TRACKING SOURCES OF PARTICULATE PHOSPHORUS IN RIVER WATERS: A CASE STUDY IN EAST CREEK, A CHESAPEAKE BAY WATERSHED

by

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ABSTRACT

Incomplete understanding of phosphorus (P) forms and their recalcitrance under imposed environments coupled with methodological limitations to track P sources are obstacles preventing accurate assessment of nutrient loads released to open waters. In this thesis research, the East Creek Watershed in Crisfield, MD, an agricultural runoff dominated watershed, was selected to understand the fate and stability of P from various sources during transport from East Creek to the Chesapeake Bay. To achieve this, P concentrations and phosphate oxygen isotope ratios were compared between sequentially extracted Hedley P pools from potential sources and suspended particulate matter. A transect study from an agricultural field to a drainage ditch was also conducted to identify potential P release pathways. Transect study results suggested soil erosion is a plausible P source to ditch sediments after crop harvest, although dissolved P was the prominent P form in the ditch water column during summer algal blooms which drive sedimentary P release. Comparative analyses of P pools in suspended particulate matter in creek water suggest that particulate P is primarily carried in loosely sorbed and Fe/Al oxide-bound forms. Isotope results suggest loosely sorbed P is bioavailable whereas Fe/Al oxide-bound P and Ca-P precipitates were not accessed by microbes during particulate P transport through the creek. Agricultural field soil, streambank material, and sediments were important sources of legacy P, although mechanism of P release and contribution differed among them. These findings improve understanding on sources and fate of particulate P and are expected to be useful for nutrient management in soils and waterways.
Chapter 1
INTRODUCTION AND LITERATURE REVIEW

1.1 Role of Phosphorus in Chesapeake Bay Water Quality

The Chesapeake Bay Watershed is home to over 17 million people, and the bay itself carries an economy of around one trillion dollars (Figure 1a). Thus, water quality issues in the bay have caused resounding environmental and economic impacts (DEEP, 1989; CBP, 2004). Varying degrees of late-spring to early-summer surface water eutrophication and bottom water hypoxia have caused the bay water quality to suffer since the mid-1900s, fueled by both point and nonpoint source nutrient loadings (Hagy et al., 2004). Eutrophication-related consequences include increased turbidity, algal blooms, and decreased submerged aquatic vegetation and fisheries production. Despite decades of remediation efforts, Chesapeake Bay water quality has not shown significant improvement. Many rivers, particularly on the Eastern Shore of the Chesapeake Bay, including parts of Delaware, Maryland, and Virginia (Figure 1b), carry P loads exceeding water quality standards recommended for aquatic organisms (USEPA, 2000; Ator and Denver, 2015). These conditions warrant the need for further research on primary culprits of eutrophication in the Chesapeake Bay and its watershed.
Figure 1: The Chesapeake Bay watershed (a) and Eastern Shore region of the watershed (b) that disproportionately releases a high nutrient load to the bay. Eastern Shore streams are classified and color-coded to reflect excess nutrient release (Ator and Denver, 2015).

Phosphorus (P) is an essential element because there is no P substitute in sustaining life and food production. Because of the low stoichiometric need for P among other nutrients (106C:16N:1P; Redfield, 1958), small changes in P concentration could promote eutrophication and cause severe impacts on water quality.
in receiving catchments. The nitrogen to phosphorus (N:P) ratio in the Chesapeake Bay is higher than the Redfield ratio throughout the year, suggesting P limitation (Prasad et al., 2010). However, during months of high primary productivity like June through September, P and N are co-limiting (Prasad et al., 2010). During this highly productive season, phytoplankton flourish with access to an amount of P above stoichiometric need. When these phytoplankton die and sink, their decomposition in bottom waters cause oxygen consumption. Hypoxic or anoxic bottom water conditions generated as a result of this oxygen consumption can cause fatality of aquatic organisms that require oxygen and rely on the benthos for some portion of their lifespan, such as submerged aquatic vegetation, crustaceans, striped bass (Breitburg et al., 1997), and bottom feeding fish (Caddy, 1993). Thus, P plays an undeniable role in localized dead zones that have limited the diversity and distribution of micro- and macro-species compositions in the Chesapeake Bay.

1.2 Speciation of Phosphorus in Soils

In soil, both inorganic P (Pᵢ) and organic P (Pₒ) forms contribute to the total P pool, and the composition and concentration of these forms depends largely on soil type, pH, mineralogy, and other biogeochemical factors such as redox potential and composition of (micro)organisms synthesizing or degrading Pₒ species. By virtue of its chemistry, P is readily fixed in soil. Pᵢ can be loosely sorbed to soil surfaces, attached through cation bridges or hydrogen bonding to form a semi-labile P pool. Phosphorus sorbed onto iron (Fe) and aluminum (Al) oxides is generally considered unavailable for plant uptake, although desorption or reductive dissolution can release Fe and Al oxide-bound P into the soil solution, thus becoming available for subsequent uptake.
by biota. Phosphorus can also precipitate in mineral forms such as apatite (Ca-phosphate), which is generally considered recalcitrant, or less available, P.

Continuous application of P, such as with fertilizer or manure in agricultural soils, promotes both P buildup within these chemical pools (Lehmann et al., 2005) and interspecies transfer and transformations among P pools (Joshi et al., 2016). For example, loosely sorbed P could become gradually fixed and accumulated in chemically distinct P pools, such as Fe oxide-bound P or Ca-P precipitates. Although P is commonly fixed in soils, dissolved P in the soil solution sometimes constitutes a substantial bioavailable fraction of total P, particularly when sorption sites are saturated (e.g., Sharpley et al., 2013).

Figure 2: Comparison of dissolved P, and phytate P pools in water and phytate P in sediment showing dominance of phytate P in water in various sites in the East Creek Watershed, Crisfield, MD (Stout et al., 2016).

$P_o$, while not as commonly studied due to methodological limitations (Fitzsimmons et al., 2011), is also an important component in the total soil P pool. $P_o$ can consist of various proportions of nucleic acids, inositol phosphates, sugar phosphates, condensed $P_o$, phospholipids, amino phosphoric acids, and others. $P_o$ can
be hydrolyzed by extracellular and intracellular enzymes before biological uptake and cycling. Soil P\textsubscript{o} is more bioavailable than it was originally thought, and the role of P\textsubscript{o} on overall P cycling are thought to be important but still uncertain (Rees et al., 2009). P\textsubscript{o}, such as phytate P, can also be mobilized to waterways and mineralized to cause environmental concern (Figure 2; Stout et al., 2016).

1.3 Legacy Phosphorus

Continual P applications to agricultural soil in excess of plant uptake results in buildup of soil P reserves often called legacy P. The legacy P can remain fixed in the soil, desorb and leach to deeper soils or groundwater, or remobilize during erosion and runoff. Legacy P mobilized from agricultural fields can subsequently be deposited in wetlands, forests, lowlands, or streambanks (Kleinman et al., 2011; Sharpley et al., 2013) before reaching larger water bodies. Recently, legacy P has been attributed as an explanation for the lack of reduction in P loading and thus lack of water quality improvements despite implementation of best management practices in many watersheds, including the Chesapeake Bay watershed (Kleinman et al., 2011; Sharpley et al., 2013; Sharpley and Wang, 2014).

Environmental consequences of legacy P are exacerbated in areas hydrologically connected to nearby waterways. In fact, legacy P is now projected to act as a long-term and chronic source of P release to the Chesapeake Bay (Sharpley et al., 2013) because of the risk of loss through surface waters. For example, in the East Creek watershed in Crisfield, MD, a predominantly agricultural watershed which drains directly to the Chesapeake Bay, sedimentary P release was found to occur under existing physicochemical and biological conditions, likely acting as a chronic P source to the water column (Upreti et al., 2015). Further research is necessary to examine
similar critical source areas that could potentially promote P release from legacy P reserves such as wetlands and streambanks. Identifying whether legacy P reserves are currently releasing P and specific physicochemical and biological conditions that could promote P retention or release in the future could warrant creation of appropriate management approaches to minimize P loading to the bay.

1.4 Legacy Phosphorus in the Eastern Shore

Legacy P reserves are of particular interest on the Eastern Shore of the Chesapeake Bay. The Eastern Shore, including parts of Delaware, Maryland, and Virginia (Figure 1), accounts for approximately 7% of land in the Chesapeake Bay watershed (Ator and Denver, 2015). Agriculture has been the predominant land use in the Eastern Shore since the mid-18th century, a shift from the previously forested landscape (Allen, 2009). Agriculture remains the dominant land use, with corn and soybean as the primary row crops (Staver and Brinsfield, 2001) and poultry the primary livestock. For example, >550 million broiler chickens were raised on the Eastern Shore in 2012 (USDA, 2014). Soils in this region are typically sandy, yet poorly drained due to flat, low lying landscapes.

More than half of the P load to the Chesapeake Bay originates from agricultural sources (Taylor and Pionke 2000) and is primarily derived from fertilizer and manure applied to agricultural fields (Taylor and Pionke 2000; Brietburg et al., 2001; CBP, 2012). Non-point P sources on the Eastern Shore are estimated to be responsible for one quarter of total manure P load and one third of fertilizer P load in the entire Chesapeake Bay watershed (Ator et al., 2011). Lack of effective manure redistribution from local poultry operations has caused this regional nutrient imbalance; nutrient inputs are greater than outputs, creating localized sub-basins of P-
saturated soils. Many farmers applied poultry manure to fields based on N requirements for decades, resulting in P over-applications due to the low N:P ratio in manure (Sims et al., 2000). Although P applications are conducted at more reasonable rates today, legacy P accumulated in soil during fifty-plus years of agricultural practice has increased P in many Eastern Shore soils to levels above optimum for crop requirements (i.e., 50-100 mg/kg of Mehlich-3 P; Sims, 2000; Buda et al., 2010; Ator and Denver, 2015).

1.5 Phosphorus Transport from Agricultural Lands to Waterways

High P levels in soil become an environmental concern during eventual entry to waterways through runoff, erosion, or leaching. Many soils on the Eastern Shore are artificially drained, lowering the water table to avoid submergence that can harm crop growth (Brisson et al., 2002). Artificial drainage, typically in the form of constructed ditches or channelized streams, provides a direct hydrological connection from fields to waterways (Kleinman et al., 2007).

Phosphorus release from cropland as overland flow is typically higher after large storm events than at base flow conditions (Grant et al., 1996). Heavy rainfall can also inundate P-saturated topsoil with water, contributing to dissolved P runoff (Vadas et al., 2007). Subsurface flow can also potentially transport dissolved P to ditches during base flow if ditch depth is lower than water table depth (Vadas et al., 2007). Although leaching is typically thought of as a less prominent transport mechanism for P, leaching can occur in both dissolved and particulate form (de Jonge et al., 2004). Soil type influences the proportion of dissolved P and particulate P (PP) loss from agricultural fields, with dissolved P found to be the dominant P form lost through artificially drained sandy loam soils (Eastman et al., 2010). Poorly drained, sandy soils
common on the flat topography of the Eastern Shore (Needelman et al., 2007) have lower erosion potential and lower sorption capacity for P, promoting higher losses in the form of dissolved P.

By virtue of the phosphate anion and soil chemistry, PP transport is generally expected to be more common than dissolved P due to its high affinity of soil minerals, particulate matter, and colloidal particles (Heathwaite and Johnes, 1996). In fact, over 90% of P is transported through world’s rivers to larger water bodies in particulate form (Meybeck, 1982; Follimi 1996). Likewise, PP is the dominant form of P transported by rivers to the Chesapeake Bay (Conley et al., 1995). Suspended particulate matter is made up of both sediment and seston, with sediment consisting of sand, silt, clay, and colloidal particles which settle in water at different velocities, and seston consisting of bacteria, phytoplankton, and other biological material which are typically less dense than water and do not easily sink (Fitzsimons et al., 2011). Thus, suspended particulate matter is a prominent carrier of both P<sub>1</sub> and P<sub>o</sub> through waterways. Differences in both residence time and mobility of P<sub>1</sub> and P<sub>o</sub> carried by suspended particulate matter is also possible, yet such selective mobility of particulate matter is still widely unknown (Fitzsimons et al., 2011).

Parameters such as tidal height, pH, and salinity in open waters influence the concentration (Figure 3; Upreti et al., 2015) and proportion of dissolved P and PP, and thus potentially alter the ultimate fate of P. Increases in salinity, due to tidal influence or transport of suspended particulate matter along a river-to-estuary gradient, can cause desorption of P from sorption sites and thus cause increase in dissolved P in the water column (e.g., Spiteri et al., 2008). Sedimentation along the salinity gradient can be a promising PP sink; however, reductive dissolution of Fe oxides can cause P,
release into the water column (e.g., Chambers et al., 1995; Jordan et al., 2008). Overall, biogeochemical cycling of P in rivers is complex, with biotic and abiotic factors playing variable roles in the ultimate fate and bioavailability of different PP forms.

Figure 3: Diurnal variation in tidal height, $\delta^{18}O_w$, and dissolved P concentration over a 24 hr. sampling period in East Creek, a tidal creek draining to the Chesapeake Bay (Upreti et al., 2015).

