s Bridge, while the dominant phylum was *Deltaproteobacteria* (Figure 3.4). Within the *Deltaproteobacteria* is the genus *Geobacter*, which is an iron reducer (Childers et al., 2002), and was the 9th most abundant genus observed in the sampled legacy sediment across all sites. *Geobacter* was detected in these samples at the depths closest to being in contact with the water. They thrive in the anoxic and reduced conditions (Childers et al., 2002) that are predominant in the bottom layer of these banks. For the lowest depth sampled at Brandywine Zoo, (BZ_152), 1.17% of the total sequences measured were *Geobacter*. They are the most abundant Fe(III) reducing microorganism in environments where Fe(III) reduction is actively taking place (Lovely et al., 2011 and references therein).

The relative abundance of microbial communities also varied with depth. The NMDS results demonstrated that 60% of bacterial communities classified within the bottom layer group showed separation from other samples (Figure 3.3b). There are
two potential reasons for this separation. The first reason relates to the moisture content and the saturation of sediment at the lower portion of the stream banks. The sediment located at this depth may frequently come into contact with the stream water, and thus limit oxygen availability at these depths. This sediment may become more anoxic and see a more abundant amount of anaerobic bacteria than in the upper portions where oxygen availability is abundant. Studies have found phylum-level shifts due to hydrologic connectivity in floodplain sediment (Arigroff et al., 2017). Anaerobic microbes increased in abundance when hydrologic connectivity increased while aerobic microbial phyla decreased (Arigroff et al., 2017). Specifically, *Firmicutes* increased in relative abundance, *Actinobacteria* and *Acidobacteria* diminished as soil habitat conditions shifted towards saturation and anoxia (Argiroff et al., 2017). The second reason is attributed to the presence or absence of the precolonial hydric layer. This organic layer rich in carbon is a remnant of a wetland-like environment (Walter and Merritts 2008; Merritts et al., 2011). The presence of this layer may explain why nine of the fifteen sites showed variation in the bottom layer (Figure 3.3b). The precolonial layer was not visually present at all the sites and may have been unknowingly incorporated when sampling. As such, the carbon in this layer may also have attributed to the differences in microbial community structure at the bottom depth.

### 3.4.2 Nitrogen transformation communities and genes in legacy sediments

Functional gene abundances better represent the functional roles of microbial communities with regards to nutrient processing compared to 16S rRNA genes which provide insight on community structure and function (Kan 2018). Nitrification and denitrification are essential pathways for ammonia oxidation and nitrate reduction
(Thamdrup 2012; Kan 2018). Ammonia monooxygenase gene amoA, composed of both AOA and AOB, aid in ammonia oxidation (Herrmann et al., 2011; Leininger et al., 2006), while nosZ aids in the last step in the denitrification process by reducing nitrous oxide to N₂ (Zhang et al., 2016). Our results show that the nosZ gene was more abundant compared to the combined Bacteria and Archaea amoA genes at all sites and depths. This was contrary to our expectations. We initially expected to see amoA functionals genes to be more abundant than nosZ in the near surficial depths because of the aerobic favoring environment and nosZ to be most abundant than amoA at the lowest depths due to the anaerobic conditions of the deepest depths.

Forested sites had the lowest abundance of nitrifier functional genes, while the urban sites had the highest abundance and were significantly different from each other (p= 0.006). Forested sites lack the amount of additional anthropogenic influence an urban location would have. Other studies have shown that urbanization and agricultural input will have an impact on sediment microbial communities (Zhang et al., 2016). Zhang et al. (2016) found statistically greater differences between the abundance of nosZ in an agricultural zone compared to industrial, and residential zones. However, our results did not show a significant difference in functional gene abundance between agricultural, suburban, and urban sites. In general, urban sites had higher concentrations of heavy metals compared to sediment from other land use types, but aside from M3-P, nutrient concentrations were also not significantly different across sites (Lutgen 2019). Another analyzed element, M3-Ca, was significantly different between urban and forested locations (p = 0.014). Increased concentrations of calcium can promote higher nitrification rates in sediment due to the increased alkalinity (Amatya et al., 2011). We observed a positive correlation with
M3-Ca and the *amoA* functional genes (Table 3.3). Nitrification rates are highest when the pH is more alkaline (7.3 – 8.0) (Amatya et al., 2011). Various organisms that convert ammonium to nitrate prefer more alkaline conditions (Amatya et al., 2011).

The low abundance of AOB found within the stream banks may be attributed to the low NH$_4$-N concentrations within the stream banks (mean: 3.90 mg/kg). Similar results have been found in other studies where AOB is more abundant when there are high concentrations of NH$_4^+$, while AOA functional genes are more abundant when NH$_4^+$ concentrations are low (Höfferle et al., 2010; Herrmann et al., 2011). Höfferle et al. (2010) study demonstrated that there was a greater abundance of AOB at a polluted wetland site, while it was below the detection limit in a low ammonium wetland. Contrary to this, Brandywine Zoo was one of two sites in which AOB was detected at all depths despite low mean NH$_4$-N concentrations (mean: 1.68 ± 0.26 mg/kg). One potential reason for this is the location of the stream bank. In addition to the Brandywine River receiving input from factories, the stream bank site is located next to a combined sewer overflow (CSO). The CSO transports industrial wastewater and domestic sewage into the Brandywine River during high flows. One study looked at the influence of wastewater discharge on microbial communities in river sediment (Li et al., 2016). The wastewater discharge increased dissolved organic carbon, ammonia, nitrite, chloride, and phosphate concentrations in downstream locations (Li et al., 2016). Additionally, they found an increase in the relative abundance of genes that were capable of metabolizing nitrogen, among other elements when directly exposed to wastewater discharge (Li et al., 2016). The wastewater may be stimulating bacterial growth and attribute to the presence of AOB in the legacy sediments from Brandywine Zoo despite low NH$_4$-N concentrations.
Nitrospira is the fifth most abundant genera found within the stream banks, and aids in the second step of nitrification. Every member of the genus is thought to have the nitrite oxidoreductase gene which functionally allows them to act as nitrite-oxidizers in nitrification (Pester et al., 2014). Our results showed a positive correlation between Nitrospira and AOA functional genes (Appendix A12). This also has been observed in soil samples and proposed that there was possible interaction, or a shared habitat preference for the nitrifiers (Pester et al., 2014). Microbes containing the amoA gene along with Nitrospira may promote nitrification within the stream banks.

Another genus Candidatus Solibacter usitatus observed in our stream bank sediment was the second most abundant genus in the samples. The phylum for this genus is Acidobacteria, which was the most abundant phyla in our samples. Functionally, this genus produces an enzyme that breaks down organic carbon and participates in nitrate and nitrite reduction (Pearce et al., 2012). Nitric oxide reductase genes have been observed in this genus (Kielak et al., 2016), but no evidence of denitrification has been observed (Pearce et al., 2012). The physiology of this community remains mostly unknown (Kielak et al., 2016), but its functionality could have implications for the nutrient processing of the stream banks.

Nitrifier functional gene abundance was highest near the surficial layer compared to the other depths measured for 10 of the 15 stream banks. The abundance may be attributed to the unsaturated conditions of the stream bank layers found near the surface. Similar results were found in Weitzman et al., (2014) where the nitrifier population index was significantly higher in the surface layer compared to the mid-layer and relic hydric layer at the Big Spring Run site. Additionally, only two of the sixty-seven samples from our study had undetectable amounts of nosZ, which supports
the notion that the legacy sediments have a high potential to metabolize nitrogen compounds. If denitrification is actively occurring in the sediment, then the high abundance of nosZ may attribute to the overall low concentrations of nutrients within the stream banks compared to upland sources.

