

**CHARACTERIZATION OF PHOSPHORUS SOURCES AND
BIOAVAILABILITY AT DIFFERENT HYDRODYNAMIC GRADIENTS IN
COASTAL ENVIRONMENTS**

by

Qiang Li

A dissertation submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Plant and Soil Sciences

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ABSTRACT

Phosphorus (P) over-loading is a leading cause of surface water eutrophication and bottom water hypoxia. Response to P loading on water quality varies with watershed size and other physico-chemical and biological parameters. As the watershed size increases, nutrient loading augments and often results in complex and non-linear response to loading and corresponding biogeochemical interactions. The limited understanding between different P pools and their bioavailability from sources to sinks has limited the development of appropriate nutrient management strategies aimed to improve water quality. To address this knowledge gap, this dissertation research investigated P sources and transformation pathways at small (~62 km²) Love Creek watershed in Rehoboth Bay and part of larger Susquehanna River estuary (~777 km²) in Chesapeake Bay. In both watersheds, multiple stable isotopes and geochemical proxies were applied to analyze colloidal, dissolved, and soil/sediment P pools and associated elements along the continuum from the sources and sinks.

In the Love Creek watershed, NaOH-P_i was found to be the most dominant P pool among colloids and soil fractions. Based on the trends of carbon (C) and nitrogen (N) isotopes, concentration, and relative sizes of P pools, major colloidal source in the non-tidal section was most likely to be non-agricultural sources, such as plant debris and forest soils. Along the salinity gradient, the contribution of terrestrial sources gradually decreased from the non-tidal section to the tidal section of the creek. In the Susquehanna River estuary, NaOH-P_i was still the most dominant P pool. Deer Creek, a small tributary, contributed a disproportionately high amounts of colloids to the

River. Oxygen isotope values of phosphate ($\delta^{18}\text{O}_\text{P}$) in the NaOH-P_i and $\text{HNO}_3\text{-P}_\text{i}$ pools of different colloidal size fractions were much heavier than equilibrium values in ambient water, suggested that these two pools were resistant to biological uptake and aided to identify the potential sources of colloids. Combined C, N, and P_i isotopic compositions of colloids showed that the contribution of terrestrial sources of colloids decreased downstream.

Spatiotemporal analyses of colloidal and dissolved P in the Susquehanna River estuary identified the hotspots areas that appear to be controlled from seasonal loading and recycling within the water column. To further investigate loading vs internal cycling, permanently suspended particulate matter (PSPM; $< 40\ \mu\text{m}$) and resuspended particulate matter (RSPM; $> 40\ \mu\text{m}$) were studied in controlled erosion experiment in the laboratory and paired with the water column in the field. Comparison of C and N isotopes and P pools in colloids in eroded and in-situ sediments and water column confirmed that the sediment resuspension was a minor source for particulate and colloidal P in the water column in the estuary. Overall, these findings provided an improved understanding of P sources, sinks, and internal cycling in the water column in both watersheds. These results are expected to aid in further investigations of nutrient cycling in comparable watersheds and estuaries.

Chapter 1

INTRODUCTION AND RESEARCH OBJECTIVES

1.1 Introduction

Phosphorus (P) is a nonrenewable resource and an essential element for agricultural productivity. Geological phosphate reserves are continuously mined to produce fertilizers and have created a largely unidirectional flow of phosphorus, from mines to fertilizers for crop production, and then to processing foods and fodders for human and animal consumption [Suh and Yee, 2011]. In this transport, chiefly driven by anthropogenic activities, a significant fraction of extracted P is lost to the environment and could run off into open waters. Globally, nutrient enrichment has been a major cause of impaired freshwater and coastal ecosystems.

Eutrophication is the process whereby water bodies enriched with nutrients support excessive primary productivity, triggering various deleterious effects. The nutrient loads could be in the dissolved or colloidal phases. Colloids encompass all solid phases suspended in water bodies; particulate P is a major contributor (75–90%) of total P transferred from terrestrial to aquatic ecosystems [Burwell et al., 1977; Sharpley et al., 1987]. Colloidal P contains both a readily bioavailable and less bioavailable P pool [Van Moorleggem et al., 2013]. Under certain biogeochemical conditions, the bioavailable P can be mobilized, allowing it to be a P source for aquatic life [Carignan and Kalff, 1980; Jordan et al., 2008; Wildung et al., 1977].

Chesapeake Bay receives an estimated 9.74×10^6 kg/year of P from its watershed and 28% of it is contributed by the Susquehanna River alone [Ator et al.,

2011]. Small creeks such as Deer Creek (Figure 1.1) are dominated by agricultural runoff and are expected to contain high concentrations of nutrients. They contribute a proportionally higher nutrient load per unit volume of water, which is readily apparent where they join the main river channel. A creek-river-bay continuum provides an opportunity to explore how temporal and spatial variations of nutrients and their bioavailability serve as a function of their loading and flow rate. The transformation of P between different forms in the environment adds complexity to the interpretation of results. Recycled nutrients, mainly nitrogen and phosphorus, contribute to late summer bottom water hypoxia [Testa and Kemp, 2012]. The geographical extent and longevity of seasonal hypoxia is still increasing even after reduced nutrient load from improved watershed land management beginning in the 1990s [Sprague et al., 2000; Zhang et al., 2013]. Quantification, source tracking, and determining bioavailability of colloidal P pools at different size scales in an estuary provides information essential to discriminating sources and forms of P that may negatively impact water quality, and may aid in the development of appropriate watershed nutrient management plans.

Phosphate oxygen isotope ratios ($\delta^{18}\text{O}_\text{P}$) have been increasingly used to better understand sources and cycling of P in a variety of ecosystems such as agricultural soils, sediments, and water bodies [Angert et al., 2012; Davies et al., 2014; Gooddy et al., 2016; Markel et al., 1994; Tamburini et al., 2014; Joshi et al., 2016]. In biological reactions driven by enzymes, oxygen isotope exchange between water and phosphate ions is rapid and results in an isotopic composition determined by ambient water oxygen isotope ratios ($\delta^{18}\text{O}_\text{W}$) and temperature [Blake et al. 2001; Blake et al., 2005; Chang and Blake, 2015]. Oxygen isotope exchange between water and phosphate is extremely slow at low temperatures and circumneutral pH in abiotic systems

[(Kolodny et al. 1983; Lecuyer et al., 1996; O’Neil et al., 2003; Jaisi et al., 2010)].

These fundamentally distinct properties of $\delta^{18}\text{O}_\text{P}$ make it ideal for tracking P sources and bioavailability in complex ecosystems.

Using $\delta^{18}\text{O}_\text{P}$ for tracking environmental P sources is in its infancy [Davies et al., 2014]. The fluid residence time of different P sources in water bodies question the fidelity of its oxygen isotopic signature because longer residence times allow for more biological activity, which may result in isotopic overprinting [Tonderski et al., 2017]. Source tracking is more linear in small creeks with short residence times of water where potential sources can be identified through high-resolution sampling. In contrast, a substantial difference in magnitude and response to nutrient loading is found in larger water bodies because of non-linear processes and estuary-specific ecological interactions [Cloern, 2001; Kemp et al., 2005]. This challenge can be addressed from starting at a small watershed with detailed and systematic investigation of P source tracking and bioavailability and upscaling to larger and more complex water bodies.

1.2 Research Objectives and Questions

This dissertation seeks to investigate the sources and bioavailability of phosphorus in different hydrodynamic gradients in the Love Creek (Figure 1.2) and Susquehanna River estuaries. This is accomplished through analysis of distinct phosphorus pools, multiple stable isotope proxies, and erosion experiments of different size fractions of particulate matter.

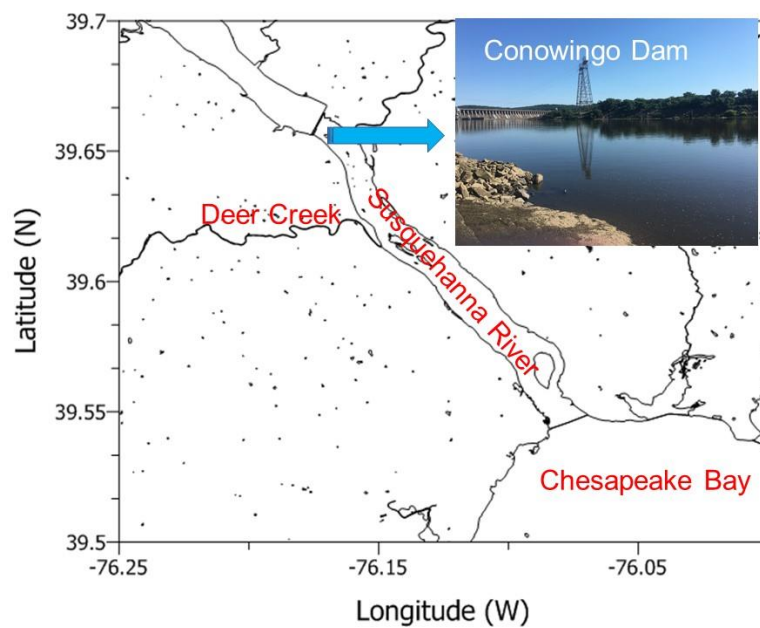


Figure 1.1. Location of sampling sites in Deer Creek, the lower Susquehanna River, and the mouth of Chesapeake Bay.

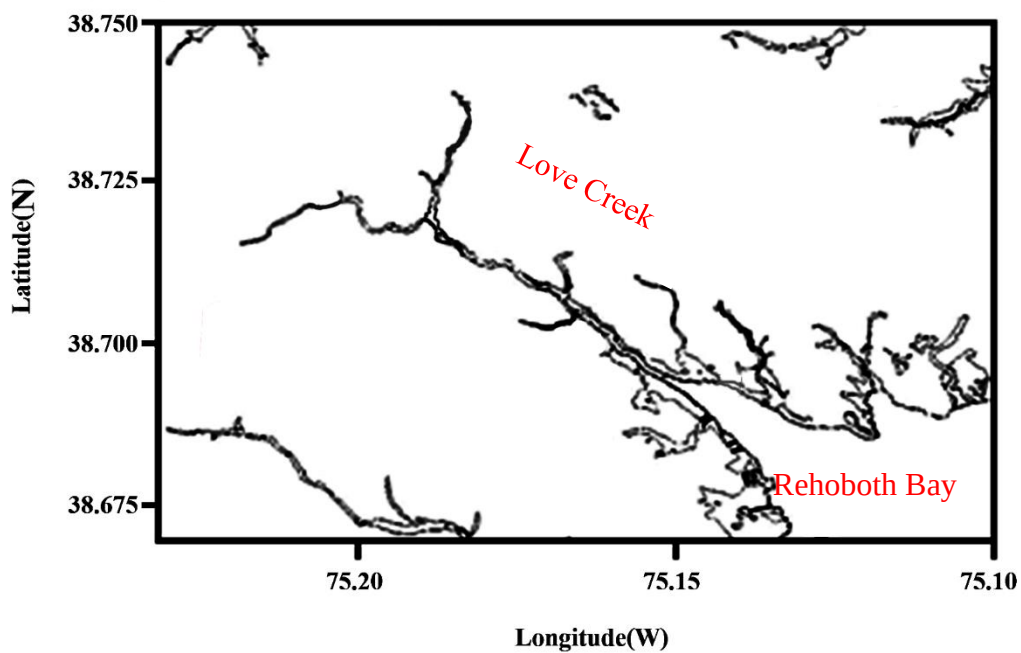


Figure 1.2. Location of sampling sites in Love Creek Watershed.

Objective 1: Trace the sources of phosphorus in the Love Creek watershed

- Do different terrestrial P sources have distinct isotopic signatures and are their signatures retained during transport?
- Do isotopic values vary among different colloidal sizes, both in source soils and those transported through water?
- How does the composition of sources and the sediment-water interaction change from upstream to downstream?

Objective 2: Investigate the temporal and spatial variability and bioavailability of phosphorus in the Deer Creek-Susquehanna River estuary

- How do the organic and inorganic fractions of colloidal and dissolved phosphorus pools vary with hydrological gradients in small and large estuaries?
- Can the bioavailability of phosphorus vary with turbidity and if so, how does it impact primary productivity?
- Can combined isotopic data using carbon, nitrogen, and phosphate oxygen provide a reliable assessment of terrestrial sources of nutrients in the Deer Creek-Susquehanna River estuary?

Objective 3: Identify the role of sediment resuspension on phosphorus supply to the water column in the Susquehanna River estuary

- Can the current-derived sources of the suspended particulate matter be differentiated from remobilized sediment in the water column?

- What are the relative contributions of permanently suspended particulate matter (PSPM) and resuspended particulate matter (RSPM) sources of P under different shear stress conditions?
- Can the results from controlled laboratory erosion experiments and field water columns be compared to distinguish the loading vs internal cycling in the field?

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Chapter 2

TRACING THE SOURCES OF PHOSPHORUS IN THE LOVE CREEK WATERSHED

2.1 Introduction

Nutrient contamination in coastal waters worldwide has compromised ecosystem values through decreasing natural goods and services and in turn, reducing tourism and recreational incomes. Federal, state, local, and private agencies actively seek solutions to minimize nutrient-related water quality reduction, but their success has been limited. The identification of nutrient load contributions from different sources is required to develop an effective management plan to reduce nutrient loads. Due to the spatial and temporal variations in phosphorus (P) flux, its sourcing, and active and variable transformation among its forms, understanding the sources and processes of P in estuarine settings is a near-impossible task if the research approach is limited to traditional methods.

Oxygen isotopic ratios of phosphate ($\delta^{18}\text{O}_\text{P}$) have been increasingly used as a powerful tool to trace P sources in a variety of ecosystems such as agricultural soils, fresh waters, and marine ecosystems [Angert et al., 2012; Bauke et al., 2018; Bi et al., 2018; Davies et al., 2014; Gooddy et al., 2016; Joshi et al., 2016; Hacker et al., 2019; Tamburini et al., 2012; Siebers et al., 2018; Tonderski et al., 2017]. The fundamentally distinct properties of $\delta^{18}\text{O}_\text{P}$ from biotic and abiotic sources enable it to serve as a tracer to identify P cycling and transformation in complex ecosystems. Regardless, slight variations in the isotopic signature of P from multiple sources and overprinting of the

signatures during P transformation in soils and waters complicate the precise identification of sources [Gross and Angert, 2015; Joshi et al., 2016; Granger et al., 2017]. Coupling $\delta^{18}\text{O}_\text{P}$ with other reliable stable isotopic tools, including those from carbon (C) and nitrogen (N), may counteract false sourcing.

Losses of colloidal and dissolved P from soils can occur along surface and subsurface pathways [McDonnell et al., 2010; Sharpley et al., 2015], and may conserve their isotope source signal. The correct interpretation of oxygen isotope ratios in phosphate requires understanding all related processes and reactions leading to the alteration of primary isotopic compositions or the fractionation of oxygen isotopes and the factors that govern these processes.

Soil samples are essentially always treated as whole samples by combining all soil fractions. This ignores that erosion preferentially removes finer-sized particles, which are more enriched in P than coarser-sized particles, complicating source tracking. Potential isotopic fractionation between source and sink may result in a range of soil grain sizes with different isotopic signatures. Very few investigations have been made into the oxygen isotopic effects associated with P of different colloidal sizes.

The relatively small number of potential P sources and sampling sites needed in a comparatively small watershed makes it ideal to test hypotheses before further application in larger watershed. Love Creek, located upstream of Rehoboth Bay, is a small tributary of Delaware's Inland Bays Watershed and is designated a water of Exceptional Recreational or Ecological Significance (ERES). Its environmental health could benefit substantially from improved precision in identifying nutrient pollution sources. Therefore, our objectives are to 1) identify whether the isotopic signature of

soil fractions vary with particle size and 2) track the source of P in the Love Creek watershed.

2.2 Research Objectives

The major objective is to identify the isotopic integrity of different upstream sources of P during transport in the Love Creek watershed. Research questions to be addressed are:

- Do different terrestrial P sources have distinct isotopic signatures and are their signatures retained during transport?
- Do isotopic values vary among different colloidal sizes, both in source soils and those transported through water?
- How does the composition of sources change from upstream to downstream?

2.3 Study Area

Love creek is a coastal tidal estuary located in the eastern part of Delaware and upstream of Rehoboth Bay (Figure 2.1 A). It drains into the Atlantic Ocean. The upstream part of the creek is freshwater and covers about 45% of its area. The tidal portion includes the majority of the creek, upstream to the dam at Goslee Pond where Route 277 (Robinsonville Road) crosses it. Downstream of the dam, the salinity steadily increases toward its mouth in Rehoboth Bay. The Love Creek watershed drains approximately 62 km² of land (Figure 2.1). The northern part of the creek is covered primarily by agricultural farms interspersed with small forested patches and a few residential areas. The southern part is primarily comprised of wetland.

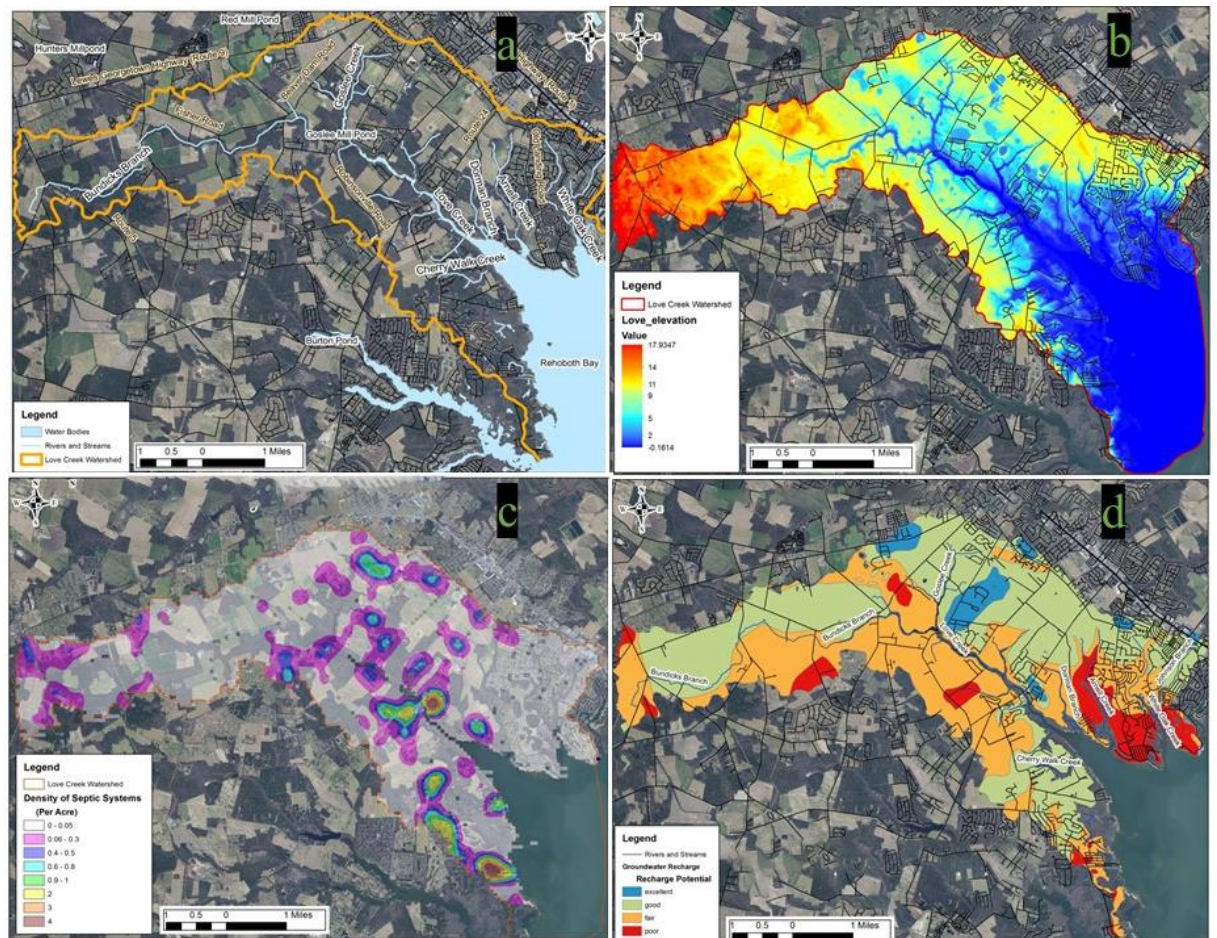


Figure 2.1. Map of A) Love Creek watershed, B) elevation map of the watershed, C) density of septic systems in the watershed, and D) groundwater recharge distribution potential (source: DCIB, 2018).

Love Creek watershed has undergone significant changes in land use. An increase in land development for housing and recreational purposes is reflected in the high density of septic tank systems in residential areas near the creek (Figure 2.1 C).

The mildly elevated topography and relatively sand-rich soil in the upstream watershed is a groundwater recharge area as well as contains groundwater discharge potential to the downstream creek.

2.4 Materials and Methods

2.4.1 Collection of Soil, Septic Waste, Leaf Litter, and Water

Based on the land use and development in the watershed, potential terrestrial P sources include agricultural runoff, forest soil drainage, leaf residue, and septic systems. The isotopic signatures of any particular land use may not necessarily match one other. To test this possibility, multiple sampling sites were chosen from each land use type. The site selection was determined based on P concentration, a factor for extent of P loss; land elevation, a geomorphic feature that determines the extent of soil erosion; soil type, as the presence of other elements such as iron and calcium affect P cycling; and groundwater discharge potential. Agricultural soils were sampled more often due to high nutrient input from crop productivity. Forest, leaf litter, and other geomorphic features were also explored. Most soil sampling sites were selected proximal to Love Creek as well as small rivulets and brooks that discharge into the creek. To obtain representative soil prone to erosion and mobilization during storm events, topsoil (0 to 10 cm depth) was collected from three farms (from LC1 to LC19) located on the headwaters and middle section of Love Creek. Within those 19 samples, LC16 and LC19 are fallow soils missing several years of recent fertilizer application, while the remainder of the soils (LC1-5; LC8-12; LC13-19) are planted with a soybean-corn rotation. Depending on the depth of agronomically relevant soil, subsoil

samples (10 to 20 cm) were collected from sites LC9, LC10, LC11 and LC17. Additionally, forest soils (LC6, LC7) were collected from the representative forest patches in the headwaters of the Love Creek watershed. For each sample, five subsamples were taken and consolidated into one representative soil sample per site.

Site selection for sewage waste from septic systems was limited due to accessibility, but efforts were made to sample private septic tanks proximal to the creek and to include a variety of tank sizes and densities in neighborhoods of various income statuses. As shown in Figure 2.1 C, septic tank density is high in the middle stretch of the watershed and immediately surrounding Rehoboth Bay. Septic tank systems with a density larger than 4 units per acre (equal to 60 units per hectare) and within 100 m from water bodies are considered to negatively impact water quality [NESM, 1973]. Therefore, sampling sites were selected within a 100 m proximity to Love Creek where the septic tank density is also high. Only one site meeting both these criteria was accessible based on landowner approval. Septic tank waste (6 L) in duplicate were chilled on ice immediately after collection and during transportation and then stored at 4 °C before processing.

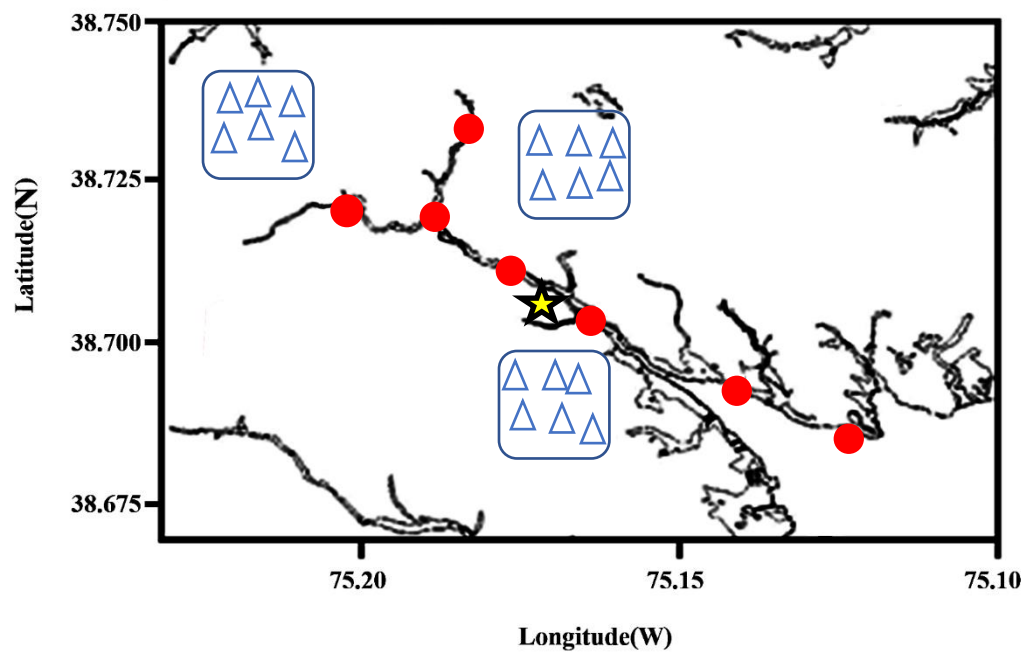


Figure 2.2. Sampling sites in the Love Creek watershed: blue triangles within rectangles indicate soil sampling sites; red circles denote water sampling sites, and the septic tank collection site is represented by a yellow star. Please note locations of septic tank and soil sampling sites are tentative and representative only.

Based on the tidal effect from the Inland Bays, Love Creek can be divided into non-tidal and tidal segments. To represent both the tidal and non-tidal portions of the creek, seven sites were selected for water samples across a salinity gradient (Figure 2.2). Those sites include Bundicks Non-tidal 1 (BN), Goslee Non-tidal 1 (GN), Love Non-tidal 1 (LN), Love Tidal 1 (LT1), Love Tidal 2 (LT2), Love Tidal 3 (LT3) and Love Tidal 4 (LT4). Sites BN and GN are located at Bundicks Branch and Goslee Millpond, respectively, two small tributaries upstream of the creek. Because of the high density of septic systems and agricultural land, nutrient loads from these tributaries was expected to be high. Based on preliminary data on P concentrations, 48

to 56 L of water from each site was collected during baseflow conditions over a period of six months. The procedure and timing of water sampling minimized the possibility of sediment resuspension and colloidal import via tidal action because the surface water was carefully collected under low tide. Plant debris was also collected in the immediate vicinity of water collection sites. All water samples and plant debris were chilled on ice immediately after collection and during transportation and then stored at 4 °C before processing. Water temperature, dissolved oxygen concentrations, turbidity, and salinity were recorded at the time of sample collection. Other water parameters were characterized either by the Environmental Laboratory at the Department of Natural Resources and Environmental Control (DNREC) or at the University of Delaware.