1.6 Bioavailability of Particulate Phosphorus

Lack of water quality improvement and thus increased environmental concern in aquatic ecosystems raises an important question on PP loads—which PP pools are bioavailable and thus provide warrant for targeted nutrient management plans. Bioavailability is defined based on the potential microbial or plant uptake of a particular nutrient form in a particular ecosystem (Peignenburg and Jager, 2003) from the point of origin or entry to the ecosystem, such as river waters, to the point of export or ultimate sink. Thus, bioavailable P refers to whatever fraction of total P
microorganisms can assimilate plus already assimilated P in cells (Reynolds and Davies, 2001). Understanding PP bioavailability can provide insight into both the function of the total P pool in waterways and provide knowledge necessary to devise proper management plans (Reynolds and Davies, 2001). If a PP pool is biologically unavailable, it causes less immediate concern for water quality and thus could be disregarded from active management plans.

Determining the bioavailability of a particular P pool is not straightforward because P pools are impacted by site specific chemical conditions such as pH, salinity, redox conditions, and composition and activity of (micro)organisms in the environment. This is further complicated by methodological challenges and variations thereof in the literature in determining P bioavailability. For example, various indirect methods of quantifying bioavailability include dissolved/soluble reactive P plus biomass P (Reynolds and Davies, 2001), bioassays (Ellison and Brett, 2006), and sequential extractions (Pacini and Gatcher, 1998; Reynolds and Davies, 2001). Defining PP bioavailability based on operationally defined sequential extractions can provide useful information and comparisons among literature; however, multi-scale interactions among PP pools and physicochemical and biological processes demand for more innovative methods that go beyond operationally defined P pools based on soil P tests and extraction methods (Kruse et al., 2015) to discriminate bioavailable PP pools from recalcitrant PP pools.

1.7 Phosphate Oxygen Isotope Ratios: A Tool to Understand Bioavailable P and P Sources

Since P has only one stable isotope ($^{31}$P), stable P isotopes cannot be used as source tracers. However, P is strongly bound to oxygen and most commonly present as
orthophosphate (PO$_4^{3-}$). P-O bonds are not impacted by abiotic processes such as sorption, desorption, mineral transformation, and transport under low temperatures (<70°C) and pH ranges in most natural environments (Lecuyer et al., 1999; O’Neil et al., 2003; Blake et al., 2005; Jaisi et al., 2010; Jaisi, 2013). The association of P with O and the stability of the P-O bond enables the use of stable oxygen isotope ratios ($\delta^{18}O_P$) in phosphate to trace P sources in the environment.

In contrast, biotic processes cause measurable isotopic fractionations, which provide understanding of biological P cycling and thus bioavailability in the environment. For example, microbial cycling can cause complete O isotope exchange between PO$_4$ and ambient H$_2$O causing a resultant equilibrium isotopic composition (Longinelli and Nuti, 1973; Blake et al., 1997; Stout et al., 2014; Chang and Blake, 2015). An equilibrium isotopic composition of phosphate oxygen isotopes, the resultant $\delta^{18}O_P$ values after complete microbial P cycling, can be calculated from ambient water oxygen isotopes ($\delta^{18}O_W$) and temperature. Thus, $\delta^{18}O_P$ values allow discrimination of P pool bioavailability; microbial utilization is realized with excursion of $\delta^{18}O_P$ values of a cycled P pool towards equilibrium $\delta^{18}O_P$ values in contrast with unavailable P pools retaining $\delta^{18}O_P$ values of an original source.

Hydrolysis of P$_o$ imprints kinetic fractionation by a series of phosphohydrolase enzymes (Blake et al., 1997; Jaisi et al., 2014). Subsequently, released P$_i$ undergoes pyrophosphatase-catalyzed isotope exchange through microbial cycling and thus $\delta^{18}O_P$ values shift towards equilibrium isotopic compositions (Blake et al., 2005). $\delta^{18}O_P$ values have been utilized to successfully understand biogeochemical P cycling as well as to trace P sources in different environments, such as rivers (McLaughlin et al., 2006), lakes (Elser et al., 2009), marine sediments (Jaisi and Blake, 2010), and
atmospheric particles (Gross et al., 2013). Combining sequential extractions and isotope measurement of P pools in soils (Zohar et al., 2010; Joshi et al., 2016) and sediments (Jaisi and Blake, 2010; Joshi et al., 2015) allows greater certainty in quantifying sources, internal cycling, interspecies transfer of different P pools, and differentiating between bioavailable and recalcitrant P pools.

1.8 Research Objectives

The major research objectives were to:

(I) Identify isotopic signatures of inorganic P pools and P$_i$ sources within the East Creek watershed;

(II) Differentiate bioavailable and unavailable inorganic P pools during transport along an environmental gradient from sources within the watershed to ultimate export in the Chesapeake Bay, and

(III) Track suspended particulate matter and PP to its origin in the East Creek watershed using $\delta^{18}$O$_P$ values and multi-element sediment fingerprinting.
Chapter 2
MATERIALS AND METHODS

2.1 Study Site

2.1.1 East Creek Watershed, Crisfield, Maryland

East Creek is a tidal tributary located in Somerset County, MD and drains directly to the lower Chesapeake Bay (Figure 4). The creek is approximately 10 km long, draining a watershed of approximately 67 km$^2$ within the Coastal Plain Physiographic Province. Agriculture is the primary land use in the northern half of the watershed and includes poultry farms and agricultural fields with predominantly corn-soybean rotations. Agricultural fields in this watershed are artificially drained by open ditches. Agricultural fields are interspersed with small forested patches. The southern half of the watershed is primarily surrounded by wetland, and a fraction of the wetland remains saturated after rain events and during high tide. Applications of poultry manure in excess of P requirements have contributed to excessive soil test P levels in the region (Cabrera and Sims, 2000; Sims et al., 2000). Direct drainage to the Chesapeake Bay contributes to environmental concern from this critical source area (Coale and Layton, 1999).

Diurnal P concentrations, as well as physicochemical and biogeochemical processes, are dynamic in East Creek due to tidal influences, temperature, and P load variability. As water depth decreases with receding tides, dissolved P concentration
can double in creek water (e.g., 6.5 to 12.9 µmol P/L; Upreti et al., 2015).

Figure 4: Location map showing the Chesapeake Bay and its watershed (a). The circle represents East Creek. The zoomed in panel (b) shows East Creek and its tributaries and sampling sites in the watershed. Black circles along the creek show water and sediment sampling locations. Small squares represent approximate locations of wetland soil sampling locations; open squares are locations where streambank and wetland samples were both collected. Triangles enclosed in boxes represent forest soil sampling locations. Boxes with horizontal lines represent fields where agricultural soil was collected. Note that multiple samples collected from each land use were used for multi-element sediment fingerprinting, and selected samples were processed for isotopic analysis. Map modified from Stout et al. (2016).
Tides also influence salinity and thus impact P sorption and release. East Creek sediments were found to be a P source to the water column under ambient bacterial activity, pH, salinity, and temperature (Upreti et al., 2015). Furthermore, dissolved P concentrations in creek water at the mouth of East Creek are about two times higher than Chesapeake Bay P concentrations (Prasad et al., 2010) suggesting that P is exported from the creek to the bay (Upreti et al., 2015).

2.2 Collection and Analysis of Water, Sediment, and Soil Samples

Water, sediment, and soil samples were collected from the East Creek watershed (Figure 4) to identify P speciation and determine P pools that are either recalcitrant or bioavailable and to connect these pools to potential land sources. Details of sampling are included below:

2.2.1 Water and Sediment Sampling from the Mainstem and Streambanks of East Creek

Sampling sites along the creek were chosen across a salinity gradient from a drainage ditch near an agricultural field to the mouth of creek at the Chesapeake Bay. The 14 sites are located approximately 0 m (site A), 690 m (site B), 1415 m (site C), 1915 m (site D), 2495 m (site E), 3685 m (site F), 4395 m (site G), 5405 m (site H), 6260 m (site I), 7530 m (site J), 7900 m (site K), and 8420 m (site L) from the mouth of the creek. Sites B, D, and E are smaller tributaries that drain largely single-land use basins before joining the mainstem creek. Sites K and L are located in a ditch that drains an agricultural field with corn-soybean rotations. Site L, the closest to the agricultural field, is least influenced by high and ebb tide. Site J1 was located at the confluence of the ditch to the mainstem East Creek channel.
Water samples were collected in June 2015 from the center of the creek flow line during baseflow in receding tidal conditions so that suspended particulate matter predominantly originated from land or upstream sources. During the time of sampling, in-situ water temperature was recorded. Volume of collected water ranged between 8 L (at site L) to 64 L (at site A) based on a pilot study to determine the amount of suspended particulate matter required for isotopic analysis (around 10 µmol P per sample).

Paired creek bed sediment cores were collected from immediately below the water sampling site (from six sites: A, C, F, H, J, and K) with a suction corer. One core was collected per site. A piston was pulled inside a 7.6-cm core liner, simultaneously aspiring a sediment core into the liner. This method was effective to hold sediment and pore water intact and minimize disturbance at the sediment-water interface (Hartzell et al., 2010). In most cases, water overlying the retrieved core was clear, suggesting that the liner insertion did not impact the sediment/water interface. The top 2 cm of the core were collected; this depth should be representative of settled particulate matter and plays a major role in P exchange with the water column. Samples were immediately stored on ice and placed in coolers during transportation to the laboratory for analysis.

Streambank sample sites were selected based on the water flow direction with particular focus on scour channel in the mainstem. These include banks next to sites F, H, and J. One streambank material sample per location was collected from 0-2 cm depth, stored in a sealed tube, and immediately placed on ice.
2.2.2 Soil Sampling across the East Creek Watershed

Surface soil samples were collected from the East Creek watershed in June 2015 (at the same time of paired water-sediment samples collection) and October 2015 (at the time of soil transect studies). Sampling locations were selected based on the analyses of land use maps, aerial photographs, and site visits to target areas of distinct land use patterns and/or active soil erosion. Agricultural fields, forested areas, and wetlands were the dominant land uses. About 20 single soil samples were collected from the top 0-5 cm from each land use and land cover type for multi-element sediment fingerprinting. Sampling locations were spread out as far as possible in the watershed as permitted by access constraints (Figure 4b). Agricultural fields were all managed the same with manure the primary P fertilizer and corn-soybean crop rotations. Forested lands varied from predominantly deciduous, well-mixed deciduous and coniferous, and predominantly coniferous; all forest types were represented with the sampling methodology. Wetland samples were collected approximately 5 m inland from sites selected for streambank sampling because these areas were of close proximity and direct hydrologic connectivity to East Creek (Figure 4b).

Soil samples were also taken for P pool differentiation and isotopic analysis; at least two samples per land cover were collected separately and processed for this purpose. To obtain a representative sample for isotopic analyses, five subsamples were collected within an approximately 5m x 5m area from the top 0-5 cm of soil with a clean shovel and composited by thorough mixing in a clean bucket. Only the top 0-5 cm of soil was collected from all sites based on the notion that surface soil is most vulnerable to erosion and could be easily mobilized during runoff, contributing to the PP in the mainstem channel. Soil samples were collected following this procedure from different sites in the agricultural fields (Field A and B), forest (Forest A, B, and
C), and wetland (Wetland A and B). Fields A and B were collected from two different fields managed similarly to each other adjacent to site L, the ditch draining to East Creek. Forest A was also located near site L, whereas Forest B and C were in different forested patches further south in the watershed. Given that poultry litter (hereafter called manure) is the most common source of P input in regional agricultural fields and the sole external input of P to the agricultural fields near site L, manure samples (Manure A, B, and C) were also collected with the same procedure from an area where manure had been previously stockpiled before spreading into the agricultural field. Please note that it is likely that manure samples were a mixture of manure and soil. Sampling methodology did not statistically overcome natural variability within soil and thus results should be interpreted with caution.

2.2.3 Soil Sampling from Agricultural Field-Ditch Transect

A transect study was also conducted in the agricultural field near site L in October 2015 to test distribution of soil P pools in the field, at the edges of the field, and in the drainage ditch (Figure 5). Sampling was conducted after crop harvest. Transect direction was chosen based on the expected hydraulic gradient that depends on general slope of the graded agricultural field and location of the ditch network to capture the major flow of dissolved P and PP both as overland flow (such as during rain and storm events) and subsurface flow to the ditch. The transect expanded 45 m, with 30.5 m located in the flat agricultural field and ~10 m from the ditch bank to the middle of the ditch. Ten transect sampling locations (T1 through T10) were chosen with sites T1-T5 uniformly distanced at 7.63 m apart in the agricultural field. The spacing of sites T6-T10 sites varied consecutively at approximately 4.88 m, 1.22 m, 0.61 m, 0.61 m, and 1.22 m apart ending on T10 (Figure 5). Soil sampling from T1
(agricultural field) to T9 (ditch bank) was conducted following previously explained methods with one sample per location among transect collected at 0-5 cm, but T10 was collected from the bottom of the ditch under standing water. At the time of soil sampling, the depth of the water column in the ditch was approximately 15 cm.