Interestingly, a vast abundance of nosZ was present in one of the lower depths (7.15 x 10^6 copy # * g^-1) at the Gramies Run location. This sample at the forested site makes up the precolonial layer sediment and is high in total carbon (41479 mg/kg). The high amount of carbon found in the sediment may have a direct impact on the functional gene abundance. Another site that showed positive trends with nosZ was the Scotts Mill 3 location, where nosZ gene abundance increased as stream bank depth decreased towards the surface water (Figure 3.6c). This site had observable saturated conditions near the bottom layer which may be indicative of anoxic conditions. A similar observation could be made with the nutrient concentrations of this stream bank. The percentage of total carbon increased moving down the depth profile while NO3-N decreased with depth (Lutgen 2019). The decrease of NO3-N may highlight denitrification occurring in the stream bank. However, the microbial functional gene abundance was site-specific and did not show this similar pattern across all locations indicating the tremendous amount of spatial variability between the microbial communities and functional genes. nosZ gene abundance had the strongest correlation with percent total carbon. This also makes sense as the amount of carbon within the sediment is going to influence denitrification.

3.4.3 Implications for nutrient processing and water quality

Stream banks comprised of legacy sediments may act as a source or a sink for nitrogen in aquatic systems. Weitzman et al. (2014) found that extracellular enzyme
activities were low in legacy sediment. Despite the relic hydric layer having high soil C concentrations, there was low microbial N cycling activity in this and other subsurface layers. Denitrification potentials were several orders of magnitude lower than in surface sediment despite the presence of nosZ (Weitzman et al., 2014). Thus, they concluded that legacy sediments might act as potential sources of NO\textsubscript{3} pollution into waterways (Weitzman et al., 2014). Inversely, Kim et al. (2016) studied the distribution and activities of microbial populations involved with N-cycling in riparian and stream sediments and found that riparian sediments act as sinks for inorganic nitrogen loads from non-point sources. Their riparian sediment was characterized as having a higher organic matter content and extracellular enzyme activities compared to stream sediment and thus higher rates of nitrification and denitrification occurring within the sediment (Kim et al., 2016).

While nitrification and denitrification are potentially central functions within legacy sediments, the interactions between environmental conditions and microbial communities are intricate. Aside from the studies above, several studies have looked at the effects of nutrients on the abundance and activity of amoA genes and have been met with conflicting results (Herrmann et al., 2011; Liu et al., 2014; Wang et al., 2014; Zhao et al., 2013). For instance, Liu et al. (2014) determined that AOA abundance negatively correlated with NH\textsubscript{4}-N and positively correlated with NO\textsubscript{3}-N in lake sediment. However, no significant correlations were found for AOB (Liu et al., 2014). On the other hand, Wang et al. (2014) found AOB diversity for agricultural and reservoir sediment to be positively correlated with total nitrogen. AOA diversity, however, was negatively affected by NO\textsubscript{3}-N and NH\textsubscript{4}-N (Wang et al., 2014). We did not observe any significant correlations between AOA, AOB, or nosZ and NH\textsubscript{4}-N and
NO$_3$-N in our samples. This highlights a need to study microbial communities on a site-specific basis.

One caveat of this study is that we only sampled one denitrifier functional gene. However, many other functional genes are involved with different steps in denitrification. These genes, $nirS$, and $nirK$ reduce nitrite to nitrous oxide during the first step of denitrification (Zhang et al., 2016). Future studies will need to analyze the presence and abundance of these genes to see the complete denitrifier functionality in legacy sediments. A broader knowledge of microbial communities and their functionality is needed and necessary to understand the nitrogen cycling processes within legacy sediments.

![Image of conceptual model](image_url)

Figure 3.7 Conceptual model highlighting various factors that could influence microbial community composition within legacy sediments.
3.5 Conclusions:

This study contributes to the current knowledge regarding microbial community composition and their potential roles in nitrogen processing and provides a foundation for future studies to build on. These microbial communities in legacy sediment may be influenced by contemporary land use and variations in depth, among other factors. Legacy sediment from our sites showed similar dominant phyla across all sites, but abundances varied between sites. To our knowledge, this is the first study that identifies the detailed bacterial communities residing in legacy sediments.

On the other hand, functional gene analysis indicates that the sampled stream banks have microbes that may functionally perform denitrification and nitrification, but this may not necessarily be influenced by bank depth or contemporary land use. While the qPCR results do not describe the actual turnover rates of nitrogen compounds in legacy sediments, additional investigations determining enzymatic activity and gene expression in legacy sediments may provide insights into comparing the presence of functional groups with their activities and turnover rates. Future research should further explore the relationship between the presence and absence, or removal of milldams and its potential effect on microbial communities and nutrient processing. Ultimately, understanding microbial communities in legacy sediment could lead to a better understanding of the fate of nutrients in legacy sediments.
REFERENCES


Kloos, Karin et al. (2001). "Denitrification Within the Genus Azospirillum and Other Associative Bacteria." *Functional Plant Biology*. 28.9 Print


Chapter 4
THE FATE OF NUTRIENTS ASSOCIATED WITH LEGACY SEDIMENTS IN MIDATLANTIC STREAMS

Abstract

Legacy sediments contribute a substantial amount of sediment into Mid-Atlantic watersheds. Despite this, little is known about the fate of legacy sediment once it is deposited into aquatic ecosystems. We conducted a series of laboratory and field experiments to assess whether legacy sediments act as a nutrient source or sink. Legacy sediment samples were available from 15 stream banks in the mid-Atlantic from multiple bank depths and four different land use types (forested, suburban, urban, agriculture). An additional field experiment collected and analyzed sediment at one of the sampling locations from January – September 2018. Results highlighted that fine (< 63µm) fraction legacy sediments have a high P sorption capacity, allowing them to adsorb a high amount of phosphorous from a system. Despite this, results from determining the Equilibrium Phosphorus Concentration (EPC\textsubscript{0}) for each of these 15 sites indicated that eight of the fifteen sites were potential sources for phosphorous. P-release from sediment was higher in anoxic conditions than in oxic conditions. These results indicate that legacy sediments can either act as a source or a sink for nutrients depending on the environmental conditions in which it is deposited. These legacy sediments will be mobilized during storm events allowing for them to be potentially carried to the Chesapeake Bay. Future best management practices and water quality models should account for legacy sediments and their nutrient inputs.
4.1 Introduction:

Input of sediments to streams has become an increasing concern for scientists and managers in Mid-Atlantic watersheds, such as the Chesapeake Bay (Chesapeake Bay Program 2018). Sediments in suspension can decrease light penetration and degrade aquatic health, reducing the primary productivity of aquatic vegetation (Henley et al., 2000; Hotzel and Croome 1994). Increases in sedimentation can reduce dissolved oxygen levels in the water column, reducing respiratory functions in fish (Goldes et al., 1988; Horkel and Pearson, 1976; Waters, 1995). The Chesapeake Bay Program ranks sediment pollution as the second most harmful polluter to the health of the Bay (Chesapeake Bay Program 2018) following nutrients. Another cause for concern are the nutrients that sediments carry downstream. Inputs of nitrogen (N) or phosphorous (P) into aquatic systems can enhance the production of harmful algal blooms and cause eutrophication in aquatic systems (Schindler 1977; Smith et al., 2003).