2.4.2 Size Separation of Soil

All collected soils were stored at -12 °C and freeze-dried. Because of the potential low mass of small soil fractions from each separate site (0.10 to 0.45 µm), the same size fraction of freeze-dried samples was combined within same farm (LC1-5; LC8-12; LC13-18) and usage purpose (LC6-7; LC16-19). Dry samples were dissolved in water (1:10 mass:volume) and separated with a 53 µm sieve. The fractions smaller than 53 µm were further diluted to make a 1% (1:100 m:v) suspension before centrifugation to separate < 0.1 and < 0.45 µm size fractions to make them comparable with the size fraction of colloids suspended in water (see below). The 1% m:v soil suspension was centrifuged using Stokes' Law of Settling to obtain < 0.1 and <0.45 µm fractions. For each soil fraction, carbon, nitrogen and phosphate oxygen isotopes were measured. The limited soil particle size range in forest soils prevented the separation of the 0.10 to 0.45 µm fraction in LC6-7.

Soil, Colloids and Plant Debris Characterization

Revised sequential extraction methods [Hedley et al., 1982] were used to differentiate and quantify different P pools ($\text{H}_2\text{O}-\text{P}_i$, $\text{NaHCO}_3-\text{P}_i$, $\text{NaOH}-\text{P}_i$, and HNO_3-P_i) in soils and suspended colloids in the creek water. To recover extracted P adsorbed onto the residual solid phase, additional steps of 0.5 mol/L NaHCO_3 extraction and H_2O rinse were added after the NaOH and HNO_3 extractions. The concentration of P_i in each pool was measured using the phosphomolybdate blue method [Murphy and Riley, 1962]. For all soils and colloids, the solid: solution ratio and other extraction conditions (time, temperature, shaking speed) was kept constant, while plant debris was treated based on Pfahler et al. [2013]. Plant debris was oven-dried, grinded and then treated with 0.3M trichloroacetic acid (TCA) [Hawkins and Polglase, 2000] to extract the TCA-soluble reactive P (TCA-P) that is rapidly cycled within plant cells. Subsequent extraction with 10M nitric acid resolves total P [Pfahler et al., 2013]. The concentrations of total P and other elements in all extraction solutions was measured by using inductively coupled plasma optical emission spectrometry (ICP-OES).

2.4.4 Measurement of C and N Isotopes

Approximately 10 mg of freeze-dried and size fractionated soil, colloids, and plant debris was used to measure carbon isotope ratios ($\delta^{13}\text{C}$) and nitrogen isotope ratios ($\delta^{15}\text{N}$) in an Elemental Analyzer (EA) (Costech, CA, USA) connected with a Delta V IRMS (ThermoFisher Scientific, Germany). All $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were calibrated against two USGS isotope standards: USGS40 ($\delta^{13}\text{C} = -26.39\text{‰}$ and $\delta^{15}\text{N}$

= -4.52‰) and USGS41 ($\delta^{13}\text{C} = +37.63\text{‰}$ and $\delta^{15}\text{N} = +47.57\text{‰}$). For data uniformity and comparison with literature values, all measured N and C isotope values were reported in standard delta notation in per mil (‰) unit with respect to atmospheric air and Pee Dee Belemnite (VPDB), respectively.

2.4.5 Purification of P Pools and Measurement of Oxygen Isotopes of Water and Phosphate

Sequentially extracted P pools were further processed to remove contaminants and concentrate Pi before the precipitation of silver phosphate (Ag_3PO_4) for phosphate oxygen isotope ($\delta^{18}\text{O}_\text{P}$) analyses following the method described in Joshi et al. [2018]. All extracted solutions were first treated with nonionic, microporous DAX 8 Superlite resin to remove the bulk of dissolved organic matter. The clear solution produced was further processed to reduce the volume and to concentrate the Pi by the Mg-induced co-precipitation (MagIC) method [Karl and Tien, 1992]. MagIC pellets were dissolved and then evaporated to attain a final Pi concentration of 500 to 1000 μM before ammonium phosphomolybdate (APM) precipitation. APM precipitation at low pH allowed removal of contaminants that are acid-soluble, while the following stage of magnesium ammonium phosphate (MAP) precipitation at high pH eliminated alkaline-soluble contaminants. The MAP crystals were then dissolved, pH neutralized, and further treated with a cation resin to remove cations. Silver amine solution was used to precipitate silver phosphate. Crystals were dried out after 12–24 hours at 50 °C in the dark. The bright yellow silver phosphate crystals were filtered and then further dried at 110 °C before measuring their isotopic composition. Duplicate phosphate standards

with known isotopic values were processed in parallel with field samples to assess the veracity of sample processing and final isotopic composition.

Around 300 μg of silver phosphate was prepared into silver capsules to measure $\delta^{18}\text{O}_\text{P}$ values using Thermo-Chemolysis Elemental Analyzer (TC/EA) coupled to a Delta V continuous-flow isotope ratio measuring mass spectrometer (IRMS; ThermoFisher Scientific, Bremen, Germany). The measured $\delta^{18}\text{O}_\text{P}$ values were calibrated against YR series standards (-5‰ and +33‰) that were originally calibrated using the conventional fluorination method.

The oxygen isotopic composition of water ($\delta^{18}\text{O}_\text{w}$) was measured from all watershed waters, including porewater extracted from soils and other potential P sources. For standard measurement, about 0.3 mL of water was equilibrated with CO_2 at 26.0 °C in borosilicate vials for 24 h. The isotopic composition of headspace CO_2 after equilibration was measured using a GasBench II coupled to IRMS (ThermoFisher Scientific, Bremen, Germany) and calibrated using two USGS standards (W67400 and USGS W32615 with $\delta^{18}\text{O}_\text{w}$ values of -1.97‰ and -9.25‰, respectively). Triplicate standards were analyzed with precision of < 0.10‰. A temperature-dependent equilibrium fractionation between CO_2 ($\delta^{18}\text{O}_{\text{CO}_2}$) and H_2O ($\delta^{18}\text{O}_{\text{H}_2\text{O}}$) [Cohn and Urey, 1938] was used to calculate $\delta^{18}\text{O}_\text{w}$ values. All oxygen isotopic values of Pi and water are reported in standard delta notation in per mil (‰) with respect to Vienna Standard Mean Ocean Water (VSMOW).

To identify the extent to which P pools in soils and colloids are biologically cycled, the equilibrium isotope composition was calculated using the temperature dependent fractionation equation developed by Chang and Blake [2015]:

$$\delta^{18}\text{O}_p = (\delta^{18}\text{O}_w + 10^3) \cdot e^{\frac{[(14.43 \cdot \frac{10^3}{T}) - 26.54]}{10^3}} - 10^3$$

where $\delta^{18}\text{O}_p$ and $\delta^{18}\text{O}_w$ refer to oxygen isotope ratios of Pi and ambient water, respectively, and T denotes the water temperature in degrees Kelvin (K).

2.4.6 Measurement of the Elemental Composition of Sources and Sinks

Multiple conservative elements have been used to calculate the individual contribution of land-derived sources of these elements [Massoudieh et al., 2013]. This multi-element fingerprinting may serve as an independent method to identify sources of colloids as well as complement the results from isotope modeling for source partitioning. This requires systematic analyses of both individual sources and sinks at various temporal and spatial scales for comprehensive comparison. For this analysis, a subsample of each source and sink were analyzed for the concentration of fingerprinting elements using inductively coupled plasma optical emission spectrometry (ICP-OES). Conservative elements best suited for the study site were selected for multiple element sediment fingerprinting using the method outlined in Mingus et al. [2018]. P concentrations and other conservative elements were used for Principal Component Analysis (PCA).

2.5 Results

2.5.1 Salinity and Chlorophyll a

Water salinity from seven different sites was measured from March 2017 to September 2017 (Figure 2.3). Salinity in the non-tidal segment of Love Creek (GN, BN and LN) hovered around 0 ppt throughout the measurement time period, while it increased significantly along the tidal segment of Love Creek (LT4, LT3, LT2 and LT1) until reaching a maximum of about 35 ppt. The high salinity of LT1 indicated that water from this site is no different from that of Delaware Bay. The concentration of chlorophyll a was almost zero in the non-tidal section, while its concentration seemed to increase significantly from the non-tidal to tidal section with its peak concentration occurring in the middle of Love Creek (LT4 and LT3). High monthly variation in the chlorophyll a concentration was also observed along the salinity gradient. For example, its concentration in LT4 went from 1.89 $\mu\text{g/L}$ in March to as high as 382 $\mu\text{g/L}$ in July.

2.5.2 Variation of colloidal P, different size fractions of soil and plant debris in the Love Creek Watershed

Concentrations of P_i in different colloidal P pools over three different months are shown in Figure 2.4. Colloidal samples collected in July have a relatively higher P_i concentration of all pools from all sites than those collected in May and September. Colloidal P_i concentrations collected in September 2017 tend to be higher than those of May 2017. Generally, in all study sites, the order of concentration of P_i is NaOH- P_i

$\text{NaHCO}_3\text{-P}_i > \text{HNO}_3\text{-P}_i > \text{H}_2\text{O-P}_i$. As illustrated in Figure 2.4., P_i concentrations in upstream colloids were higher than those downstream and tended to decrease in concentration with increasing salinity. Unexpectedly high colloidal P_i concentration was observed at the tidal-non-tidal boundary site, LT4.

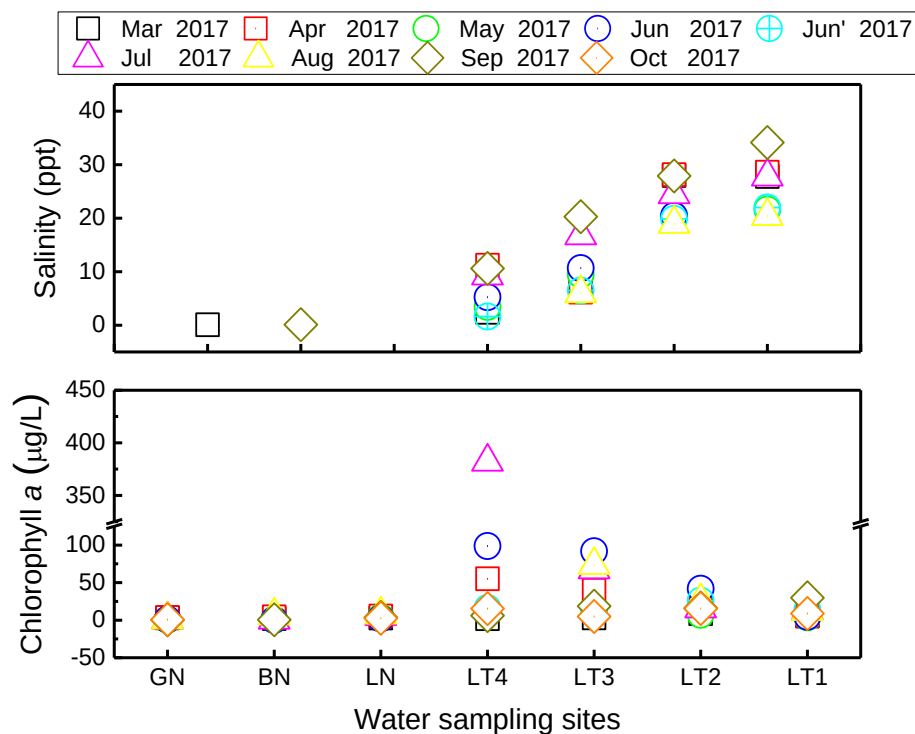


Figure 2.3. Salinity (A) and Chlorophyll *a* (B) concentration of water samples collected from the Love Creek watershed.

Concentrations of P_i in different P pools of different size fractions of combined surface soils ($\geq 53 \mu\text{m}$; $0.45 - 53 \mu\text{m}$; $0.10 - 0.45 \mu\text{m}$) are shown in Figure 2.5. Concentration of P_i of different P pools decreased with the increasing soil size fraction. Soil from the smallest size fraction ($0.10\text{--}0.45 \mu\text{m}$) had the highest P_i

concentration in all pools compared to the other two soil size fractions. Compared to soils from farms, forest soils (LC6-7) had relatively lower concentrations of P_i . P_i concentrations of septic tank samples were one or two orders of magnitude higher than the ones of soils (Figure 2.5). As illustrated in Figure 2.6, P_i concentrations in TCA- P_i of plant debris tended to increase with increasing salinity, while no pattern was observed for HNO_3 - P_i along an increasing salinity gradient. Comparatively, TCA- P_i contributed around 55–70% of P in plant debris, indicating that a large portion of P in plant debris is bioavailable to microorganisms. The ratio of NaOH- P_i to HNO_3 - P_i for colloids is usually similar to that of forest soil (LC6-7) and relatively low compared to farm soils, while unexpectedly high values were observed in the middle of Love Creek (LT4). With decreasing soil size, the ratio of NaOH- P_i to HNO_3 - P_i increases (Figure 2.7).

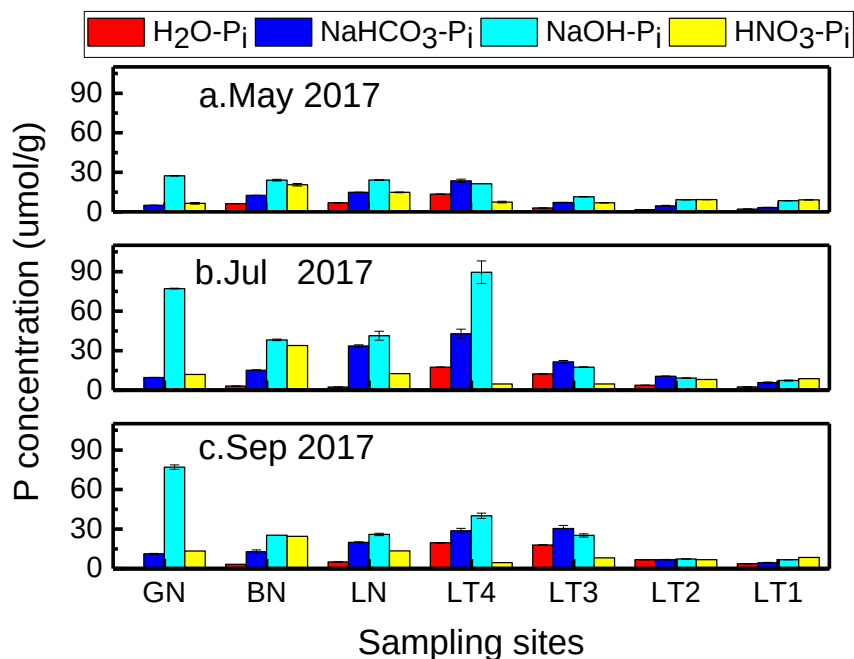


Figure 2.4. Concentrations of colloidal P_i pools in (A) May 2016, (B) Jul 2017, and (C) Sept 2017.

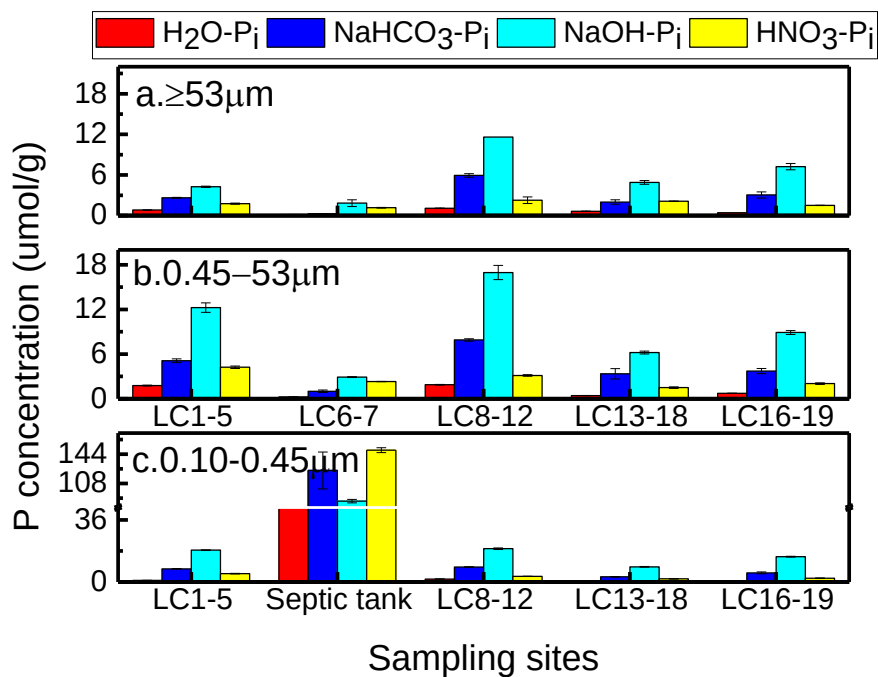


Figure 2.5. Concentrations of P_i pools of different size soil fractions: (A) $\geq 53 \mu\text{m}$; (B) $0.45 - 53 \mu\text{m}$; (C) $0.10 - 0.45 \mu\text{m}$. Due to the small total mass of all soil from LC6-7, there are only two size fractions from this site. Please note septic tank sample was not separated in different sizes ($\geq 0.10 \mu\text{m}$).

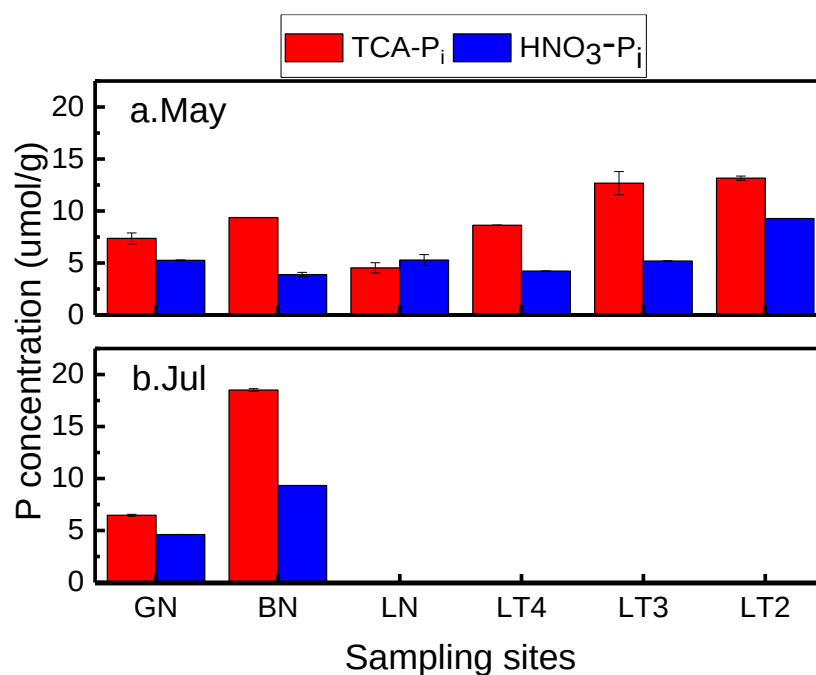


Figure 2.6. Concentrations of P_i pools of plant debris in (A) May 2017 and (B) July 2017. Plant debris at sites from LN to LT2 was not available.

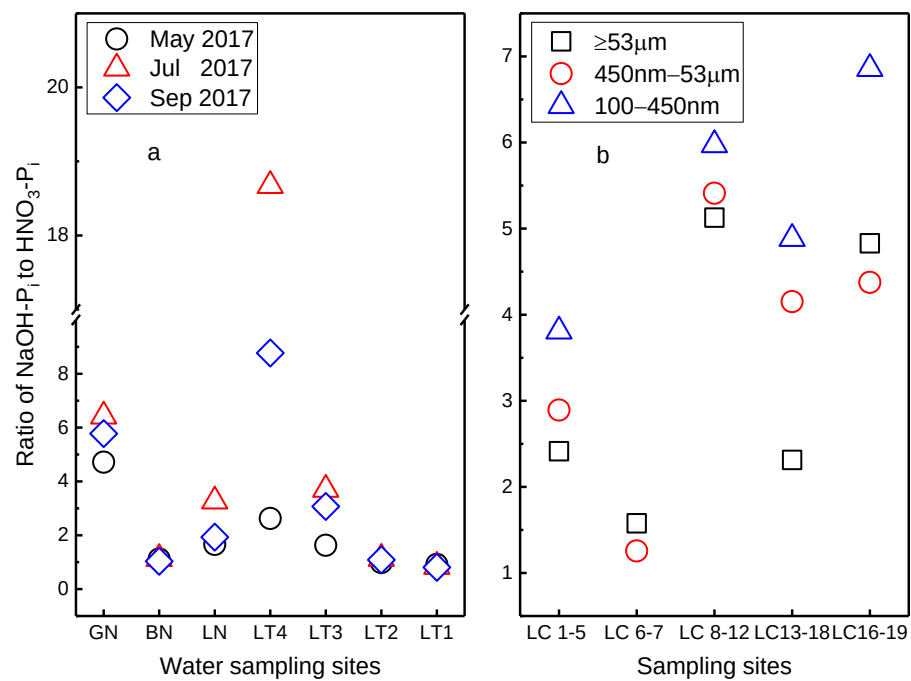


Figure 2.7. Ratio of NaOH- P_i to HNO_3 - P_i for colloids (A) and combined surface soils of different size fractions (B).

2.5.3 Isotope Composition of Carbon and Nitrogen and C/N Ratio

From March 2017 to September 2017, $\delta^{13}\text{C}$ of colloids enriched gradually downstream from -28.6‰, the monthly average for GN, to -21.3‰, the monthly average for LT1. Colloids in LT4 had the lightest $\delta^{13}\text{C}$ ($\approx$ -30‰) in September 2017 compared to other sites, which may be the result of an exceptionally high concentration of chlorophyll a in LT1 (Figure 2.3). Similarly, the $\delta^{15}\text{N}$ of colloids mirrored those of $\delta^{13}\text{C}$ along the water flow direction. From LT4, the average colloidal $\delta^{15}\text{N}$ steadily became lighter downstream from 9.2 to 6.5‰ in the Love Creek Watershed (LT1) (Figure 2.8 B). Seasonal comparison shows the variability in $\delta^{15}\text{N}$ was relatively less than that of $\delta^{13}\text{C}$. The highest difference was observed in site BN with $\delta^{15}\text{N}$ of 2.2‰ and 5.3‰ in July and September, respectively. The same site showed high variability in $\delta^{13}\text{C}$, with values of -27.8 in July and -17.4‰ in September 2017. Comparison of C/N ratios along the flow direction showed an analogous pattern with $\delta^{13}\text{C}$ values; specifically, the ratio steady decreases with increasing salinity (Figure 2.8 C). The exceptionally low C/N value of colloids was observed at site LT4, which corresponded to the high concentration of chlorophyll a compared to other sites.

$\delta^{13}\text{C}$ of plant debris gradually enriched downstream from -31.5‰ (GN) to -19.2‰ (LT1). Similarly, $\delta^{15}\text{N}$ of plant debris had an analogous pattern along the water flow direction with that of $\delta^{13}\text{C}$. From site BN the average $\delta^{15}\text{N}$ of plant debris steadily enriches downstream from -4.7 to 6.0‰ at LT2 (Figure 2.8 E). Comparing C/N ratios along the flow direction showed an opposite pattern with $\delta^{13}\text{C}$, as the ratio steadily decreased with increasing salinity (Figure 2.8 F). The exceptionally high C/N

ratio of plant debris was observed at site LT4, which mirrors its high concentration of chlorophyll *a* compared to other sites.

$\delta^{13}\text{C}$ of two different size fractions of all surface soils from farms were relatively constant with values ranging from -23‰ to -21‰, while the ones from forests (LC6, LC7) had distinctly lighter $\delta^{13}\text{C}$ values at ca. -27‰ (Figure 2.9). For soils from different depths (0–10 cm and 10–20 cm), $\delta^{13}\text{C}$ values were also consistent (-23‰ to -21‰) (Figure 2.9a). Similar patterns appeared for $\delta^{13}\text{C}$ of soils combined from the same farms and forests among three different size fractions ($\geq 53\ \mu\text{m}$; 0.45–53 μm ; 0.10–0.45 μm) (Figure 2.9 B). There was no difference in $\delta^{13}\text{C}$ between different soil size fractions irrespective of sampling depth and usage purpose. Similarly, $\delta^{15}\text{N}$ of two different size fractions of all surface soils from farms were around +6‰, while the ones from forests (LC6 and LC7) had distinctly lighter $\delta^{15}\text{N}$ values at ca. +2‰ (Figure 2.10). For soils from different depths (0–10 cm and 10–20 cm), $\delta^{15}\text{N}$ values were also stable at ca. +6‰ (Figure 2.10a). A similar pattern emerged for $\delta^{15}\text{N}$ values of soils combined from the same farms and forest with three different size fractions ($\geq 53\ \mu\text{m}$; 0.45–53 μm ; 0.10–0.45 μm) (Figure 2.11). A comparison of C/N ratios with size fractions showed a distinct pattern in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, as C/N ratios steadily increased with decreasing size fractions (Figure 2.11 C). Generally, no significant difference in C/N ratio was observed between forest and farm soil.

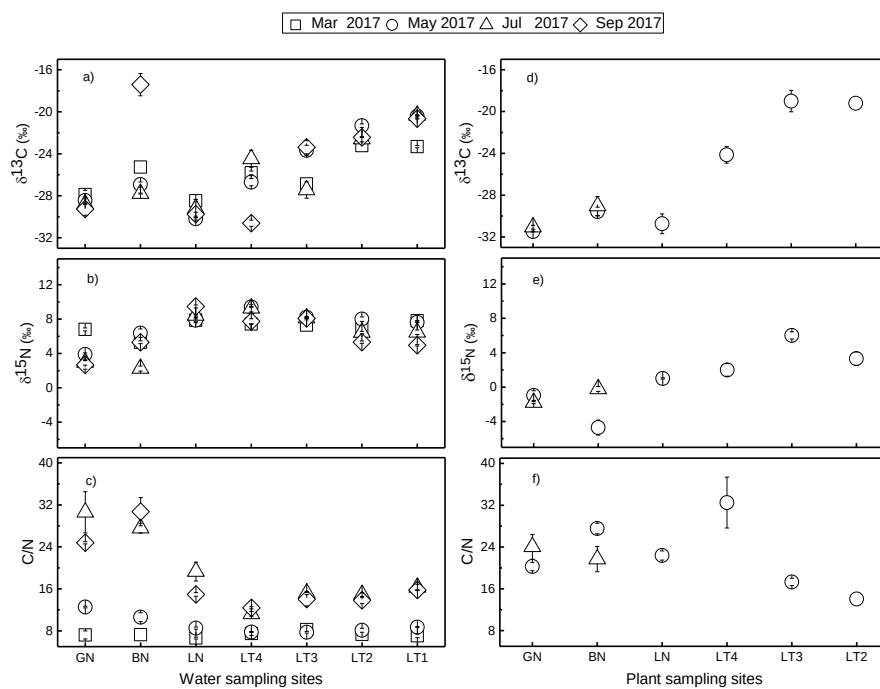


Figure 2.8. (A) Measured $\delta^{13}\text{C}$, (B) $\delta^{15}\text{N}$, and (C) C/N ratios of colloids in March, May, July and September 2017. (D) Measured $\delta^{13}\text{C}$, (E) $\delta^{15}\text{N}$, and (F) C/N ratios of plant debris in May and July 2017. GN, BN and LN are freshwater sites and LT1-4 are tidal sites.