![Figure 5: Schematic diagram showing the locations of soil sampling sites (T1-T10) in the agricultural field-to-ditch transect study. Note that the vertical scale of the ditch was exaggerated to enhance clarity. The depth of the ditch from the cropland (1.52 m) is approximate.](image)

2.3 Pre-Processing Suspended Particulate Matter and Source Samples

Water samples that had been stored on ice after collection were processed upon arriving at the laboratory at the University of Delaware. Suspended particulate matter was separated from creek water via centrifugation (Sorvall LYNX 6000), and Stoke’s Law of settling was used to obtain target particle size ≥100 nm. Samples-in-waiting were stored at 4 °C until centrifugation. Conductivity was measured using an EC meter and salinity was measured using a commercial kit (LaMotte Salinity 0-20 ppt Kit) on the ‘as-received’ water samples. Pelleted particulates were washed twice with
de-ionized (DI) H$_2$O to remove salts for the accuracy of determining particulate mass that otherwise would be impacted by variable salinity among sampling sites.

Pore-water was separated from sediment and all other solid samples via centrifugation and saved for water oxygen isotopes ($\delta^{18}O_W$) analysis. The pH of creek water was measured directly with a pH meter. For solid samples, pH was measured with 1:1 soil to DI H$_2$O ratio. Sediment, soil, manure, and streambank samples (after porewater removal) were freeze dried, homogenized, and size-separated (<212 µm) using a mechanical sieve and stored until further analyses.

2.4 Separation and Quantitation of Phosphorus Pools

To differentiate and quantify four different P pools in solid (soil, suspended particulate matter, manure, streambank, and sediment) samples, a sequential extraction technique originally developed by Hedley et al. (1982) and revised by Tiessen et al. (1984) was used with slight modifications. These modifications were aimed at restricting redistribution of extracted P onto residual solid surfaces (Joshi et al., 2016). Additional rinsing steps with 0.5 M NaHCO$_3$ and/or DI H$_2$O were incorporated after each extraction reagent was used (Figure 6), analogous to the sediment P extraction method (Ruttenberg, 1992). To ensure that inorganic P (P$_i$) from any particular pool was not carried over to subsequent P pools, supernatant from the extraction and each rinse step in a particular P pool was collected separately and quantified individually.

For all extraction and rinsing steps, the solid-to-solution ratio was kept constant at 1:100. The first extraction step was conducted with DI H$_2$O (without pH adjustment) by shaking with the sample in a reciprocating shaker for 2 hr at room temperature (22 ± 0.5 ºC). The solid and supernatant were separated by centrifugation using Stoke’s Law (as above) with target particle size of 100 nm (assuming <100 nm
as dissolved P). Please note the choice of this target size is arbitrary but expected to account for more accurate dissolved P than filtration by using 450 nm filter. Residual soil was then treated with 0.5 M NaHCO₃ and shaken for 16 hr. before separation of extracted P via centrifugation. To recover any mobilized P that was subsequently resorbed back onto residual minerals, residual soil was washed with DI H₂O for 2 hr. followed by separation of supernatant and residual soil by centrifugation (as above). Supernatants from extraction and rinsing/washing steps were collected separately for the quantitation of both P₀ and Pᵢ. Next, soil was treated with 0.5 M NaOH, in which the soil-reagent suspension was shaken for 16 hr., centrifuged, and the supernatant separated. Following the NaOH extraction, the soil was rinsed first with 0.5 M NaHCO₃ followed by a DI H₂O rinse (2 hr. shaking for each rinse step). Supernatants from extraction and rinsing steps were collected separately. The final extraction step was 1.0 M HNO₃, in which the residual soil was treated with 1.0 M HNO₃ for 16 hrs. Please note 1.0 M HNO₃ was used instead of 1.0 M HCl in the original Hedley et al. (1982) extraction to avoid complication of silver chloride precipitation (from Cl⁻ in acid) during sample processing for isotopic analyses. The residual soil was washed using 0.5 M NaHCO₃ followed by DI water (as above).

Concentrations of Pᵢ in all extracted supernatants were quantified separately using the molybdate blue method after pH adjustment to 7 ± 0.5 (Murphy and Riley, 1962). Samples were measured on a spectrophotometer at 883 nm wavelength. Persulfate digestible P, referred to hereafter as total P, in all extracted pools was determined using the acid persulfate digestion method (Eisenreich et al., 1975), in which all P₀ is oxidized to Pᵢ and then quantified by measurement with the molybdate blue method. Concentration of P₀ was calculated as the difference between total P and
Pi. Please note that total P (Pi or Po) concentration was calculated from the sum of all Pi (or Po) in each extracted P pools. Residual P in soils (after all extraction steps) was not accounted for. All extracted supernatants were stored at 4°C for one week or less before processing and purification for isotopic analyses.

Figure 6: Modified Hedley et al. (1982) extraction method used in this study. Extraction steps are shown in solid boxes and rinsing steps are in open boxes in the left panel.

2.4.1 Justification for Modification on Hedley Extraction Method

As discussed in Section 2.4, additional rinsing steps were added to the Hedley extraction method (Figure 6). These additional steps, analogous to those in the
Ruttenburg (1992) sediment extraction method, ensured removal of P resorbed to soil during extraction steps. It was found that both 0.5 M NaHCO$_3$ and DI H$_2$O rinsing steps removed quantitatively significant amounts of P$_i$ that had been resorbed back to minerals during the extraction. In selected samples (Table 1), rinsing steps included additional ~ 11% (Streambank (Site F), NaOH extraction) to 366% (Forest A, HNO$_3$ extraction) P$_i$ compared to P$_i$ removed by the original extractant. This means if washing steps are not incorporated, a significant amount of P could be carried into the following extraction step, potentially causing significant under- or over-estimation of P concentration in certain P pools. This can also cause mixing of isotopic signatures, thus producing erroneous data.

Table 1: P$_i$ extracted from Hedley extraction and rinse steps.

<table>
<thead>
<tr>
<th>Extraction/Rinse</th>
<th>Streambank F</th>
<th>Forest A</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 M NaHCO$_3$</td>
<td>1.61</td>
<td>0.95</td>
</tr>
<tr>
<td>H$_2$O Rinse</td>
<td>0.51</td>
<td>0.25</td>
</tr>
<tr>
<td>0.5 M NaOH</td>
<td>4.25</td>
<td>2.18</td>
</tr>
<tr>
<td>NaHCO$_3$ Rinse</td>
<td>0.32</td>
<td>0.36</td>
</tr>
<tr>
<td>H$_2$O Rinse</td>
<td>0.15</td>
<td>0.11</td>
</tr>
<tr>
<td>1 M HNO$_3$</td>
<td>0.89</td>
<td>0.08</td>
</tr>
<tr>
<td>NaHCO$_3$ Rinse</td>
<td>0.61</td>
<td>0.22</td>
</tr>
<tr>
<td>H$_2$ORinse</td>
<td>0.17</td>
<td>0.07</td>
</tr>
</tbody>
</table>

2.5 Sample Processing and Purification for Isotope Analyses

Dissolved P samples from East Creek (water samples after separation of suspended particulate matter) and all extracted P pools from soil, sediment, and particulate matter were further processed to remove contaminants and concentrate P$_i$ for isotope analyses. Different P pools contain variable amount of P$_o$, particularly
NaHCO$_3$-P and NaOH-P pools. Separation of P$_o$ is an essential purification step for O-isotope analysis because hydrolysis of P$_o$ compounds can occur when exposed to acidic and alkaline reagents used in subsequent purification steps. This hydrolysis incorporates light water O-isotopes with negative fractionation factors (Liang and Blake, 2006, 2009) in the released P$_i$ and compromises the isotopic signature of P$_i$. Thus, the extracted solution was first treated with a non-ionic, macro-porous DAX 8 Superlite resin to trap organic compounds (Tamburini et al., 2010) in a column or a bottle, depending on the nature of the sample and P$_o$ content. The DAX resin is moderately polar and is suitable for removal of surfactants, fulvic and humic acids, and other hydrophobic organic compounds with molecular weight up to 150,000 Da.

The clear solution produced after DAX treatment was further processed to reduce sample volume and concentrate P$_i$ using the magnesium induced co-precipitation (MagIC) method (Karl and Tien, 1992). MagIC pellets containing sorbed P were dissolved by gradually adding 0.5 M HNO$_3$ dropwise while shaking the sample container to disperse MagIC pellets and facilitate proton consumption from acid as quickly as possible. Samples with remaining visible impurities after MagIC, most notably in the form of dark brown color from acid-insoluble humic acid and other recalcitrant organic matter, underwent a second treatment with DAX resin to remove remaining P$_o$ and organic contaminants. Samples were further treated with cation-exchange resin (AG50W-X8, BioRad) to remove salt ions in the solution. The sample volume was then reduced by evaporation in a water bath <70 °C to concentrate P$_i$ for ammonium phosphomolybdate (APM) precipitation.

Precipitation of APM and subsequent steps followed methods described in Liang and Blake (2006) and Jaisi and Blake (2010, 2014). APM precipitation was
conducted to remove ions and contaminants that are soluble at low pH. A 0.1 µm polysulfone filter (Pall Scientific) was used to separate and rinse APM precipitates with 5% ammonium nitrate solution before dissolving in ammonium citrate solution. Inorganic P was then precipitated as magnesium ammonium phosphate (MAP) at high pH. The MAP crystals were separated by filtration (as above) and rinsed with 5% ammonium hydroxide solution to remove ions and contaminants that are soluble at high pH. Dissolved MAP solution, after pH adjustment to neutral with 2 M HNO₃, was passed through a vertical fixed-bed column packed with cation-exchange resin (AG50W-X8, BioRad) to remove cations (primarily Mg²⁺ and NH₄⁺) and transform Pᵢ into protonated forms (HPO₄²⁻ and H₂PO₄⁻). Finally, silver phosphate (Ag₃PO₄), the ultimate analyte for measurement of phosphate oxygen isotope ratios, was precipitated by adding silver amine solution in the concentrated Pᵢ solution. A pair of internal phosphate standards with different δ¹⁸Oₚ values were processed in parallel with sample extracts to validate sample processing as well as to confirm the reliability of isotope analyses.

2.6 Measurement of Phosphate Oxygen Isotope Ratios

A Thermo-Chemolysis Elemental Analyzer (TC/EA) coupled to a Delta V continuous flow isotope ratio monitoring mass spectrometer (IRMS, Thermo, Germany; precision of 0.3‰) was used to measure phosphate oxygen isotope ratios (δ¹⁸Oₚ) at the Stable Isotope Laboratory, University of Delaware. Oxygen yield was monitored against the theoretical O content in Ag₃PO₄ (15.4 %). This is important because contaminants associated with Ag₃PO₄ decrease O yield if percentage of O content in the contaminant is less than that of Ag₃PO₄. For example, AgO could be a major contaminant if excess silver amine is used and precipitating solution is almost
evaporated. Similarly, nitrate could be a contaminant due to incomplete washing of Ag₃PO₄ crystals. Presence of such contaminants produces erroneous isotope values and could be a cause of incorrect data, thus skewing interpretation (Colman, 2002; Jaisi and Blake, 2014). Each run sequence included triplicate Ag₃PO₄ capsules from each sample and an internal phosphate standard (in triplicate), benzoic acid (in duplicate), and two isotope standards (YR 1-1a and YR 3-2 with δ¹⁸O_P values of −5.49 and +33.63‰, respectively) (in triplicate).

In certain cases, small quantity of Ag₃PO₄ precipitation inhibited ability to run samples in triplicate, in which case the sample was run in duplicate when possible and in rare cases as a single sample. Suspended particulate matter samples with only one capsule analyzed included NaHCO₃-Pᵢ from site A, C, F, and J1, NaOH-Pᵢ from site J, and HNO₃-Pᵢ from site C from 2015 sampling. Limited samples from 2013 and 2014 were collected and processed with the same methodology as 2015 and reported for comparison, with only one capsule analyzed for 2014 NaHCO₃-Pᵢ at site K and NaOH-Pᵢ at sites J1 and K. For 2013 particulate matter samples, only one capsule was analyzed for NaHCO₃-Pᵢ at sites J and L. All other samples were run in duplicate or triplicate. Isotope standards were included at the beginning and end of each run. The measured δ¹⁸O_P values were calibrated against YR1-1a and YR3-2 standards that were originally calibrated using conventional fluorination method (Vennemann et al., 2002). All measured oxygen isotope compositions hereafter are reported relative to the Vienna standard mean oceanic water (VSMOW) standard in units of permil (‰).
2.7 Measurement of Water Oxygen Isotope Ratios and Calculation of Equilibrium Isotope Composition

Because biological reactions imprint equilibrium isotope composition in P, identification of biologically cycled P requires knowledge on equilibrium isotope values. For this, δ^{18}O_w should be known. All surface water and porewater from sediments and soils (when possible) were analyzed for δ^{18}O_w values. Measurements of δ^{18}O_w values were conducted using a Finnigan™ GasBench II coupled with IRMS: a small amount of CO_2 in the sample vial headspace was introduced into the IRMS and measured for δ^{18}O_{CO_2} values after complete equilibrium with the water sample at 26 °C (>24 hours). The δ^{18}O_w values were calibrated against standards USGS W67400 (-1.97‰), USGS W32615 (-9.25‰), and three internal standards EBL101 (-7.05‰), EBL202 (-1.25‰), and EBL303 (8.71‰). Typical precision for replicate standards is <0.06 ‰. δ^{18}O_w values are reported following standard delta notation relative to Vienna Standard Mean Ocean Water (VSMOW).