Large deposits of legacy sediment exist in the valley-bottoms of mid-Atlantic watersheds due to historic erosion from land clearing and agriculture and the simultaneous construction of mill dams on first to third order streams in the region (Merritts et al., 2011, 2013; Walter and Merritts, 2008;). Many of the milldams have now breached leaving incised streams vulnerable to bank erosion (Donovan et al., 2015; Merritts et al., 2011). Not surprisingly, recent studies reveal that stream bank erosion of legacy sediments could be a significant contributor to watershed sediment loads (Gellis et al., 2009, 2018; Cashman et al., 2018). As much as 50 to 100% of the watershed fine sediment exports are being attributed to stream bank sediment sources with legacy sediment forming a major component of these loadings (Banks et al., 2010; Gellis and Noe, 2013).
Beyond sediments, we know even less about the content and fate of nutrients (e.g., nitrogen (N) and phosphorus (P)) associated with legacy sediments. Very few studies have investigated the concentrations of N and P for legacy sediments and how these concentrations change once the sediments are deposited in the streams and other water bodies. A recent report by the Chesapeake Bay Science and Technology committee (STAC, Miller et al., 2019) highlighted the lack of information on nutrients as a major knowledge gap. Until recently, the Chesapeake Bay model (version 5.3.2) and associated total maximum daily load (TMDL) assessments did not even consider inputs of legacy sediment and associated nutrients in their budgets and water quality assessments. Without consideration of legacy sediments and associated nutrients, we may be misrepresenting the watershed loadings and misallocating sediment and nutrient reductions and management resources.

Our interest here was to address these knowledge gaps and better understand the fate of nutrients associated with legacy sediments. Specifically, we were interested in studying: the capacity of legacy sediments in sorbing nutrients, the changes in nutrient concentrations following legacy sediment deposition in stream waters, and the relative shifts in nutrient concentrations under oxic and anoxic sediment conditions. A combination of laboratory incubations and field experiments were performed. For laboratory incubations, legacy sediments were available from 15 stream bank sites in the mid-Atlantic. In-situ analysis for legacy sediments was conducted over an eight-month period. The specific questions that were addressed were:

1. Are legacy sediments a source or sink for nutrients?

2. How do legacy sediment nutrient concentrations vary under oxic and anoxic conditions?
4.2 Samples for laboratory and in-stream/field incubation:

For laboratory incubations, legacy sediment samples were available from 15 sites in the mid-Atlantic. The collection and processing of the samples are described in Chapter 3 and hence not repeated here. The primary focus of the laboratory incubations was to evaluate the changes in legacy sediment P (ortho-P) for various conditions. The field/in-stream experiment was conducted in the Big Elk Creek in Cecil County Maryland. The primary interest here was to explore the changes in N species in legacy sediments.

Laboratory Experiment 1 – Phosphorus Sorption Index (PSI) for legacy sediments:

The intent of this experiment was to determine the maximum sorption capacity of the legacy sediments and compare them against literature values for other types of sediments. Sediments from all 15 stream bank sites and depths (total samples = 67) were used. These samples were partitioned into fine (< 63 microns) and coarse fractions and were replicated twice for a total of 67 x 2 x 2 = 268 samples. Soils were treated with a 75mg P/L “phosphorus sorption solution” created from dissolving 0.3295g of monobasic potassium phosphate (KH$_2$PO$_4$) in 1L of deionized water following the protocol of Sims (2000) based on Bache and Williams (1971). About one gram of sediment (the exact amount was noted) was placed in a 50mL centrifuge tube along with 20mL of the 75mg P L$^{-1}$ sorption solution. This provides a ratio of added P to soil of 1.5g P kg$^{-1}$ soil. Two drops of chloroform were added to each solution to kill and inhibit microbial activity. Tubes were placed in an end-over-end shaker and shaken for 18 hours. The temperature was recorded at the start and end of this interval. After 18 hours, samples were centrifuged at 2000 rpm for 30 minutes and then filtered through 0.45 μm filters using a Millipore filtration unit into 40mL amber
glass vials. The filtered solution samples were determined for orthoP using the method of EPA-118-A Rev 5 on an AQ2 Discrete Analyzer (Seal Analytical, Mequon, Wisconsin).

The PSI value was determined using the equation:

$$\text{PSI (L kg}^{-1}) = \frac{X}{\log C}$$

In which $X$ is the amount of P sorbed (mgP/kg) = \(\frac{(75 \text{mgP L}^{-1} - C)x (0.020L)}{(0.001 \text{ kg soil})}\)

Where $C$ is the equilibrium P concentration after 18 hours (mg L\(^{-1}\)). Statistical analysis was performed to determine how PSI values varied with land use and particle size class (fine versus coarse fraction). Pearson correlations were also determined to investigate any relationships between PSI values and the Al and Fe contents of legacy sediments. All statistical analyses were performed in Excel Statistical Package (Office 16) and JMP (Version 14.0).

**Laboratory Experiment 2 – Equilibrium Phosphorus Concentration (EPC\(_0\)) of Stream bank Legacy Sediments:**

The intent of this experiment was to determine the EPC\(_0\) values for legacy sediments. EPC\(_0\) is the concentration at which there is no net sorption or desorption from the sediment James et al., 2002; Froelich 1988). If the EPC\(_0\) values of sediments are lower than that of the stream water concentrations, the sediments will sorb nutrients from stream water and will be considered net sinks for nutrients or P. Inversely, if the sediment EPC\(_0\) value is greater than stream water concentrations, they
will desorb P and thus act as net P sources. Since sorption assays had to be performed with multiple P solution concentrations, we limited this analysis to only one legacy sediment sample (one depth) from each of the 15 stream bank sites. Furthermore, analyses were limited to only the fine sediment fraction. With the inclusion of two replicates the total sediment samples that were analyzed were = 15 x 1 depth x 1 fraction x 2 replicates = 30 sediments. Using a 7.5ppm Phosphorus sorption solution that was made from dissolving 0.03295g of monobasic potassium phosphate in 1L of filtered stream water, three 1L solutions with varying orthoP concentrations (0.25mgP L⁻¹, 0.50mg P L⁻¹, & 2.0mg P L⁻¹) were created. A fourth 1L solution that was treated as a control was composed of only filtered stream water (~ 0.00mgP L⁻¹). A subsample of each of these stock solutions was analyzed for orthoP (EPA-118-A Rev 5) on the AQ2 Discrete Analyzer (Seal Analytical, Mequon, Wisconsin).

About one gram of sediment (exact weight was noted) was added to a 50mL centrifuge tube to which twenty milliliters of P solution were added. This mixture was created for each of the four P solution concentrations. Two drops of chloroform were added to the solution to inhibit microbial activity. Samples were placed on an end-over-end shaker and incubated for 24 hours at 25±2° C. Once incubated, samples were centrifuged at 2000 rpm for 30 minutes. Using a Millipore filter and Sterlitech Glass Microfiber 0.7-micron filters, the centrifugate was filtered into 40mL amber vials. The solution P concentrations after the 24-hour equilibration were then measured colorimetrically using EPA-118-A Rev 5 on the AQ2 Discrete Analyzer (Seal Analytical, Mequon, Wisconsin).
The EPC$_0$ was computed as described below (House and Denison, 2000; Roberts and Cooper 2018). The change in solution P concentration ($\Delta C$) after a 24 hours period was calculated using the following equation:

$$\Delta C = \frac{V}{m} C_0 - C_{24}$$

Where $\Delta C$ is the change in P concentration (mg P/L) of the solution after 24-hours, $V$ is the total volume of the solution in (L), $m$ is the mass of dry sediment in (g), $C_0$ is the concentration of solution prior to incubation and $C_{24}$ is the concentration of solution after the 24-hour incubation. $\Delta C$ was plotted against $C_{24}$ and the data points were fitted with a non-linear least squares Freundlich isotherm given as:

$$\Delta C = K_f C_{24}^{1/n}$$

Where $K_f$ is the Freundlich sorption affinity constant and $n$ is the Freundlich isotherm constant. Additionally, several regressions were made to test the significance of relationships between the EPC$_0$ value and other factors such as the stream water concentrations, particle size fractions, land use, and depth using JMP software (Version 14.0).

