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Understanding the Role of Recalcitrant Organic Phosphorus Mineralization on Harmful Algal Blooms at the Sediment-Water Interface of Freshwater System

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UNDERSTANDING THE ROLE OF RECALCITRANT ORGANIC PHOSPHORUS
MINERALIZATION ON HARMFUL ALGAL BLOOMS AT THE SEDIMENT-WATER
INTERFACE OF FRESHWATER SYSTEM

by

TASNUVA FARNAZ

Under the Direction of

FRANCISCO CUBAS SUAZO

ABSTRACT

Over the past few decades, an enormous number of resources have been allocated to reduce the amount of phosphorus (P) entering the environment from both point and non-point sources. Nevertheless, the decrease in external P loading has not always resulted in a simultaneous decrease in the occurrence of harmful algal blooms (HABs) in waters contaminated with phosphorus. A two-part study is currently being conducted to investigate and measure the role of legacy recalcitrant P forms in the overall pool of soluble reactive phosphorus (SRP). However, the objective of this particular study is to evaluate the potential of Organic Phosphorus (PO) mineralization towards the SRP pool. Results from field data and lab-scaled experiments, showed that under both initial low and high OP levels sediments from streams subject to legacy P loadings released OP and organic P when subject to different environments. The total phosphorus (TP) levels at the Occoquan and RAC sample sites were primarily influenced by redox reactions and organic phosphorus associated with iron-bound sediments. However, in the Ogeechee River sample stations, the regulation of TP was not just affected by OP because there was no presence of Fe-bound OP, but also by organic P. Furthermore, while anaerobic conditions improve the overall process of PO mineralization, it is evident that the OP/TP ratio also changes when exposed

to aerobic environments. Thus, results from this study invariably indicate that recalcitrant organic P forms are important sources for OP as microorganisms are required to mineralize organic P under low dissolved OP conditions.

INDEX WORDS: Recalcitrant Organic Phosphorus, Soluble Reactive Phosphorous, Harmful Algal Blooms, Mineralization, Low and High OP environment

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MASTER OF SCIENCE

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DEDICATION

This thesis book is dedicated to Zoharin, my only child, who is among the most beautiful, smart, witty, talented, and yet kind-hearted individuals I have ever met. Mummy loves you to the moon and back!

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I would like to express my appreciation to my advisor, Dr. Francisco Cubas, for his unwavering support throughout my research endeavor. The support and motivation he provided greatly bolstered my confidence to undertake and carry out all the tests and analysis. I would like to express my gratitude to KM Risaduzzaman and Iffat Tasnim for their valuable contributions in assisting me with the current research led by Dr. Francisco Cubas. I would also like to express my gratitude to Md. Nizam Uddin for his voluntary efforts during the sampling stage. Additionally, I would like to appreciate Keiner Sanchez and Osely Perea from the 100,000 Strong in the Americas Exchange Program for their exceptional involvement in the setup and operation of two Occoquan reservoir reactors. Finally, I am grateful to Dr. Lewis Stetson Rowles and Dr. Rocio L. Perez for helping me comprehend the intricate details that pertain exclusively to my research.

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CHAPTER 1

INTRODUCTION

Harmful algal blooms (HABs) provide a significant environmental challenge in every state in the United States. These are simple aquatic plants that proliferate excessively in both marine and freshwater environments, resulting in the production of toxic substances that can have detrimental impacts on humans, fish, and the whole biodiversity. As per the US Environmental Protection Agency (EPA), HABs have the potential to generate highly toxic poisons that can cause illness or death in both humans and animals. Additionally, they can lead to the formation of dead zones in water bodies, increase the costs associated with treating drinking water, and negatively impact industries reliant on clean water. Although N as a nutrient contributes to algae growth, scientific investigations and experiments have proved that P is the primary component responsible for nuisance algal bloom. Including both flowing and lentic waters, P transport and availability to aquatic systems is a key driver of biological processes. There are many types of organophosphorus compounds that serve as SRP sources in freshwater. Common forms include orthophosphate di and monoesters (including inositol phosphate (IP), phosphonates, and biological polyphosphates, which may be readily available for biological use or recalcitrant in nature. OP has consistently been the primary focus of attention due to its high abundance from external sources, its bioavailability, and the simplicity of measurement (Ni et al., 2022). Despite the implementation of strict rules and regulations to regulate external loading, the status of HABs has not improved globally. According to the classical viewpoint on phosphorus cycling in lakes and streams, when phosphate enters the system, it is quickly taken in by both living and non-living suspended particles. The non-living particles then settle at the bottom of the lake. Once deposited in the