The theoretical isotopic equilibrium values (expected after complete microbial turnover of orthophosphate and complete exchange of oxygen between phosphate and ambient water; Blake et al., 1997, 2005) can be determined using phosphate-water fractionation equations. In this study, we compared data with two equations. The most commonly used equation was derived from observed O-isotope fractionations between phosphate and water in biogenic phosphate minerals (Longinelli and Nuti 1973),

\[ T (°C) = 111.4 - 4.3 (\delta^{18}O_{Eq} - \delta^{18}O_w) \]  

Similarly, a newly determined equation for equilibrium O-isotope fractionations between dissolved phosphate and water (fractionation factor \( \alpha_{(PO_4 - H_2O)} \)), which is more relevant to P precipitation as Ag_3PO_4 and measurement in online TC/EA (Chang and Blake 2015):
\[1000 \ln \alpha(PO_4 - H_2O) = 14.43 \cdot 1000 /T (K) - 26.54\]  
(2)

Since the equilibrium equation contains temperature and \(\delta^{18}O_W\) values, variation in both \(\delta^{18}O_W\) values and temperature of sampling location must be accounted for. Thus, the range of equilibrium was calculated based on the minimum and maximum temperature within one month of the sampling date. Equilibrium isotope calculations reported in this communication are based on the Chang and Blake (2015) equation.

2.8 Multi-Element Suspended Particulate Matter Fingerprinting

Multi-element fingerprinting offers an independent method to trace sources of suspended particulate matter, as well as verify results obtained from stable isotopes. Multiple conservative elements in suspended particulate matter samples were compared to potential sources of particulates to track their origin (Massoudieh et al., 2013; Stewart et al., 2015). At least twenty samples were collected from each endmember, or potential source of suspended particulate matter, including agricultural land use, wetland, forested land, and streambanks, and six samples from surface sediment in the creek (see Section 2.2.2).

These samples were subjected to modified Hedley extraction (as described in Section 2.4). However, supernatants from different P pools were combined in 1:1:1 ratios for NaHCO\(_3\):NaOH:HNO\(_3\). Since the suspended particulate matter samples were rinsed before quantitation of mass (to remove salts), the first DI H\(_2\)O extraction was not performed. Thus, to be consistent with suspended particulate matter, DI H\(_2\)O extractions were performed and discarded for source samples. Also, DI water rinses for NaOH and HNO\(_3\) pools were discarded to ensure consistency of sediment to solution ratio. Concentrations of 20 elements (Table 2) with potential to behave
conservatively were measured on combined extracted solutions on the inductively coupled plasma optical emission spectrometry (ICP-OES) at the Soil Testing Laboratory at the University of Delaware.

Table 2: Selected elements chosen for measurement for multi-element suspended particulate matter fingerprinting.

| Elements chosen for multi-element suspended particulate matter fingerprinting |
|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| Aluminum (Al) | Cadmium (Cd) | Potassium (K) | Lead (Pb) |
| Arsenic (As) | Cobalt (Co) | Magnesium (Mg) | Sulfur (S) |
| Boron (B) | Chromium (Cr) | Manganese (Mn) | Selenium (Se) |
| Beryllium (Be) | Copper (Cu) | Nickel (Ni) | Vanadium (V) |
| Calcium (Ca) | Iron (Fe) | Phosphorus (P) | Zinc (Zn) |

Given that different elements in suspended particulate matter and these source sites behave differently, it was essential to differentiate conservative elements for corresponding P pools. To identify conservative elements in suspended particulate matter from the mainstem channel of East Creek, bivariate plots were created to compare each element against salinity for all sites within the main channel and the ditch (sites A-L). Elements showing linear relationships with salinity along the flow line were considered conservative. In addition, elements coupled to P cycling were plotted against each other in bivariate scatter plots to identify any linear relationships.

Subsequently, conservative elements in suspended particulate matter were compared to elements measured in potential source samples. A qualitative comparison was conducted, comparing the average and standard deviation of conservative elements in suspended particulate matter to that of different sources. Potential endmembers with no overlap between standard deviation of conservative elements in particulate matter were considered to be unlikely sources suspended particulate matter,
and those with overlap were considered to be potential suspended sediment sources. Please note that these elements will be run on a Bayesian model as in Massoudieh et al. (2013) at a later date, and a detailed statistical analysis is beyond the scope of this research. However, the purpose of analysis in this study is to loosely support or reject findings on the source of PP from isotope analysis in East Creek with comparison of conservative elemental profiles.
Chapter 3

RESULTS

3.1 Phosphorus Mobility and Transport in Dissolved and Particulate Forms in East Creek

3.1.1 Agricultural Field-Ditch Transect

Along the agricultural field-ditch transect in the East Creek watershed, pH varied slightly more than 2 units. For example, agricultural field sites (T1-T5; Figure 5; Table 3) had slightly acidic pH, gradually increasing towards neutral near the edge of the agricultural field (5.61 and 6.99 at T1 and T5, respectively). In contrast, soils collected on the steeply sloping ditch bank (T6-T8) had much lower pH, ranging between 4.73 and 4.97. Ditch sites (T9-T10) exhibited pH values between the agricultural field and ditch bank, falling between 5.29 and 5.39.

Table 3: Elevation and pH along the agricultural field-ditch transect.

<table>
<thead>
<tr>
<th>Location</th>
<th>Agricultural Field</th>
<th>Ditch Bank</th>
<th>Ditch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>T1</td>
<td>T2</td>
<td>T3</td>
</tr>
<tr>
<td>pH</td>
<td>5.61</td>
<td>6.34</td>
<td>6.79</td>
</tr>
<tr>
<td>Elevation (m)</td>
<td>2.13</td>
<td>2.44</td>
<td>2.13</td>
</tr>
</tbody>
</table>

*Elevation estimated for T7-T10 based on a ~ 1.52 m difference from the bottom of the ditch to the field.

Total P<sub>i</sub> in the transect samples showed an anticipated pattern with high P<sub>i</sub> in the agricultural field, decreasing sharply on the ditch bank, and increasing again in ditch sediments (Figure 7a). Among four different P pools analyzed (H<sub>2</sub>O-P<sub>i</sub>,

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NaHCO$_3$-P$_i$, NaOH-P$_i$ and HNO$_3$-P$_i$), concentrations of NaHCO$_3$-P$_i$ and NaOH-P$_i$ pools in the ditch sediment were within the range of NaHCO$_3$-P$_i$ and NaOH-P$_i$ pools in the field. However, H$_2$O-P$_i$ (1.63-2.86 µmol P/g) in the crop field was about double than that in the ditch sediment (0.65-1.43 µmol P/g). HNO$_3$-P$_i$ was the only P pool exhibiting substantially lower P$_i$ concentrations in ditch sediment (0.43-0.97 µmol P$_i$/g) than in field soil (3.09-9.51 µmol P$_i$/g). Interestingly, sites with higher pH had relatively higher HNO$_3$-P$_i$ concentrations.

Measurement of P$_o$ in extracted samples allowed comparison between P$_i$ and P$_o$ in the transect samples. In contrast to the clear trend in P$_i$ (Figure 7a) with pH, P$_o$ did not show any particular trends along the transect (Figure 7b) although, in general, P$_o$ made up a higher percentage of TP on the ditch bank than in the agricultural field and ditch. Relative distribution of P$_o$ among different P pools was largely similar, with H$_2$O-P$_o$ and HNO$_3$-P$_o$ pools being low (<0.5 and <0.18 µmol P/g, respectively) at all sites and NaHCO$_3$-P$_o$ and NaOH-P$_o$ being high accounting for up to 73% and 87% of total NaHCO$_3$-P and NaOH-P, respectively.

### 3.1.2 Dissolved and Particulate P Pools in East Creek Water Column

Along the stretch of East Creek studied, proportion of dissolved and particulate inorganic and organic P pools (P$_i$ and P$_o$ and PP$_i$ and PP$_o$, measured as the sum of NaHCO$_3$-P, NaOH-P, and HNO$_3$-P pools) varied along the salinity gradient (Figure 8). All sites where water was collected from the ditch next to the agricultural field (J1, K, and L) were dominated by dissolved P with this pool comprising 61-83 % of total P. These sites had an order of magnitude higher PP than sites in the main channel. Dissolved P$_o$ in all sites did not constitute a major portion of total dissolved P. On the other hand, PP in these sites was composed largely of P$_o$. However, in the main
channel of East Creek, all sites, except site I, consisted of >50% total P as PP with PP₀ still the major PP species.

Figure 7: Speciation of P into four Pᵢ pools along the crop field-ditch transect (a) and corresponding P₀ pools (b). Cross hatching is used to differentiate P₀ from Pᵢ but the color for particular P pool is same. Please note that T1-T5 were in the agricultural field, T6-T8 were on the slope of the ditch bank, and T9-T10 were in the ditch.
For receding tidal conditions, measured salinity and pH along sampling sites in East Creek showed a consistent trend (Table 4). As shown, pH ranged from near neutral to 9.10 (at site L) and salinity ranged from 17.8 to 0.5 PSU at sites A and L, respectively.

Additional analyses of relative P pool size among sites provided interesting information regarding PP pool distribution (Figure 9). Ditch sites J1, K, and L had an order of magnitude higher PP than sites in the main channel, with NaOH-P_i and P_o both dominating total P pools. NaHCO₃-P_i and P_o were the second most dominant pools. HNO₃-P_i and P_o were the smallest and most consistent pools among sites (with the exception of Sites A and L where HNO₃-P concentrations were much lower and
higher, respectively). In the main channel, both NaHCO$_3$ and NaOH extracted pools were dominant PP forms. In general, NaHCO$_3$-P$_i$ and NaOH-P$_i$ pools at any site in the main channel were relatively similar. As expected, NaOH typically extracted the most P$_o$. Surprisingly, HNO$_3$-P$_o$ made up about half of the total HNO$_3$-P pool at most sites despite incorporated washing steps and the common held belief that this pool contains negligible P$_o$.

Table 4: Variation of pH and salinity in water samples collected along East Creek. Please note that site A is nearest to the bay and J is furthest upstream. Sites J1, K, and L sites are located in the agricultural ditch.

<table>
<thead>
<tr>
<th>Site</th>
<th>A</th>
<th>C</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
<th>J</th>
<th>J1</th>
<th>K</th>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.66</td>
<td>7.69</td>
<td>7.65</td>
<td>7.72</td>
<td>7.68</td>
<td>7.51</td>
<td>7.50</td>
<td>7.51</td>
<td>8.01</td>
<td>9.10</td>
</tr>
<tr>
<td>Salinity (PSU)</td>
<td>17.8</td>
<td>11.8</td>
<td>8.0</td>
<td>7.2</td>
<td>4.0</td>
<td>2.9</td>
<td>2.2</td>
<td>1.8</td>
<td>1.0</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Figure 9: Speciation of particulate P into three major P pools in the main channel of East Creek (a) and ditch sites that drain to the main channel (b). The color coding of P$_i$ and P$_o$ follows Figure 7. Note the order of magnitude scale difference of total particulate P between the in the main channel and the ditch.
3.1.3 Phosphate Oxygen Isotopes of Dissolved and Particulate P Pools

Dissolved $\delta^{18}O_P$ values were obtained for water samples nearest the agricultural field (Figure 10). Sites J and L were around 18 ‰, whereas sites I, J1, and K ranged between 20 ‰ and 23 ‰. Sites J and L are within the equilibrium range, as typically expected for bioavailable dissolved P. However, sites I, J1, and K were above equilibrium, suggesting that dissolved P$_i$ pool had not been completely cycled by microorganisms. Note that sites J1 and K also had high dissolved P concentration at this time, which could suggest an influx of P$_i$ that has not yet been microbially cycled.

![Figure 10: Isotope composition of dissolved P$_i$ (data points) and equilibrium isotope composition (solid black line) calculated from Chang and Blake (2015) and calculated range of equilibrium values based on temperature variation within a month of sampling shown in the shaded gray area.](image-url)
Different PP$_i$ pools typically demonstrated $\delta^{18}$O$_P$ values within slightly overlapping ranges (Figure 11), with NaHCO$_3$-P$_i$ typically between +16 ‰ and +20 ‰, NaOH-P$_i$ typically between +18 ‰ and +22 ‰, and HNO$_3$-P$_i$ between +20 ‰ and +23 ‰. Interestingly, NaOH-P$_i$ $\delta^{18}$O$_P$ values closely corresponded to dissolved P.
δ^{18}O_P values at sites J1 and K. NaHCO₃-Pi δ^{18}O_P values were also similar at site K. In contrast, at sites J and L, dissolved P δ^{18}O_P values were lower than NaOH-Pi δ^{18}O_P values.