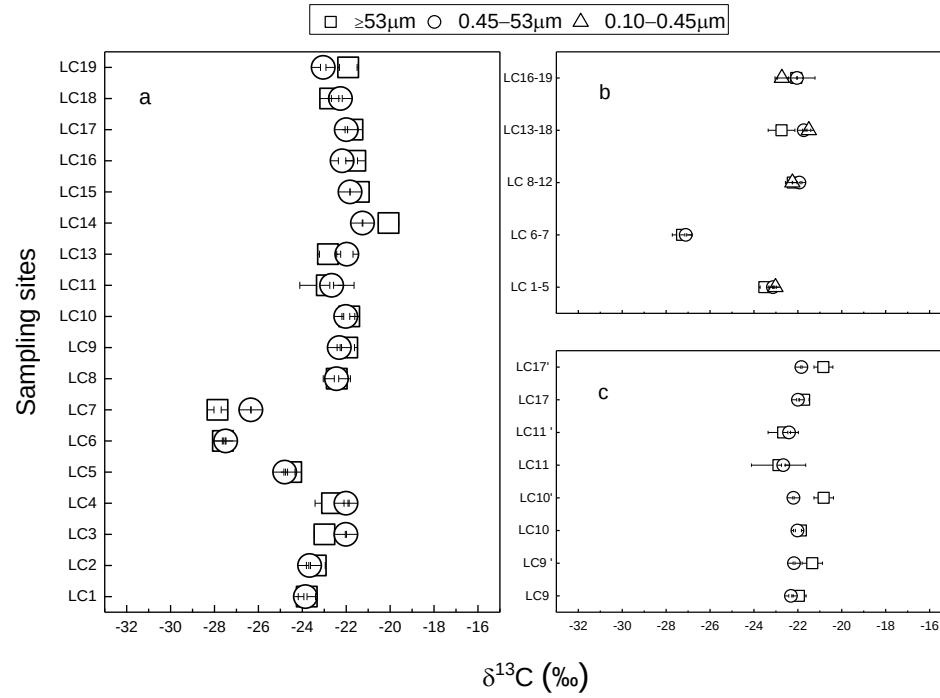


Figure 2.9. (A) Measured $\delta^{13}\text{C}$ values of two different size fractions of all surface soils. (B) Measured $\delta^{13}\text{C}$ values of three different size fraction of combined surface soils. (C) Measured $\delta^{13}\text{C}$ of two different size fractions of soils from different depths (e.g., LC9 indicates depth from 0 to 10 cm and LC9' represents depth from 10 to 20 cm).

2.5.4 Isotope Composition of Carbon and Nitrogen and C/N Ratio

To determine the correlation among water samples and the relative importance of different variables, PCA was used to identify two significant components ($\lambda > 1$) that collectively explained 53% of the variance in surface water with water flow. The loading plot and correlation matrix resulting from the PCA showed a wide gradient in

nutrient species distribution in surface water over 8 months upstream and downstream in Love Creek (Figure 2.2; Figure 2.12). Total phosphorus (TP) and chlorophyll a in the water column were strongly correlated with each other (Group 1). Dissolved inorganic phosphate (DIP) and ammonium were located near the intersection point, which suggest that either they were not correlated at all or that they were not explained by two components alone. Moreover, TP and total nitrogen (TN) were orthogonal to each other, indicating that they were also not related. The clear separation between N and P forms in the water column suggests that they may originate from different sources with water flow.

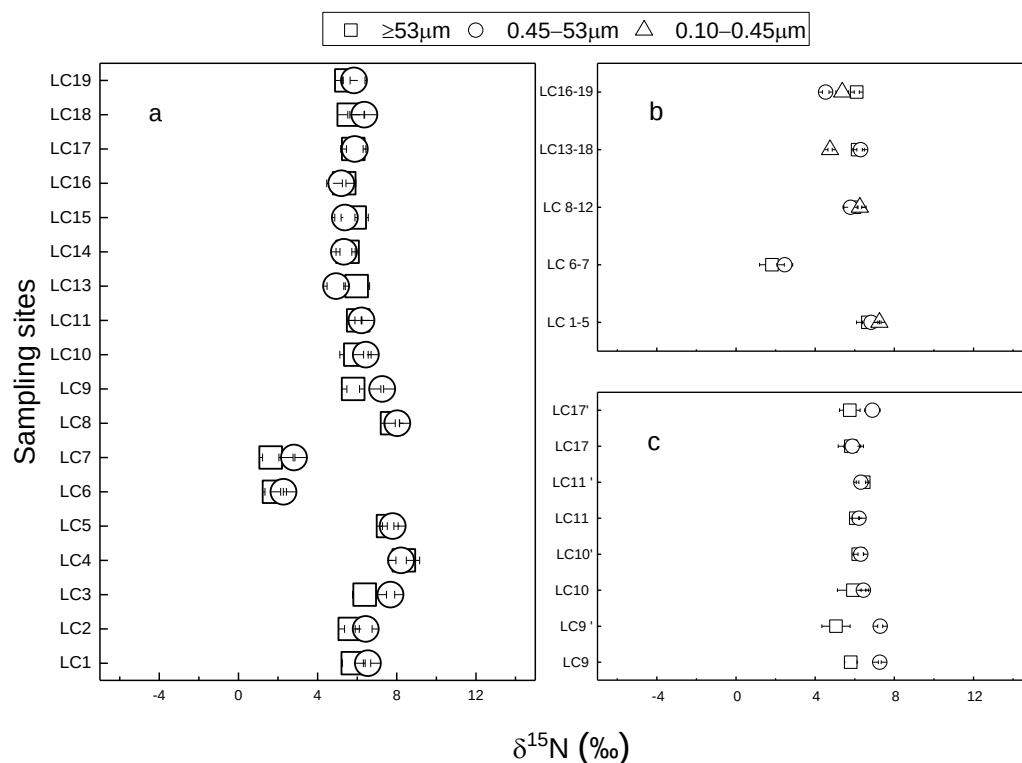


Figure 2.10. (A) Measured $\delta^{15}\text{N}$ of two different size fractions of all surface soils. (B) Measured $\delta^{15}\text{N}$ values of three different size fractions of combined surface soils. (C) Measured $\delta^{15}\text{N}$ values of two different size fractions of soils from different depths (0–10 cm and 10–20 cm).

2.6 Discussion

2.6.1 Salinity and chlorophyll a

Primary productivity is reflected through chlorophyll a concentration, suggesting oligo-mesotrophic and eutrophic conditions in the non-tidal and tidal segments of Love Creek [KDHE, 2011], respectively. The contribution of phytoplankton to particulate matter requires careful consideration due to the complexity between its inorganic and organic components. For instance, the relative relationship between chlorophyll a and phytoplankton biomass can vary. Chlorophyll a contributes from 0.08% to 1.88% to phytoplankton biomass in shallow lakes [Vörös and Padisak, 1991] while this value ranges from 0.308% to 0.702% across a broad trophic gradient of lakes from oligotrophic to highly eutrophic [Kasprzak et al., 2008].

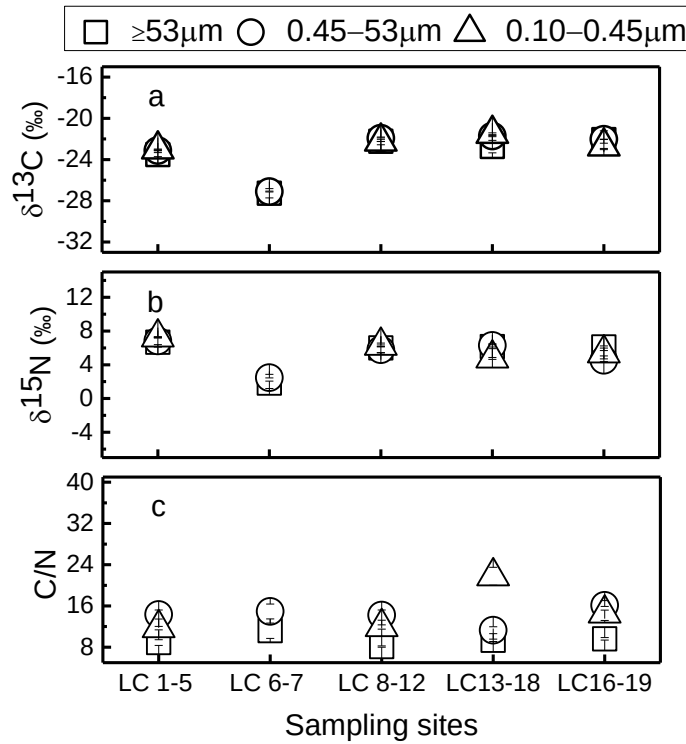


Figure 2.11. (A) Measured $\delta^{13}\text{C}$, (B) $\delta^{15}\text{N}$, and (C) C/N ratios of three different size fractions of combined surface soils. Due to the small total mass of all soil from LC6-7, there are only two size fractions from this site.

Phytoplankton in LN and LT4 contributed to more than 45% of total particulate matter, while their contribution was less than 15% for other sites when the ratio between chlorophyll a and phytoplankton is selected at 1%. Please note that this calculation is based on the wet weight of phytoplankton and thus their contribution would be much less if based on their dried weight. The influence of phytoplankton on particulate matter in other sites is likely negligible, while the impact in LN and LT4 is considerable. This may also suggest a unknown nutrient source or different physicochemical reactions at the tidal-non-tidal boundary site.

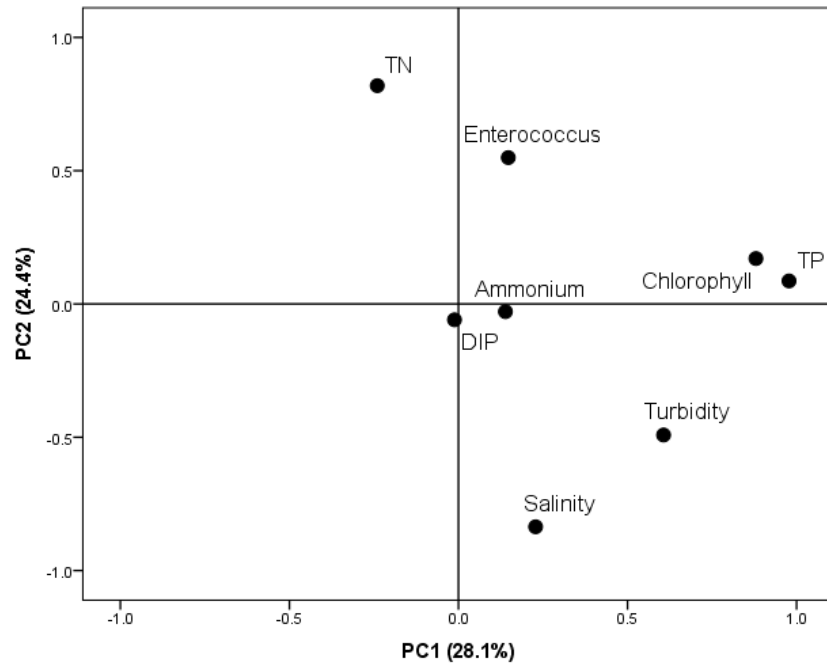


Figure 2.12. Loading plot of basic parameters of surface water based on PCA.

2.6.2 Variation of colloidal P, different size fractions of soil and plant debris in the Love Creek Watershed

The ranges in colloidal P concentrations in the study sites were also similar to those in the mouth of East Creek, which drains into Chesapeake Bay [Mingus et al., 2018], and in the Deer Creek-Susquehanna River continuum [Li et al., 2019]. While there could be various reasons for the sharp increase in colloidal content downstream as compared to upstream, it is likely that the major reason was increasing salinity toward the creek's mouth, as supported by the monthly salinity variation (Figure 2.3). Total P and total colloid concentrations were comparable to the Patuxent estuary of the

Chesapeake Bay, such as along the Pennsylvania-Maryland border north of Baltimore [Jordan et al., 1997].

In general, the order of the concentration of P_i in different P pools of colloids and soils was $\text{NaOH- } P_i > \text{NaHCO}_3\text{- } P_i > \text{HNO}_3\text{- } P_i > \text{H}_2\text{O- } P_i$ (Figures 2.4 and 2.5). The high concentration of P_i found in the NaOH pool, which corresponds to P tightly bound with Fe and Al minerals [Hedley et al., 1982] indicates that this pool was the major P sink for colloids. This was similar to the P pools in the Susquehanna River [Li et al., 2019], East Creek [Mingus et al., 2018] and agricultural soils [Joshi et al., 2016]. This similarity implies that one potential source of colloids in the surface water were soils. One exception was that NaOH- P_i in GN (July and September) contributed more than 80% of total colloidal P, while NaOH- P_i in other sites contributed around 40%, indicating that the composition of colloids in GN could be significantly different from other sites. For most sites, NaOH- P_i contributed around 50% of total colloidal P. This contribution of NaOH- P_i to total colloidal P was slightly less than that in Jordan et al. [2008] in the Patuxent estuary. This could be due to differences in salinity and separation methods (i.e., visible plant debris and algae were removed before centrifugation in this study). The relatively gradual depletion of P in colloids from May to July and to September may reflect compositional differences in colloidal sources upland and in biogeochemical process that alter colloidal P speciation as temperature and salinity increase in the water column (Hartzell et al., 2017).

Significant differences in P concentrations of different P pools were observed between farm and forest soils. The continuous fertilization in farm soil results in the growth of legacy P; the amount of applied fertilizer varied from one farm to another (Figure 2.5). With decreasing size fraction, P content increased in both farm and forest

soils. The P concentration in the smallest soil size fraction (0.10 – 0.45 μm) was ca. 50% higher than that of the larger fractions ($\geq 53 \mu\text{m}$) (Figure 2.5). This may have resulted from unique mineral compositions among differing soil size fractions. With much higher specific surface area, precipitation could preferentially mobilize small colloids into runoff and increase their proportion in the total colloidal mass. In contrast, larger colloids with low P content are only mobilized through high-intensity precipitation [Sharpley et al., 1998].

P_i concentrations of septic tank samples were one or two orders of magnitude higher than the ones of soils in all four P pools (Figure 2.5). In general, the order of the concentration of P_i in different P pools of septic tank samples is $\text{HNO}_3\text{-P}_i > \text{NaHCO}_3\text{-P}_i > \text{NaOH-P}_i > \text{H}_2\text{O-P}_i$ (Figure 2.5). The highest concentration of P_i is found in the HNO_3 pool, which corresponds to P tightly bound to Ca minerals [Hedley et al. 1982], indicating that this pool was the major colloidal P sink. It differed significantly from the colloidal P composition in the water column and from the different soil size fractions (Figure 2.5). The second highest P_i concentration found in the NaHCO_3 pool suggested that the large portion of colloidal P in septic tank samples were bioavailable to microorganisms. Note that the location of the septic tank sampling site was near water sampling site LT4, which has monthly outliers in a variety of different parameters, such as colloidal P and chlorophyll *a*.

Comparatively, TCA- P_i contributes around 55–70% of P in plant debris, which was much higher than $\text{HNO}_3\text{-P}_i$, indicating that the large portion of P in plant debris was bioavailable to microorganisms. Slight revision was made to the extraction methods for plant debris such that 10M HNO_3 was used to assess the total and structural P content in the same plant debris after TCA extraction. P_i concentrations in

TCA and HNO_3 extracts are lower than similar research [Pfahler et al., 2013]. Plant debris in this study was composted and dried, while Pfahler et al. [2013] used fresh leaves. Furthermore, there is a compositional difference in plant debris along the salinity gradient, as evidenced by the fact that P_i concentrations in TCA- P_i of plant debris tended to increase with increasing salinity while no pattern was observed for HNO_3 - P_i . A direct comparison in P pools between plant debris and colloids may not be possible due to different extraction methods. The high Fe/Al concentrations observed in plant debris extracts potentially make it possible to explain the discrepancy in the unexpected high contribution of NaOH- P_i to total colloidal P in GN in July and September as the composition of colloids in GN could be significantly different from other sites.

2.6.3 Isotope Composition of Carbon and Nitrogen and C/N Ratio

Both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of forest and farm soils in this study are similar to those of Awiti et al. [2008]. Specifically, $\delta^{13}\text{C}$ values in farm soils are more enriched compared to forest soils. This is mainly due to yearly crop rotation of C4 plants (maize) with C3 plants (soybean), whose $\delta^{13}\text{C}$ values discriminate one from the other [Lamb et al., 2006; Smith and Epstein, 1971]. Similar $\delta^{15}\text{N}$ enrichment between farm and forest soil may result from relatively closed N cycles in natural forests, as opposed to the open cycles in cultivated fields [Eshetu, 2004]. Due to the preferential uptake of lighter N isotopes by microorganisms during soil biogeochemical processes such as mineralization and nitrification, losses of soil nitrogen following cultivation could lead to soils that have heavier $\delta^{15}\text{N}$ signatures [Hogberg, 1997; Awiti et al., 2008]. No significant difference in both C and N isotopic signatures were observed among different soil size fractions irrespective of sampling depth and usage purpose,

including farm and forest. One potential reason was that the shallow plow depth of ~20 cm and sandy texture make it difficult for plants to adsorb nutrients, which get redistributed unevenly [Djodjic et al., 2004]. These same characteristics could later benefit in source tracking of colloidal P.

$\delta^{13}\text{C}$ of plant debris had a similar pattern to that of colloids across the salinity gradient with a similar isotopic range (Figure 2.8 A; Figure 2.8 D). For example, the light $\delta^{13}\text{C}$ values of colloids (-30‰ to -26‰) in freshwater sites (GN, BN and LN), were ca. -6‰ lighter than the ones of different size fractions of farm soils, indicating that plant debris (-32‰ to -29‰) are the major colloidal source in upstream Love Creek (Figure 2.8 A). The $\delta^{13}\text{C}$ values of C3 plants ($\approx$ -32 to -21‰) are isotopically depleted compared to those of C4 plants ($\approx$ -17 to -9‰) [Lamb et al., 2006; Smith and Epstein, 1971]. For aquatic plants such as phytoplankton, $\delta^{13}\text{C}$ values are between -42 and -24‰ with an average of -30‰ [Hamilton and Lewis, 1992; Kendall et al., 2001]. Similarly, C/N ratios of autochthonous sources such as plankton are low (5–8) [Li et al., 2016] compared to land-derived vascular plants (>15) [Meyers, 1994; Savoye et al., 2003] while that of soil organic matter lies in the intermediate range (8–15) [Brady and Weil, 1990]. The low concentration of chlorophyll *a* (less than 1 µg/L in May and July; Figure 2.3) rules out the possibility that algae may have been an important source which would decrease the $\delta^{13}\text{C}$ values of colloids. The $\delta^{15}\text{N}$ values of colloids in the freshwater sites (GN and BN) were significantly lighter than that of the tidal zone (LT2 and LT1). Similarly, the C/N ratios of colloids in the freshwater sites were quite high compared to the ones of plant debris (Figure 2.8 F) and decreased sharply before becoming almost steady with water flow. This further confirmed that the major source of colloids upstream was plant debris, whose $\delta^{15}\text{N}$ values and C/N

ratios are much lower than the ones of farm soils and higher than those of septic tanks (Figure 2.10 and Figure 2.11). Forest soils could serve as one of major sources of colloids because of their low C/N ratios in May 2017 (ca. 12), while the relatively high C/N ratio of colloids preclude the potential of forest soils as the major source of colloids in July 2017. This may indicate variability in the proportion of different sources of colloids in upstream Love Creek over time. Some outliers, such as the $\delta^{13}\text{C}$ values of colloids in BB and LT4 in September, could be explained by seasonal variation of different sources or switching of tree leaves to corn debris under certain circumstances. In essence, the colloids of upstream Love Creek were dominated by non-agricultural sources.

In contrast to the general trend in estuarine environments, $\delta^{13}\text{C}$ values of colloids enriched gradually downstream from -28.6‰ to -21.3‰, implying variability in the proportion of different sources or even switching of the major source with salinity. For example, colloids in LT4 had intermediate $\delta^{13}\text{C}$ ($\approx$ -26.91‰) and $\delta^{15}\text{N}$ (+8.50‰) values as compared to the ones of forest soils ($\delta^{13}\text{C}$: -27.80‰; $\delta^{15}\text{N}$:+2.00‰), farm soils ($\delta^{13}\text{C}$: -22.00‰; $\delta^{15}\text{N}$:+5.00–8.60‰) and plant debris ($\delta^{13}\text{C}$: -24.00‰; $\delta^{15}\text{N}$:+2.00‰), confirming that the contribution of plant debris decreased downstream relative to upstream. Meanwhile, the low C/N ratios of colloids (9.7) reduced the possibility that plant debris (32.5) served as a major source of colloids in LT4. Moreover, as discussed in section 2.6.1, phytoplankton in LT4 contributed more than 45% of total particulate matter and so the major source of colloids in LT4 was phytoplankton. Furthermore, colloids in LT2 had similar $\delta^{13}\text{C}$ ($\approx$ -21.4‰) and $\delta^{15}\text{N}$ (+6.7‰) values to those of farm soils (ca. 3‰ different from the ones of plant debris), proving that the major source of colloids in water column had been switched from

plant debris upstream to phytoplankton and farm soils downstream with increasing salinity. Septic tanks ($\delta^{13}\text{C}$: -24.35‰; $\delta^{15}\text{N}$: +2.0‰) and sediments may also contribute to bulk colloids with variable temporal and spatial scales because of their similar isotopic signatures to colloids. This assertion could be validated further through phosphate oxygen isotopic signatures and redundancy analysis of conservative elements from different sources.

2.6.4 Sources of colloids and associated P in water

Colloidal P encompasses solid phase of P suspended in water and is derived from land sources as well as in-situ production. Colloidal P can be partly bioavailable at different temporal scales because it includes readily bioavailable P pools among other non-bioavailable P pools [Mingus et al., 2018]. The extent of bioavailability depends on the composition of the different P pools, and ambient physical and chemical processes, residence time, and microbial community structure and activity. The major source of the colloids in Love Creek can be identified with the help of multiple stable isotopes. The high similarity in C and N isotopic signatures and C/N ratios between plant debris and colloids upstream strongly validate that plant debris was a major source of colloids in Love Creek. Although P concentrations in different P pools are not directly comparable due to different extraction methods, high Fe and Al concentrations supplement the isotopic results. In upstream Love Creek, one major source of colloidal P could be plant debris, such as leaves and grass clippings, whose P_i can be released into water column as they degrade [Cowen and Lee, 1973; Zhang et al., 2017]. This conclusion may differ from the ones from Goody et al., [2016] and

Mingus et al. [2018], whose main P source are agricultural sources for watershed. This may potentially change the directions of enactment of nutrient management plan in Love Creek watershed.

Based on C and N isotopic results, farm soil can be excluded as the major colloidal source in the water column whereas forest soil may significantly contribute to colloids. The P load contribution from agricultural fields was expected to be higher than soils under other land covers due to soil erosion vulnerability and high initial P content. The P concentration of farm soil in different P pools was much higher than the ones of forest soils from similar size fractions (Figure 2.4; Figure 2.5). Greater similarity is observed between P concentration in farm soil and colloidal P in water column compared to forest soils. This discrepancy can be explained by P transport mechanisms, as finer-sized soil particles, which contain much more P, are preferentially moved compared to coarser-sized particles [Haygarth and Sharpley, 2000]. The P content and reactivity of eroded material is usually greater than the source, confirmed by the increasing of P concentration with soil size fraction (Figure 2.5). The enrichment of soil test P and total P content of sediment in surface runoff ranged from 1.2 to 6.0 and 1.2 to 2.5, respectively [Sharpley, 1985]. The ratio of NaOH-P_i to HNO₃-P_i pools of colloids and farm soil was similar in all sites besides sites with high chlorophyll a concentration, such as LT4. Coupled with C and N isotopic results, this could indicate similar physicochemical processes driving the size of P pools or mobilizing farm soil into water bodies. Chlorophyll a and TP are grouped together through PCA (Figure 2.12), indicating that phytoplankton play an important role in colloidal composition, especially when chlorophyll concentration is high (LN, LT4).

NaOH- P_i and HNO_3 - P_i pools are normally considered to be recalcitrant P pools and may carry distinct P source signatures [Mingus et al., 2018; Li et al., 2019], implying that they have not been cycled by (micro)organisms and thus are suitable for source tracking. As shown in Figure 2.7, there was a gradual depletion of the NaOH- P_i / HNO_3 - P_i ratio in colloidal P from upstream (2-4) to downstream (0.95), suggesting physicochemical processes such as source mixing and/or partial removal of specific P pools along an increasing salinity gradient [Jordan et al., 2008]. One major reason may have been the release of Fe-oxide bound P and paired precipitation of Ca-P induced by high concentrations of P_i and Ca, high pH, and high salinity [Li et al., 2017]. The proportion of HNO_3 - P_i (Ca-mineral pool) increases gradually from the non-tidal section to tidal section of Love Creek. The dilution of terrestrially derived colloids upstream by the import of colloids from tidal action may also have caused the depletion in the NaOH- P_i / HNO_3 - P_i ratio. Regardless, the salinity data (Figure 2.3) exclude the possibility of colloids imported from the Bay reaching upstream. The unexpected high phytoplankton concentration at the tidal-non-tidal boundary site of Love Creek is still not confidently explained. Please note that our result can prove the organic matter in colloids could result from non-agricultural sources. However, due to the fact that the P in colloids seems to be mainly in inorganic forms, which could originate from different places besides the organic matter. Given that agricultural soils become enriched in P it is possible that much of the P in colloids comes from farmlands. Therefore, more research is needed to characterize the sources and release mechanisms of P in runoff originating from urban landscapes such as septic tanks.