**Laboratory Experiment 3 – Legacy sediment sorption under anoxic and oxic conditions:**

The intent of this experiment was to study how legacy sediment P sorption/desorption varies with anoxic and oxic conditions. Legacy sediment samples
from four stream bank sites, one depth each, and only the fine fraction were selected. A control solution was also included where no sediment was added. Three replicates were evaluated for each sample. Since the legacy sediments had very low initial inorganic P, the sediments were exposed to elevated solution P (10 mg L\(^{-1}\)) prior to the experiment. Thirty grams of each sediment was placed in an acid-washed, ethanol cleaned glass tray and 600mL of 10 ppm PO\(_4\) solution was added to saturate the sediment. Sediment was then incubated on a shaker table for 24 hours at 100rpm and then placed under a dry hood until any excess liquid was evaporated. The sediment was then removed and placed in a sterile Ziploc bag and frozen until we were ready for the second part of the experiment.

To maintain oxic conditions, the cap was left off the amber vial to ensure oxygen would not be depleted in the water. Anoxic conditions were created by adding one gram of sodium sulfite (Na\(_2\)SO\(_3\)) to the solution. The vial was then sealed once the sediment was added to it. Sodium sulfite was used as an oxygen scavenger that removed the oxygen from the solution.

Two grams P-saturated legacy sediments were added to 40 mL of filtered stream water (no P) in an amber vial (with oxic and anoxic conditions) and placed on a shaker table for 24 hours at 112 rpm (n=30). After 24 hours, samples were then filtered using a Millipore filter and Sterlitech glass microfiber 0.7um filters. The sample solutions were then measured for their solution PO\(_4\) concentrations (EPA-118-A Rev 5) on an AQ2 Discrete Analyzer (Seal Analytical, Mequon, Wisconsin). A Tukey-Kramer t-test was used to determine the significant differences between the oxic and anoxic treatment groups.

**In-Situ Stream Incubation Experiment**
Site Description

This study was conducted in the Big Elk Creek in Cecil County, Maryland. Legacy sediments were collected from near the Scotts Mill location (39.6896, -75.8268) and incubated in the stream at its junction with two tributaries. One site location named SMA was located on a tributary on the west side of the Big Elk Creek while SMB was located in another small tributary near a trail on the east side of Big Elk Creek.

Figure 4.1 Incubation Experiment in Field: Modified milk crates placed in the SMA tributary. Sediment was incubated from January to September and sampled monthly.

Methodology
Sediment samples were collected from the bank depth at 137cm from the top of the bank. About 2.54cm (1in) of surficial sediment was scraped away and where possible, a 2.85cm (1 and 1/8in) diameter auger core was used to collect the sample. When a bank was unable to be sampled using the auger, a hand trowel was used, and the sample was collected as a “chunk.” Subsamples were collected from the banks to characterize both chemical and microbial composition.

At both sites, sediment cores or chunks are placed into modified plastic milk crates that were anchored in secondary flow channels (Figure 4.1). Crates were monitored frequently for any major physical changes/disturbances that may impact the experiment. Eight crates were deployed with four each for SMA and SMB. Each crate contained at least 12 cores or “chunks” but was filled halfway with sediment chunks to reduce the impact of losing sediment over time. Each month, one core or chunk was randomly selected from each crate and composited with a nearby paired crate. The homogenized samples/cores were subsampled for chemical analyses. Samples used for chemical analysis were placed in a sterile Ziploc bag and placed on ice and then later frozen for future analyses. This process of collection spanned over an eight-month period with 34 cores being analyzed in total between the two sites.

Chemical Analysis

Crate sediment samples were analyzed for any changes in total carbon, total nitrogen, nitrate-nitrogen, ammonium-nitrogen, and Mehlich-3 phosphorous (STPM105-01) over the eight-month period at the University of Delaware Soil Testing Lab (Newark, DE). Nutrient analysis for nitrate (NO₃-N) and ammonium (NH₄-N) was
done via KCl extractions (STPM111-01). Total carbon and total nitrogen were determined by combustion process.

### 4.3 Results and Discussion:

**Laboratory experiment 1: Phosphorus Sorption Index (PSI) for legacy sediments:**

Solution concentrations of PO$_4$ after 18 hours of legacy sediment incubation were lowest for the fine fraction (< 63µm) (43.79 ± 8.52 mgP L$^{-1}$). In comparison, solution concentrations for the coarse fraction (> 63µm) were higher (61.58 ± 10.94 mgP L$^{-1}$), indicating lower sorption for this sediment class. Three samples within the coarse fraction had solution concentrations greater than the starting solution of 75mgP L$^{-1}$ (n = 67) (Figure 4.2).
Figure 4.2: Solution PO₄ concentrations after 24-hour incubation; Coarse fraction sediment is represented by the top figure (a), while figure (b) represents the fine fraction. The line represents the initial starting concentration of the solution (75 mgP L⁻¹).
The mean PSI value for both coarse and fine fractions was $472.33 \pm 270.40 \text{ mg kg}^{-1}$. For the coarse fraction the mean PSI value was $292.65 \pm 224.40 \text{ mg kg}^{-1}$, while the mean PSI for the fine fraction was $652.01 \pm 177.30 \text{ mg kg}^{-1}$. There was a significant difference in PSI values between the coarse and fine size fractions (t-test, p < 0.001). There was a strong positive correlation between PSI values for the coarse fraction and m3-Al ($r^2 = 0.75$; p < 0.001) and a weak but significant positive correlation with M3-Fe ($r^2 = 0.16$; p < 0.001). For the fine fraction, the relationship between M3-Al is slightly weaker, but still positive ($r^2 = 0.49$; p < 0.01) and a significant positive correlation with M3-Fe ($r^2 = 0.07$; p = 0.029).