To determine whether PP was bioavailable, isotope data were compared against existing equilibrium equations (Longinelli and Nuti, 1973; Chang and Blake, 2015). When comparing data to the Chang and Blake (2015) equation (Eq. 2), nearly all NaHCO₃-Pi δ^{18}O_P values lied within the range of equilibrium isotopic composition in both agricultural and wetland dominated sites in East Creek. In stark contrast, however, δ^{18}O_P values of the NaOH-Pi pool were heavier than equilibrium (+18 ‰ – +22 ‰) across the salinity gradient. HNO₃-Pi pools were also above the equilibrium range.

3.2 Potential Land Sources of Phosphorus in the Creek Water and their Isotope Signatures

3.2.1 Soils from Agricultural Field, Forest, and Wetland

Among potential land source soils for P release to creek, agricultural field soils had numerically higher Pᵢ than wetland and forest soils (Figure 12). Among different P pools, NaOH-Pᵢ and HNO₃-Pᵢ were dominant in the agricultural field, although NaHCO₃-Pᵢ was still quantitatively important. In forest soils, the general trend of Pᵢ pool dominance was NaOH>NaHCO₃>HNO₃>H₂O. Wetland soils were similar in composition to forest soils albeit with NaHCO₃-Pᵢ dominating.

Isotopic composition of Pᵢ pools from the agricultural field and manure piles mirrored each other (Figure 13a) as expected because manure is the primary P input to the fields. In all cases, NaHCO₃-Pᵢ and NaOH-Pᵢ δ^{18}O_P values ranged between +22 ‰ and +26 ‰ but were all above the calculated equilibrium range calculated based on
Chang and Blake (2015). Interestingly, $\delta^{18}O_{P}$ values of $H_2O-P_i$ lied within the equilibrium range for two samples (Field A and Manure A), but was heavier for other samples, falling within similar range of NaHCO$_3$-P$_i$ and NaOH-P$_i$. HNO$_3$-P$_i$ $\delta^{18}O_{P}$ values were within or near the equilibrium range.

Figure 12: Speciation of $P_i$ into four pools for soil that could act as potential $P$ sources to East Creek, including agricultural fields, wetland, and forest. The color coding for $P_i$ pools follows Figure 7.

Isotopic composition of $P_i$ pools from wetland and forest soil are shown in Figure 13b. Please note that low $P_i$ concentration and high impurities, primarily organic matter, complicated processing of samples for isotopic analysis and thus required repetition and extra time to generate acceptable results. In general, $\delta^{18}O_{P}$
values of NaOH-P$_1$ pool from wetland and forest soils are heavier than equilibrium (ca at +20‰ to +24‰).

Figure 13: $\delta^{18}O_P$ values are shown for soils under different land cover that could be potential P sources to East Creek, including an agricultural field and manure (a) and wetland and forest (b). The black line represents equilibrium values (Chang and Blake, 2015), and the gray shaded region is variation within a month of sampling.
3.2.2 River Bottom Sediments

Sediment $P_i$ and $P_o$ concentrations were higher in the ditch (site K) than in the main channel (Figure 14), as expected due to proximity to agricultural fields. Proportionally, sediment $P$ pools varied more than other potential $P$ sources. For example, NaOH-$P$ was dominant at sites F, H, and K. In contrast, NaHCO$_3$-$P$ was dominant at site C, and HNO$_3$-$P$ was dominant at sites A and J. Thus, NaHCO$_3$-$P$, NaOH-$P$, and HNO$_3$-$P$ were all proportionally important $P$ pools in the main channel. Proportion of $P_o$ to $P_i$ was relatively low in sediments, especially when compared to PP and that of soil in transect studies. In the main channel, total $P$ pools (combined Hedley extracted pools) were $<10 \ \mu$mol/g at all sites, much lower than total $P$ pools in agricultural field soil and suspended particulate matter.

![Figure 14: Speciation of four important $P$ pools in sediments. Color coding follows Figure 7. Cross-hatched bars indicate $P_o$ and solid bars $P_i$, but the color is the same for each particular $P$ pool.](image-url)
Sediment isotope data show a surprising trend, with Sites H and K exhibiting NaOH-P\textsubscript{i} \(\delta^{18}O_P\) values within but on the heavy end of the range typically found in natural environments (+26 ‰ - +27 ‰) (Figure 15) (Jaisi and Blake, 2014; Paytan and McLaughlin, 2011). At both of these sites, NaHCO\textsubscript{3}-P\textsubscript{i} \(\delta^{18}O_P\) values were lighter than NaOH-P\textsubscript{i} \(\delta^{18}O_P\) values, trending towards equilibrium. Site F shows a similar trend, with NaOH-P\textsubscript{i} \(\delta^{18}O_P\) values (+22 ‰) heavier than NaHCO\textsubscript{3}-P\textsubscript{i} (+21 ‰), yet these values are within typical natural range. Sites J and H displayed HNO\textsubscript{3}-P \(\delta^{18}O_P\) values within or near equilibrium range. NaOH-P\textsubscript{i} particulate pools seem to be in a similar range of \(\delta^{18}O_P\) values, as do HNO\textsubscript{3}-P\textsubscript{i} (Figure 15).

Figure 15: \(\delta^{18}O_P\) values of three important P\textsubscript{i} pools in East Creek sediment. The black line represents equilibrium (Chang and Blake, 2015), and the gray shaded region represents variability within one month of sampling.
3.2.3 Streambank sediment from the East Creek

Among potential sources of P within the water column, two streambank samples had much greater total P than river bottom sediments. Streambank samples at sites F and J had high amounts of Hedley extracted P concentrations (Figure 16). Sites F and J followed the general trend NaOH-P > NaHCO$_3$-P > HNO$_3$-P > H$_2$O-P. Site H had very low P$_i$ pools and negligible P$_o$, likely because site H is in an area of the creek that is relatively straight as opposed to F and J which were taken at meanders. As expected, the creek structure has strong influence in areas where legacy P is likely to accumulate.

![Figure 16: Speciation of P into four different P pools in East Creek streambank samples, with cross-hatched bars representing P$_o$ and solid bars P$_i$.](image_url)
Streambank sites had similar trends in $\delta^{18}O_P$ values (Figure 17). At both sites F and J, $\delta^{18}O_P$ values of NaOH-P$_i$ pool were heavier than equilibrium (+22 ‰ - +24 ‰). However, $\delta^{18}O_P$ values of NaHCO$_3$-P$_i$ pools were lighter than that of NaOH-P$_i$ pools at both sites but closer to the equilibrium range. Interestingly, $\delta^{18}O_P$ values of NaHCO$_3$-P$_i$ and H$_2$O-P$_i$ pools were nearly identical at site J, as expected because P can easily be exchanged between these pools (Jaisi et al., 2010, 2011).

![Figure 17: Isotope values of different P$_i$ pools in streambank sediment. The black line represent equilibrium isotopic composition (Chang and Blake, 2015) and the shaded gray region represents range in equilibrium.](image)

3.2.4 Multi-element fingerprinting of phosphorus sources

Conservative elements suited for East Creek were selected for multiple element sediment fingerprinting based on an inert nature of these elements along the salinity gradient. Elements exhibiting relatively linear relationships with salinity
included aluminum (Al), calcium (Ca), copper (Cu), iron (Fe), and zinc (Zn) (Table 5). Please note that sites in the ditch and site J were excluded from this correlation analysis due to high variability in concentration and strong influence from the agricultural field sources. Concentrations of Al, Ca, Cu, Fe, and Zn were thus selected for subsequent comparison to potential source sites. Please note that all comparisons are highly qualitative due to the lack of rigor in analysis and absence of appropriate constraints.

Table 5: Correlation between potentially conservative elements and salinity from bivariate pots.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Al</th>
<th>Ca</th>
<th>Cu</th>
<th>Fe</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson's R</td>
<td>-0.87</td>
<td>0.88</td>
<td>0.88</td>
<td>-0.67</td>
<td>-0.54</td>
</tr>
<tr>
<td>$R^2$ (adj.)</td>
<td>0.70</td>
<td>0.71</td>
<td>0.70</td>
<td>0.31</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Large variability existed among sites for elemental analysis (Table 6). In the suspended particulate matter, the average for Al concentration matched closest with average Al in streambanks and forest sites, although large standard deviation included overlap in all samples except sediment. Mean Ca concentrations were higher than all potential sources but with large variability, and little information can be gleaned from such comparisons to Ca. Cu was near/below detection limit for most potential sources, however its concentration in the agricultural field was similar with the suspended particulate matter. Mean Fe concentrations were similar among suspended particulate matter, streambank, and wetland samples, although standard deviation in was much high. Finally, Zn (which was the least conservative of elements in analysis) in suspended particulate matter overlapped with streambank and wetland sites. Thus, the preliminary analyses suggest streambanks, wetland, forest, and agricultural fields...
could all potentially contribute to suspended particulate matter in the creek water, although sediment is not a likely source.

Table 6: Elemental concentrations of suspended particulate matter and potential sources.

<table>
<thead>
<tr>
<th>Sample Group</th>
<th>Al</th>
<th>Ca</th>
<th>Cu</th>
<th>Fe</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg kg(^{-1}) ± std. dev.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Particulate</td>
<td>2628 ±1148</td>
<td>147847 ± 99960</td>
<td>36 ± 12</td>
<td>10972 ±7058</td>
<td>75 ±29</td>
</tr>
<tr>
<td>Streambank</td>
<td>2532 ±1165</td>
<td>2108 ± 2870</td>
<td>BDL</td>
<td>7262 ±3147</td>
<td>38 ±17</td>
</tr>
<tr>
<td>Wetland</td>
<td>3107 ±864</td>
<td>4384 ± 3535</td>
<td>BDL</td>
<td>9969 ±3244</td>
<td>54 ±18</td>
</tr>
<tr>
<td>Forest</td>
<td>2516 ±1471</td>
<td>844 ± 1778</td>
<td>BDL</td>
<td>2051 ±3527</td>
<td>BDL</td>
</tr>
<tr>
<td>Agricultural Field</td>
<td>1709 ±466</td>
<td>1870 ± 2590</td>
<td>26 ± 31</td>
<td>562 ±213</td>
<td>20 ±7</td>
</tr>
<tr>
<td>Sediment</td>
<td>720 ±409</td>
<td>1371 ± 1438</td>
<td>BDL</td>
<td>1323 ±1215</td>
<td>10 ±6</td>
</tr>
</tbody>
</table>

*BDL = Below Detection Limit*

In addition to source tracking of particulate matter, certain elements were found to correlate with P content in the particulate matter. Al, Fe, and Zn exhibited linear trends with P and among each other in bivariate scatter plots (Table 7). Please note that the bivariate scatterplots excluded Fe in site L because of extremely high concentration of Fe in this site and was considered an outlier.

Table 7: Correlation between related or otherwise coupled elements with P in bivariate analysis.

<table>
<thead>
<tr>
<th>Elements</th>
<th>Al</th>
<th>Fe</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>R²</td>
<td></td>
</tr>
<tr>
<td>Fe</td>
<td>0.893</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.751</td>
<td>0.918</td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>0.848</td>
<td>0.951</td>
<td>0.927</td>
</tr>
</tbody>
</table>
Chapter 4

DISCUSSION

4.1 Soil Phosphorus in Agricultural Fields and Potential Loss to Ditches

4.1.1 Surface and Subsurface Flow of Phosphorus

Chronic P losses from agricultural soil P pools accumulated over long-term agronomic management have been increasingly recognized as a major reason for degraded water quality (Shapley et al., 2013). Understanding P transport in agricultural drainage ditches is essential to improving nutrient management and water quality in local streams and larger water bodies. Even though soils generally have a high capacity to sorb P, soil-bound P can be mobilized and released from land via erosion, surface runoff, or subsurface transport. In coastal plain soils and relatively flat land surfaces such as the East Creek watershed (generally 0-2% slope), subsurface transport of P has been increasingly realized as a primary pathway of P loss from agricultural soils to ditches (Djodjic et al., 2000; Kleinman et al., 2007), with overland flow being a minor component of P loss (<8%) in several Delmarva ditches (Kleinman et al., 2007). Therefore, an agricultural field-to-ditch transect study on soil P pools paired with dissolved P and PP speciation in a drainage ditch could provide important information to constrain potential mechanisms of P loss from agricultural fields.