2.7 Conclusions

Concentrations of inorganic P (P_i) pools in colloids along the salinity gradient vary in the order $\text{NaOH- } P_i > \text{NaHCO}_3\text{- } P_i > \text{HNO}_3\text{- } P_i > \text{H}_2\text{O- } P_i$. The $\text{NaOH- } P_i$ is the most dominant P pool in colloids, soils, and river bank sediments. Relative size of P pools in different size fractions in farm and forest soils are comparable but the P_i concentration of each P pool decreases with increasing size fraction and salinity. Comparison of C and N isotopes, P pools and other biogeochemical parameters suggested that the major colloidal source in the non-tidal section of Love Creek is non-agricultural sources such as plant debris with a portion from forest soil. Along the salinity gradient, the contribution of terrestrial sources gradually decreased. These results collectively suggest that multiple stable isotopes provide complementary information on the identification of relative contribution of colloidal P from different land uses as well as help assess the bioavailability of the different P_i pools in a watershed.

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Chapter 3

LOADING AND BIOAVAILABILITY OF PHOSPHORUS IN THE DEER CREEK-SUSQUEHANNA RIVER TRANSECT

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Loading and Bioavailability of Colloidal Phosphorus in the Estuarine Gradient of the Deer Creek - Susquehanna River Transect in the Chesapeake Bay

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3.1 Abstract

Phosphorus (P) overloading is a major cause of surface water eutrophication and bottom water hypoxia. The incomplete understanding of different P pools and their corresponding bioavailability in the continuum from sources and sinks has limited the development of appropriate nutrient management strategies. Here we apply multistable isotope proxies to track colloids and identify whether specific P pools in colloids are biologically cycled at the Deer Creek-Susquehanna River mouth stretch. Results showed that NaOH-P_i is the most dominant P pool in the summer and winter seasons. Oxygen isotope values ($\delta^{18}\text{O}_\text{P}$) of NaOH-P_i and HNO₃-P_i pools of different size fractions of colloids are much heavier than the ranges of equilibrium values in the ambient water, which suggest that these two pools are recalcitrant against biological uptake. It further means isotopic signatures of these P pools could be used

to identify the sources of colloids. Carbon (C) and nitrogen (N) isotope compositions of colloids showed that the contribution of terrestrial sources gradually decreases downstream of the river toward the bay and Deer Creek contributes disproportionately high amounts of colloids to the Susquehanna River. These findings provide valuable information on the loading of colloids and relative bioavailability of colloidal P pools in estuarine ecosystems.

3.2 Introduction

Phosphorus (P) is a nonrenewable resource and an essential element for agricultural productivity. In the land-water continuum, excess P input from the point and/or non-point external source into the aquatic ecosystem is dominated by colloids because phosphate anion has high affinity to solid surfaces [Burwell *et al.*, 1977]. Colloidal P encompasses solid phase of P suspended in water and is derived from land sources as well as in-situ production. Colloidal P can be partially bioavailable because it includes readily bioavailable P pools among other non-bioavailable P pools [Mingus *et al.*, 2018]. The extent of bioavailability depends on the composition of different P pools, and ambient physical/chemical processes, residence time, and community structure and activity of (micro)organisms. Therefore, understanding of colloidal P bioavailability in a multi-scale ecosystem can provide insight into the role of colloidal P on water quality and provide information necessary to devise effective management plans.

The Chesapeake Bay, the most productive estuary in the U.S., is suffering from varying degrees of water quality issues due to excess nutrients from both point and nonpoint sources. It receives an estimated 9.74×10^6 kg of P per year from its watershed and 28% of is contributed by Susquehanna River alone [Ator *et al.*, 2011]. Small tributaries such as Deer Creek are expected to contain higher concentrations of nutrients than rivers and thus contribute a proportionally higher nutrient load per unit

volume of water. Because of complex non-linear and estuary-specific ecological interaction [Cloem, 2001; Kemp *et al.*, 2005], there are substantial differences in magnitude and trajectory of responses to nutrient loads for various estuaries as well as within a portion of an estuary. With the increase in the size of an estuary, potential P sources and P transformation pathways may increase exponentially. Research on spatial and temporal variability in the abundance of divergent allochthonous and autochthonous sources of colloidal P may either allow identifying their original sources or are efficiency at which they are recycled and transformed into more bioavailable forms due to long residence time in the water.

Phosphate oxygen isotope ratios ($\delta^{18}\text{O}_\text{P}$) have been applied to advance mechanistic understanding of P cycling as well as tracing sources in a variety of environments such as agricultural soils, fresh waters, and marine systems [Angert *et al.*, 2012; Davies *et al.*, 2012; Goody *et al.*, 2016; Joshi *et al.*, 2016; Tamburini *et al.*, 2012]. In a biological system, oxygen isotope exchange between water and phosphate is rapid and results in isotope equilibrium, which is determined by the ambient water oxygen isotope ratios ($\delta^{18}\text{O}_\text{w}$) and temperature [Blake *et al.*, 1997; Longinelli and Nuti, 1973]. However, in abiotic systems, the isotope exchange is negligible at low temperature and circumneutral pH conditions [O'Neil *et al.*, 2003]. These fundamentally distinct properties of $\delta^{18}\text{O}_\text{P}$ make it a unique tracer to identify P cycling and transformation in various environments. Similarly, stable isotopes of carbon (C) and nitrogen (N) have long been used as reliable tools to trace the source of particulate organic matter (POM) in a variety of ecosystems such as creeks, rivers, and coastal water bodies [Kendall *et al.*, 2001]. Application of multiple isotope techniques adds confidence on source identification if multiple sources have an overlapping signature for one isotope or if the signature is altered due to biogeochemical processes. For example, phosphate oxygen isotope is coupled with dual nitrogen and oxygen isotopes in nitrate to track the input sources and the downstream fate of nutrients [Goody *et al.*, 2016]. In this chapter, multi-stable isotopes (O, C, and N) are used to track sources of colloids and identify whether specific P pools in the colloids are biologically cycled at

the Deer Creek–Susquehanna River mouth transect. To realize this objective, water samples were collected along the physicochemical and hydrodynamic gradients and colloids were separated. Different P pools of colloids were systematically investigated using modified Hedley sequential extraction method and isotope ratios of phosphate oxygen and carbon and nitrogen of the colloids were measured. Our results suggest that multi-isotopes can provide complementary information to identify loading of colloids and bioavailability of colloidal P in estuarine ecosystems.

3.3 Materials and Methods

The major objective of this section is to investigate the temporal and spatial variability and bioavailability of phosphorus in the estuarine gradient in the Deer Creek-Susquehanna River transect:

- How organic and inorganic fractions of colloidal and dissolved phosphorus vary with hydrological gradients in small and larger estuaries?
- Can the bioavailability of phosphorus vary with turbidity and how does it impact primary productivity?
- Can multi-isotopes of carbon, nitrogen, and phosphate and their isotope mass balance provide a semi-quantitative assessment on terrestrial sources of nutrients in the Susquehanna River estuary?

3.4 Materials and Methods

3.4.1 Study Area and Collection of Water Samples

In this study, a stretch of lower Susquehanna River downstream of the Conowingo Dam to its mouth in the Chesapeake Bay and Deer Creek, a small tributary to the Susquehanna River (Figure 3.1), was chosen to represent a creek-river-

bay continuum. The major land use is agricultural crop fields amid forest patches. Five sites chosen along the water flow direction are Deer Creek (DR1: 39°37.363' N, 76°09.846' W), the lower Susquehanna River (CB1.0: 39°39.125' N, 76°10.195' W, and CB1.0-1: 39°35.88' N, 76°07.66' W; SR1: 39° 33.285' N, 76°05.10' W), and the Chesapeake Bay (CB1.1: 39°32.443' N, 76° 5.05' W) (Figure 3.1). Water samples were collected in three different seasons (winter, spring, and summer) in Dec 2016, Jun 2017, and Aug 2017. The site SR1 was abandoned after Dec 2016 sampling and the new site (CB1.0-1) was established to provide a reasonable spatial coverage among sites. Water sampling at different sites (CB1.0, DR1, CB1.0-1, SR1, and CB1.1) was done with specific time lag as follows: CB1.0 ($t_0 = 0$ min), DR1 ($t_1 = 1$ hr 5 min), CB1.0-1 ($t_2 = 2$ hr), SR1 ($t_3 = 3$ hr 51 min), and CB1.1 ($t_4 = 5$ hr 11 min). The lag time was chosen based on the flow rate of water so that the same surface water transported downstream is collected in each site. About 80 L of surface water was scooped in carboys from each site and chilled in ice until storing at 4 °C cold room before processing (described below). The depth of water column in each site was at least 110 cm, so possibility of mobilizing colloids from sediment-water interface was not expected. Water temperature and pH were measured in-situ. The concentrations of total P and selected elements (N, C, Fe, Mn, Ca, and Mg) in the as-received water samples were measured by inductively coupled plasma optical emission spectrometer (ICP-OES) (Thermo iCAP 7000, USA).

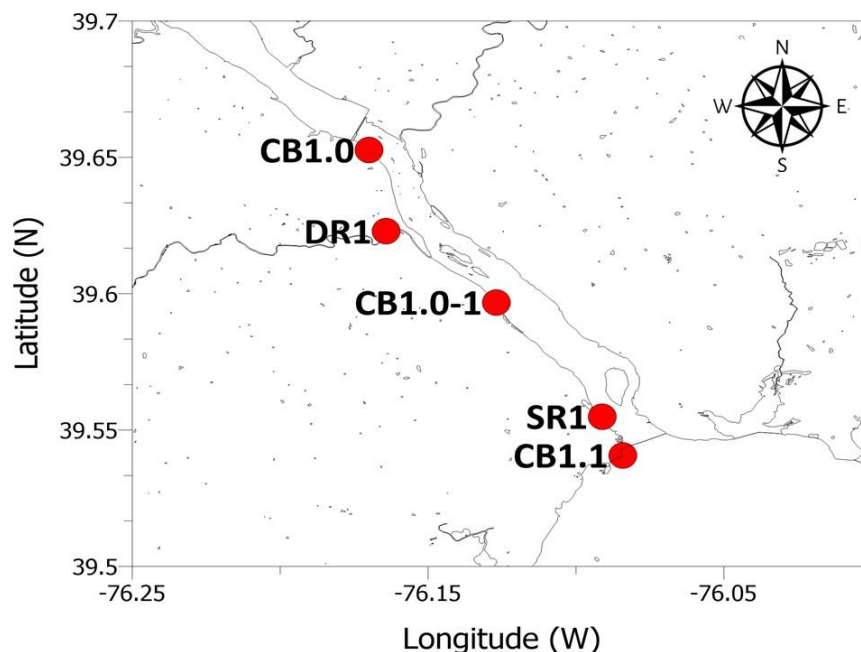


Figure 3.1. Location of sampling sites in Deer Creek, the lower part of Susquehanna River, and the mouth of the river in the Chesapeake Bay.

3.4.2 Separation of Colloids

Colloids in the water samples were separated into three size ranges (50–100, 100–450, and ≥ 450 nm) using centrifugation (Sorvall LYNX 6000, Thermo) based on Stokes' Law of settling. Prior to separation, visible plant debris and algae filaments were removed using pipettes. The supernatant was decanted and pelleted colloids were freeze-dried. Since the salinity of all sites was <1.0 PSU, no washing or salinity correction was needed to obtain the true mass of colloids.

3.4.3 Measurement of C and N Isotopes

Freeze-dried colloids collected from river water were weighted into tin foil capsules for carbon and nitrogen isotope determination as described in section 2.4.5.

3.4.2 Extraction of Different P Pools and Measurement of Phosphate Oxygen Isotopes

P pools in the colloids were sequentially extracted following the modified Hedley's Sequential extraction as described in the section 2.4.6. P pools were individually quantified by using the phosphomolybdate blue method [Murphy and Riley, 1962] before processing and purification of each pool for isotope analysis.

All P pools extracted from the colloids were processed to remove contaminants and precipitated as silver phosphate. Protocols for sample processing and silver phosphate precipitation for all P pools and isotope measurements will be the same as described in Chapter 2. Similarly, $\delta^{18}\text{O}_w$ values of as received water was measured as described in the section 2.4.8. Elemental concentrations in water column and colloids extraction solution were measured (using ICP-OES).

3.5 Results

3.5.1 Seasonal Variation of Colloids and Inorganic P in the Deer Creek-Susquehanna River Transect

The physicochemical properties of water samples collected from five different sites in winter, spring, and summer seasons (Dec 2016, Jun 2017, and Aug 2017) are included in Tables 3.1-3.3. The concentration of ammonium in all waters was below detection limit ($<0.55 \mu\text{mol/L}$). Nitrate and total N concentrations in Deer Creek (DR1) were 2–4 times higher than that in Susquehanna River (CB1.0 and CB1.0-1) and Chesapeake Bay (CB1.1) in different sampling seasons. The range of total (bulk) P in as received samples (i.e., before separation of colloids) in all waters over different seasons varied from 0.19 to 1.61 $\mu\text{mol/L}$. The colloid P concentration varied from 0.10

to 1.06 $\mu\text{mol/L}$, with the highest colloidal P concentration in Deer Creek, which accounted for 85% of total P in June 2017. High variability of colloid content was observed in Deer Creek over seasons than other sites and the content in summer much higher than that in winter and spring seasons (Dec 2016 vs. Jun and Aug 2017) (Figure 3.2a). For example, colloids content increased significantly from 2.8 mg/L (Jun 2017) to 17.7 mg/L (Aug 2017). The Susquehanna River sites generally followed the winter-summer pattern but the variability and total change were low. For example, the colloids content of CB1.0 slightly increased from 4.9 mg/L (Dec 2016) to 5.9 mg/L (Jun 2017) and then to 6.5 mg/L (Aug 2017). The CB1.1 had a distinct outlier (19.8 mg/L) in Dec 2016 but otherwise increased from 2.5 mg/L (Jun 2017) to 3.2 mg/L (Aug 2017). This outlier is commonly not expected and the reason for is yet unknown. This difference in variability in creek and river are often reported to be due to the different discharge and the total area of surface water in the catchment (de Klein and Koelmans, 2011). Generally, the distribution of different size ranges of colloids was consistent among different sites. For example, colloid size ranging from 100 to 450 nm had the highest fraction in the total colloidal mass, while colloids ranging from 50 to 100 nm contributed the least ($\sim 15\%$) (Figure 3.2b).

Table 3.1. Physicochemical properties of as recived water from differnty sites in the Deer Creek-Susquehanna River transect in Dec 2016.

		DR1	CB1.0	SR1	CB1.1
		Dec 2016	Dec 2016	Dec 2016	Dec 2016
pH		7.4	7.5	7.4	7.1
EC	(mmhos/cm)	0.2	0.34	0.32	0.3
NH ₄ -N	($\mu\text{mol/L}$)	<0.55	<0.55	<0.55	<0.55
NO ₃ -N	($\mu\text{mol/L}$)	59.8	11.0	12.3	13.7
TIC	($\mu\text{mol/L}$)	307	1242	1132	978
TC	($\mu\text{mol/L}$)	490	1950	1784	1341

TOC	($\mu\text{mol/L}$)	183	708	653	363
TN	($\mu\text{mol/L}$)	228	143	136	101
Al	($\mu\text{mol/L}$)	0	0.37	0.37	20
B	($\mu\text{mol/L}$)	0.91	1.8	2.7	1.8
Ca	($\mu\text{mol/L}$)	345	766	735	684
Cu	($\mu\text{mol/L}$)	0	0	0	0
Fe	($\mu\text{mol/L}$)	0.36	4.3	0.36	6.8
K	($\mu\text{mol/L}$)	61.8	64.1	61.5	62.8
Mg	($\mu\text{mol/L}$)	280	379	369	335
Mn	($\mu\text{mol/L}$)	0	0.91	0	0.73
Na	($\mu\text{mol/L}$)	512	828	775	768
P	($\mu\text{mol/L}$)	0.84	0.19	0.48	0.65
S	($\mu\text{mol/L}$)	101	397	388	376
Zn	($\mu\text{mol/L}$)	0	0	0	0

Concentrations of P_i in different P pools of colloids over three different months and among distinct size fractions (50–100, 100–450, and >450 nm) are shown in Figures 3.3 and 3.4, respectively. Colloids in winter seasons have a relatively higher P_i concentration in all pools than that in summer months, while P_i in colloids collected in June tends to be higher than the one of Aug 2017. As illustrated in Figure 3.4, P_i concentrations in smaller size fractions of colloids (50–100 and 100–450 nm) are higher than that of larger fraction (≥ 450 nm) in Dec 2016. In all study sites, the general order of concentration of P_i is $\text{NaOH-}P_i > \text{NaHCO}_3\text{-}P_i > \text{HNO}_3\text{-}P_i > \text{H}_2\text{O-}P_i$. High concentration of P and colloids in Deer Creek suggests that it contributes a higher nutrient load per unit volume of water compared to that from upstream Susquehanna River.

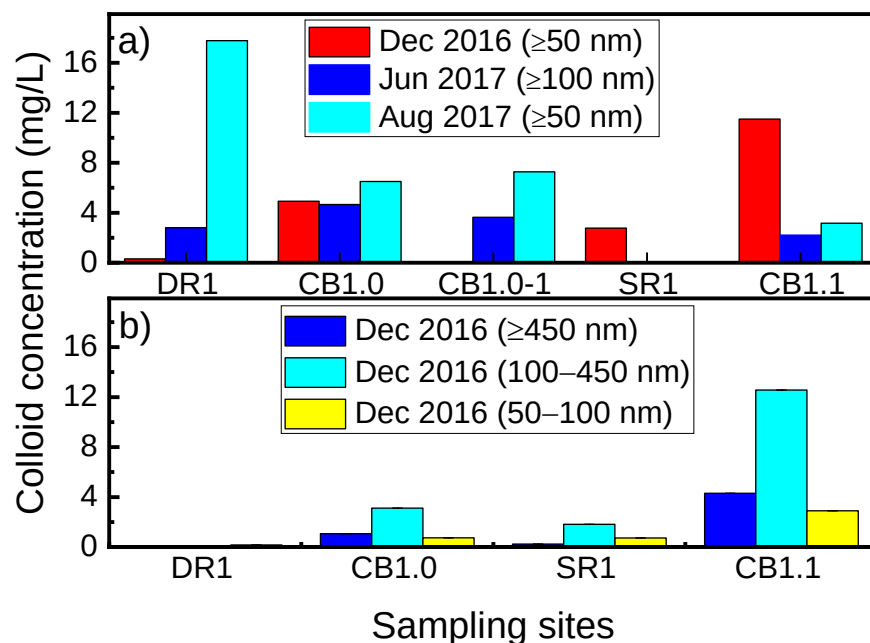


Figure 3.2. The concentration of colloids (mg/L) in different sampling sites in Dec 2016, Jun 2017, and Aug 2017 (a) and in different size ranges of the colloids in Dec 2016 (b). Concentration of different size ranges of the colloids in DR1 are 0.17 mg/L (50–100 nm), 0.10 mg/L (100 – 450 nm) and 0.03 mg/L (≥ 450 nm), respectively. Similarly, the concentration of colloids (≥ 50 nm) in DR1 in Dec 2016 is 0.30 mg/L.

3.5.2 Isotope Composition of Carbon and Nitrogen and C/N Ratio

In Jun and Aug 2017, $\delta^{13}\text{C}$ values (-26.1‰ to -20.7‰) of colloids at DR1 site are heavier compared to that of colloids collected from CB1.0 and CB1.0-1 sites (Figure 3.5). Along the flow direction of water from Deer Creek and lower Susquehanna River to the Chesapeake Bay, the $\delta^{13}\text{C}$ of colloids generally became heavier. For a single site, for example at CB1.0-1 site, the $\delta^{13}\text{C}$ value of colloids enriched from -28.4‰ to -26.6‰ from June to Aug (Figure 3.5a). Similarly, the $\delta^{15}\text{N}$ values of colloids had an analogous pattern along the water flow direction with that of $\delta^{13}\text{C}$ values. For example, from DR1 site, average $\delta^{15}\text{N}$ values of colloids steadily

became heavier from 4.9 to 8.4 ‰ to the mouth of the Chesapeake Bay (Figure 3.5b). Comparing among seasons, the variability of the $\delta^{15}\text{N}$ values was relatively less than that of $\delta^{13}\text{C}$ values. For example, the highest difference was observed in DR1 site (with $\delta^{15}\text{N}$ values of 4.0 and 5.7 ‰ in Jun and Aug, respectively), the same site which showed higher variability of $\delta^{13}\text{C}$ values. Comparison of C/N ratio along the flow direction showed steady decrease of the ratio (Figure 3.5c) but $\delta^{13}\text{C}$ values showed positive relationship with C/N ratios (Figure 3.5d).

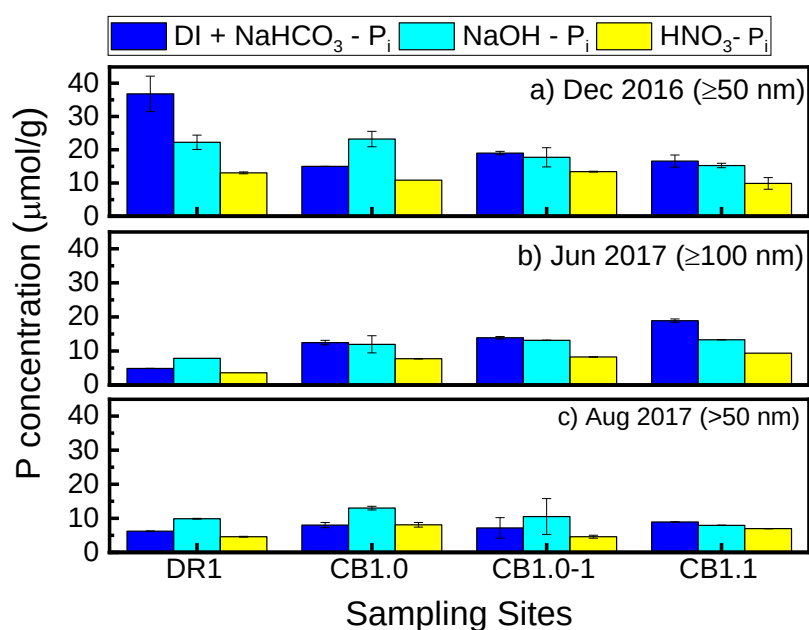


Figure 3.3. Concentrations of P_i pools of colloids in Dec 2016, Jun 2017, and Aug 2017: (a) ≥ 50 nm in Dec 2016; b) ≥ 100 nm in Jun 2017; c) ≥ 50 nm in Aug 2017.

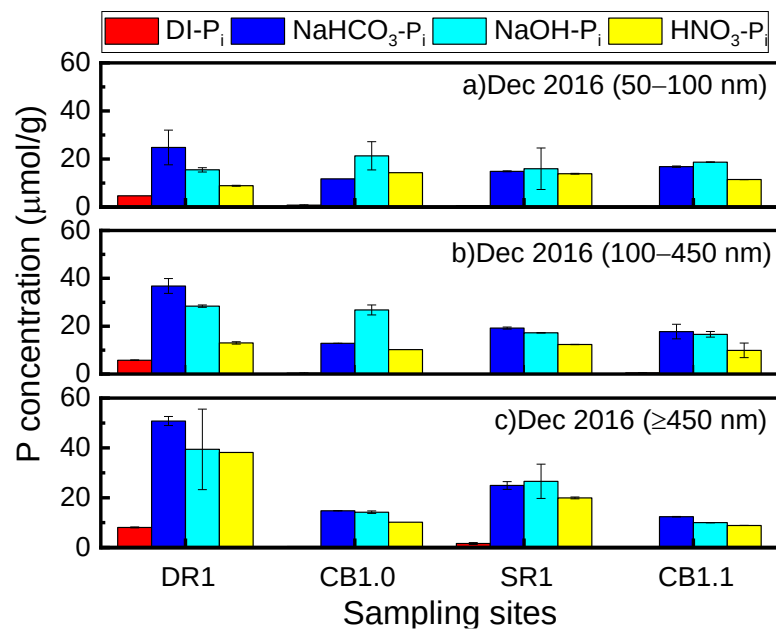


Figure 3.4. Concentrations of P_i pools in different size ranges of the colloids in Dec 2016: (a) 50 – 100 nm; b) 100 – 450 nm; and c) ≥ 450 nm.

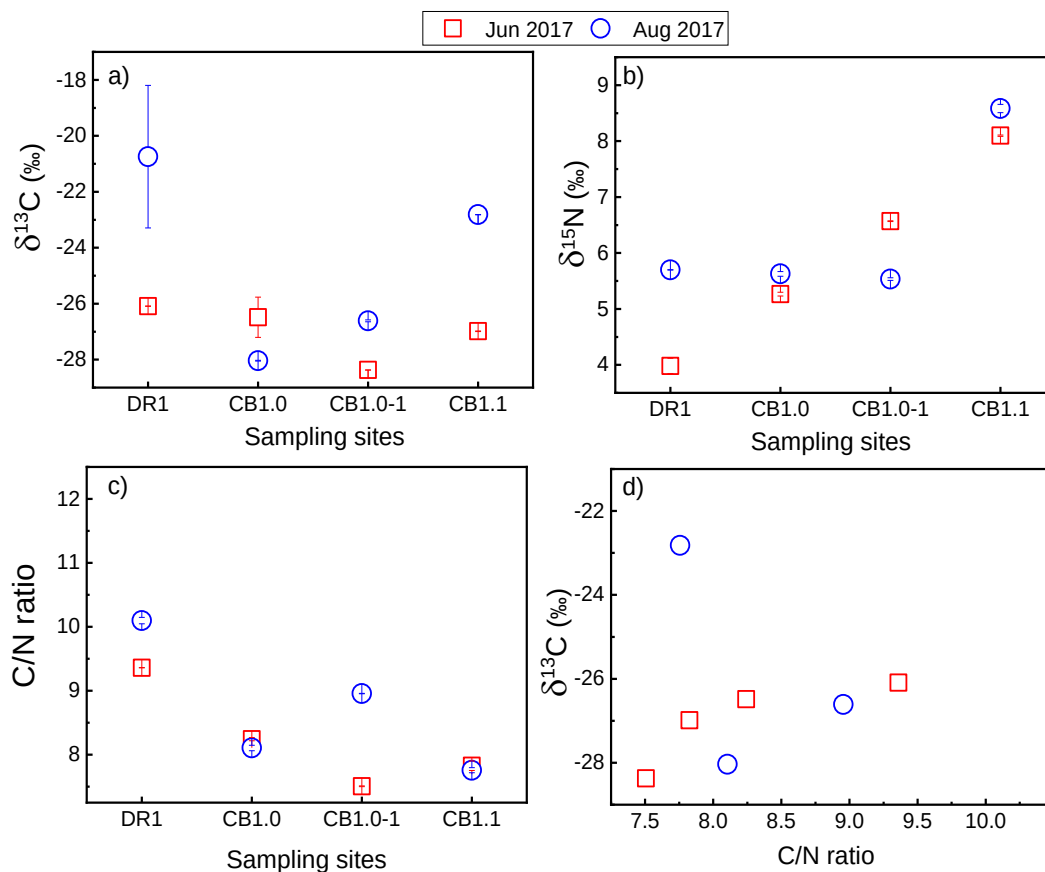


Figure 3.5. Measured $\delta^{13}\text{C}$ values (a), $\delta^{15}\text{N}$ values (b), and C/N ratios (c) of colloids in Jun 2017 and Aug 2017. Relationship of C/N and $\delta^{13}\text{C}$ values (d) of colloids in Aug 2017.