The PSI experiment demonstrated that there is a difference in sorption capacity between the coarse and fine fractions of legacy sediment. The fine sediment had a higher mean PSI value and sorbed more PO$_4$ from the solution compared to the coarse fraction. This high sorption capacity could be due to the abundance of Al and Fe within the fine sediments. Our PSI values are compared against values measured for agricultural soils from Delaware (Mozaffari and Sims, 1994). The PSI value for the fine legacy sediments was greater than most of the agricultural soils (e.g., Evesboro loamy-sand (136-263 mg kg$^{-1}$) and Pocomoke sandy clay loam (95-714mg kg$^{-1}$)). However, the Matawan sandy loam had a higher sorption capacity (588-2564mg kg$^{-1}$) (Mozaffari and Sims, 1994). Another study looked at an exposed stream bank and the submerged bank sediments within an agricultural catchment in central Pennsylvania (McDowell & Shapley, 2002). Their PSI values were 259mg kg$^{-1}$ and 214mg kg$^{-1}$ respectively which were lower than our fine legacy sediment fraction, but comparable to our coarse fraction values.
Table 4.1 PSI values for soils in the Mid-Atlantic Region

<table>
<thead>
<tr>
<th>Location</th>
<th>Soil Depth</th>
<th>Soil Type</th>
<th>PSI (mg/kg)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple northern Delaware</td>
<td>0-20 cm</td>
<td>Stream bank legacy sediments (Coarse)</td>
<td>293</td>
<td>Sienkiewicz 2019</td>
</tr>
<tr>
<td>watersheds</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20-40 cm</td>
<td>Evesboro loamy-sand (Ag)</td>
<td>136</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40-60 cm</td>
<td>Evesboro loamy-sand (Ag)</td>
<td>217</td>
<td></td>
</tr>
<tr>
<td>Delaware Inland Bays Watershed</td>
<td>60-80 cm</td>
<td>Evesboro loamy-sand (Ag)</td>
<td>263</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0-20 cm</td>
<td>Matawan sandy loam (Ag)</td>
<td>588</td>
<td>Mozaffari &amp; Sims 1994</td>
</tr>
<tr>
<td></td>
<td>20-40 cm</td>
<td>Matawan sandy loam (Ag)</td>
<td>2083</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40-60 cm</td>
<td>Matawan sandy loam (Ag)</td>
<td>2564</td>
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<td></td>
<td>60-80 cm</td>
<td>Matawan sandy loam (Ag)</td>
<td>1886</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0-20 cm</td>
<td>Matawan sandy loam (Field Border Area)</td>
<td>434</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20-40 cm</td>
<td>Matawan sandy loam (Field Border Area)</td>
<td>1562</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40-60 cm</td>
<td>Matawan sandy loam (Field Border Area)</td>
<td>2000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60-80 cm</td>
<td>Matawan sandy loam (Field Border Area)</td>
<td>1923</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0-20 cm</td>
<td>Pocomoke sandy clay loam (Ag)</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20-40 cm</td>
<td>Pocomoke sandy clay loam (Ag)</td>
<td>714</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40-60 cm</td>
<td>Pocomoke sandy clay loam (Ag)</td>
<td>212</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60-80 cm</td>
<td>Pocomoke sandy clay loam (Ag)</td>
<td>303</td>
<td></td>
</tr>
<tr>
<td>Mahantango Creek Catchment</td>
<td>0-20 cm</td>
<td>Agricultural catchment exposed stream</td>
<td>259</td>
<td>McDowell &amp; Sharpley 2002</td>
</tr>
<tr>
<td>(Central PA)</td>
<td></td>
<td>bank</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20-40 cm</td>
<td>Agricultural catchment submerged bank</td>
<td>214</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40-60 cm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>60-80 cm</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Field Border areas separate crop fields from drainage ditches**
Laboratory Experiment 2: Equilibrium Phosphorus Concentration (EPC$_0$)

EPC$_0$ values ranged from 0.005 – 0.236 (Mean: 0.036 ± 0.05) mg L$^{-1}$ across the 15 sites. There was no significant difference in EPC$_0$ values with land use. Eight of fifteen sites had EPC$_0$ values greater than the baseflow stream water concentration indicating that the legacy sediment could be a potential nutrient source if deposited into the channel (Table 4.2). Five of the fifteen sites had EPC$_0$ values that were less than the stream water concentrations indicating that the sediment would act as a potential nutrient sink if deposited into the channel (Table 4.2). Two sites, Cottage Mill (RH) and Brynes Mill (BYR) had EPC$_0$ values that indicate they could act as either a source or a sink for those specific samples (Table 4.2). No significant correlations were observed between the EPC$_0$ concentration and %fine, %sand, %silt, and %clay (p > 0.05).

Our EPC$_0$ values were within the range of values reported by other studies (Table 4.3). A study in Maryland that investigated legacy sediments reported an EPC$_0$ value of 0.010 mg L$^{-1}$ (Sobotka 2011). Stream bank sediment from Lake Pepin had an EPC$_0$ value of less than 0.1mg L$^{-1}$ (Grundtner et al., 2014), whereas another study determined the EPC$_0$ for sediments (0.085 mg L$^{-1}$) downstream of sewage treatment works in the River Wensum Catchment in the UK (Roberts and Cooper 2018).

The EPC$_0$ values for our sediments were highest for the Brandywine Zoo and Cooch’s Bridge locations (Table 4.2) compared to the other thirteen sites. These two sites were in urbanized areas where the stream water nutrient concentrations were also high.
Table 4.2: EPC\textsubscript{0} concentrations for the 15 sites sampled and whether they are source or sinks for phosphorous

<table>
<thead>
<tr>
<th>Site Name</th>
<th>Sample</th>
<th>EPC\textsubscript{0} (mg/L)</th>
<th>Stream Water Concentration (mg/L)</th>
<th>Land Use</th>
<th>Sink or Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gramies Run</td>
<td>GMT_107</td>
<td>0.018</td>
<td>0</td>
<td>Forest</td>
<td>Source</td>
</tr>
<tr>
<td>Middle Run</td>
<td>MR_91</td>
<td>0.019</td>
<td>0.036</td>
<td>Forest</td>
<td>Sink</td>
</tr>
<tr>
<td>Cider Mill</td>
<td>CDM_183</td>
<td>0.017</td>
<td>0.01</td>
<td>Suburban</td>
<td>Source</td>
</tr>
<tr>
<td>Casho Mill</td>
<td>CM_102</td>
<td>0.012</td>
<td>0.005</td>
<td>Suburban</td>
<td>Source</td>
</tr>
<tr>
<td>Cottage Mill*</td>
<td>RH_76</td>
<td>0.001</td>
<td>0.001</td>
<td>Suburban</td>
<td>Sink/Source</td>
</tr>
<tr>
<td>Byrnes Mill*</td>
<td>BYR_163</td>
<td>0.017</td>
<td>0.023</td>
<td>Urban</td>
<td>Sink/Source</td>
</tr>
<tr>
<td>Brandywine Zoo</td>
<td>BZ_76</td>
<td>0.203</td>
<td>0.064</td>
<td>Urban</td>
<td>Source</td>
</tr>
<tr>
<td>Cooch’s Bridge</td>
<td>COB_38</td>
<td>0.102</td>
<td>0.004</td>
<td>Urban</td>
<td>Source</td>
</tr>
<tr>
<td>Woolen Mill</td>
<td>WM_61</td>
<td>0.002</td>
<td>0.205</td>
<td>Urban</td>
<td>Sink</td>
</tr>
<tr>
<td>Big Elk Bridge</td>
<td>BEB_122</td>
<td>0.020</td>
<td>0.004</td>
<td>Agriculture</td>
<td>Source</td>
</tr>
<tr>
<td>Camp Bonsul Road</td>
<td>CB_137</td>
<td>0.011</td>
<td>0.066</td>
<td>Agriculture</td>
<td>Sink</td>
</tr>
<tr>
<td>Nature Center Beach</td>
<td>NCB_114</td>
<td>0.023</td>
<td>0.032</td>
<td>Agriculture</td>
<td>Sink</td>
</tr>
<tr>
<td>Scotts Mill 2</td>
<td>SM2_122</td>
<td>0.012</td>
<td>0.002</td>
<td>Agriculture</td>
<td>Source</td>
</tr>
<tr>
<td>Scotts Mill 3</td>
<td>SM3_183</td>
<td>0.021</td>
<td>0.002</td>
<td>Agriculture</td>
<td>Source</td>
</tr>
<tr>
<td>Tweeds Mill</td>
<td>TM_81</td>
<td>0.017</td>
<td>0.039</td>
<td>Agriculture</td>
<td>Sink</td>
</tr>
</tbody>
</table>

5 Sinks; 8 Sources; 2 either a source or sink
Table 4.3: Comparison of our EPC_{0} values (mean value in bold) against those reported in the literature.