sediment, the PO is liberated from the sediments by bacterial decomposition, which takes place when the bottom waters or sediments of a lake or stream become depleted of oxygen. The contribution of PO recalcitrant forms to the pool of SRP has historically been overlooked due to the challenging nature of characterizing PO (Macintosh et al., 2018). However, the release of P from internal sources, primarily due to the breakdown of organic matter, may play a significant role in the initiation and persistence of HABs, especially since external sources of phosphorous have been recently regulated. Numerous researchers additionally pointed out that internal phosphorus loading in reservoirs with limited external contributions hinders the improvement of water quality (Moura et al., 2020) by delaying the regeneration of waterbodies by approximately 10–15 years (Paytan et al., 2017). A two-part study has been conducted to comprehensively analyze and measure the extent to which legacy recalcitrant P forms contribute to the overall pool of SRP. The goal of the proposed research is to measure the potential of PO to the total pool of SRP that initiates and fuels HABs in freshwater.

1.1 Purpose of this study

Surface water management has traditionally focused on controlling nutrient species that create an immediate impact on water quality, ignoring or minimizing the long-term negative effects of other species. In the case of P, common sampling protocols used to assess P effects on eutrophication focus mainly on measuring SRP and TP in water samples and sporadically in sediments, while less information is collected on OP production from PO. Additionally, in the last few decades, huge efforts to control OP discharges from point and nonpoint sources have effectively decreased external loadings, but the effects of having high concentrations of P in freshwaters are still present. Massive HABs, eutrophication, and surface water hypoxia fueled by high P concentrations are still persistent water quality and economic problems affecting the US

and the world. This research will advance current surface water management practices by changing our fundamental understanding of the P cycle and by providing quantitative evidence demonstrating that it is critical to control long-term P sources to prevent HABs and eutrophication in freshwaters.

1.2 Goals and Objectives

The objectives of this part of the study are: 1) assessing the capacity of PO found in particulate forms in freshwater sediments in OP production through the microbial mineralization; 2) determining the surrounding environmental conditions (i.e., OP and TP ratios, pH and redox levels) in which PO is preferentially mineralized by biological degradation process. The primary goal of the comprehensive study is to make precise measurements of Inositol Phosphate (IP) dephosphorylation. This will provide a deeper understanding of the P cycle by quantifying the formation of OP from PO that are typically regarded as non-labile. Understanding IP mineralization dynamics will also help to determine how recalcitrant PO forms trigger HABs in different environmental conditions.

1.3 Hypothesis

Due to the lack of tools to precisely measure OP extraction from PO forms and the abundance of OP in eutrophic waters; the contribution from PO recalcitrant forms to the biologically available soluble reactive phosphorus (SRP) pool has traditionally been ignored. The internal Phosphorous (P) loading, which is mostly driven by PO mineralization, may have a substantial role in initiating and maintaining HABs as the external loading has recently been controlled through regulations. On that note, in both high and low OP situations where external phosphorus inputs are restricted, the PO present in sediment has the potential to release OP through

mineralization and thus the sediment can act like a sink for soluble SRP with high phosphorus repository.

CHAPTER 2

LITERATURE REVIEW

The transportation and delivery of phosphorus to aquatic systems play a crucial role in stimulating biological activity in both flowing water and static water. Eutrophication of a freshwater body can occur when there is an overabundance of phosphorus and nitrogen, in particular, flowing into the water from nearby watersheds. Eutrophication is a prevalent issue in rivers, estuaries, lakes, and coastal environments. It occurs when these bodies of water become excessively enriched with nitrogen and phosphorus, leading to numerous detrimental impacts on aquatic ecosystems.



Figure 1. A harmful red-tide algal bloom at offshore of San Diego County, California
(Source: <https://arstechnica.com/>)



Figure 2. An aerial view of Lake Okeechobee in Florida shows a cyanobacterial bloom
(Source: www.tcpalm.com)

An adverse consequence is the heightened proliferation of nuisance algae, such as cyanobacteria (also known as blue-green algae) (Wetzel 2001). These blooms are responsible for

several water-related issues such as fish die-offs in the summer, unpleasant smells, toxins, degradation of drinking water quality, difficulties in operating water treatment facilities, and the creation of trihalomethane during water chlorination in treatment plants (Hallegraeff., 2003). Algal blooms in marine environments, often known as red or brown tides, have detrimental effects due to the release of toxins and the depletion of oxygen caused by the decomposition of dead algae (Cooke et al. 2005, Carpenter et al. 1998). This necessitates the introduction of more effective strategies to control phosphorus loads originating from urban centers and excessively fertilized agricultural regions.