3.5.3 Phosphate Oxygen Isotope Ratios of Different P Pools

The measured $\delta^{18}\text{O}_\text{P}$ values of different P pools of the colloids and the theoretical equilibrium values calculated using eq 1 (see above) by *Chang and Blake* [2015] are presented in Figure 3.6. The $\delta^{18}\text{O}_\text{P}$ values for $\text{HNO}_3\text{-P}_\text{i}$ pool of different size ranges of colloids at CB1.1 site (50–100, 100–450, and >450 nm) in Dec 2016 were heavier than the calculated equilibrium values by ca. 1.5–6.0‰, as was the one of NaOH-P_i pool of colloids collected at CB1.1 site (100–450 nm) in Dec 2016. The $\delta^{18}\text{O}_\text{P}$ values for NaOH-P_i pools of colloids collected at three sites (DR1, CB1.0, and

CB1.0-1 site) in both Jun 2017 and Aug 2017 were heavier than the calculated equilibrium values by ca. 3–10‰, while the one of NaOH-P_i pool of colloids collected at CB1.0 site (100–450 nm) in Jun 2017 were lighter than the calculated equilibrium values by ca. 1.8‰.

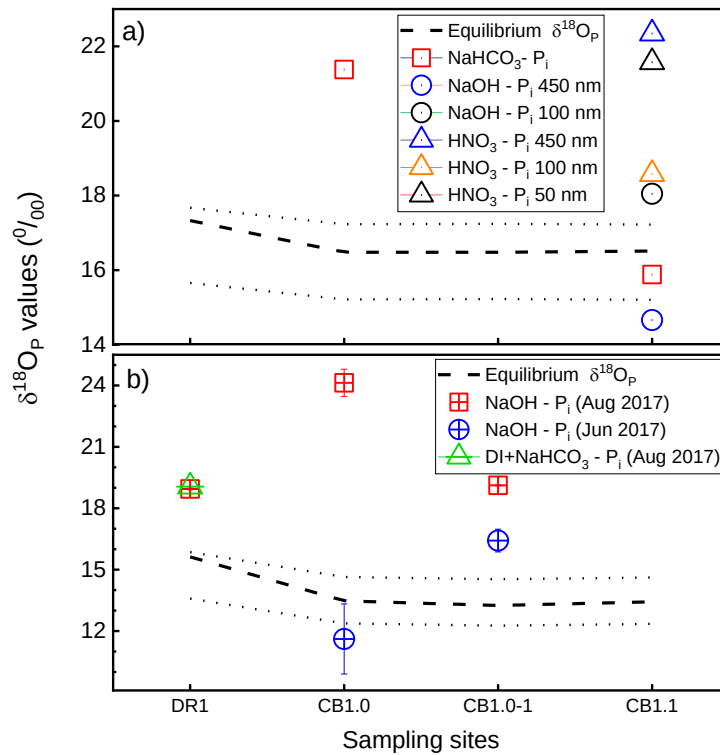


Figure 3.6. $\delta^{18}\text{O}_\text{P}$ values of different P pools of colloids in Dec 2016 (a) and Jun 2017 and Aug 2017 (b). The heavy dashed line represents the equilibrium $\delta^{18}\text{O}_\text{P}$ values calculated from measured temperature and water $\delta^{18}\text{O}_\text{W}$ values on the day of sampling and region bracketed between two dotted lines represents the variability of equilibrium $\delta^{18}\text{O}_\text{P}$ values within a month of the sampling date.

3.6 Discussion

3.6.1 Seasonal Variation of Colloids and P in the Deer Creek-Susquehanna River Transect

The ranges of colloidal P concentration in the study sites are similar to that in mouth of East Creek drained into Chesapeake Bay [Mingus *et al.*, 2018]. While there could be various reasons for the sharp increase in the colloidal content, but it is likely that the intensity of rainfall in the season which increases soil saturation and soil erosion could be a major reason. This can be supported by the increase in total precipitation in study site from Dec (3.68 cm) to June (5.61 cm) to August (22.35 cm) [US CD, 2019]. Total P and total colloids concentration in study site are comparable to the ones of Piedmont watersheds of Chesapeake Bay, such as along the Pennsylvania-Maryland border north of Baltimore, Maryland [Jordan *et al.*, 1997].

In general, the order of the concentration of P_i in different P pools is $\text{NaOH-}P_i > \text{NaHCO}_3\text{-}P_i > \text{HNO}_3\text{-}P_i > \text{H}_2\text{O-}P_i$ (Figures 3.3 and 3.5). The highest concentration of P_i in NaOH pool (which corresponds to P tightly bound with Fe and Al minerals; Hedley *et al.*, [1982]) indicates that this pool is the major P sink in the colloids. This result is similar to East Creek draining to the Chesapeake Bay [Mingus *et al.*, 2018] and agricultural soils [Joshi *et al.*, 2016] and this similarity implies that the source of colloids in the surface water may be upland soils. However, NaOH- P_i contributes around 50% of total colloidal P, which is slightly less than that in Jordan *et al.* [2008] in Patuxent estuary, the difference could be due to the different salinity and separation methods (e.g., plant debris and algae were removed before centrifugation in this study). Comparing among seasons, colloids in winter season has a relatively higher concentration of P_i in all pools than that in summer months (Figure 3.3). However, the total P load carried by colloids in summer months is still much higher than that in winter because of higher mass of colloids (Figure 3.3a). This trend is comparable to Seine estuary in France [Némery and Garnier, 2007] and long-term data from non-tidal portion of the Susquehanna River [Zhang *et al.*, 2013], upstream

of the current study site. The relatively gradual depletion of P in colloids from Dec to Jun and to Aug may reflect the compositional differences of the sources of colloids in the upland and biogeochemical process that alters the P speciation in colloids as the temperature increases (by around 17°C) in the water column. The June and August sampling postdated the precipitation in the watershed. The high-intensity precipitation could mobilize colloids with bigger size range into runoff and increase their proportion in the total colloidal mass and bigger size fraction is low in P content compared to small size fraction [Sharpley *et al.*, 1998], which has a much higher specific surface area. Higher P concentration in small size fraction of colloids (50–100 and 100–450 nm) than that of larger fractions (≥ 450 nm) in the study sites (Figure 3.4) supports this trend.

3.6.2 Seasonal Variation of Colloids and P in the Deer Creek-Susquehanna River Transect

The concentration data along the transect clearly suggest that the contribution of colloids from site DR1 to site CB1.0-1 increases significantly from June to August and this means a lesser influence of colloid loads from other sources. Moreover, the flow flux rate ratio between Deer Creek (3.3–17.1 m³/s) and Susquehanna River (173 m³/s) was comparable on Aug 2017 sampling date (USGS, 2019). The sampling plan on CB1.0-1, SR1, and CB1.1 sites accounted for the flow rate to collect the same surface water transported to different sites. This approach help isolate other compounding variables but limit to the effect of ambient biogeochemical processes on the colloids transported downstream. This includes any interference in the upstream both natural and man-made (such as water release from Conowingo dam).

The C/N ratios in the colloids showed a positive correlation with $\delta^{13}\text{C}$ values (Figure 3.5d). The relationship of $\delta^{13}\text{C}$ values and C/N ratios are used as an effective marker to identify the relative proportions of terrigenous and aquatic sources of

organic carbon [Careddu *et al.*, 2015; Yu *et al.*, 2010]. This is because $\delta^{13}\text{C}$ values of C3 plants (≈ -25 to -28‰) is isotopically depleted compared to those of C4 plants (≈ -10 to -14‰) [Smith and Epstein, 1971]. As to plankton, $\delta^{13}\text{C}$ values are reported between -42 and -24‰ with average of -30‰ [Hamilton and Lewis, 1992]. Similarly, C/N ratios of autochthonous sources such as plankton are low (5–8) [Li *et al.*, 2016] compared to land-derived vascular plants (>15) [Meyers, 1994] and that of soil organic matter lie in the intermediate range (8–15) [Brady and Weil, 1990]. The heavier $\delta^{13}\text{C}$ values and higher C/N ratio of colloids in DR1 site may indicate relative higher abundances of land-derived sources of colloids such as from C4 plant debris compared to autochthonous sources. The lighter $\delta^{13}\text{C}$ values (-27.0‰ to -22.8‰) and the corresponding lower C/N ratios (7.8–7.8) of colloids at CB1.1 compared to upstream sites indicate relatively higher proportion from autochthonous sources. Along the water flow direction from Deer Creek and lower Susquehanna River to its mouth in the Chesapeake Bay, overall the linear trend between $\delta^{13}\text{C}$ values and C/N ratios suggest that the contribution of land-derived sources gradually diminish and is replete with autochthonous sources.

3.6.3 Seasonal Variation of Colloids and P in the Deer Creek-Susquehanna River Transect

Heavier phosphate oxygen isotope ratios of colloidal NaOH-P_i and $\text{HNO}_3\text{-P}_i$ pools than equilibrium line (for the day of sampling) and zone (within the month of sampling) indicate that these two P pools are not microbially cycled. It means their oxygen isotopic composition may be suitable for source tracking. However, the $\delta^{18}\text{O}_p$ values of $\text{NaHCO}_3\text{-P}_i$ pools at CB1.1 site are within the calculated equilibrium range. Intriguingly, the $\delta^{18}\text{O}_p$ values of $\text{NaHCO}_3\text{-P}_i$ pool at CB1.0 site were away from equilibrium value and the reason for this outlier is not clear.

For the same NaOH-P_i pool, $\delta^{18}\text{O}_\text{P}$ values were found to be enriched with the decrease in particle size (from 14.6 to 18.1‰) (Figure 3.6a). In contrast, depletion of isotope values was found with the decrease of size range in the HNO₃- P_i pools. While these data indicate that isotope values of different size ranges of specific P pools of the colloids can be distinct, the reason for this difference remains unknown. A likely possibility could be because the preferential loss and transport of finer-size of particles during erosion [Haygarth and Sharpley, 2000] causing a mixture of larger and smaller size particles with different mineralogy and potential land sources which could have originally; distinct isotopes. For example, both C and N isotopic values of ultrafiltrates (<1.2 nm), and colloids (1.2 nm to 400 nm) and colloids and larger suspended particles (> 400 nm) are reported to be varied by as high as 5.6‰ and 5.1‰, respectively, in the Potomac River in late spring [Sigleo and Macko, 2002]. The preferential loss of mineralogically distinct colloids during runoff is indirectly supported from the enrichment of P content in colloids in surface runoff versus soil by a factor of 1.2 to 2.5 [Sharpley, 1985]. SEM analyses of different size fractions revealed compositionally distinct biological and abiotic components. The higher P content in small size fraction of particulate matter (50–100 and 100–450 nm) than that in a larger size (≥ 450 nm) (Figure 3.4) is consistent with this notion. However, changes in phosphate oxygen isotope values of colloids with different size range may also be caused due to differential settling of particles due to decrease in flow rate when the river widens toward the Bay. A detailed study on how different mineralogy and other physico-chemical characteristics of colloids under a variety of size ranges and their relationship with isotope values can point towards different P source or processes.

3.6.4 Relative Contribution of Colloids from Deer Creek and Upstream Susquehanna River

The two-endmember linear mixing model can be used to calculate the individual contribution of colloids load from Deer Creek (DR1) and upstream Susquehanna River (CB1.0) to the lower Susquehanna River site (CB1.0-1) as follows:

$$M_{CB1.0-1} = f_{DR1}M_{CB1.0} + f_{CB1.0}M_{DR1} \quad (2)$$

where symbols M_{DR1} , $M_{CB1.0}$ and $M_{CB1.0-1}$ refer to the specific parameter such as physio-chemical or isotopic values at Deer Creek (DR1), upstream Susquehanna River (CB1.0), and lower Susquehanna River (CB1.0-1) sites, respectively. Similarly, f_{DR1} and $f_{CB1.0}$ (where $f_{DR1} + f_{CB1.0} = 1$) denote fractional contribution of DR1 and CB1.0 sites, respectively.

Table 3.2. Physicochemical properties of as recived water from differnty sites in the Deer Creek-Susquehanna River transect in Dec 2016.

Parameter	Jun 2017		Aug 2017	
	DR1	CB1.0	DR1	CB1.0
$\delta^{15}\text{N}$	—	—	—	—
$\delta^{13}\text{C}$	—	—	22.7	77.3
C/N	—	—	43.0	57.0
$\text{NO}_3\text{-N}$	9.1	90.9	6.8	93.2
TIC	25.5	74.5	6.1	93.9
TC	11.0	89.0	5.1	94.9
TOC	3.8	96.2	29.6	70.4
TN_b	6.8	93.8	48.7	51.3
$\text{NaHCO}_3\text{-P}_i$	—	—	45.3	54.7
NaOH-P_i	—	—	79.5	20.5
$\text{HNO}_3\text{-P}_i$	—	—	99.0	1.0
Discharge	1.6–1.9	98.1–98.4	2.8–9.8	90.2–97.2
Average	11.2 (± 8.4)	88.8 (± 8.5)	19.2 (± 19.4)	80.7 (± 19.4)

Colloids load at CB1.0-1 site contributed by DR1 and CB1.0 sites were calculated using the equation above is shown in Table 3.4. Based on the isotope parameters $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values the DR1 contribution ranges 22.7% and 0–100%, respectively. The relative contribution calculated based on other nutrient parameters were found to vary highly. For example, comparison of $\delta^{18}\text{O}_\text{P}$ values of NaOH- P_i pool among three sites DR1 (18.9‰), CB 1.0 (24.1‰), and CB1.0-1 (19.1‰) (Figure 3.5b) provides some insights into relative contribution. Using the mixing model, contribution of Deer Creek to colloidal P in CB1.0-1 site is ~80%. This result is supported by the high amount of colloids loading from DR1 (Figure 3.2) and is also consistent with the results from $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values and C/N ratios. The fact that NaOH- P_i pools remained largely recalcitrant against biological cycling in creek-river continuum provides information useful for source tracking of P.

Considering the average values, the DR1 site contributes 11.2 (± 8.4) % in Jun 2017 and 19.2 (± 19.4) % in Aug 2017. While this variability is significant, the general range is somehow expected in small tributaries like Deer Creek. A note of caution, however, is that the different parameters are not mutually related or are not necessarily conservative and could have pronounced non-linear relationship across the continuum causing higher variability in the contribution.

3.7 Conclusions and Environmental Implications

Concentrations of inorganic P (P_i) pools vary in the same order: $\text{NaOH-}P_i > \text{NaHCO}_3\text{-}P_i > \text{HNO}_3\text{-}P_i > \text{H}_2\text{O-}P_i$ in both seasons, showing that $\text{NaOH-}P_i$ is the most dominant P pool in colloids. Oxygen isotope ratios of $\text{NaOH-}P_i$ and $\text{HNO}_3\text{-}P_i$ pools of different size ranges of colloids are much heavier than the equilibrium value range in the water column, which suggests that $\text{NaOH-}P_i$ and $\text{HNO}_3\text{-}P_i$ pools are recalcitrant against biological uptake. Comparison of $\delta^{18}\text{O}_P$ values of $\text{NaOH-}P_i$ pool among three sites DR1 (18.9‰), CB 1.0 (24.1‰) and CB1.0-1 (19.1‰) and isotope mixing suggest the higher contribution of Deer Creek to colloidal P in CB1.0-1 site. Carbon (C) and nitrogen (N) isotope compositions of colloids showed that the contribution of terrestrial sources gradually decreased downstream of the river towards bay and Deer Creek contributed disproportionately high amounts of colloids to the Susquehanna River in Summer months.

Colloidal P load contributes a significant fraction to the overall P load to the Chesapeake Bay, which thus cannot be ignored in geochemical models and nutrient management plans particularly identifying sources that requires remediation. However, gaps in scientific knowledge to connect sources and sinks have limited our understandings of the origin, sources, and routes of P transformation in the environment. Those gaps could be better filled in from detailed studies on how colloidal P varies from hydrologic gradients and identify whether specific P pools in colloids are biologically cycled at the Deer-Creek-Susquehanna River transect. The multi-stable isotope proxies used in this study provided complementary information on the relative contribution of colloidal P loading and bioavailability from different sources in estuarine ecosystem. These results are expected to provide a foundation to further investigate P sources and biogeochemical processes in estuary environment such as in the Chesapeake Bay and beyond.

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Chapter 4

DEGRADATION OF GLYPHOSATE BY MN-OXIDE MAY BYPASS SARCOSINE AND FORM GLYCINE DIRECTLY AFTER THE C–N BOND CLEAVAGE

4.1 Introduction

Colloidal phosphorus (P) includes all solid P phases and is the major contributor (75–90%) of P transferred from terrestrial to aquatic ecosystems [Burwell *et al.*, 1977; Sharpley *et al.*, 1985; Sharpley *et al.*, 1998]. Colloidal P provides a stable P source since it includes readily bioavailable P pools [Moorlegghem *et al.*, 2013] that can release P for aquatic plant growth through certain physical and chemical processes and reactions. Therefore, understanding colloidal P variation at the ecosystem scale provides insight into the effects of colloidal P on water quality and paves the way to devise effective nutrient management plans.

As the most productive estuary in the U.S., the Chesapeake Bay suffers from numerous water quality issues due to surplus nutrients from multiple point and nonpoint sources of different scales in its watershed. The Bay receives an estimated 9.74×10^6 kg of P per year from its watershed, with 28% of this contributed by the Susquehanna River alone [Ator *et al.*, 2011]. Mixing and dispersion of N- and P-containing colloids and corresponding reactive processes that occur during different flow regimes have received increasing attention as this assists with river water management and risk assessment in hazardous waste storage [Meybeck, 1982; Howarth *et al.*, 1996; Vitousek *et al.*, 1997; Mayer *et al.*, 1998]. Solute dispersal and mixing are strongly affected by spatial variability in the flow field as a result of spatial heterogeneity of subsurface hydraulic properties [Pool and Dentz, 2018]. Flow

heterogeneity in creek-river-bay systems, and fluctuations due to weather may significantly influence flow and transport over time scales of various lengths. There is a paucity of chemical and hydrologic data in large rivers that explain temporal changes in colloidal P composition [Fitzsimons *et al.*, 2012]. Accurate characterization of colloidal and dissolved P with respect to current is essential to understanding flow and transport processes of P. A map of spatial and temporal colloidal and dissolved P concentrations provides useful insight into the role of heterogeneity on the dispersion of different P sources and their connectedness to one another through mixing. Moreover, this information helps link P biogeochemistry with aquatic ecology since variations in dissolved P dispersal affects aquatic primary productivity, impacting food webs with multiple trophic levels.

In this chapter, water sampling sites were selected along physicochemical and hydrodynamic gradients in Deer Creek, the lowermost Susquehanna River, and Chesapeake Bay. Large quantities of water were collected across this area to characterize spatial and temporal colloidal and total dissolved phosphorus (TDP) variation.

4.2 Research Objectives

The major objective of this section is to investigate the temporal and spatial variability of colloids and total dissolved phosphorus (TDP) concentrations in the estuarine gradient in the Deer Creek-Susquehanna River transect:

- How do organic and inorganic fractions of colloidal and total dissolved phosphorus vary with hydrological gradients in small and large estuaries?
- Can colloids and DIP hotspots be correlated? Do river geometry and hydrodynamics impact hotspots?

4.3 Materials and Methods

4.3.1 Study Area and Collection of Water Samples

Deer Creek, a small tributary to the Susquehanna River, and a stretch of the lower Susquehanna River downstream of the Conowingo Dam to its mouth in the Chesapeake Bay (Figure 3.1), was selected to represent a creek-river-bay continuum. Most land is used for agriculture, with occasional forest patches interspersed. Fifty-five sites chosen along the creek, river, and Bay provide reasonable spatial coverage (Figure 4.1). Water samples were collected in November 2017, April 2018, and July 2018, representing winter, spring, and summer. Random errors resulting from sampling potentially different water masses upstream and downstream were minimized through time lag methods. Sites near Deer Creek and Conowingo Dam were sampled separately by another group. Water current speed was accounted for before sampling where Deer Creek drains into Susquehanna River, ensuring collection from the same initial water mass. About 1 L of surface water was placed in carboys from each site and chilled on ice during transport. Water was stored at 4 °C until further processing. Water column depth for all sites was at least 110 cm, so the possibility of mobilizing colloids from the sediment-water interface was not expected.

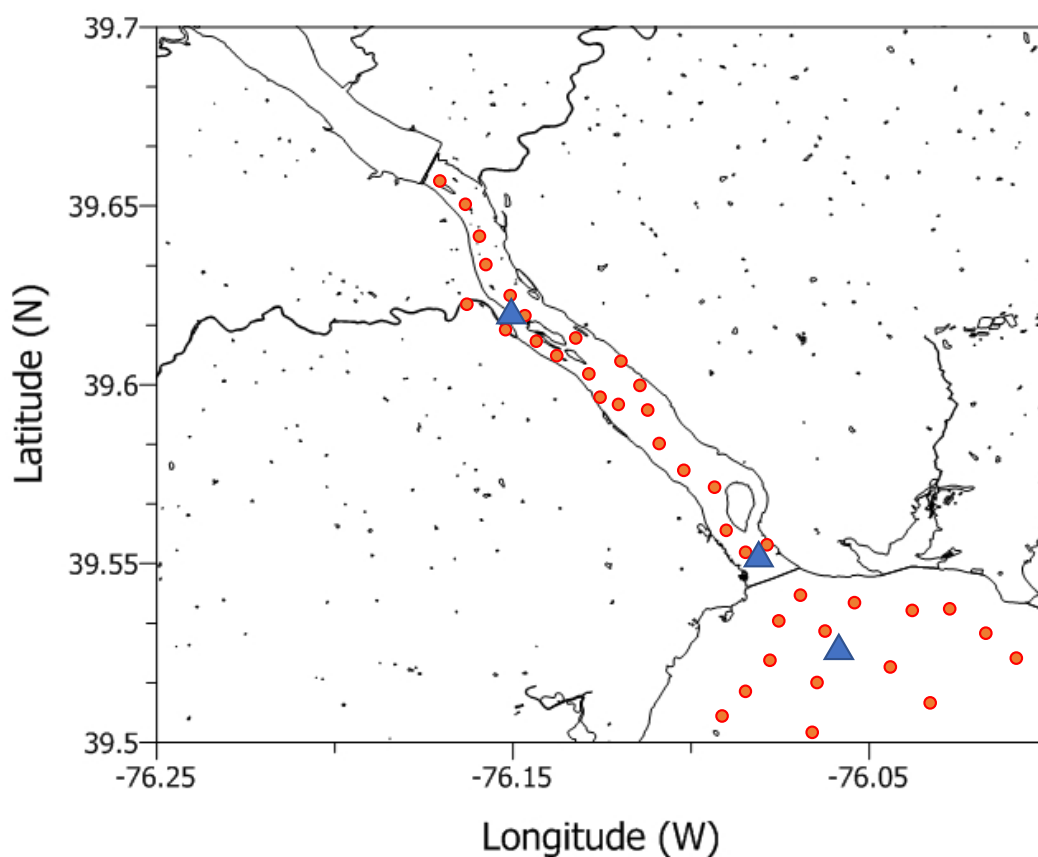


Figure 4.1. Site map showing representative sampling sites in creek-river-bay continuum: red circles indicate surface water sampling sites and blue triangles represent connections of creek-river-bay continuum.

4.3.2 Separation of Colloids

Colloids in the water samples were separated into only one size fraction (≥ 50 nm) using centrifugation (Sorvall LYNX 6000, Thermo Fisher Scientific) based on Stokes' Law of settling. Prior to separation, visible plant debris and algae filaments were removed using pipettes. The supernatant was decanted and pelleted colloids were

freeze-dried and weighed separately. Since the salinity of all sites was < 1.0 PSU, no washing or salinity correction was needed to obtain the true mass of colloids. The concentration of total P and selected elements (N, C, Fe, Mn, Ca, and Mg) in the water samples after separation of colloids was measured by inductively coupled plasma optical emission spectrometry (ICP-OES) (iCAP 7000, Thermo Fisher Scientific, USA).

4.4 Results and Discussion

4.4.1 Seasonal Variation of Colloids in the Deer Creek-Susquehanna River Transect

As shown in Figure 4.2, colloids had high spatial and temporal variability in the creek-river-bay continuum. In November 2017, the colloidal concentration distribution was relatively even from Conowingo Dam to Chesapeake Bay, except for a maximum concentration at the mouth of Deer Creek. Colloidal content near Deer Creek (Figure 4.2a) was around 30 mg/L, while colloidal concentration was around 20mg/L in the remainder of the estuary. Similar patterns were observed in April 2018 (Figure 4.2b, black oval). The drainage of Deer Creek had the highest colloidal concentration at 50 mg/L. In July 2018, the colloidal concentration at the mouth of Deer Creek was still high, with the addition of high colloidal concentration on the opposite bank. This consistency indicates a colloidal “hotspot” where Deer Creek drains into the Susquehanna River.

The colloidal concentration in winter (November 2017) was lower overall than during spring (April 2018) and summer (July 2018), consistent with the results in Chapter 3. Noteworthy was the lower colloidal concentration in water collected in July 2018 than that in April 2018, potentially a result of colloidal dilution from the precipitation encountered during sampling in July 2018. Total measured colloidal

concentrations were comparable to previous Deer Creek and Susquehanna River data collected in 2016 and 2017 (Chapter 3), East Creek [Mingus *et al.*, 2018] and the Piedmont watershed of Chesapeake Bay at the Pennsylvania-Maryland border north of Baltimore, Maryland [Jordan *et al.*, 1997]. The large increase in colloidal concentration during the spring is likely due to intense rainfall which increases soil saturation and soil erosion (as discussed in Chapter 3). This is supported by the amount of total precipitation at the study site from November (0.94 cm) to April (8.90 cm) to July (4.93 cm) (US CT, 2020).

Precise sampling following water flow may help isolate confounding variables but also limits the effect of ambient biogeochemical processes on transported colloids. This includes any interference upstream, both natural and artificial, such as water release from the Conowingo dam. Another colloidal “hotspot” in April 2018 located downstream of the mouth of Deer Creek, corresponding to CB1.0-1 in Chapter 3 (Figure 4.2b). Comparison of flow flux rates between Deer Creek and Susquehanna River is not enough to create a colloidal hotspot at the mouth of Deer Creek [USGS, 2019]. However, there was another colloidal “hotspot” in July 2018 (Figure 4.2b) located the other bank of Susquehanna River. The underlying reason was not clear.