Phosphorus speciation in agricultural soils with long-term manure and fertilizer application has been studied to identify the relationship among different P pools and to
assess P leaching (Lehman et al., 2005). Results of P pool speciation from the East Creek transect study (Figure 7) show high P in both readily bioavailable (H$_2$O-P$_i$ and NaHCO$_3$-P$_i$) and relatively less bioavailable (NaOH-P$_i$ and HNO$_3$-P$_i$) soil P pools in agricultural fields, demonstrating build-up of soil P over time. Interestingly, concentrations of NaHCO$_3$-P$_i$ and NaOH-P$_i$ pools and soil pH are relatively similar in the agricultural field and the ditch (see 3.2.1). A potential explanation for this similarity could be that ditch sediments originated from agricultural soils. In these sites, NaOH-P$_i$ is a dominant P pool and accounts 43-56% of total P in agricultural fields near East Creek. Runoff or leaching of PP could be one potential fate of accumulated topsoil P, particularly when small (colloidal) sizes of Fe and Al oxides are mobilized. The agricultural fields draining to site L appears to have been graded, with elevation slightly decreasing towards the ditch. This could direct overland flow during storm events, mobilizing P-rich topsoil to the ditch. Phosphorus leaching could also be facilitated in particulate form, although increased PP leaching has been found to correlate with increased clay content (de Jonge et al., 2004) not typically expected of soils in the East Creek watershed. The agricultural field by site L is primarily (>60 %) classified as Othello silt loam (NRCS, 2016). Silt loam should have slightly higher P retention capacity than sandy (or sandy loam) soils due to greater surface area and thus sorption capacity. However, sandy/silty loam is the most common soil throughout the Eastern Shore. The transect study was conducted in October after crop harvest, and it is likely that surface erosion could have been enhanced due to direct exposure of soil to potential impact of wind and water erosion, which could thus mobilize loose soil to the ditch. Thus, even if similar P-pool profiles in the field and ditch suggest PP as a potential carrier of P from agricultural fields, this pathway of P loss and transport
might not be representative for all seasons unless other factors facilitate heavy overland flow.

$\text{HNO}_3\text{-P}_i$ behaved differently among the transect, and it was proportionally much lower in ditch sediments than agricultural soil. On average, $\text{HNO}_3\text{-P}$ made up of 14.5 (±5.3) % total $\text{P}_i$ in the field contrasting with just 3.1 (±0.2) % of total $\text{P}_i$ in the ditch sediments. Selective decrease of $\text{HNO}_3\text{-P}_i$ is most likely caused by proton-promoted dissolution of Ca-P minerals under acidic pH (at the time of transect sampling). In addition, $\text{HNO}_3\text{-P}_i$ might be less easily mobilized from agricultural field soils than NaOH-$\text{P}_i$, because Fe and Al oxides are more likely to exist in colloidal form than Ca-P precipitates, thus selectively facilitating transport of particles enriched with particular P pools. These speculations require further validation.

Often, soil test P (such as Mehlich-3 P) in ditches and adjacent fields are compared to identify whether PP or dissolved P is the prominent P carrier from fields (e.g., Sims and Ritter, 1993; Kleinman et al., 2007). Past studies have found that the P content (measured as Mehlich-3 P) in ditch sediments in Delmarva was five times less than surrounding fields. They suggested that overland flow is not a dominant P transport mechanism in Delmarva agricultural fields receiving manure applications for over 20 years (Kleinman et al., 2007). Please note that the agricultural fields near East Creek are, in general, similar in both management and soil type to the soils included in these studies and thus likely behave similarly.

Several sub-watersheds in the Chesapeake Bay watershed have shown high dissolved P concentrations (e.g., Kleinman et al., 2007; Vadas et al., 2007). This trend was apparent in East Creek as well. For example, ditch sites J1, K, and L showed dissolved P (defined arbitrarily here as <100 nm) as a dominant P pool (>60 %) in the
water column despite evidence that colloidal and particulate matter is suspected as a mechanism of P loss, at least in certain seasons (see above). High dissolved P in the ditch could potentially be related to soil type and agronomic history. Delmarva landforms are generally flat with low erosion potential, and soils are sandy with fewer sorption sites than clay rich soils. For example, the agricultural field by site L has 0-2% slope and negligible runoff capacity (NRCS, 2016), warranting conditions that could favor dissolved P transport as the dominant pathway of P loss. Variable findings amongst studies suggest that primary P transport could be heavily impacted by site specific conditions such as local hydrology, soil type, macro-, micro-, and nano-pore structures (de Jonge et al., 2004), flow regime, catchment size (Jordan et al., 2005), and storm events (Heathwaite and Johnes, 1996).

4.1.2 Role of Algal Blooms on Sedimentary Phosphorus Release

Influence of cyanobacteria blooms on internal nutrient loading and sediment biogeochemistry is particularly prominent because such blooms are accompanied by abnormally high pH, dissolved oxygen content, and dissolved phosphorus concentrations (Gao et al., 2014). Of particular interest is the highly variable P concentration in the ditch site L among different sampling times, suggesting various sources and biogeochemical processes contribute to P concentrations. For example, dissolved P was the dominant P pool and was an order of magnitude higher during 2015 sampling than 2014 sampling (>50 and 5 µmol/L, respectively) at site L. Similar variability (11 and 40 µmol/L in late spring and summer in 2012 and 2013) has been recorded in this site before (Upreti et al., 2015). High dissolved P, generally attributed to subsurface flow from agricultural lands, can also result from biogeochemical processes in the ditch itself as observed in site L. The mainstem sampling was
conducted on a hot June day when water temperature at the site was 34°C. Visual algal blooms suggested high primary productivity in a eutrophic water column, accompanied by high pH (9.10) within range of observed pH in areas of algal blooms (Seitzinger, 1991; Jensen and Anderson, 1992; Gao et al., 2014). This pH increase is caused by CO₂ removal from water for photosynthesis during high primary productivity. With persistently high (yet diurnally fluctuating) pH in the eutrophic water column and organic matter inputs from sedimented algal detritus, flux rates of dissolved P from the sediment-water interface are significantly higher as has been documented in other regions of the Chesapeake Bay and its watershed (e.g., Gao et al., 2014; Joshi et al., 2015; Li et al., 2016).

pH is a master variable for P release. For example, results from controlled laboratory experiments on sediments from site L have shown that approximately 1.2 μmol Pᵢ/g would be released at pH 9.0 (Upreti et al., 2015). Fe and Al oxides have a point of zero charge (PZC) between 5.6 and 9.0 (Sparks, 2003) and thus become negatively charged when pH>PZC, facilitating desorption of P from sediments. Similar results have been observed in eutrophic estuarine ecosystems. For example, the water column pH in the Potomac Estuary increased to 9.5-10.0 in algal bloom affected areas. Controlled laboratory experiments from estuarine sediments identified pH driven sedimentary P release from Fe and Al oxides, promoting a positive feedback loop where released P sustained algal growth (Seitzinger, 1991). In a more detailed study from a lower catchment to estuarine environment in the upper Sassafras River, Gao et al. (2014) demonstrated that high pH during algal blooms can penetrate into the sediment and drive P release. Since NaOH-P is high in both ditch sediments and suspended particulate matter in East Creek, substantial P release is expected under
elevated pH. Additionally, sediment metabolism is expected to increase during blooms due to abundant labile organic matter and supersaturated dissolved oxygen promoting fast decomposition, exacerbating the positive feedback loop by further sustaining the bloom (Gao et al., 2014). Since algal mats are common on shallow, low flow Delmarva ditches, this phenomena could help explain a primary mechanism of P release from ditch sediments when dissolved P concentrations cannot be accounted for entirely by subsurface flow (e.g., Kleinman et al., 2007).

Sedimentary P release and P recycling in the ditch were reflected in corresponding $\delta^{18}$O<sub>P</sub> values of P pools in the ditch because such processes imprint unique isotope compositions. At site L, $\delta^{18}$O<sub>P</sub> values of dissolved P were close to or within the equilibrium range, whereas NaOH-P<sub>i</sub> $\delta^{18}$O<sub>P</sub> values of PP were only slightly heavier (Figures 10 and 11a). Site K showed the same trend: dissolved P<sub>i</sub> $\delta^{18}$O<sub>P</sub> values were similar to particulate NaHCO<sub>3</sub>-P<sub>i</sub> and NaOH-P<sub>i</sub>. This reflects rapid microbial cycling of P in the ditch causing isotope excursion towards equilibrium (Blake et al., 2005; Stout et al., 2014) as the isotope values of sites in the ditch are distinctly different from sites in the main channel. Since high P release and cycling was occurring at this time, it is likely that PP pools were undergoing ion exchange with dissolved P, causing dissolved and particulate $\delta^{18}$O<sub>P</sub> values to converge (Jaisi et al., 2011). Alternatively, new PP pools were being formed in the ditch. Despite expected P release from Fe and Al oxides, the particulate NaOH-P pool was very high. High pH promotes P release, but high concentration of dissolved P also promotes P sorption. Thus, P release is not a one-way reaction, and the relationship between $\delta^{18}$O<sub>P</sub> values of dissolved and particulate NaOH-P<sub>i</sub> pool in this scenario would cause measured values of NaOH-P<sub>i</sub> to trend towards equilibrium, imprinting a semi-cycled signature that
might not be representative of a source signature that it might hold in a different season or year if the algal bloom was not present.

4.1.3 Methodological Limitation Separating Particulate and Dissolved Phosphorus

It is relevant to note that separation between PP and dissolved P is arbitrarily defined by filter size or, in this case, settling size of centrifugation. Dissolved P (anything <100 nm), as measured in this study, could thus include substantial amounts of P in colloidal (1 nm to 1 mm) and nano (<100 nm) (Vold and Vold, 1983) size particles. Small size and large surface area lead to high reactivity with and ability to sorb and retain P, and thus colloids and nanoparticles facilitate P transport in the water column. Size fractionation of PP at site L displayed > 90% of P less than 450 nm size was actually in the <10 KDa size fraction (Jing Yan, unpublished data). This suggests that P at site L was closer to truly dissolved P than at site A, where only <10% of P less than 450 nm size was also <10 KDa (Jing Yan, unpublished data). This supports the expected mechanism of pH driven sedimentary P release generating high dissolved P at Sites K and L. At a different sampling time, particles smaller than typical filter sizes (≥20 nm) were also found to contain substantial P fractions at site L, supporting underestimation of truly particulate-bound P when using arbitrary size separation (Figure 18; Upreti et al., 2015). Particulate and colloidal fractions of different sizes constitute a complex mixture of minerals and organic substances (Fitzsimmons et al., 2011). Thus, results obtained from arbitrary selection of filter size (or other separation methods) should be interpreted and compared amongst other studies with caution.
Figure 18: Inorganic phosphorus (P$_i$) concentration in particulate matter of particular size ranges showing a significant amount of P in smaller size fractions (Upreti et al., 2015).

4.2 Phosphorus Transport in the Main Channel and Export to the Bay

Phosphorus in the water column may undergo biological uptake and cycling, sedimentation onto or remobilization from streambanks and river bottoms, or release from sediments or suspended particulate matter due to ion exchange or reductive dissolution. The ultimate fate of P is largely determined by the properties of suspended particulate matter, the chemical form of associated P, and ambient conditions in the water column including primarily pH, redox condition, and salinity.

Along the salinity gradient in the main channel of East Creek from the agricultural ditch (site L) to the creek mouth at the Chesapeake Bay (site A), P concentrations decreased, with the dominant P pool switching from dissolved P to PP (Figures 6 and 7). Particulate P concentrations are an order of magnitude lower in the main channel of East Creek than the ditch, suggesting the contribution from a critical
source area causing P influx from artificially drained fields with high soil test P (see section 4.1; Coale and Layton, 1999; Upreti et al., 2015; Stout et al., 2016). Particulate P concentration was much lower in the main channel and decreased towards the bay. At sites with higher salinity (e.g., site A with salinity 17.8 PSU), salts are expected to influence P speciation. Increasing salt concentration has been found to increase P release from East Creek sediments (Upreti et al., 2015) due to P displacement by ions such as Cl\(^-\) and SO\(_4^{2-}\), and similar results have been observed in other studies (e.g., Sundareshwar and Morris, 1999; Jordan et al., 2008). For example, Jordan et al. (2008) found Fe oxide-bound PP decreased from 30-55 µmol P/g in freshwater sediments to 15-25 µmol P/g when salinity was near 7 ppt.

Understanding speciation of P pools within suspended particulate matter provides additional information on P formation, removal, and sources. In the main channel, NaOH-P (corresponding to Fe and Al oxide-bound P) was the dominant P pool. In the Patuxent Estuary, Fe oxide bound-P has also been found a major P pool, accounting for about 50-90% total P (Jordan et al., 2008). A dominant NaOH-P pool is consistent with a strong positive correlation of PP with both particulate Fe and Al concentrations in East Creek (Table 7). Fe and Al oxides can be more dominant carriers of P than clay minerals (Manning and Wang, 1995). Additionally, the correlation of Zn with Fe and P, could suggest bonding through a hydroxyl or an organic complex such as humic or fulvic acid (Manning and Wang, 1995). These authors attributed this to a ferric oxide-organic matter assemblage that was considered relatively unavailable in suspended particulate matter in river water. A note of caution, however, is that changing biogeochemical conditions, such as sulfate concentration.
and redox conditions, play major roles in P speciation in estuarine environments (Jordan et al., 2008).

In East Creek, NaHCO$_3$-P (corresponding to loosely sorbed P) was the second major PP form. Variable concentrations along the channel might reflect that this pool is undergoing constant exchange with dissolved P in the water column during transport. In the Kleine Aa catchment in Switzerland, loosely sorbed P has been found to dominate particulate matter during baseflow conditions, with a switch to NaOH-P in storm events, which has been interpreted as shift in the source of P from remobilization of sedimentary PP to topsoil erosion (Pacini and Gatcher, 1999). This suggests changing P speciation during different weather events and flow conditions can potentially impact size and bioavailability of PP pools.