<table>
<thead>
<tr>
<th>Location</th>
<th>EPC0 Points (mg/L)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Various Delaware Legacy Sediment (Mean)</td>
<td>0.033</td>
<td>This study (2019)</td>
</tr>
<tr>
<td>Redwood River Suspended Solids</td>
<td>0.074</td>
<td>James et al. (2002)</td>
</tr>
<tr>
<td>Minnesota River Suspended Solids</td>
<td>0.117</td>
<td>James and Larson (2008)</td>
</tr>
<tr>
<td>Lake Pepin Sediment</td>
<td>0.155</td>
<td>James and Barko (2004)</td>
</tr>
<tr>
<td>Minnesota River Basin Agricultural Soil</td>
<td>0.34</td>
<td>Fang et al (2002)</td>
</tr>
<tr>
<td>Eau Galle River</td>
<td>0.129</td>
<td>James and Barko (2005)</td>
</tr>
<tr>
<td>Lake Pepin Stream bank Sediment</td>
<td>&lt;0.1</td>
<td>Grundtner et al (2014)</td>
</tr>
<tr>
<td>River Wensum Catchment (UK)</td>
<td>0.085</td>
<td>Roberts and Cooper (2018)</td>
</tr>
<tr>
<td>Mahantango Creek Catchment (Central PA)</td>
<td>0.02</td>
<td>McDowell &amp; Sharpley (2002)</td>
</tr>
<tr>
<td>Courthouse Creek Sediment VA</td>
<td>0.090</td>
<td>Sobotka; n.p ms thesis (2011)</td>
</tr>
<tr>
<td>Kimages Creek Sediment VA (Legacy sediment)</td>
<td>0.010</td>
<td>Sobotka; n.p ms thesis (2011)</td>
</tr>
<tr>
<td>Rathbun Lake Watershed (Iowa):</td>
<td></td>
<td>Hongthanat (2010)</td>
</tr>
<tr>
<td>Pre-Illinoian Till (Cut Bank)</td>
<td>0.013</td>
<td></td>
</tr>
<tr>
<td>Nodaway Fine-Silty mix (Cut Bank)</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>Colo Fine-silty mix (Floodplain)</td>
<td>0.232</td>
<td></td>
</tr>
</tbody>
</table>
Laboratory Experiment 3: Legacy sediment sorption under anoxic and oxic conditions:

The final solution PO$_4$ concentrations for the anoxic treatment group (mean $1.11 \pm 0.62$ mgP L$^{-1}$) were significantly greater (t-test, $p < 0.001$) than those measured for the oxic treatment group ($0.22 \pm 0.20$mgP L$^{-1}$) (Figure 4.3).

The oxic/anoxic experiment results showed that the P-saturated sediment released more PO$_4$ under anoxic conditions than under oxic conditions. Sediment enriched with phosphorous will release it in anoxic conditions, effectively acting as a source. Anoxic conditions result in Fe(III) reduction to Fe(II) (Chambers and Odum 1990; Roden and Edmonds 1997). This releases the tightly bound PO$_4^{3-}$ from the sediment (Kleinman et al., 2007). Reduced conditions also allow Fe (II) to preferentially bind to sulfide, releasing the PO$_4^{3-}$ ions (Nair and Reddy 2013). This suggests that legacy sediment in aquatic systems could release nutrients under anoxic conditions and could become an internal source. Future studies are needed to further explore this aspect for sediments.
Figure 4.3: \( \text{PO}_4 \) solution concentrations after a 24-hour incubation in oxic and anoxic conditions.

**In-situ Stream Incubation Experiment**

Nutrient and microbial community structure varied through the nine-month study period. For site SMA, the sediment nutrient concentrations did not show much change over the study period (Figure 4.4). For site SMB, there was an increase in sediment concentrations over the spring and summer, particularly for TC and TN and then a decrease later in the fall (Figure 4.4a,b).
b.
Discharge (m^3/s) vs Date and Time

- Scaled down Discharge m^3/s
- SMA
- SMB

NH_4^+ - N (mg/kg)
Discharge (m$^3$/s)

Date and Time

Scaled down Discharge m$^3$/s

□ SMA  ○ SMB

NO$-$N (mg/kg)
Figure 4.4 Crate incubation time series from January to September 2018. Samples from SMA are labeled as squares while SMB samples are labeled as circles. Each graph indicates the scaled-down USGS discharge for Scotts Mill and: (a.) TC, (b.) TN, (c.) NH$_4$-N, (d.) NO$_3$-N, and (e.) M3-P

Results from a one-way ANOVA indicated that several nutrient and microbial characteristics were significantly different between the two sites. Visually, SMA received additional sediment input after large storm events. This was due to the erosion of nearby stream banks. This would in some instances, completely bury the crates at this location. They were dug out and cleaned up as much as possible. On the other hand, the right tributary SMB, received very little, if any additional sediment
input from the time the crates were placed at the location. As the months progressed, the legacy sediment samples from in the crates in SMB shifted from a light-brown color to greyish black. This color shift is believed to come from the additional organic inputs that the crate sediments were subjected to each month. This potentially explains the increase in total carbon and nitrogen over time within the sediment.

Sediment NH$_4$-N concentrations were relatively low at the SMA location compared to SMB throughout the duration of the incubations (Figure 4.4). This also held true for SMB for the winter months an increase was observed starting in April and reaching peak concentrations in May. Sediment NO$_3$-N decreased gradually over the months at both locations from the initial concentration measured from the stream bank. We believe that this decrease is due to denitrification processes. The lowest NO$_3$-N concentrations were sampled on July 24, 2018, for SMA and July 31, 2018 for SMB. This coincides with one of the larger storm events of that year which took place on July 23, 2018. The concentrations were only 0.55mg/kg and 0.86mg/kg respectively. The following month the concentrations measured around their previous averages of 3.55mg kg$^{-1}$ and 3.94mg kg$^{-1}$ respectively. This decrease could be directly influenced by the storm event where we observed a dilution pattern in the sediment concentration. Peak discharge for that event was 155.6 m$^3$s$^{-1}$. One potential reason for the dilution pattern could be attributed to the flushing of microbial communities from the sediment during these storm events. Little variation was observed with M3-P sediment concentrations. The concentration fluctuated each month, but the increases and decreases were not significant.

This initial assessment into the temporal aspects of legacy sediments seems to indicate that if the sediment is not mobilized it will try to reach equilibrium with the
environment, it is deposited in. At the locations where this experiment was conducted results indicate that the legacy sediment acted as nutrients sink.