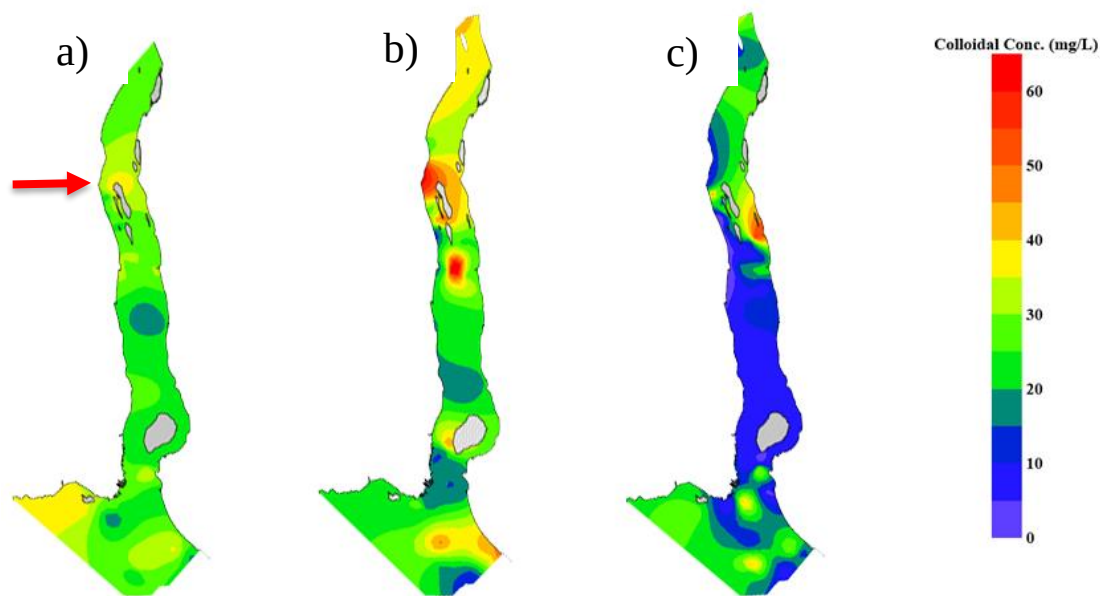


Figure 4.2. Spatial distribution of colloidal concentration in the continuum in Nov 2017 (a), Apr 2018 (b), and Jul 2018 (c). Please note that the red arrow indicates the confluence of Deer Creek with Susquehanna River.

4.4.2 Seasonal Variation of TDP in the Deer Creek-Susquehanna River Transect

As shown in Figure 4.3, high spatial and temporal variability of TDP was observed in the creek-river-bay continuum. In November 2017, the overall P concentration was relatively low and was evenly distributed from the Conowingo Dam to the Chesapeake Bay. As shown in Figure 4.3b, TDP concentration continued its roughly even distribution in April 2018 except where Deer Creek drains into Susquehanna River. This P “hotspot” is similarly located to the high colloidal P in April 2018. Near Deer Creek (black oval), P content was around 0.06 mg/L and colloidal concentration was around 0.02 mg/L.

The discrepancy between colloidal distribution and TDP indicates seasonal variation in the contribution of colloidal P to total P. In November 2017, TDP was

consistently low across the continuum, with a negligible contribution from Deer Creek, while high colloidal concentration was observed near the “hotspot.” In April 2018, TDP and colloidal concentrations were much higher than the ones in November 2017, with concurrent peaks at the mouth of Deer Creek. This indicates that the contribution of Deer Creek to colloidal P and TDP were high in April 2018, a potential result of higher seasonal variability in the creek than the river. Deer Creek itself had a higher seasonal variability of colloidal content than other sites, with the content in summer much higher than that in winter and spring. The Susquehanna River generally followed the winter-summer patterns but its internal variability was low. The contrasting variability in creeks and rivers is often attributed to differential discharge and total water surface area in the catchment [*de Klein and Koelmans*, 2011]. Mixing and dispersion of colloids and their corresponding reactive processes in outflow from Deer Creek could strongly affect colloidal P and TDP concentrations in the area near the drainage of Deer Creek.

Though colloids in winter had a relatively higher concentration of P_i in all pools than those in the summer, the total P load carried by colloids in the summer is still much higher because colloids in the summer have a larger mass. Similar trends to data collected in the summer in 2016 and 2017 was observed in July 2018. This is comparable to the Seine estuary in France [*Némery and Garnier*, 2007] and long-term data from a non-tidal portion of the Susquehanna River [*Zhang et al.*, 2013] upstream of this study site. One potential reason for the high TDP concentration in the water column is the mineralization of bioavailable colloidal P pools as temperature increases by around 10 °C in the summer, enhancing biological activity. The other potential reason is enhanced runoff from nonpoint sources, such as farms, due to variability in precipitation [*Sharpley et al.*, 1998; *Haygarth and Sharpley*, 2000]. This is supported by precipitation totals at the study site as discussed in section 4.4.1. Note that the ratio between colloidal P and TDP in this study may be different from the long-term monitoring database of the Chesapeake Bay Program. The filter size used to separate

colloids here is 50 nm, while the Chesapeake Bay Program used 0.70 μm [CBP, 2017]. Comparing TDP here with similar research should account for this factor.

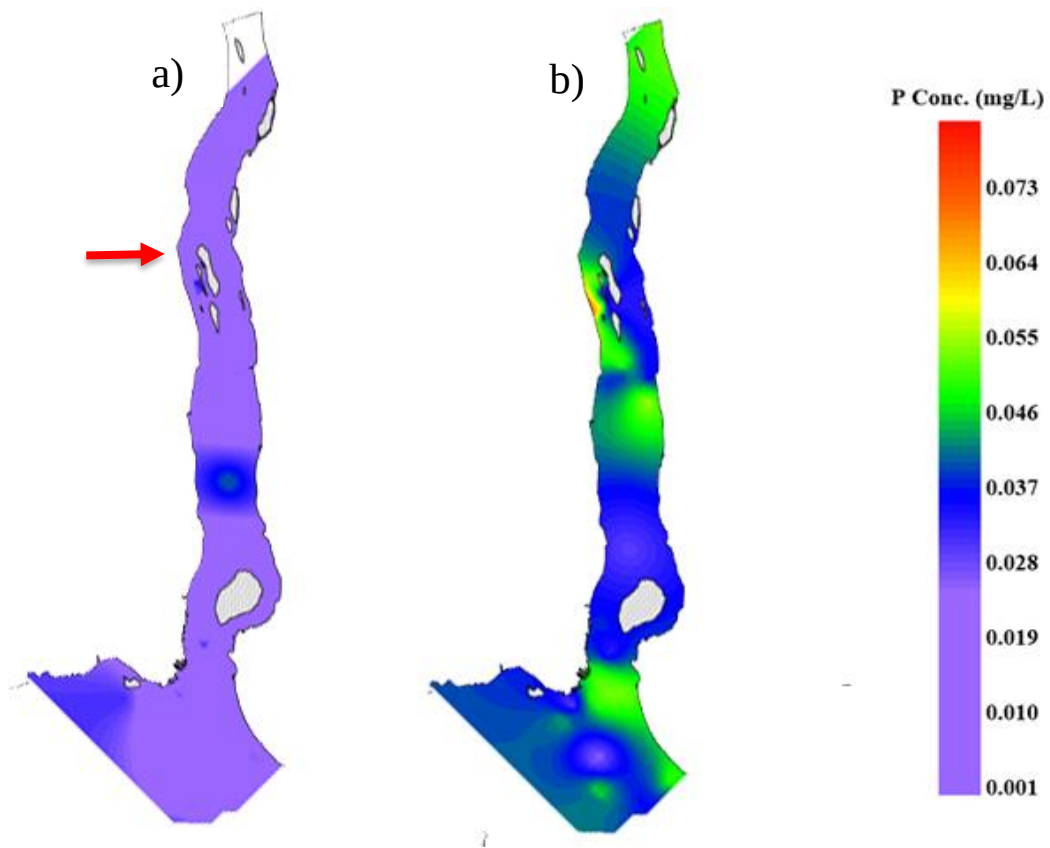


Figure 4.3. Spatial distribution of P concentration in the continuum in Nov 2017 (a) and Apr 2018 (b). Please note that red arrow indicates the confluence of Deer Creek with Susquehanna River.

4.5 Conclusions

The high-resolution Spatio-temporal analyses of colloids and TDP showed that the loading of colloids from Deer Creek to the Susquehanna River was consistently high in all three seasons. In general, a strong correlation of colloids and TDP was found along the transect. The mouth of Deer Creek was the prominent hotspot of colloids and TDP. This result is comparable with that from a few isolated sampling studies performed in the transect (chapter 3), which showed that the Deer Creek contributes a higher nutrient load per unit volume of water compared to that of upstream Susquehanna River. Other hotspots are likely to be caused by river geometry and hydrodynamics- details of which are unclear. Three to five folds changes in colloid and TDP concentrations in the transect is intriguing and highlights the need for careful scrutiny on spatiotemporal variability for both scientific investigation and regulatory measures.

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Chapter 5

ROLE OF SEDIMENT RESUSPENSION ON THE WATER COLUMN PHOSPHORUS DYNAMICS AT THE SUSQUEHANNA RIVER ESTUARY

5.1 Introduction

The sediments of rivers and lakes have increasingly become phosphorus (P) repositories. Under certain biogeochemical circumstances, sediment P can be recycled and diffused up to surface waters, potentially stimulating algal blooms. Due to differences in external P loading, biotic productivity, morphology of the water body, and the physical and chemical characteristics of the sedimentary layer, the contribution of sediment P flux from sediment into the water column to the overall total phosphorus load in lakes varies dramatically from 3% (3–35% for 22 lakes; *Nurnberg*, [1984]) to 80% [*Ryding and Forsberg*, 1977; *Larsen et al.*, 1981]. In some lakes, internal loading may exceed the input from external sources, which is more likely to occur in smaller lakes. Shallow lakes are more sensitive to localized weather, such as rainfall and wind, which affects wave speed and direction, and currents, all factors that could release more P [*Somlyódy*, 1983].

Waves (wind) and currents (tides or flow) create bottom shear stress and cause sediment resuspension. The relative contribution of waves and currents in producing shear stress depends on a variety of parameters such as water depth, physical properties of sediment (composition, diagenetic condition, and texture) and micro-topography. Wind greatly influences the resuspension of sediment in shallow water (< 2 m) such as in intertidal flats [*de Jonge and van Beusekom*, 1995] but its effect is limited in deep water and channels (>7 m). Waves, the product of wind, are more effective at resuspension in shallow water [*Green and Coco*, 2007]. As water depth decreases, such as in salt marshes (water depth around 1 m), current-driven shear

stress overwhelms sediment suspension. In contrast, during storms waves contribute most of the combined shear stress (up to 81%) with suspended sediment concentration as much as three times greater than in base flow conditions. Once critical flow conditions within the water column exceeds bottom shear stress, as induced by waves and currents [Dyer 1986; Sanford *et al.*, 1991], sediments are eroded and remain in suspension. This process causes P sequestered in bottom sediments to be released into the water column.

Resuspended materials can be operationally defined based on their size as dissolved phase (< 1 nm), colloids (1 nm – 1 μ m) and suspended particulate matter (SPM; > 1 μ m) [Lead and Wilkinson, 2007]. Unlike the dissolved phase, neither the colloids nor SPM are in a thermodynamically distinct stage [Fitzsimons *et al.*, 2012]. The distinction between colloids and SPM is always operational in nature [Bauer and Blodau, 2009]. More than 90% of P transported by rivers to estuaries and coastal waters is in colloidal and SPM phases [Föllmi 1996]. SPM can be divided into two groups based on settling velocity and transport distance: i) resuspended particulate matter (RSPM, > 40 μ m), which settles down easily and relatively quickly and ii) permanently suspended particulate matter (PSPM; < 40 μ m), which remains in the water column because of its small particle size, allowing it to be transported a greater distance [Fitzsimons *et al.*, 2012]. Colloidal P can be considered PSPM based on this definition. Studies on the gravitational settling of estuarine suspension from the Ogeechee Estuary have shown that generally PSPM has significantly higher proportions of particulate organic carbon (POC) and chlorophyll *a* and phaeopigments as well as enhanced bacterial hydrolytic activity [Alber, 2000]. Similar settling experiments performed using sediments from the Humber Estuary showed that the amount of PSPM increased as the concentrations of SPM increased [Williams and Millward, 1999; Fitzsimons *et al.*, 2012]. Due to differences in the hydrodynamic properties of RSPM and PSPM, they may originate from different sources of P and differ in their fates in natural water bodies. Information on potential variation of P

sources in RSPM and PSPM therefore provide insight into P source contribution, dynamics, and stability during transport.

Because of fundamental processes at the sediment-water interface such as adsorption, desorption, ion exchange, and dissolution, sediment-derived P can be a considerable nutrient source for aquatic plant growth because it contains a readily bioavailable P pool [Van Moorlehem *et al.*, 2013; Carignan and Kalif, 1980; Sharpley *et al.*, 1991]. The extent of P release due to sediment resuspension is affected by both the sediment composition and its diagenetic conditions. There are two major processes, remineralization of organic matter and remobilization of P in associated sediments, that generate dissolved P (DIP) in the water column [Joshi *et al.*, 2016]. Since DIP is the most bioavailable P form to phytoplankton and all other organisms, it exerts a relatively higher influence on the productivity and health of water bodies. Therefore, it is a matter of great importance to determine the contribution that sediment resuspension makes to DIP in riverine systems. Proper quantification of DIP input from sediment resuspension, including vertical and horizontal heterogeneity, provides useful information that could be used to estimate spatial and temporal variation of DIP in the water column.

Both resuspension and transport of SPM are driven largely by hydrodynamics at the sediment-water interface. This controls particle exchange between the sediment bed and water column [El Ganaoui *et al.*, 2004]. Controlled studies aimed at investigating the erodibility of cohesive sediments have used erosion chambers (primarily the Gust chamber test) to measure the magnitude and nature of suspension under specified bottom stress gradients [Kleeberg *et al.*, 2007]. Pairing sediment resuspension studies with isotope tracers and elemental modeling could provide information useful in identifying sources and biogeochemical cycling pathways. For example, carbon (C) and nitrogen (N) isotopes have been used to trace the source of particulate organic matter (POM) in creek, river, and coastal water bodies [Kendall *et al.*, 2001]. A combination of multiple stable isotopes may provide additional insights into C, N, and P cycling within aquatic ecosystems. This method has been applied in

preliminary work combining N and phosphate oxygen isotopes ($\delta^{18}\text{O}_\text{p}$), which has revealed nutrient coupling in eutrophication [Goody *et al.*, 2016].

The total P in SPM is composed of resuspended sediment and terrestrial-derived matter carried by river currents. This chapter employed results from field measurements and controlled flux experiments using intact sediment cores collected along a transect from Deer Creek to the Susquehanna River. A combination of sediment geochemistry, erosion experimental results, in-situ hydrodynamics and multi-stable isotope proxies provides a more complete understanding of sources and pathways of P fluxes in the water column.

5.2 Research Objectives

The major objective of this section is to identify the role of sediment resuspension in P dynamics in the water column at the Susquehanna River estuary under environmentally relevant conditions:

- Can the current-derived sources of the suspended particulate matter be differentiated from remobilized sediment in the water column?
- What are the relative contributions of permanently suspended particulate matter (PSPM) and resuspended particulate matter (RSPM) sources of P under different shear stress conditions?
- Can the results from controlled laboratory flux experiments and field water column be compared to distinguish the loading vs internal cycling?

5.3 Methods

5.3.1 Study area and choices of sampling sites

The study area is located along the lower Susquehanna River downstream of the Conowingo Dam to its mouth in Chesapeake Bay and includes Deer Creek which drains into the Susquehanna (Figure 5.1). The creek-river-bay continuum provides a span of hydrological gradients and bottom shear stress conditions. The mean salinity, measured from 1985 to 2018 at site CB1.1 (Figure 3.1, located at the mouth of Susquehanna River), is virtually zero. The concentration of dissolved oxygen (DO) in the bottom water decreases to about 50% saturation in the summer (June to August) compared to other seasons. The proximity of the lower Susquehanna to the seasonally anoxic Bay results in the largest variation of DO here of the sampled sites. The depth of water column in Deer Creek (SS1, the lower part of Susquehanna River (SS2), and mouth of Susquehanna River (SS3) is 1 m, 4 m and 1.5 m, respectively.

Sampling sites along the continuum was determined based on nutrient concentration, dominant components of bottom shear stress, and water depth. Deer Creek has a relatively high concentration of nutrients in both water and suspended particulate matter compared to that of Susquehanna River. SS1 is located where Deer Creek feeds into the Susquehanna River. SS2 is at the inner mouth of Susquehanna River where water downstream of the Conowingo Dam mixes with tidal water. Site SS3 at the mouth of Susquehanna River is shallow with distinctly dominant bottom shear stress (Figure 5.1). Sediment cores and water samples were taken during the summer (June to September) because low dissolved oxygen in bottom water, high biological activity, and potentially extreme weather conditions may capture one end member of bottom shear stress. While tide-driven currents are common, higher river flow velocity at the sediment interface is expected to contribute more to shear stress. Water normally flows through the spillway at Conowingo Dam at flow flux rate of $1900 \text{ m}^3/\text{s}$ (Figure 5.1), impacting flow dynamics and bottom shear stress. To minimize these effects, sampling was done during periods of minimal water release

from the Conowingo Dam. To account for tidal effects in flux experiments, samples were collected during low tide under baseflow conditions.

5.3.2 Collection of Sediment Cores and Waters

All sediment cores were collected using a sediment corer designed for retaining the sediment-water interface in shallow water bodies [Upreti *et al.*, 2015]. Precautions were taken to ensure the integrity of the sediment surface during collection. Sediment cores that were disturbed or not representative were discarded. Paired water column samples were collected simultaneously at three different depths using a peristaltic pump (Geotech Geopump) proximal to sediment core sampling sites but before the coring to avoid resuspension. Large quantities of water (24 L to 40 L) were taken at each depth, ranging from the bottom to surface. More detailed sampling at shorter depth intervals using 1 L of water covered the heterogeneity of water column. Water was pumped in carboys and chilled on ice until storage at 4 °C before further chemical processing. Ancillary parameters including water column temperature were measured on site. The concentrations of total P and selected elements (N, C, Fe, Mn, Ca, and Mg) in the water samples were measured by inductively coupled plasma optical emission spectrometer (ICP-OES) (iCAP 7000, Thermo Scientific, MA, USA). Turbidity of all water samples were measured within two days of sampling.

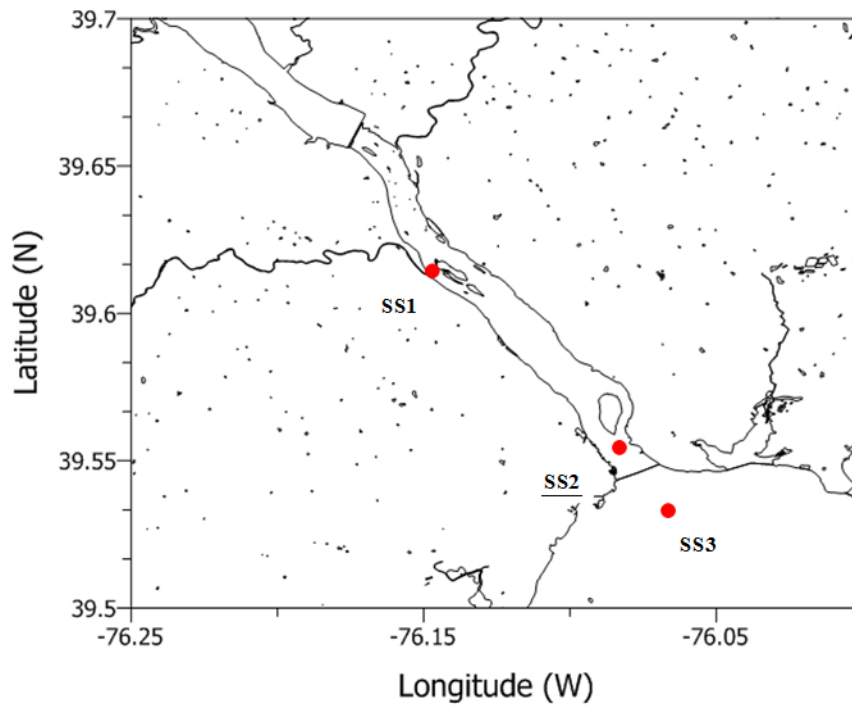


Figure 5.1. Location of water and sediment core sampling sites in the creek-river-bay continuum.

5.3.3 Set Up of Sediment Erosion and Resuspension

The U-GEMS device (analogous to the Gust chamber) is designed to test the erodibility of undisturbed sediment cores under variable shear stresses and to mimic field conditions. This device generates a nearly uniform shear stress distribution across the entire sediment surface through the combination of a spinning disk and a central

suction. The U-GEMS device eliminates the artefacts that are generated by vane-type rotor resuspension devices. The U-GEMS system is designed for 10 cm diameter cores and includes a rotating head, turbidimeter, water handling system, and computer software that controls the rotation rate and pump speed (Figure 5.2).

River sediments are highly heterogeneous and variability among cores taken from a single site is expected. To avoid the impact of such heterogeneity, two cores were used for erosion experiments and compared to an undisturbed control core. The control core along with the two processed cores were sliced (0-0.5 cm, 0.5-1 cm and 1-2 cm) and analyzed for basic physio-chemical properties. To minimize excess shear stress in the center of sediment cores undergoing erosion experiments, a circular plate was placed in their center as suggested by the manufacturer before the experiment.

Calibration of shear stress in the U-GEMS device were conducted using published methods [Gust, 1990; Tengberg *et al.*, 2004]. The relationship between the revolution speed of the stirring disc and shear velocity at a constant flow rate was used to calibrate the system. This ensures the peristaltic pump can mimic hydrodynamic conditions in the field. In typical erosion experiments, suspended colloids in the overlying sediment cores were removed by flushing, then shear stress was applied in consecutive stages: 0.01, 0.05, 0.1, 0.2, 0.25, 0.30, 0.35, 0.40, 0.45 and 0.50 Pa. Each shear stress level was held constant for 20 minutes, the amount of time for erodible colloids to be flushed from the system according to constant turbidity values [Kleeberg *et al.*, 2007; Kleeberg *et al.*, 2008].

Effluent water from the experiment was collected at different times. During each erosion experiment, motor speed output and turbidimeter data were recorded and used in data analysis. For each water sample used in an erosion experiment, SPM (≥ 50 nm) was removed through centrifugation (Sorvall LYNX 6000, Thermo). This minimized the contribution of particles from the flow-through water in the SPM collected at the outflow [Porter *et al.*, 2013; Seelen *et al.*, 2018]. Water pumped out of the chamber along with eroded SPM passed through the turbidimeter before

collection. The SPM in outflow water was separated through centrifugation and prepared for further analysis.

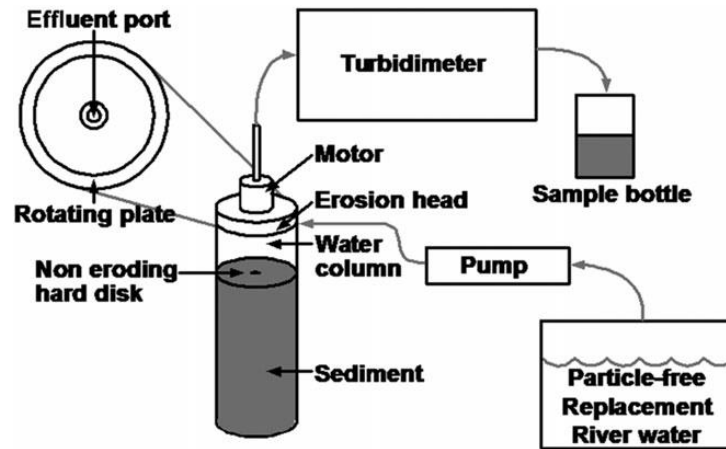


Figure 5.2. Principle of the U-GEMS system with core setup including duel pump and turbidity meter [Wiberg et al., 2013; Legout et al., 2018].

5.3.4 Separation of RSPM and PSPM and Extraction of P Pools

SPM was separated from water samples collected at three different depths in the field and outflow water from the erosion experiments into two size ranges ($0.05\text{--}40\text{ }\mu\text{m}$ and $\geq 40\text{ }\mu\text{m}$) via centrifugation using Stokes' law of settling. A split of water was saved for as-received characterization. Visible plant debris and algae were removed carefully with pipettes before size separation. The separated colloids from each site were combined and twice washed with DI water to remove salts and to calculate an accurate mass. Colloids were separated from septic tank samples with similar methods. All colloids were freeze-dried and stored until laboratory characterization.

The different P pools in the sliced cores from bulk sediment and SPM of the water column and outflow from experiments were sequentially extracted following the modified Hedley extraction to differentiate and quantify pools ($\text{H}_2\text{O-P}_i$, $\text{NaHCO}_3\text{-P}_i$,

NaOH-P_i, and HNO₃-P_i). To recover extracted P adsorbed onto the residual solid phase, an additional 0.5 mol L⁻¹ NaHCO₃ extraction and H₂O rinse were added after the NaOH and HNO₃ extractions. The concentration of P_i in each pool was measured using the phosphomolybdate blue method [Murphy and Riley, 1962]. Each P pool (organic and inorganic) and elemental composition (N, C, Fe, Mn, Ca, and Mg) in each extract were individually quantified before processing and selected P pools were purified through organic matter removal for isotope analysis. A fraction of residual SPM and sediment after sequential extraction was used to quantify the amount of P in each P pool and for P mass balance calculation.

5.3.5 Measurement of C and N Isotopes

Approximately 10 mg of freeze-dried SPM collected from river water, erosion experiments and bulk sediment was used to measure carbon isotope ratios ($\delta^{13}\text{C}$) and nitrogen isotope ratios ($\delta^{15}\text{N}$) in an Elemental Analyzer (EA) (Costech, CA, USA) connected to a Delta V IRMS (Thermo, Germany). All $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were calibrated against two USGS isotope standards: USGS40 ($\delta^{13}\text{C} = -26.39\text{‰}$ and $\delta^{15}\text{N} = -4.52\text{‰}$) and USGS41 ($\delta^{13}\text{C} = +37.63\text{‰}$ and $\delta^{15}\text{N} = +47.57\text{‰}$). For data uniformity and comparison with literature values, all measured N and C isotope values were reported in standard delta notation in per mil (‰) with respect to atmospheric air and Pee Dee Belemnite (VPDB), respectively.