Among the P pools studied in particulate matter, HNO$_3$-P was the smallest pool in East Creek (Figure 7). Similar results have been found in Patuxent Estuary in which both detrital and authigenic apatite in particulate matter accounted for <10% total P (Jordan et al., 2008). The ratio of HNO$_3$-P$_i$:NaOH-P$_i$ could be a proxy in estuarine environments to identify sources of suspended particulate matter since both pools are considered less bioavailable. In the main channel, this ratio increased from the agricultural lands to the bay, following a similar observed trend along a salinity gradient where Fe bound-P was more dominant in fresh water, but Ca-P precipitates became more dominant in a salt marsh (Paluden and Morris, 1998).

Depending on site-specific parameters, P$_o$ or P$_i$ can dominate PP pools (Conley et al., 1995; Fitzsimons et al., 2011). Organic P in particulate matter has been less studied and is often ignored in literature (Fitzsimons et al., 2011). It is of note that East Creek PP consists predominantly of P$_o$ (41-80% of total P), particularly dominant in
the NaOH-P pool but also as a major component in NaHCO$_3$-P and HNO$_3$-P pools. Large proportions of PP$_o$ are not surprising due to high P concentration promoting P assimilation by algae and bacteria that contribute to the P$_o$ pool. For all three P pools, relative concentration of P$_o$ increased downstream. In limited studies, PP$_o$ has been found to be both retained on particulate matter (Deborde et al., 2007) and slowly mineralized (Nemerey and Garnier, 2007) along a salinity gradient in differing estuarine environments.

Various proportions of nucleic acids, phytate or inositol phosphates, sugar phosphates, condensed P$_o$, phospholipids, and amino phosphoric acids, among others, can contribute to the PP$_o$ pool. Among them, phytate, an important P$_o$ form in soils (Golterman, 2006) and manure (Hill and Cade-Menun, 2009), has been reported as an important source of P$_o$ in East Creek (Stout et al., 2016). Quantitatively, phytate-P has been found to dominate P$_o$ among all sites along East Creek, ranging from 27 µmol phytate-P/L at site A to 60 µmol phytate-P/L at Site L (Figure 2; Stout et al., 2016). Furthermore, > 95% of isolated bacteria from site L were able to mineralize phytate, and higher amounts of phytate mineralizing bacteria were found in the water column than the sediment (Stout et al., 2016). While composition of other P$_o$ forms have not been studied in East Creek, understanding P$_o$ composition and transformations are likely to be important in P cycling within the creek as well as in transport to the bay. Thus, further research is needed to determine the fate of PP$_o$ in East Creek.
4.3 Bioavailability of Particulate Phosphorus in the Water Column during Transport

4.3.1 Bioavailable Phosphorus in Suspended Particulate Matter

In East Creek, P concentrations are much higher than the recommended minimum for the criteria to sustain healthy aquatic life (1 µM P; EPA, 2000). Microorganisms have been found to cycle bioavailable P even if P concentration is in excess of their biological requirement, thus imprinting a distinct isotope signature on bioavailable P pools (e.g., Blake et al., 2005; Stout et al., 2014). This allows the application of $\delta^{18}O_P$ values to assess whether certain P pools have been microbially accessed and cycled. Depending on the isotopic composition of the original source and its flux in relation to the rate of biological cycling, imprinted isotope values vary between original source values and equilibrium isotopic compositions in the natural environmental. Recently, determining PP bioavailability has been scrutinized as a larger concern than quantifying total PP loading to water bodies (e.g., Ishee et al., 2015). Therefore, $\delta^{18}O_P$ values of sequentially extracted P pools could help constrain whether certain P pools are accessed by microbes in various environmental conditions and thus determine their bioavailability.

$\delta^{18}O_P$ values of PP provided a clear distinction of bioavailable and unavailable P$_i$ pools (Figure 11). As shown, NaHCO$_3$-P$_i$ $\delta^{18}O_P$ values lie within the equilibrium range at most sites, demonstrating that this PP$_i$ pool has been microbially cycled. This means that a significant albeit variable amount of PP transported from agricultural areas to the Chesapeake Bay is bioavailable and could directly impact water quality. NaHCO$_3$-P, corresponding to loosely sorbed P, is considered bioavailable P in soils (Olsen, 1954; Tiessen and Moir, 1993) and in controlled paired isotope and concentration studies (Jaisi et al., 2011). Thus, this result verifies that NaHCO$_3$-P is a
suitable proxy to help quantify bioavailable PP, which is important since sequential extractions and bioassays are the most common tools used to assess PP bioavailability.

Bioavailability of PP can be influenced by a variety of factors in any given setting, although most studies show that a significant amount of PP is bioavailable. In one study, 25-70% of PP from one storm event was interpreted to be either bioavailable or at risk to become bioavailable after deposition (Pacini and Gatcher, 1999). Particulate P bioavailability was found to remain around 24% in a Puget Sound agricultural catchment regardless of baseflow or stormflow conditions (Ellison and Brett, 2006). Understanding how stormflow impacts PP bioavailability in East Creek would thus be an interesting next step because stormflow often mobilizes a substantial P load in a short amount of time, so this could be more quantitatively important information for nutrient management.

Isotopic analysis provided insight into bioavailability for P\textsubscript{i} in suspended particulate matter. However, many forms of PP\textsubscript{o} could also be differentially accessed by microbes. Depending on the stoichiometric nutrient need, uptake of P\textsubscript{o} can be facilitated (Rees et al., 2009). Mineralization of other, more labile, P\textsubscript{o} could also greatly increase amount of bioavailable P (Hedley and Stewart, 1982; Rees et al., 2009). As mentioned above (section 4.2), active phytate mineralizing bacteria have been found in the East Creek water column (Stout et al., 2016) and could actively degrade phytate, and similar cases are expected for other PP\textsubscript{o} compounds. Although phytate is not generally considered an available P source, its mineralization would increase bioavailable P in the water column. Thus, phytate or other P\textsubscript{o} could be a greatly underestimated source of P\textsubscript{i} in East Creek.
4.3.2 Biologically Unavailable Phosphorus in Suspended Particulate Matter

NaOH-P, corresponding to Fe and Al oxide bound P, has been defined as moderately or conditionally bioavailable depending on both efficiency of organisms to access NaOH-P; and relative P concentrations in different P pools (Tiessen et al., 1983; Zhang and MacKenzie, 1997). However, imposed biogeochemical conditions, such as reducing environments or high pH, may cause P release from this pool into a bioavailable P pool. $\delta^{18}O_P$ values of NaOH-P; and HNO$_3$-P; pools were heavier than the equilibrium composition in almost all cases (Figure 11) and were distinctly different from the NaHCO$_3$-P; pool. This demonstrates that particulate NaOH-P; and HNO$_3$-P; pools have clearly not been microbially cycled.

Discrimination of unavailable P is significant because NaOH-P; is a dominant PP pool and might be less of an immediate environmental concern than NaHCO$_3$-P;.

This study shows that NaOH-P; has not been biologically cycled once in the main channel of East Creek, nor is it likely formed in the main channel of East Creek. This means that NaOH-P; is unavailable along the entire salinity gradient in East Creek. Questions about the ultimate fate of NaOH-P; in estuarine environments, such as after export to the Bay and following its deposition, are beyond the scope of this research but have been previously researched (see Joshi et al., 2015; Li et al., 2016a, b).

Another significant finding is that since NaOH-P; remained largely uncycled in East Creek, this pool allows source tracking of PP in the water column (see section 4.4).

Isotopic composition of HNO$_3$-P; was similar to that of NaOH-P; and was distinctly heavier than the equilibrium range. It also appeared to be less likely formed from interspecies transformations between P pools (Joshi et al., 2016) in the water column. HNO$_3$-P;, corresponding to Ca-P precipitates, is not directly bioavailable. Thus, this PP pool is both quantitatively and qualitatively less of an environmental
concern in East Creek. Furthermore, out-of-equilibrium isotopic compositions of this pool allows identification of its source (see section 4.4.). In summary, two PP pools identified as unavailable for microbial uptake along the entirety of East Creek are NaOH-P\textsubscript{i} and HNO\textsubscript{3}-P\textsubscript{i}.

4.4 Phosphorus Sources, Sinks, and Cycling in East Creek

Isotope ratios in phosphate have been increasingly applied to identify P sources and track P in natural environments (e.g., McLaughlin et al., 2006; Elsbury et al., 2009; Jaisi and Blake, 2010; Gross et al., 2013). However, this method has not yet been applied to source tracking of PP. Tracking sources using $\delta^{18}$O\textsubscript{P} values is based on isotope fidelity either (i) remaining the same in the sink as in the source, or (ii) imprinting a new isotope value on the sink, but following a known pathway distinctive of a certain source, process, or enzymatic reaction (Jaisi et al., 2011; Jaisi and Blake, 2014; Wu et al., 2015). While identification of source(s) in the first case is straightforward, the second case often requires calculations and certain assumptions (Jaisi et al., 2014). In both cases, however, isotopic partitioning methods require a discernible difference between isotopic signatures of the start (source) and finish (sink) (Stout et al., 2014). If there is more than one potential P source, source signatures must be distinct in order to separate their contribution to the sink. In some cases, even if source signatures overlap, the use of $\delta^{18}$O\textsubscript{P} values in more than one P pool can help constrain the P source.

In East Creek, two P pools were suitable for source tracking: NaOH-P\textsubscript{i} and HNO\textsubscript{3}-P\textsubscript{i}. These P pools were heavier than equilibrium throughout the mainstem channel. The only plausible explanation causing $\delta^{18}$O\textsubscript{P} values of PP to lie above the equilibrium range is that these P pools have not been cycled by microorganisms and
carry a distinct P source signature. Thus, both NaOH-P<sub>i</sub> and HNO<sub>3</sub>-P<sub>i</sub> pools could serve as source tracers in East Creek. When using these P pools as source tracers, it is important to keep in mind that physicochemical and biogeochemical processes along East Creek can alter these pools due to source mixing and/or partial removal of certain P pools. For example, increased salinity could cause Fe oxide-bound P release, and Ca-P is likely to precipitate if P<sub>i</sub> concentration is high in the presence of high Ca and low Mg (Li et al., 2015).

Potential sources of PP in the East Creek watershed, including soil from various land cover, manure, streambank, and sediment, have partially overlapping δ<sup>18</sup>O<sub>P</sub> values in both NaOH-P<sub>i</sub> and HNO<sub>3</sub>-P<sub>i</sub> pools (Figure 19). While particulate δ<sup>18</sup>O<sub>P</sub> values of NaOH-P<sub>i</sub> in some sites lie partially within potential source δ<sup>18</sup>O<sub>P</sub> values, other values trend lighter than source signatures (Figure 19a). The opposite is true for δ<sup>18</sup>O<sub>P</sub> values of HNO<sub>3</sub>-P<sub>i</sub> as some data are slightly heavier than source values (Figure 19b). Overlap of δ<sup>18</sup>O<sub>P</sub> values between sources and suspended particulate matter could mean that PP is derived from a mixture of P sources in varying proportions, including agricultural soil as well as legacy P sources such as streambanks (e.g. Massoudieh et al., 2013), wetlands, or forest (Sharpley et al., 2013). Differentiating proportional contribution from multiple sources with overlapping δ<sup>18</sup>O<sub>P</sub> signatures is a next step and beyond the scope of this research. However, based on the relative concentrations of P pools, corresponding δ<sup>18</sup>O<sub>P</sub> values, composition of conservative elements in source sites, and knowledge of site specific physicochemical and biogeochemical conditions, inferences can be made about potential PP sources. Details of these parameters are discussed below.
Figure 19: Measured $\delta^{18}O_P$ values of various P sources in the East Creek Watershed for NaOH-P (a) and HNO$_3$-P (b). Note that “Bank” and “Sed” in (a) refer to streambank and sediment, respectively.
4.4.1 Role of topsoil Erosion Contributing to East Creek Suspended Particulate Matter

The agricultural fields actively contribute a large P load to the ditch and thus East Creek (see section 4.1). Phosphorus concentration of Hedley pools is much higher in agricultural fields than wetland or forest soils, suggesting that agricultural field soil is a more likely contributor to PP in East Creek than soil under other land cover. For context, Mehlich-3 soil test P in the agricultural field by site L was 16.81 µmol/g (521 ppm) in 2013, far above the agronomic optimum of 1.61-3.23 µmol/g (50-100 ppm) (Sunendra Joshi, unpublished data). Evidence from $\delta^{18}$O$_P$ values of agricultural soil and multi-element sediment fingerprinting suggest that agricultural fields could contribute to a portion of PP in suspended particulate matter.

$\delta^{18}$O$_P$ values of NaOH-P$_i$ pools in manure and agricultural soil are slightly heavier (typically +22‰ to +24‰) than particulate NaOH-P$_i$ pools (+18‰ to +22‰). Interestingly, measured $\delta^{18}$O$_P$ values of H$_2$O-P$_i$ pools in agricultural soils range around +20‰ to +25‰. If dissolved P was transported from P rich topsoil in agricultural fields to the ditch via subsurface flow and subsequently bound to Fe oxides before microbial cycling could fully imprint equilibrium isotope compositions, $\delta^{18}$O$_P$ values would be similar to measured values: lighter than the agricultural field $\delta^{18}$O$_P$ values yet not quite equilibrated in the creek. While this condition is likely, it is unclear whether this pathway is significant. In general, this pathway is anticipated to be of lesser importance than temporal and site-specific conditions of high primary productivity and pH promoting dissolved P release at the time of sampling (see section 4.1.2).