**Implications for TMDLs**

The Chesapeake Bay commission seeks to reduce nitrogen inputs by 25%, phosphorous by 24%, and sediment loads by 20% per year (USEPA, 2010). The Chesapeake Bay watershed model’s goal is to reduce nutrient loads within the Bay; however, until recently, the TMDL plan did not account for legacy sediment input (Chesapeake Bay Program 2017). The reason why water management agencies are not reaching TMDL goals could be due to the additional inputs of legacy sediment (STAC, Chesapeake Bay Program 2017). Climatic variability will increase the intensity of storms (Melillo 2014), and thus the amount of sediment and nutrients that are mobilized through storms and exported into the Bay (Dhillon and Inamdar 2013; Gellis et al., 2017; Inamdar et al., 2018). Our results indicate that while legacy sediments are relatively low in nutrients compared to other upland sources, whether sorption or desorption/leaching occurs will depend on oxic and anoxic conditions of the sediments. Oxic conditions could favor sorption while anoxic conditions could result in the reductive dissolution of P and sorbed nutrients. It however, also needs to be recognized that organic N and P in the sediments could also be mineralized under oxic conditions and release nutrients. Thus, whether legacy sediments serve as an overall source or sink for nutrients will depend on the balance of processes such as sorption-desorption and mineralization and immobilization. Future studies and TMDL should account for legacy sediment input if they want to achieve the reduction of nutrients and sediment goals by 2025 (Chesapeake Progress 2019).
Figure 4.5: Conceptual model illustrating how legacy sediments could act as either source (via desorption or reductive dissolution) or sinks (via sorption) for N and P if exposed to either oxic or anoxic conditions.

4.4 Conclusions

Through a series of laboratory experiments and an in-situ/stream incubation study, we show that legacy sediments deposited in streams have the potential to act as a source or a sink of nutrients. This source/sink behavior will, however, vary with site conditions and sediment characteristics and will vary with processes such as sorption and desorption and mineralization and immobilization. Understanding these fluxes is critical to understanding the role of legacy sediments in aquatic ecosystems. Nevertheless, legacy sediments need to be considered in nutrient budgets and watershed models being used to develop watershed management plans and TMDLs.
REFERENCES:


Environmental Protection Agency (EPA). *Chesapeake Bay TMDL Executive Summary*. N.p., 2010. Print.

Froelich, P. N. (1988). Kinetic control of dissolved phosphate in natural rivers and estuaries: A primer on the phosphate buffer mechanism. *Limnology and


Chapter 5

CONCLUSIONS

This research expanded our understanding of how microbial communities influence nutrient processing in legacy sediments and how the nutrient concentration in legacy sediment interact with the environment once deposited into streams.

5.1 Key Conclusions

A summary of the key conclusions for this research are summarized below:

- The most abundant Phyla observed in our legacy sediments were:
  
  Acidobacteria (25-45%), Proteobacteria (15-40%), Chloroflexi (1-5%),
  
  Actinobacteria (1-10%), Nitrospirae (2-10%), Firmicutes (1-10%),
  
  Verrucomicrobia (1-5%), Gemmatimonadetes (1-5%), AD3 (1-5%),
  
  Planctomycetes (1-5%), Bacteroidetes (1-5%), and OD1 (1-3%).

- Microbial community composition and distribution varied by depth on a site-specific basis with saturation of the stream banks and oxygen availability acting as predominate contributing factors to said variation.

- The microbial communities at Brandywine Zoo and Cooch’s Bridge were significantly different compared to other sites and potentially influenced the urban site separation.

- Several weak but significant correlations with nutrients and metals indicate stream bank chemistry is driving microbial communities in legacy sediment. However, these trends were on a site-specific basis.
• Nitrification functional genes were more abundant near surficial depths, while the highest abundance of $\text{nosZ}$ functional genes found in the depths closest to contact with water. $\text{nosZ}$ was present in nearly all samples implying that legacy sediments may have high potential to metabolize nitrogen compounds.

• Legacy sediments have a high sorption capacity and can sorb a large amount of phosphorous from stream water in either high or low concentrations.

• Legacy sediments can be a sink or source of nutrients depending upon EPC$_0$ and stream water concentrations.

• Legacy sediments release more phosphorus in anoxic conditions compared to oxic conditions.

• Over time, deposited sediments will try to reach equilibrium with the water column and will act as a source or a sink to adjust accordingly.

5.2 Future Direction

This study addressed some of the key knowledge gaps, explicitly relating to microbial communities in legacy sediment. However, more questions need to be addressed. There is a high amount of spatial heterogeneity with microbial communities within stream banks. This means that we must study sites on a case-by-case basis and cannot easily group each legacy sediment stream bank. This can potentially raise issues with incorporating microbial analyses into future TMDL planning. Future research should be applied once there is more background knowledge.
on the specific processes of some microbial communities at the genera level. Additional studies can also look at the monitoring changes in microbial communities in stream banks as more mill dams are removed across the Mid-Atlantic Region. Finally, measuring the rates of nitrification and denitrification within these stream banks, pre-and post-dam removal may also help us have a better understanding on how the presence of mill dams affects the nutrient processing of these legacy sediment stream banks.

This study also taught us that legacy sediment has a high nutrient sorption capacity for phosphorus. This was specifically true for the fine fraction sediment which is mobilized during the high-intensity storm events experienced in the Mid-Atlantic Region. While this would make us believe that legacy sediments would generally act as nutrient sinks, results from our other studies show that they can either act as a source or a sink depending on environmental conditions. Factors such as the EPC₀, the oxygen levels of the water, and the nutrient concentrations of the stream water must be considered when determining if the sediment is a source or a sink. Future research should analyze how legacy sediment would react when it reaches the more saline conditions of the Chesapeake Bay.

5.3 Final Thoughts

Understanding the fate of legacy sediment is more complex and dynamic than we originally thought. There are a variety of factors that can influence both the microbial community structure and the nutrient composition of the stream banks. This may make it difficult to incorporate legacy sediments into mitigation policies and TMDLs. However, as more studies help add to our current knowledge, we will be able to address these issues with more clarity.
Appendix A

MICROBIAL COMMUNITY COMPOSITION AND NITROGEN TRANSFORMATION GENES OF LEGACY SEDIMENTS IN THE MID-ATLANTIC REGION, USA
Figure A1: Relative abundance of microbial groups (phylum level) varying depths for each site: Results from high throughput sequencing indicating variation in microbial community composition at different depths. SM2_183, SM2_224, and BEB_112 were unable to be sequenced and were omitted.
Woolen Mill

a.

12”

24”

36”

48”

Tweed’s Mill

b.

12”

32”

52”

72”
Scotts Mill 3 c.
Middle Run g.
Casho Mill
i.

Cider Mill
j.
Brandywine Zoo
k.

15”

30”

45”

60”
Camp Bonsul 1.
Cooch’s Bridge
m.
Figure A2: Stream bank depth profiles: Each of the 15 stream banks used for this experiment were documented. Camp Bonsul (l.) (Depths: 76, 137, 198, 259, 320, and 381cm) did not have the depths labeled on the picture due to not being properly able to see the markers on the yardstick. Cooch’s Bridge (m.) (Averaged depths: 18, 38, and 56) was the only site where we augured down making labeling the depths impossible.
Functional Gene Abundances by site and depth

AoA:

![Graph showing functional gene abundances by site and depth.](image)

- Site Depth (cm): 60.96, 121.92, 182.88, 259.08
- AOA copies g soil⁻¹:
  - 60.96: 89622.1933
  - 121.92: 80723.26034
  - 182.88: 146477.293
  - 259.08: 46852.96005
b. [Bar chart showing AOA copies per soil sample at different bank depths (cm): 41, 81, 122, 163, 213. Values for each depth are 1602832.546, 139365.213, 2234104.45, 31365.7115, 0, respectively.]