5.3.6 Purification of P and Measurement of Phosphate Oxygen Isotopes

Dissolved P in the water column and different P pools extracted from SPM and sediment cores were processed to remove contaminants and were precipitated as silver phosphate. Protocols for sample processing and silver phosphate precipitation for all P pools and isotope measurements were the same as described in Chapter 3. Before the separation of SPM, an aliquot of water samples was taken out and $\delta^{18}\text{O}_w$ was measured.

5.4 Results and Discussion

5.4.1 Wind and current condition

Under the effect of waves (without current), wave-induced bed shear stress τ_w is typically expressed as follows [Nielsen, 1992; Soulsby, 1997]:

$$\tau_w = 0.5\rho f_w U_{w,b}^2 \quad (1)$$

$$f_w = \exp [5.213 (k_b/A_b)^{0.194} - 5.977] \quad (2)$$

$$A_b = U_{w,b} T/2\pi \quad (3)$$

where ρ is water density, f_w is the wave friction factor, $U_{w,b}$ is the maximum horizontal over-the-wave-cycle wave-orbital speed at the bed, k_b is bed roughness, T is the wave period, and A_b is the wave-orbital semi excursion at the bed. In most cases, k_b may be estimated as $2.5D_{50}/30$, where D_{50} is the median grain size of the bed sediment [Smith and McLean, 1977]. Water depth is a key factor on wave-induced shear stress [Green and Coco, 2013]. Bed shear stress τ_w decreases significantly with increasing water depth and increases exponentially with wind speed. When the major driving force is current instead of wave action, bed shear stress τ_w can be estimated by calculating the natural mean flow velocity (U_{mean}) at 1–2 m above bottom, which scales with shear velocity (U^*) in a ratio of about 19:1 [Porter et al., 2004; Kleeberg et al., 2007].

Since it is shallow in the study site (1–2 m depth), shear stress is affected by a combination of waves and currents. Based on historical weather and water quality data

collected from a buoy located on Susquehanna River's inner mouth (39.5403° N, 76.0734° W) (Table 1), the median wind speed is 3.3 m/s with 75th percentile of wind speed is 5 m/s. Shear stress derived from wave action at the mouth of Susquehanna River is around 0.1-0.15 Pa due to the shallow (~1 m) water depth (based on V_{75}). Similarly, based on the 75th percentile of current speed (Table 2), the estimated shear stress at the sediment-water interface in the lower Susquehanna River (water depth of ~1 m) is around 0.025-0.10 Pa. The wave component of shear stress is prevalent at this site except during extreme conditions such as storm events. The estimated bottom shear stress is around 0.25 Pa during normal calm weather with maximum bed shear stress of 0.50 Pa during storm conditions, such as Hurricane Floyd in 1999.

Table 5.1. Annual distribution of wind speeds at the mouth of Susquehanna River.

	V_{50} (m/s)	V_{75} (m/s)	V_{max} (m/s)	Data points
2015	3.6	5.0	32.0	30306
2016	3.3	5.0	15.7	34100
2017	3.3	5.0	18.5	32301

V_{50} indicates median value of wind speed, V_{75} means 75th percentile of all wind speed data, and V_{max} is the maximum wind speed in a specific year [NOAA, 2019].

Table 5.2. Annual distribution of currents speed at the mouth of Susquehanna River.

	V_{50} (mm/s)	V_{75} (mm/s)	V_{max} (mm/s)	Data points
2015	76.6	163.7	377.0	5624
2016	61.2	94.6	266.6	6290
2017	88.8	193.6	415.0	5948

To compare with data from Harris et al. [2010], Root-mean-squared (RMS) values of current speed was calculated. V_{50} indicates median value of current speed, V_{75} means 75th percentile of all current speed data, and V_{max} is the maximum value of current speed in a specific year [NOAA, 2019].

Table 5.3. Annual distribution of tide height in the mouth of Susquehanna River.

	H ₅₀ (cm)	H ₇₅ (cm)	H _{max} (cm)	Data points
2017/Low tide	6	11	19	1410
2017/High tide	66	73	94	1410
2018/Low tide	7	10	21	1408
2018/High tide	66	74	93	1408

H₅₀ indicates median value of tide height, H₇₅ means 75th percentile of all tide height data, and H_{max} is the maximum value of tide height in a specific year [NOAA, 2020].

Table 5.3. Annual distribution of tide height in the mouth of Susquehanna River.

5.4.2 Turbidity and Particulate Concentration

The water column of the Chesapeake Bay exhibits strong spatial variability in turbidity. At the northernmost site (SS1; 1.5m total depth), water column turbidity was less than 1 NTU and remained relatively constant throughout the entire vertical water profile (Figure 5.3 A). There was one outlier at the surface that was excluded due to its high variability. This was confirmed by the vertical distribution of particulate matter at SS1 (Figure 5.4 A), whose concentration increased with water depth. At the middle and south sites (SS2 and SS3), turbidity remained relatively constant and then increased exponentially with depth (Figure 5.3 B; Figure 5.3 C). At SS2, turbidity increased 100-fold compared to the surface, and at SS3 it increased by 40 times. This indicated stratification within the water column. Particulate matter content was highly variable among all three sites. Its content in bottom water was much higher than that in surface water (Figure 5.2 A). As shown in Figure 5.4, particulate matter concentration increased with depth in all three sites to varying degrees. Particulate concentration increased from 2.4 mg/L to 21.2 mg/L at the site SS2, while particulate concentration increased from 6.1 mg/L to 28.3 mg/L at the site

SS3, which were comparable to ones in Li et al. [2019]. The contribution of RSPM ($\geq 40 \mu m$) to total particulate matter increased with depth in all three sites, while the contribution of PSPM decreased. The proportion of PSPM (ca. 60-80%) generally prevailed over that of RSPM (ca. 20-40%) along the depth profile. For example, the contribution from RPSM at SS3 notably decreased from surface (78%), middle (77%) to bottom (57%).

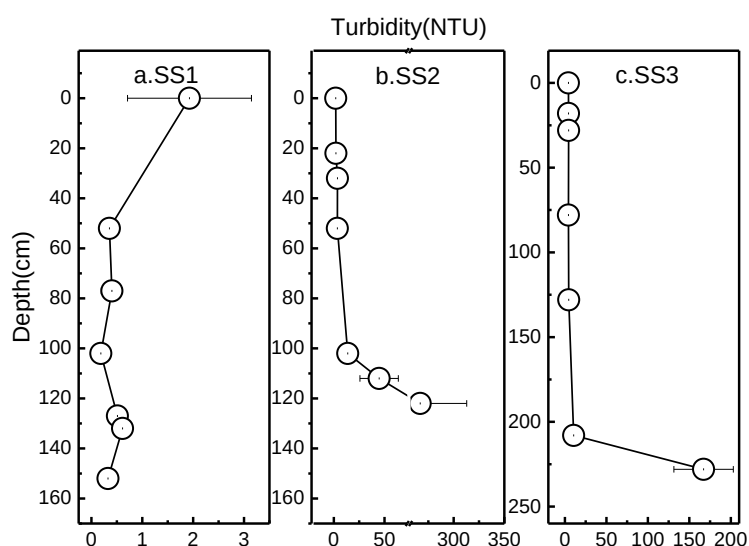


Figure 5.3. Vertical distribution of turbidity in the water column in three sites along the flow direction of the Susquehanna River: (A) SS1; (B) SS2; (C) SS3.

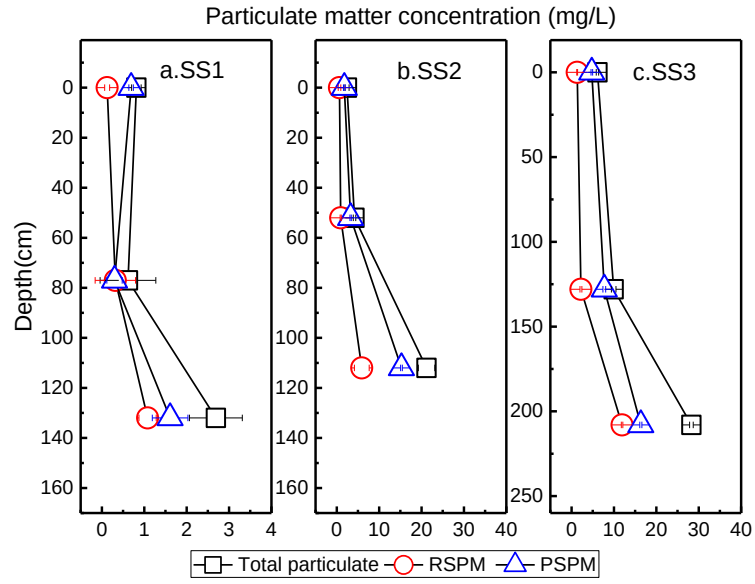


Figure 5.4. Vertical distribution in the water column of particulate matter concentrations (mg/L) from different size fractions (Total concentration; RSPM; PSPM) in different sampling sites: (A) SS1; (B) SS2; (C) SS3.

5.4.3 Sediment Erodibility

The suitability of microcosms to study river sediment erosion, resuspension, and deposition has been extensively discussed [Kleeberg *et al.*, 2007; Xu *et al.*, 2014; Legout *et al.*, 2018]. In this study, suspended colloids overlying the sediment cores were removed by flushing. Shear stresses were then applied in two consecutive ranges: i) 0.01, 0.05, 0.1, 0.2 and 0.25 Pa, which corresponded to calm weather under typical tidal cycles (75% yearly frequency) and ii) 0.30, 0.35, 0.40, 0.45 and 0.50 Pa, which corresponded to pure storm conditions.

Erosion experiments showed sediment heterogeneity, even from the same site (Figure 5.5). At SS3, enhanced particle removal was initiated at a shear stress of 0.05 Pa, peaked at 0.20 Pa, and decreased at 0.25 Pa, appropriate for normal weather conditions, before increasing again. Erodibility changed after 0.25 Pa, indicating another erosion cycle under high shear stress conditions after initial erosion under

normal conditions. Two duplicate cores from SS2 were eroded and displayed similar shear stress behavior, although a discrepancy arose at 0.45 Pa in one of the cores.

A typical river bed can be described as an aggregate of sediment in a two-layered bed model [Krone, 1962]): 1) a surficial fine-grained layer that easily gets resuspended [Droppo and Stone, 1994] and 2) an underlying consolidated layer [Thomsen and Gust, 2000]. Both the SS2 and SS3 cores confirmed the existence of two such layers at the sediment surface which were eroded with low (0.25 Pa) and high shear stress (0.50 Pa). This was further confirmed by the increasing relative contribution of large particulate matter with increasing shear stress in SS2 (except SS2#2 at 0.45 Pa) (Figure 5.5).

The final eroded mass of particulate matter undergoing increasing shear stress was around 10-20 g/m², which was slightly less than experimentally eroded sediment from the northern Gulf of Mexico on the Louisiana shelf [Mickey *et al.*, 2015] and relatively low compared to eroded material from a cobble river bed infilled with cohesive sediment [Legout *et al.*, 2018]. Sediment from the Susquehanna river may have been consolidated by removal of particulate matter before the erosion experiment. This procedural removal was believed to be very critical because of the potential for the high concentration of particulate matter at the sediment-water interface to interfere with experimental results (as shown in Figure 5.4). Increasing shear stress affected the relative contribution of larger size fractions of particulate matter between SS2 and SS3. For example, eroded particulate ($\geq 40 \mu\text{m}$) contributed around 60-80% to the total eroded mass in SS3, while eroded particulate contributed around 30-40% to the total eroded mass in SS2 in both duplicated cores. This indicated a compositional difference in the sediment of SS2 and SS3. As described by Law *et al.* [2008], sediment grain size strongly dictates surface sediment behavior when shear stress is applied since erosion preferentially removes finer-sized particles, which are relatively enriched in P, than coarser-sized particles. This process may produce a disproportionate mineral composition (sand/silt/clay) in the eroded particles compared to the parent sediment.

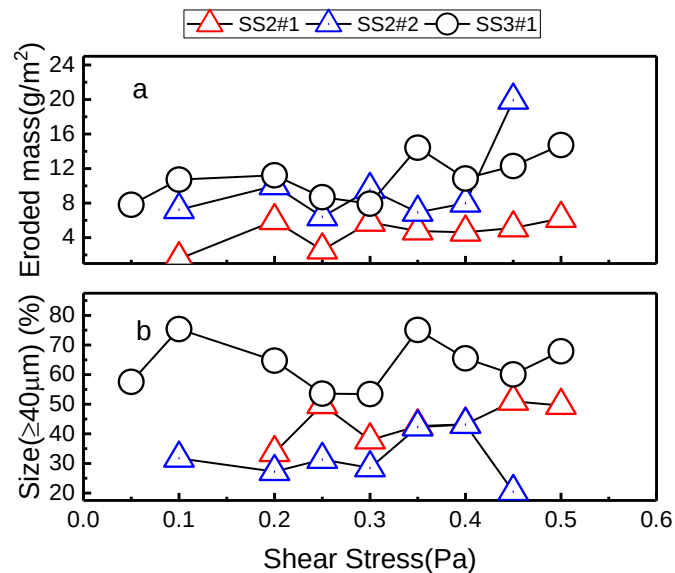


Figure 5.5. Resuspension results generated using the UGEMS for sediment cores from SS2 and SS3: (A) eroded mass of particulate at each shear stress interval for all cores; (B) distribution of eroded particulate ($\geq 40 \mu\text{m}$) over shear stress gradients. SS2#1 and SS2#2 are duplicates.

5.4.4 Variation of P in particulates in water, eroded sediment, and bulk sediment at site SS2 and SS3

Concentrations of P_i in different particulate P pools from various depths and sampling sites are shown in Figure 5.6 and Figure 5.7. Bulk sediment had elevated P concentrations upstream (SS2) relative to downstream (SS3). There was no discernable difference in sediment P concentrations before and after erosion experiments. The bulk sediment (depth 0–0.5 cm) of SS2 and SS3 had larger NaOH- P_i and HNO_3 - P_i pools compared to the other two P pools (Figure 5.6; Figure 5.7). In general, the order of the concentration of P_i in different P pools of bulk sediment was $\text{NaOH-}P_i > \text{HNO}_3\text{-}P_i > \text{NaHCO}_3\text{-}P_i > \text{H}_2\text{O-}P_i$. The highest P_i concentration was found

in the NaOH pool, which corresponded to P tightly bound to Fe and Al compounds [Hedley *et al.*, [1982], indicating that this pool was the major P sink for colloids. This was analogous to data from the Patuxent, MD estuary [Jordan *et al.*, 2008]. For most sites, NaOH-P_i contributed around 50% to total colloidal P, which was slightly less than the sites in Jordan *et al.* [2008]. This could be a result of differences in salinity and separation methods, as visible plant debris and algae were removed before centrifugation in this study.

The P pools of RSPM of a larger size fraction ($\geq 40 \mu\text{m}$) had less variability and higher P concentrations with depth compared to those of PSPM, similar Li *et al.* [2019]. For example, the concentration of P_i from different PSPM P pools decreased with increasing water depth, while RSPM remained relatively constant with depth. P transport mechanisms explain this discrepancy as higher P is found in finer-sized soil particles, which are preferentially removed over coarser-sized particles [Haygarth and Sharpley, 2000]. The P content and reactivity of particulate collected from eroded cores was usually greater than bulk source material, as confirmed by larger P concentrations in suspended matter after erosion and was particularly apparent in smaller soil size fractions. H₂O-P_i and NaHCO₃-P_i of RSPM and PSPM were greatly enriched at the surface relative to the bottom, with these two pools gradually depleting as depth increased. This resulted in similar P pool composition of RSPM and PSPM proximal to the sediment surface in both SS2 and SS3 (Figure 5.6; Figure 5.7). The PSPM in water at the surface had a much higher P concentration than in the sediment, while RSPM from bottom water had similar P concentrations to those in the sediments. Interestingly, the concentration of P in each pool in PSPM decreased with depth. The major P pools switched from H₂O-P_i at the surface to NaOH-P_i at the bottom at sites SS2 and SS3. All of these observations suggested a close association between bottom water particulate and the bulk sediment composition, as well as the disconnect between surface water particulate and bulk sediment. This disconnect reflected compositional differences in material suspended in the upper water column compared to what was eventually buried in the sediment. This suggested that influx

from additional P sources may contribute to particulate matter composition besides solely sediment resuspension and location within the water column.

The concentration of P_i of eroded sediment decreased with increasing particulate size. Particulate collected from the eroded cores at both sites from the smallest size fraction (0.05–40 μm) compared to the larger size fraction ($\geq 40\mu\text{m}$) had the highest P_i concentration in all pools. The P concentration of each P pool in the particulate from eroded sediment at SS2 and SS3 were significantly less than the suspended particulate ($p < 0.01$) but similar to the bulk homogenized sediment fraction. Moreover, the P concentrations of eroded sediment from SS2 and SS3 were much less than that of the water column (PSPM) but similar to RSPM. For eroded sediment and bulk sediment in all study sites, the general order of concentration of P_i was $\text{NaOH-}P_i > \text{NaHCO}_3\text{-}P_i > \text{HNO}_3\text{-}P_i > \text{H}_2\text{O-}P_i$, with $\text{NaOH-}P_i$ and $\text{NaHCO}_3\text{-}P_i$ as the major P pools. The concentration of P_i of PSPM and RSPM in decreasing order was $\text{H}_2\text{O-}P_i > \text{NaHCO}_3\text{-}P_i > \text{NaOH-}P_i > \text{HNO}_3\text{-}P_i$. This indicated that eroded sediment could be a major source of RSPM in the water column. While the SS2 eroded sediment was similar to bulk sediment not only in total P but also in the concentration of individual P pools, in SS3 there was slightly less total P and P in each pool after erosion of bulk sediment. This further indicated a compositional difference in the sediment between SS2 and SS3. As described by *Law et al.* [2008], sediment mineralogy strongly dictates the behavior of sediment at the surface with suspended sediment. The mineralogy of the bulk sediment here was not determined, so there was not enough information to calculate size-specific sediment mobility. Site SS3 with its much higher contribution from larger particulate matter in eroded sediment and much lower P concentration in bulk sediment attested to this. Since nutrients like P generally partition into smaller size fractions, these dynamics are an important aspect of P cycling in estuarine systems [*Li et al.*, 2019]. Additionally, flocculated material resting at the sediment surface could drive the particulate composition, especially at low shear stresses, and this flocculated fraction is important for P transport and bioavailability [*Kach et al.*, 2008].

Summed over the low shear stress conditions (0.01–0.25 Pa) and storm conditions (0.01–0.50 Pa), which encompass normal conditions (0.01–0.25 Pa), and pure storm conditions (0.25–0.50 Pa), erosion incrementally occurred with shear stress. The sediment eroded at high shear stress was slightly depleted per gram in P compared to those at lower shear stresses. The smaller size fraction of eroded material was not similar to the bulk sediment P, indicating that easily eroded material is dissimilar to the bulk sediment. This further confirmed the initial two-layer sediment model initially proposed by *Krone* [1962]. Eroded sediment P composition shadowed that of the bulk sediment whereas the suspended matter from the surface and middle depths had much larger $\text{H}_2\text{O-P}_i$ and $\text{NaHCO}_3\text{-P}_i$ pools. Again, there is little evidence that P in the water column was derived entirely from sediment production. This distinct discrepancy indicated variable sourcing of particulate matter in the smaller size fraction.

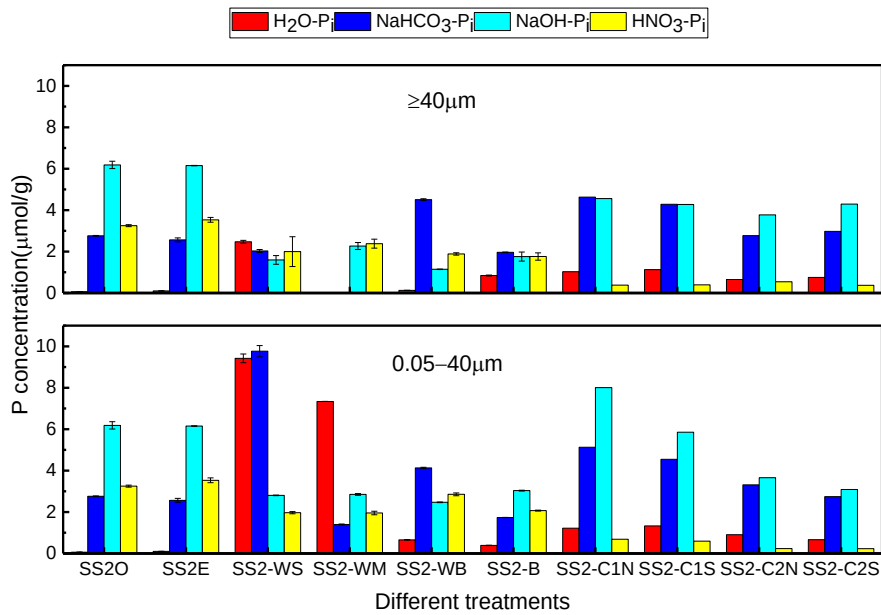


Figure 5.6. Concentrations of P_i pools of bulk samples from different treatment with two size range: 1) $\geq 40\mu\text{m}$; 2) $0.05-40\mu\text{m}$. Different treatments includes bulk sediment from original bulk sediment (SS2O; 0–0.5 cm), bulk sediment after erosion experiment (SS2E; 0–0.5 cm), bulk particulate from surface water (SS2-WS), bulk particulate from middle depth of water column (SS2-WM), bulk particulate from bottom water (SS2-WB; 20cm higher than sediment surface), bulk particulate from bottom water near to sediment surface (SS2-B; sediment surface), particulate collected from the eroded core 1 (SS2-C1N; 0.01–0.25 Pa summed shear stresses), particulate collected from the eroded core 1 (SS2-C1S; 0.01–0.50 Pa summed shear stresses), particulate collected from the eroded core 2 (SS2-C2N; 0.01–0.25 Pa summed shear stresses) and particulate collected from the eroded core 2 (SS2-C2S; 0.01–0.50 Pa summed shear stresses). Error bars represent the standard deviation. Please note that no size fractionation for both original bulk sediment (SS2O) and eroded bulk sediment (SS2E).

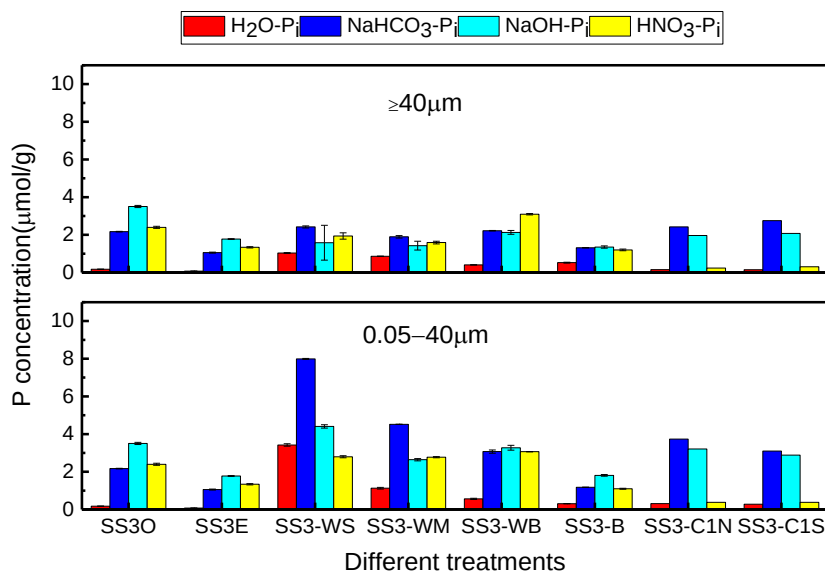


Figure 5.7. Concentrations of P_i pools of bulk samples from different treatment with two size range: 1) $\geq 40\mu\text{m}$; 2) $0.05\text{--}40\mu\text{m}$. Different treatments include bulk sediment from original bulk sediment (SS3O; 0–0.5 cm), bulk sediment after erosion experiment (SS3E; 0–0.5 cm), bulk particulate from surface water (SS3-WS), bulk particulate from middle depth of water column (SS3-WM), bulk particulate from bottom water (SS3-WB; 20cm higher than sediment surface), bulk particulate from bottom water near to sediment surface (SS3-B; sediment surface), particulate collected from the eroded core 1 (SS3-C1N; 0.01–0.25 Pa summed shear stresses) and particulate collected from the eroded core 1 (SS3-C1S; 0.01–0.50 Pa summed shear stresses). Error bars represent the standard deviation. Please note that no size fractionation for both original bulk sediment (SS3O) and eroded bulk sediment (SS3E).

5.4.5 Isotope Composition of Carbon and Nitrogen and C/N Ratio

Venturing downstream, the isotopic data between SS1, SS2 and SS3 is comparable. The $\delta^{13}\text{C}$ values of PSPM were constant, indicating similar biogeochemical processes from upstream to downstream (Figure 5.8 A). In contrast, the $\delta^{13}\text{C}$ of RSPM showed no clear pattern from upstream to downstream. Both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of particulate matter from SS2 and SS3 ranged around -25‰ and 8‰, respectively, which are comparable to values from similar research [Awiti *et al.*, 2008; Su *et al.*, 2020]. Li *et al.* [2019] confirmed that the contribution from terrestrial sources to particulate matter decreased with water flow. Different size fractions of particulate behave differently as a result of size-specific process that control transport and the relative contribution from different sources [Kleeberg *et al.*, 2008].

In the water column, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of particulate matter gradually became depleted with depth (Figure 5.8a; Figure 5.8b). The $\delta^{13}\text{C}$ of PSPM and RSPM were vertically distinct, while there was less variability in $\delta^{15}\text{N}$ of the different size fractions. The $\delta^{13}\text{C}$ values of PSPM became slightly enriched from -27.6 ‰ at the surface to -25.5 ‰ at the bottom, while the $\delta^{13}\text{C}$ values of RSPM became quite depleted from -8.6 ‰ at the surface to -22.0 ‰ at the bottom. If $\delta^{13}\text{C}$ of total particulate was calculated based on mass distribution, (Figure 5.4), there was uniform depletion with water depth similar to the case of $\delta^{15}\text{N}$.