HNO$_3$-P$_i$ pools, perhaps a more reliable tool for source tracking in East Creek, do provide some evidence that agricultural soil might be a source of PP; $\delta^{18}$O$_P$ values
of HNO3-Pi pools in suspended particulate matter overlap with those of the agricultural field soil and manure. The HNO3-Pi pool is not likely recycled, and any dissolution (from decrease in pH, Figure 7) would not change the isotopic composition of residual HNO3-Pi (Liang and Blake, 2007). Formation of HNO3-Pi pools in the ditch (specifically, sites K and L) is highly likely due to the intense algal bloom and subsequent degradation of organic debris during or after settling at the sediment-water interface (Joshi et al., 2015; Li et al., 2016). δ18O values of HNO3-Pi pools, if formed from remineralization, would be very light and are dependent on the composition of degraded Po species (Joshi et al. 2015), thus resulting in much lighter δ18O values of HNO3-Pi pool than other sites. However, low HNO3-Pi:NaOH-Pi ratios were observed in the ditch, likely discounting Ca-P precipitate formation as a dominant mechanism at sampling time. Both HNO3-Pi values demonstrating overlap between P in agricultural soil and suspended particulate matter as well as some overlap in multi-element concentrations suggest that agricultural areas likely contribute at least a portion of PP to the water column, although dissolved P transport from agricultural areas is likely an important mechanism as well.

Wetland soils may act as a source or sink of P in the East Creek watershed. The lower half of the studied watershed is entirely surrounded by wetland, but a thin strip of wetland surrounds the northern half of the creek as well. This wetland strip is flooded during high tide. However, drainage ditches in the agriculture area allow nutrients to bypass this wetland strip, which might otherwise act as a buffer and remove P or become a legacy P reserve in this watershed (Sharpley et al., 2013). This is consistent with results from concentration measurements; low P concentrations in wetlands suggest that they are not a likely P source to the water column. Wetland soils
do not appear to be the primary source of suspended particulate matter to the creek, although some elemental profiles do overlap. In addition, forest soils do not likely contribute to suspended particulate matter or PP loads in East Creek due to low soil P concentrations and relatively far proximity of forests from the creek. In general, forest soils in the East Creek watershed are not likely important legacy P reservoirs. Thus, agricultural fields are the most likely land cover that contributes to a portion of PP.

4.4.2 Remobilization of Streambank Material as a Source of Suspended Particulate Matter

Remobilization of streambank particles can occur despite best management practices in the field (Sharpley et al., 2013) and streambanks have been recently scrutinized as a dominant source of legacy PP to rivers (Massoudieh et al., 2013; Sharpley et al., 2013; Grundtner et al., 2014). However, streambank material that is under-saturated with respect to P sorption capacity could also act as a sink for dissolved P (Ishee et al., 2015). Fine particles enriched in P can be easily deposited and remobilized from streambanks. Light particles that are less likely to settle also tend to have greater surface area (such as Fe oxides) with higher capacity to sorb P. Thus, particles enriched with P might be more likely to deposit on streambanks than to settle in the sediment. Although concentration of P pools in streambank material is greater than that in sediment, HNO₃-Pᵢ:NaOH-Pᵢ ratios coincide between sediment and streambank material in sites near the bay. This could suggest that similar physicochemical processes are driving the P pool composition or that streambank material can be mobilized and deposited in sediments, contributing to the sedimentary P pool.
At sites F and J, where streambank samples were collected from meanders in East Creek, P concentration of streambank sediment is closer to agricultural fields (Figure 16) than other potential PP sources. In contrast, streambank material from site H had very low P concentration, likely because the creek is relatively straight at this site. Creek structure and hydrology influences both the buildup of legacy P reserves as a P sink and subsequently the potential of this P to become a source to the water column.

Streambanks exhibited heavier $\delta^{18}O_P$ values of NaOH-$P_i$ than NaHCO$_3$-$P_i$ (Figure 17). Interestingly, NaOH-$P_i$ $\delta^{18}O_P$ values of streambanks (+22‰ to +23.5‰) are within the range of NaOH-$P_i$ $\delta^{18}O_P$ values from the agricultural field (~+22‰ to +24‰) and are only slightly heavier than particulate NaOH-$P_i$ $\delta^{18}O_P$ values (+18‰ to +22‰). In addition, conservative element profiles of suspended particulate matter were a relatively close match to streambank material. P concentration, NaOH-$P_i$ $\delta^{18}O_P$ values, and conservative elements collectively support streambanks as a major legacy P source of PP in East Creek. On the other hand, however, these parameters could all suggest that streambanks are a current sink of suspended particulate matter deposition in meanders or during high tide.

### 4.4.3 Sedimentary Phosphorus Release more Prominent than Remobilization

Upwards flux of dissolved porewater P into the water column is likely a more dominant P source than physical remobilization of sediments in East Creek. Both the profile of porewater P concentrations in East Creek sediment (Sunendra Joshi, unpublished data) and results from controlled laboratory experiments (Upreti et al., 2015) suggest dissolved P flux into the water column occurs under natural conditions in East Creek sediment. Irrespective of the mode of release (reductive dissolution, pH
promoted dissolution or ionic exchange), the end result is an increase in water column P. Isotope values of P released from sediments depends primarily on the parental sources and extent of biological cycling. Isotope data support greater likeliness of P release from sediment than physical remobilization of particulate matter from sediments. The latter is unexpected unless flow promotes bottom scouring, which is uncommon in coastal estuaries. Very little overlap existed between conservative element profiles in sediment and suspended particulate matter, also suggesting that remobilization of sediment is not likely occurring to a significant extent. Sediment NaOH-P$_i$ $\delta^{18}$O$_P$ values are heavier than NaHCO$_3$-P$_i$ values, with NaHCO$_3$-P$_i$ trending closer towards equilibrium values (Figure 15). For example, $\delta^{18}$O$_P$ values of NaHCO$_3$-P$_i$ and NaOH-P$_i$, respectively, at Site H are 18 ‰ and 27 ‰, and K are 25 ‰ and 26 ‰.

Heavy $\delta^{18}$O$_P$ values could result from biogeochemical process occurring in the sediment. As exhibited in Jaisi et al. (2010), fractionation occurs when phosphate with lighter $\delta^{18}$O$_P$ values desorbs from Fe oxides, leaving the resultant Fe oxide pool enriched in heavier $\delta^{18}$O$_P$ values. Jaisi et al. (2010) found that continual ion exchange between heavy and light $\delta^{18}$O$_P$ values overcomes the original fractionation over time (>2000 hrs) in a closed system. In contrast, a measurable fractionation might result in a semi-open system. A portion of dissolved P$_i$ is constantly removed from the sediment through flux into the water column followed by downstream movement, potentially causing heavier resultant Fe-oxide bound P $\delta^{18}$O$_P$ values. At site H, mechanisms of sedimentary NaOH-P release could be displacement of P with increasing salinity or reductive dissolution of Fe oxides (see section 4.2). At site K,
pH>PZC drives Fe oxide-bound P release (see section 4.1.2). While this is speculative, it could explain heavy $\delta^{18}O_P$ values of sediment NaOH-Pi pools in sites H and J.

Sediment from Site F, however, exhibited much lighter $\delta^{18}O_P$ values than observed at other sediment sites (Figure 15), suggestive of deposited suspended particulate matter controlling $\delta^{18}O_P$ values in this site. This indicates that processes could be diverse among sites in this dynamic creek, and data from a few points, while useful to pinpoint local processes, might not be extrapolated or generalized across the whole creek. Nonetheless, based on P pool concentrations, isotope values, and inert element concentrations in both suspended particulate matter and sediment, physical remobilization of sediment is not a likely a significant source of PP in East Creek. Sedimentary P release, however, via remineralization or desorption is likely a continual source of dissolved P to the water column as evidenced by isotope data, porewater P profiles, and consistency with past laboratory studies.
Chapter 5
SUMMARY AND CONCLUSIONS

Suspended particulate matter is the least studied P form in aquatic environments. With increasing concern that P perhaps plays a more important role than other nutrients in coastal water quality, it is imperative that speciation of phosphorus in different forms, especially particulate and dissolved forms, be better understood. This includes different P pools within PP, both organic and inorganic. Furthermore, bioavailability of particular P pools is an important question to assess the role of varying P forms on water quality. For effective nutrient management of upland sources, identification of sources and biogeochemical conditions of P release from these sources is required. In this research, quantitation of different P pools and their corresponding isotope compositions in suspended particulate matter and potential P source sites were analyzed in East Creek, a coastal creek draining to the Chesapeake Bay.

Isotopic composition of P pools allowed discrimination of bioavailable and unavailable PP pools in East Creek. NaHCO$_3$-P$_i$, a large yet variable P pool, was found to have been cycled by microorganisms. In contrast, NaOH-P$_i$ and HNO$_3$-P$_i$ pools were not accessed by microbes throughout the entirety of East Creek, suggesting that they are unavailable P pools from the source to the ultimate sink. This means that a considerable amount of PP is not an immediate environmental concern. Since a large fraction of PP is both available and cycled even when the concentration of dissolved P$_i$ is above stoichiometric nutrient requirements, bioavailable PP pools cannot be ignored.
when calculating P loading and export out of the watershed. However, it is important to note that the $\delta^{18}O_P$ values reflected in a certain pool may more accurately reflect whether PO$_4$ that is forming a given pool has been cycled and does not necessarily reflect that microbes are actually accessing microbes in a certain pool (Joshi et al., 2016). Thus, interpreting P pools as bioavailable based on $\delta^{18}O_P$ values in equilibrium alone should be done with caution.

Among the potential upland sources of P, agricultural fields likely contribute a certain proportion to the PP pool. This contribution could vary seasonally, with post-harvest barren-soil more likely to release more PP than during cropping seasons. Soils under different land cover, such as forest and wetland, did not seem to significantly contribute as a PP source. Interestingly, streambanks were the most likely source (or alternatively, sink) of PP in the water column. Legacy P from streambank reserves could have a lasting impact on P loading to the bay, and further research is needed to understand what hydrological and biogeochemical conditions promote P release from these source sites. While physical remobilization of sediments is not expected, remineralization of P$_o$ as well P release via reductive dissolution of Fe minerals or ion exchange in sulfate rich, high salinity regions likely act as constant P source to the water column. Thus, agricultural fields, streambanks, and sediments are all important P sources to the water column, although relative contribution as well as mechanisms of P release to the creek from each source is expected to differ among source, space, and time.

Transport of PP through East Creek, as is true for similar tidal rivers or estuarine environments, is influenced by tides, salinity (and solution chemistry), and temperature. These factors control deposition and remineralization/remobilization,
sorption and desorption, and biological uptake and release. Quantitative understanding of these factors through a source vector approach is expected to contribute scientifically through mechanistic insights on processes and practical applications through translating these finding that could be helpful to devise or revise management practices to reduce or limit P loading to the bay.

5.1 Future Directions

This thesis research has highlighted important research questions for future exploration. One future research direction is to determine optimal conditions for using NaOH-P\(_i\) and HNO\(_3\)-P\(_i\) \(^{18}\)O\(_P\) values to track sources of PP. For example, NaOH-P\(_i\) might be a more effective tracer in areas or seasons where algal blooms are not present or when the receiving waters are a lake instead of an estuary that has a salinity gradient impacting the P\(_i\) pool. In addition, source tracking might be more effective for both P\(_i\) pools under storm events when suspended particulate matter is transported rapidly through creeks. This short residence time could halt alteration of the NaOH-P\(_i\) pool in particular, as Fe/Al oxides are sensitive to pH and redox driven P release. Regardless of which P pool or combination of pools is used, pairing source tracking of PP through isotopes with multi-element sediment fingerprinting can add strength to results.

Determining the source of PP during storm events is perhaps more important than during baseflow. Future research should consider the potential for change of source, bioavailability, and general composition of PP during storm events. Since P is strongly bound to soil, storm events can greatly alter P loading to the Chesapeake Bay or other receiving waters, and identifying changes in speciation and bioavailability released P from critical source areas during baseflow and storm events could have
great implications for understanding legacy P and determining how to best manage P in both agricultural soils and waterways.

Further understanding of PP\(_o\) is also necessary. Although PP\(_o\) made up >50% of TP in East Creek, assessment on its bioavailability or origin PP\(_o\) was not attainable based on methodology in this study yet is necessary to fully understand the impact of PP on water quality. As research on P\(_o\) has been gaining speed in recent years, methodological limitations aimed to understand P\(_o\) should minimize, allowing for progress in this area.

Lastly, in addition to future applications and knowledge gaps in the natural environments, laboratory experiments to help constrain P cycling and transformations would be useful to better understand and interpret \(\delta^{18}O_P\) values measured in the natural environments. This will help clarify results from data in natural environments and is the only way that the use of phosphate oxygen isotopes research will successfully move forward.
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