c. [Bar chart showing AOA copies per soil sample at different bank depths (cm): 38, 76, 114, 152. Values for each depth are 11230051.65, 4149886.784, 4734816.569, 3591955.022, respectively.]
1. Plot 1 shows the AOA copies per g of soil as a function of bank depth. The x-axis represents bank depth in cm, ranging from 61 to 224. The y-axis represents AOA copies per g of soil, ranging from 0 to 2.0E+05. The data points are as follows:

- Bank Depth 61 cm: 173705.0903 copies g soil⁻¹
- Bank Depth 122 cm: 49611.84387 copies g soil⁻¹
- Bank Depth 183 cm: 0 copies g soil⁻¹
- Bank Depth 224 cm: 0 copies g soil⁻¹

2. Plot 2 continues the analysis with AOA copies per g of soil. The x-axis represents bank depth in cm, ranging from 183 to 396. The y-axis represents AOA copies per g of soil, ranging from 0 to 5.0E+04. The data points are as follows:

- Bank Depth 183 cm: 21329.41591 copies g soil⁻¹
- Bank Depth 274 cm: 31774.16748 copies g soil⁻¹
- Bank Depth 335 cm: 40159.73549 copies g soil⁻¹
- Bank Depth 396 cm: 10338.20914 copies g soil⁻¹
Bank Depth (cm)

0.0E+00 2.0E+00 4.0E+00 6.0E+00 8.0E+00 1.0E+01 1.2E+01 1.4E+01 1.6E+01

193598.6043 29357.0933 306465.7414 1274747.036

AOA copies g soil^{-1}

n.
Figure A3. AOA functional gene abundance for each sampled stream bank: Each location is color coded based on land use type. Green = forested, orange = agriculture, pink = suburban, red = urban. AOA was undetectable at the Camp Bonsul location. Order for graphs: Big Elk Bridge (a), Brynes Mill (b), Brandywine Zoo (c), Camp Bonsul (d), Cider Mill (e), Casho Mill (f), Cooch’s Bridge (g), Gramies Run (h), Middle Run (i), Nature Center Beach (j), Cottage Mill (k), Scotts Mill 2 (l), Scotts Mill 3 (m), Tweeds Mill (n), and Woolen Mill (o).
AoB:

![Graph showing AOB copies g soil⁻¹ across different bank depths (cm)]
Figure A4. AOB functional gene abundance at legacy sediment stream banks: Only two stream banks had a detectable amount of AOB in two or more of their measured depth samples. As such, the others were not plotted. Order for graphs: Tweeds Mill (a), and Brandywine Zoo (b).
nosZ:

![Graph a.](image)

![Graph b.](image)
g. 

h.
i. 

![Graph showing nosZ copies g soil$^{-1}$ vs. Bank Depth (cm).]

- At 30 cm, the nosZ copies g soil$^{-1}$ is approximately 41,683.
- At 61 cm, the nosZ copies g soil$^{-1}$ is approximately 163,953.
- At 91 cm, the nosZ copies g soil$^{-1}$ is approximately 96,778.
- At 152 cm, the nosZ copies g soil$^{-1}$ is approximately 44,894.

j. 

![Graph showing nosZ copies g soil$^{-1}$ vs. Bank Depth (cm).]

- At 38 cm, the nosZ copies g soil$^{-1}$ is approximately 157,346.
- At 76 cm, the nosZ copies g soil$^{-1}$ is approximately 60,467.
- At 114 cm, the nosZ copies g soil$^{-1}$ is approximately 340,891.
- At 152 cm, the nosZ copies g soil$^{-1}$ is approximately 134,850.
- At 191 cm, the nosZ copies g soil$^{-1}$ is approximately 43,862.
- At 244 cm, the nosZ copies g soil$^{-1}$ is approximately 11,898.
Figure A5. nosZ functional gene abundance in legacy sediment stream banks: Each location colored in based on land use classification. Green = forested, orange = agriculture, pink = suburban, red = urban. Order for graphs: Big Elk Bridge (a), Brynes Mill (b), Brandywine Zoo (c), Camp Bonsul (d), Cider Mill (e), Casho Mill (f), Cooch’s Bridge (g), Gramies Run (h), Middle Run (i), Nature Center Beach (j), Cottage Mill (k), Scotts Mill 2 (l), Scotts Mill 3 (m), Tweeds Mill (n), and Woolen Mill (o).
Figure A6. Rarefaction curve for DADA2 analysis: Each sample had a curve generated. The samples were normalized at 50000 sequences where the coverage (x) = 0.99.
Figure A7. Comparison of functional gene abundance across all locations and samples: Copy number was log-transformed in order to show comparisons between genes. This figure shows how scarce AOB was within the sediment.
Figure A8. Variation in the phyla *Acidobacteria* between depths in legacy sediment stream banks: Results from ANOVA indicate that the difference in depths was statistically significant (*p* = 0.0015). Significant variation between the Top and Bottom layer (*p* = 0.0019) and the Upper Middle and Bottom layer (*p* = 0.0069) were observed from the results of the Tukey-Kramer HSD.
Figure A9. Variation in the phyla *Proteobacteria* between depths in legacy sediment stream banks: Results from ANOVA indicates that the difference in depths was statistically significant ($p = 0.0069$). Significant variation between the Top and Bottom layer ($p = 0.0152$) and the Upper Middle and Bottom layer ($p = 0.0155$) were observed from the results of the Tukey-Kramer HSD.
Figure A10. Correlation with the genus *Geobacter* and M3-Fe in legacy sediment stream banks: The relationship between the percentage of *Geobacter* in the stream banks and M3-Fe was statistically significant ($p = 0.0194$). The highest percentage of *Geobacter* was observed in the bottom depths where conditions at sites had a higher chance of being in reducing conditions.
Figure A11. *Candidatus Solibacter usitatus* variation between depth classification in legacy sediment: Results from a one-way ANOVA indicate that the difference in depths was statistically significant (p = 0.0037). Significant variation between the Top and Bottom layer (p = 0.0113) and the Upper Middle and Bottom layer (p = 0.0064) were observed from the results of the Tukey-Kramer HSD.
Figure A12. Correlation with the genus *Nitrospira* and the log-transformed AOA functional genes in legacy sediment stream banks: A significant positive correlation was observed between the two parameters ($r^2 = 0.40; p < 0.0001$).
Appendix B

THE FATE OF NUTRIENT ASSOCIATED WITH LEGACY SEDIMENT IN MID-ATLANTIC STREAMS
ΔC(Estimated and Experimental) (µg/L)

C24 (µg/L)

-10
0
10
20
30
40
50

0
200
400
600

ΔC(Estimated and Experimental) (µg/L)

C24 (µg/L)

0
10
20
30
40

0
10
20
30
40
50
60
70
80

NCB_114 - 1
NCB_114 - 2
TM_81 - 1
TM_81 - 2

NCB_114 - 1 Estimated
NCB_114 - 2 Estimated
TM_81 - 1 Estimated
TM_81 - 2 Estimated
\[ \Delta C(\text{Estimated and Experimental}) \ (\mu g/L) \]

\[ C_{24} (\mu g/L) \]

For the sample labeled MR_91:
- **MR_91 - 1 Estimated**
- **MR_91 - 2 Estimated**
- **MR_91 - 1**
- **MR_91 - 2**

For the sample labeled WM_61:
- **WM_61 - 1 Estimated**
- **WM_61 - 2 Estimated**
- **WM_61 - 1**
- **WM_61 - 2**
Figure B1. Phosphorous adsorption isotherms for each of the 15 legacy sediment sites sampled. The solid lines with circles are the Freundlich isotherm best fit estimated line, while the square points are the physical measurements.