Detailed vertical distribution of $\delta^{13}\text{C}$ values at SS2 and SS3 show how PSPM varied in the water column (Figure 5.9 and Figure 5.10). The $\delta^{13}\text{C}$ values of PSPM at SS3 tended to slightly enrich with water depth until a definitive enrichment in the bottom water. Particulate in the middle depths of SS2 had the lightest $\delta^{13}\text{C}$ (≈ -30 ‰) of all middle sites (Figure 5.3). Similarly, the $\delta^{15}\text{N}$ values of PSPM mirrored that of $\delta^{13}\text{C}$ at both sites excluding the deepest $\delta^{15}\text{N}$ at SS3. The $\delta^{15}\text{N}$ values of particulate matter at both sites steadily became lighter from surface water (12.0 ‰) to bottom water (7.5 ‰) (Figure 5.8 B). At SS2, $\delta^{13}\text{C}$ varied less than that of $\delta^{15}\text{N}$ and at SS3, $\delta^{13}\text{C}$ varied as much as $\delta^{15}\text{N}$, ranging from -28 to -23‰.

SS2 had a larger difference between its maximum and minimum values, with $\delta^{13}\text{C}$ increasing from -27.6 to -23.4 ‰ between the surface and bottom water and with $\delta^{15}\text{N}$ values decreasing from 11.7 at the surface to 6.0 ‰ in the bottom water. C/N ratios followed a similar pattern, decreasing to a minimum in the middle of the water column before resuming values similar to surface values (Figure 5.9 and 5.10). An exceptionally low C/N ratio of particulate matter occurred in the middle of the water column at SS2, which corresponded to the lightest $\delta^{13}\text{C}$ value of all depths and sites. These data combined indicated particulate source switching for PSPM and RSPM in the water column with depth and confirmed that PSPM and RSPM came from different sources.

Both C and N isotopic signatures were characterized to connect particulate matter in the water column with eroded sediment (Figure 5.11). The $\delta^{13}\text{C}$ of eroded sediment was highly variable under different shear stresses with values varying from -20 to -10‰. $\delta^{13}\text{C}$ values were highly scattered in eroded sediment, with no linear relationship between shear stress and $\delta^{13}\text{C}$ values in either size fraction. Sediment undergoing differential shear stress during erosion had stable $\delta^{15}\text{N}$ values of around +6‰, with no observable difference in $\delta^{15}\text{N}$ values between different size fractions (Figure 5.11 B). The relatively heavy $\delta^{13}\text{C}$ values of RSPM (-8.6‰ to -22.0‰) across all depths in SS3 were similar to the ones of different size fractions of eroded sediment (-20‰ to -10‰) (Figure 5.8 A; Figure 5.11 A). Gradual depletion of $\delta^{15}\text{N}$ of RSPM from surface to bottom (6‰), followed that of eroded sediment. This indicated that one major source of RSPM could be eroded sediment.

The $\delta^{13}\text{C}$ values of C3 plants ($\approx$ -32 to -21‰) are isotopically depleted compared to those of C4 plants ($\approx$ -17 to -9‰) [Lamb *et al.*, 2006; Smith and Epstein, 1971]. Aquatic plants such as phytoplankton have reported $\delta^{13}\text{C}$ values between -42 and -24‰ with an average of -30‰ [Hamilton and Lewis, 1992; Kendall *et al.*, 2001]. C/N ratios of autochthonous sources such as plankton are low (5–8) [Li *et al.*, 2016] compared to land-derived vascular plants (>15) [Meyers, 1994; Savoye *et al.*, 2003] while that of soil organic matter lies in the intermediate range (8–15) [Brady and Weil, 1990].

The consistently light $\delta^{13}\text{C}$ values of PSPM (ca. -27 ‰; Figure 5.9 and 5.10) ruled out the possibility that eroded sediment with heavier $\delta^{13}\text{C}$ (ca. -15 ‰; Figure 5.11) may be an important source of particulate in the water column. The $\delta^{15}\text{N}$ values of PSPM (ca. 8-12 ‰; Figure 5.10) at SS3 except those at the bottom were significantly heavier than the eroded sediment. There was no difference in $\delta^{15}\text{N}$ with increasing shear stress, indicating no or negligible isotopic fractionation during erosion between normal (0.01-0.25 Pa) and storm conditions (0.25-0.50 Pa) after summation. This could further explain the disconnect between suspended particulate and eroded sediment at SS3 though there was a slightly discrepancy concerning P pool compositions between bulk sediment and eroded sediment. Other potential particulate sources such as algae ($\delta^{13}\text{C} = -24.1$ ‰; $\delta^{15}\text{N} = +8$ ‰) and sediments may contribute to PSPM and RSPM because of their similar isotopic signatures to the measured values. Further validation through phosphate oxygen isotopic signatures and redundancy analysis of conservative elements would help with source tracking.

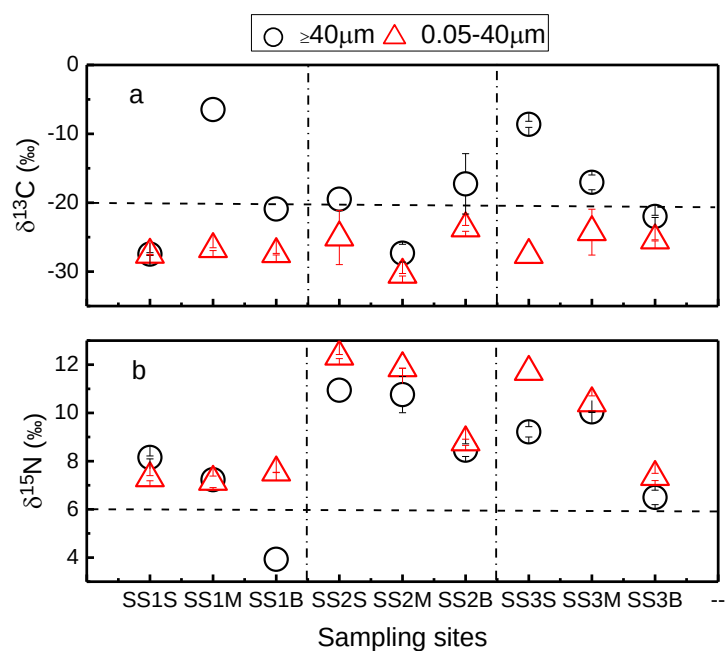


Figure 5.8. Measured $\delta^{13}\text{C}$ (A) and $\delta^{15}\text{N}$ (B) values of particulate collected from different depth (surface, middle and bottom) of water column in SS1, SS2 and SS3 with two different size range: 1) $\geq 40\mu\text{m}$; 2) $0.05-40\mu\text{m}$.

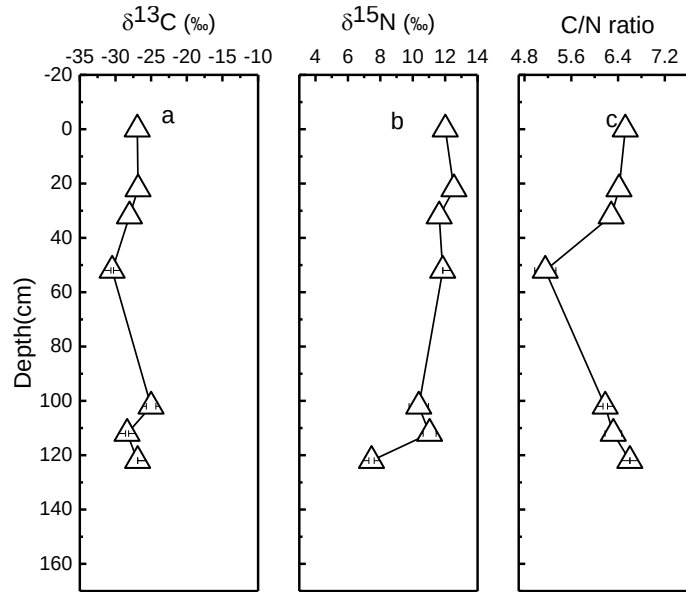


Figure 5.9. Vertical distribution of $\delta^{13}\text{C}$ values (A), $\delta^{15}\text{N}$ values (B), and C/N ratios (C) of PSPM at site SS2 for particular matter greater than 50 nm.

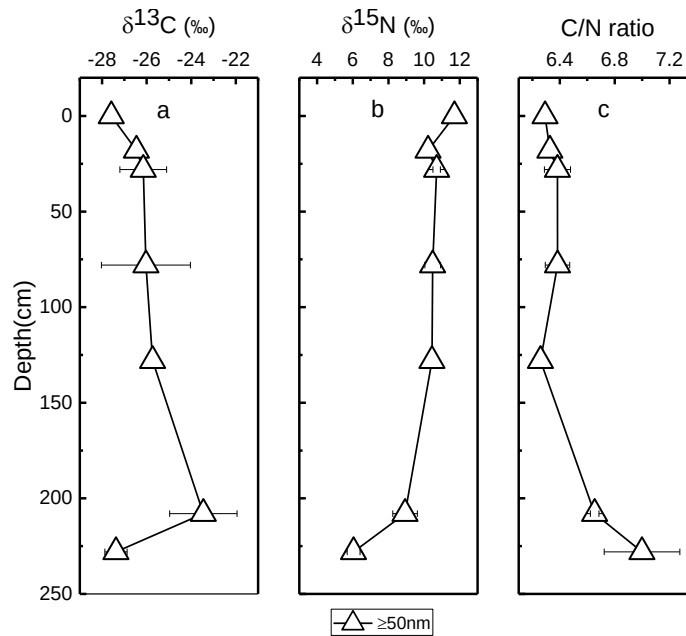


Figure 5.10. Vertical distribution of $\delta^{13}\text{C}$ values (A), $\delta^{15}\text{N}$ values (B), and C/N ratios (C) of PSPM at site SS3 for particulate matter with size greater than 50 nm.

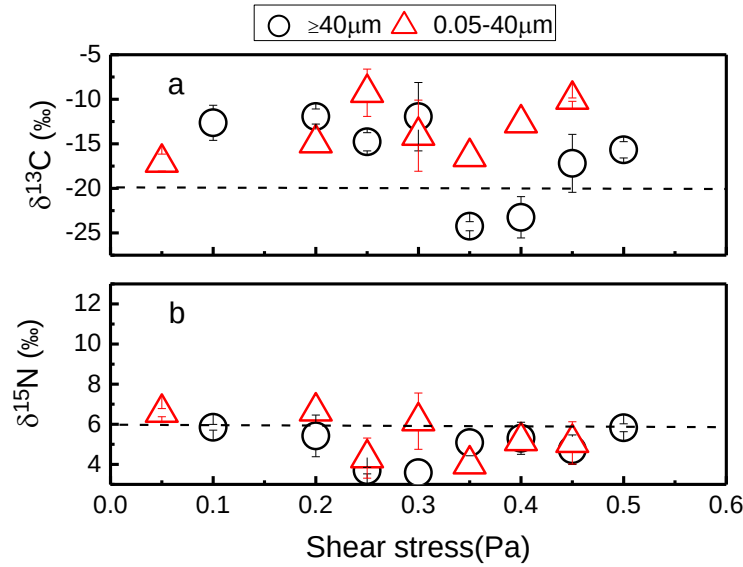


Figure 5.11. Measured $\delta^{13}\text{C}$ values (A) and $\delta^{15}\text{N}$ values (B) of two different size range of eroded sediment $\geq 40\mu\text{m}$; $0.05-40\mu\text{m}$ at SS3.

5.4.6 Role of Resuspension on Suspended Particulate Dynamics

Particulate P encompasses the suspended P solid phase in water and is derived from terrestrial allochthonous sources as well as autochthonous ones. Particulate P is partially bioavailable at different temporal scales because it includes accessible P pools among inaccessible P pools [Mingus *et al.*, 2018]. A variety of different factors such as ambient physical and chemical processes, residence time, and community structure and activity of organisms affect the extent of bioavailability of particulate P. In this study, the contribution of resuspended sediment to particulate matter in the water column was identified with the help of controlled erosion experiments coupled with multiple stable isotopic tracers.

The difference between water column values and sediment resuspension during erosion experiments indicated the contribution of surface water particles to sediment composition in addition to illuminating particle cycling between surface waters and bottom sediments. The following calculations represent the amount of P measured in the water column that came from sources other than resuspension under normal conditions (0.01–0.25 Pa) and storm conditions (0.01– 0.50 Pa). Normal conditions with low shear stress was quantified using the 75th percentiles of annual wind and current velocity and are more representative of sampling conditions. Values less than 10% provide the most confidence that sediment resuspension accounted for the majority of particulate matter in the water column and the presence of connectivity between the sediments and the water column. Higher values indicated that particles in the water column likely came from nonlocal sources or resulted from primary production. Negative numbers suggested that sediment resuspension overpredicts what was measured in the water column at the time of sampling [Seelen *et al.*, 2018].

At SS2, PSPM in surface water had a strong nonlocal signal (51.7%; Table 5.4) under normal conditions, which was stronger at high tide (60.2%) during storm conditions. On the contrary, RSPM had a strong local P signal under both normal and storm conditions from the surface (-13 %) to the bottom (-44.7%). The contribution of nonlocal sources for PSPM decreased significantly with water depth and switched to local sources at the bottom. At SS3, PSPM again dominated from other sources (> 60 %) with a clear trend between depth and shear stress conditions, suggesting the same conclusion as for SS2. However, RSPM at SS3 had a stronger nonlocal signal compared to that of SS2. The lack of change in water column particulate composition (both PSPM and RSPM) at SS3 under varying conditions further indicated that

Table 5.4. The relative importance of resuspension under different shear stresses on water column suspended particulate dynamics calculated as: $([\text{Water Column}] - [\text{Eroded}]) / [\text{Water Column}] * 100$. The values given represent the percent measured in the water column from sources not associated with the sediment surface by comparing particulate with eroded sediment on the same size fraction. ‘Normal condition’ refers to conditions approximately equal to what is experienced over normal weather and tidal conditions (summed concentrations from particles eroded during the 0.01-0.25 Pa shear stress intervals), and ‘storm conditions’ to high shear stress conditions (summed 0.01-0.50 Pa shear stress intervals).

		Normal condition (%)		Storm condition (%)	
		$\geq 40 \mu\text{m}$	0.05–40 μm	$\geq 40 \mu\text{m}$	0.05–40 μm
SS2-WS		-	51.7	-	60.2
SS2-WM	PSPM	-	14.5	-	29.6
SS2-WB		-	-14.6	-	5.8
SS2-B		-	-60.1	-	-31.8
SS2-WS		-13.2	-	-14.2	-
SS2-WM	RSPM	-96.9	-	-98.7	-
SS2-WB		-19.5	-	-20.6	-
SS2-B		-44.7	-	-45.9	-
SS3-WS		-	59.0	-	64.4
SS3-WM	PSPM	-	31.0	-	40.1
SS3-WB		-	23.5	-	33.5
SS3-B		-	-73.8	-	-51.0
SS3-WS		31.5	-	24.4	-
SS3-WM	RSPM	17.2	-	8.7	-
SS3-WB		39.1	-	32.8	-
SS3-B		-8.9	-	-20.0	-

particulate P in the water column was not connected to the surface sediments.

Therefore, PSPM with higher nonlocal signals was sourced different from that of

RSPM with higher local signal in the water column except at the bottom. Sediment signals were much stronger at the bottom water (more negative on the bottom as shown in Table 5.4). Differing physical and chemical characteristics in SS2 and SS3 and differences in transport due to particle size [Kleeberg *et al.*, 2008] likely explain the difference in source characteristics of P and may be a function of the cohesive properties of the underlying sediment.

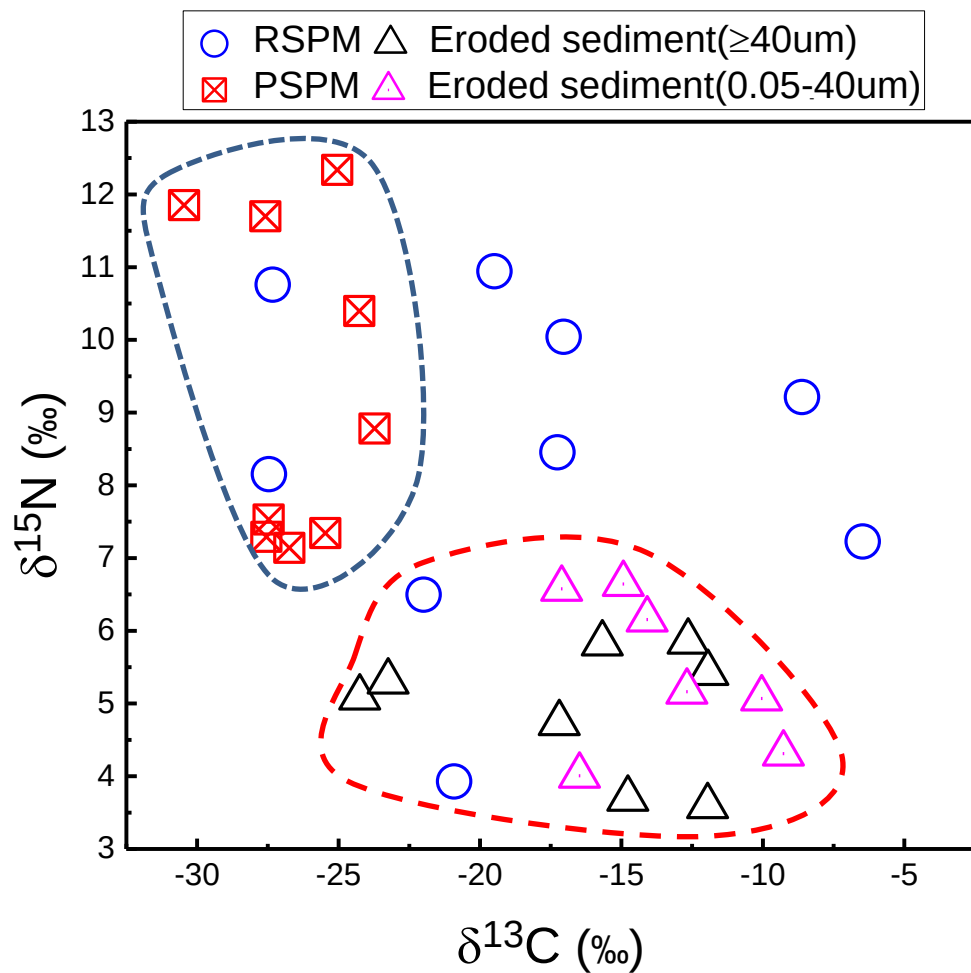


Figure 5.12. $\delta^{15}\text{N}$ vs $\delta^{13}\text{C}$ of RSPM, PSPM, eroded sediment ($\geq 40\mu\text{m}$) and eroded sediment ($0.05\text{--}40\mu\text{m}$) from all sites.

To further parse out what may be driving the similarities and differences between the two suspended particulate pools, carbon and nitrogen isotopes from the eroded and suspended particulates were analyzed, assuming that P cycling was strongly influenced by organic matter (OM) cycling. Both C and N isotopic signatures were used to categorize and establish the connectivity between suspended particulate matter and particulate matter derived from eroded sediment (Figure 5.12). Eroded sediment with both size fractions (red shape) were clearly separated from PSPM (blue shape), indicating that PSPM had no connection with eroded sediment. Although some RSPM data was located within the range of eroded sediments, the majority of their sediment signal lies in the water column. This further confirmed the conclusion about the dichotomy between the RSPM and PSPM source signals in the water column pools. Based on the fact that PSPM contributed around 80% of total particulate mass with higher P concentration, sediment resuspension was not the major process controlling particulate in mouth of the Susquehanna River.

5.5 Conclusions

Settling of particulate matters in estuaries removes the majority of P from the water column. Relative contribution of settling vs resuspension are controlled by river morphology, hydrodynamics as well as the composition and size of the suspended particulate matter. High- resolution water column study on P pools in the field paired with laboratory flux experiments revealed a poor connection between bulk sediment and suspended particulate in the water column. A high variability in the erodibility of sediment response to high shear stress was common in upstream sediments. The concentration of particulate matter in deeper waters was high and progressively increased downstream of the river- suggesting the role of resuspension increasing.

Comparative analyses of PSPM and RSPM confirms that sediment resuspension is not a major contributor to the particulate matter and P in the Susquehanna River estuary. It further attested that the terrestrial (allochthonous) sources of P carried by river currents are still the primary source in the estuary.

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Chapter 6

SUMMARY, CONCLUSIONS, AND FUTURE

6.1 Summary and conclusions

Potential P sources and transformation pathways may increase exponentially with increasing the size of estuary. To constrain P cycling in estuaries, scaling up of research from small and reasonably straightforward river systems to larger and complex watersheds could aid in systematic study in sources and processes and aid to comprehend the complexity and related biogeochemical cycling. A better understanding of different P pools and their corresponding bioavailability from source to sink and impact on water quality aids on the development of appropriate nutrient management strategies. The overarching goal of this dissertation was to investigate P sources and transformation pathways in two watersheds of contrasting sizes (62 km² Love Creek watershed in Rehoboth Bay and 777 km² lower Susquehanna River transect in the Chesapeake Bay).

In the Chapter 2, isotopic integrity of different upstream sources of P during transport in the Love Creek watershed was tested and isotope proxies were used to identify sources of colloid and associated P. The order and relative sized of P pools in colloids in the creek, different land uses (agricultural soils, septic system, and river bank sediments) as well as C and N isotopes indicated that non-agricultural source, such as from plant debris and forest soil, was the major P source in the non-tidal section of Love Creek. This finding is unconventional and points towards the need of additional high-resolution studies to corroborate these results. In general, contribution of terrestrial sources of colloids and P gradually decreased with increasing salinity indicating the role of tides to import colloids in tidal zone.

In Chapter 3, multiple stable isotope proxies were applied to track colloids and identify whether specific P pools in colloids are biologically cycled at the Deer Creek - Susquehanna River mouth stretch. Results showed that NaOH-P_i is the most dominant P pool in the summer and winter seasons. Oxygen isotope values ($\delta^{18}\text{O}_\text{P}$) of NaOH-P_i and HNO₃-P_i pools of different size fractions of colloids were found to be much heavier than the ranges of equilibrium values in the ambient water, which suggested that these two pools are recalcitrant against biological uptake. It further indicated that the isotopic signatures of these P pools could be used to identify the sources of colloids. Carbon (C) and nitrogen (N) isotope compositions of colloids showed that the contribution of terrestrial sources gradually decreases downstream of the river toward the bay and Deer Creek contributes disproportionately high amounts of colloids to the Susquehanna River.

In the Chapter 4, a high-resolution study on temporal and spatial variability of colloid and total dissolved phosphorus (TDP) concentrations in the estuarine gradient in the Deer Creek-Susquehanna River transect was performed over three seasons. In general, a strong correlation of colloids and TDP was found along the transect. The mouth of Deer Creek was the prominent hotspot of colloids and TDP, which confirmed the higher nutrient load per unit volume of water compared to that of upstream Susquehanna River (supported the results in Chapter 3). Intriguingly the ranges of colloid and TDP concentrations varied three to five folds in the transect indicating strong spatiotemporal variability in the estuary.

In Chapter 5, the role of sediment resuspension in P dynamics in the water column (in the field) studied and compared to that from controlled laboratory erosion experiments on intact sediment cores retrieved from the Susquehanna River. A detailed analyses of dissolved and particulate P, P pools and isotopes allowed to establish the connection between the underlying sediment and overlying particulate matter on the sources of particulate matter. Comparative analyses of permanently suspended particulate matter (PSPM) and resuspended particulate matter (RSPM) confirmed that role of sediment resuspension is minor. While the substantial increase

in particulate matter with water depth was found in all sites, chemical composition of suspended particulate matter was different from that of sediment. These lines of evidence attested that the terrestrial (allochthonous) sources of P carried by river currents are still the primary source in the estuary.

6.2 Future Research

While this dissertation research identified some key sources and biogeochemical processes involving P in the coastal estuary. Gaps in scientific knowledge to connect sources and sinks are still limited on the understandings of origins, sources, and routes of transformation of P in various environments. Those gaps could be better filled in from detailed and high-resolution studies on particle size from hydrologic gradients. Further research is needed to characterize whether there is systematic difference in isotopic signatures ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{18}\text{O}_\text{P}$) between different size fractions of soil from distinct soil type. Due to distinct specific surface area, P is bound to different soil size fractions may have different isotopic signatures. Moreover, surface erosion preferentially removes finer-sized soil particles which are relatively enriched in P than coarser-sized particles. The relative composition of eroded soil fractions into water bodies during erosion may vary from one soil type to another under similar climatic conditions. Identifying changes in speciation and bioavailability released P from different soil fractions could provide useful implications on understanding legacy P and source-tracking of P in soil-water continuum. This knowledge-base could guide for best management practices of P in both agricultural soils and waters.

Feasibility of combining $\delta^{18}\text{O}_\text{P}$ values of NaOH-P_i and $\text{HNO}_3\text{-P}_\text{i}$ with C and N isotopes to track sources of colloidal P and characterize P transformation pathways is explored and provided encouraging outcomes. Further tapping this outcome to larger watersheds including coastal regions could provide the additional validation and potential application of this proxy. The NaOH-P_i pool of colloids (mainly adsorbed by

Fe/Al oxides) are sensitive to pH and redox-driven P release in salinity gradients. While NaOH-P_i might be an effective tracer in areas or seasons where algal blooms are not present or when the salinity of receiving waters is low (freshwater bodies), gradual alteration of this pool occurs in the salinity gradient. Therefore, additional tracer such as C and N isotopes are needed to better characterize colloidal sources and narrow down the potential colloidal P sources. Regardless of which P pool or combination of pools is used, source tracking of colloidal P through coupling with C and N isotopes can add strength to results.

One intriguing question that remained still not very clear from this research is the detailed characterization of P and individual contribution of sediment resuspension in riverine DIP budget. The extent of P release due to sediment resuspension is affected by both the sediment composition and diagenetic conditions. Due to the fact that the DIP is the most bioavailable P form to phytoplankton and all other organisms, it feeds to primary production and adversely affects the health of water bodies. Proper identification of pathways and processes that forms DIP including inputs from sediment resuspension will resolve different pathways and help estimate spatial and temporal variation of DIP in the water column.

Knowledge gaps on the extrapolation of results from laboratory studies to natural environments still exist. An effort toward connecting them helps constrain P cycling and transformations would be useful to better understand and interpret $\delta^{18}\text{O}_\text{P}$ values measured in the natural environments. One remaining question is to identify the time for $\delta^{18}\text{O}_\text{P}$ values of dissolved P_i to equilibrate with water completely in natural conditions. The other question whether the different groups of microorganisms in all environments cast the same isotope equilibration. Answers to these questions help better interpret data from natural environments and highlight the applicability of phosphate oxygen isotopes.

Research in organic colloidal P is largely ignored. Although organic P contributed about half of TP in Love Creek and Susquehanna River, assessment on its bioavailability or source was ignored in this study due to methodological limitations.

Future research on method development on organic P speciation using advanced methods such as NMR and Orbitrap MS/MS will complement the inorganic P research.

Appendix A
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