Phosphorus sources that enter surface water can be categorized as either point sources or non-point sources. Wastewater discharges and drainage from stormwater are considered sources for point source pollution. On the other hand, phosphorus runoff from impervious urban areas and pervious surfaces like prairie, forest, and cultivated regions are examples of non-point sources of phosphorus (Novotny 2003). Phosphorus from natural sources enters surface water bodies through processes such as the disintegration of soil parent materials, the riparian vegetation, erosion of river banks, and the release of phosphorus by various aquatic organisms (Withers and Jarvie 2008, House 2003).

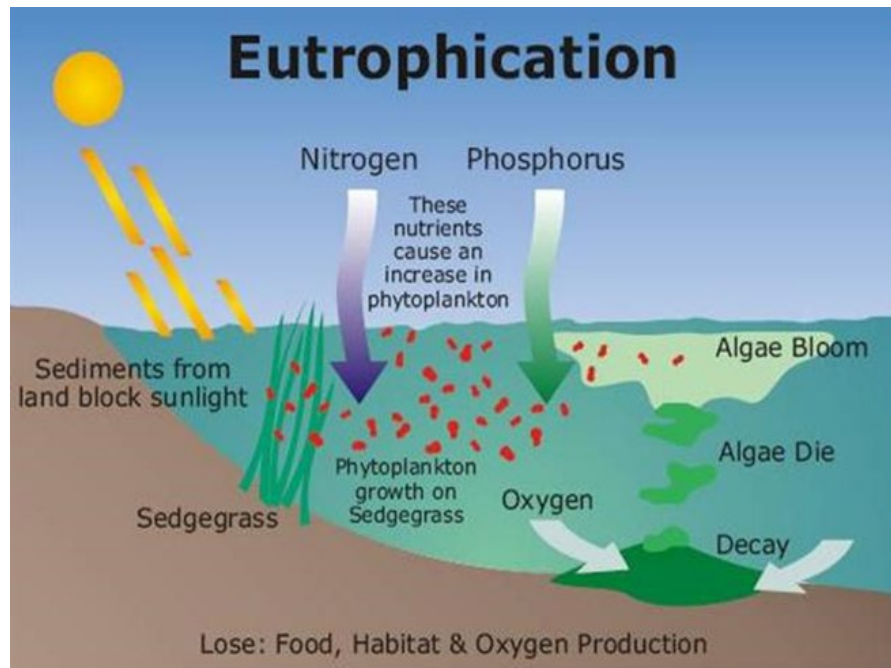


Figure 3. Role of Nitrogen and Phosphorus in eutrophication (Source: <https://indianriverlagoonnews.org/>)

Urbanization and intensified farming have caused significant pollution of surface waters in the US with excessive nutrients (Cradock et al, 2015). Phosphorus is often discussed in broad contexts, however, the most vital form for living beings is phosphate (PO_4^{3-}). Phosphate is an essential ingredient that restricts the proliferation of microbes in certain aquatic ecosystems. It is the most limiting necessary nutrient, surpassing even nitrogen. Urbanization and expanding food production have caused a persistent rise in P concentrations in surface water bodies. Eutrophication, as defined by Le Moal et al. (2019) and Withers et al. (2008), has led to a range of social, environmental, and financial difficulties at both the national and local scales. Rivers are very vulnerable to adverse effects due to their proximity to human populations, their sensitivity to alterations in land use, and their intensive exploitation (Adla et al., 2022, Walsh et al., 2005). The European Water Framework Directive (WFD) has lately been enforced, along with its regulatory guidelines, with the objective of attaining a harmonized ecological condition in different

waterbodies by 2015. Consequently, it is now imperative to understand the mechanisms by which various sources of phosphorus (P) reach rivers, their ecological importance, and the appropriate methods for monitoring them.

Agricultural and urban activities are commonly recognized as the primary contributors of phosphorus to aquatic ecosystems. Agriculture is the primary cause of nonpoint nutrient pollution, particularly phosphorus, in the United States. The amount of phosphorus transferred to surface waters is directly related to the phosphorus content of soils, increased runoff, and erosion. Phosphorus often enters a water body in a dissolved condition, but a significant portion is also carried as particulate phosphorus. Over time, this particulate phosphorus can become accessible to aquatic organisms (Sharpley et al., 1994; Carpenter et al., 1998). Phosphorus being the most crucial macronutrient in aquatic systems, often works as the main factor that limits primary production. If there is an excessive supply of phosphorus from human activities over a long period of time, it can cause an increase in algal growth, leading to undesirable eutrophication (Withers and Jarvie 2008; Jorgensen et al. 2011).

2.1 Sources and transport of phosphorus to surface waters

Sources of phosphorus from urban areas include urban litter from residential activities, domestic and industrial fertilizers, leaf fall, soil particles eroded from roadside, detergents, lubricants, etc. (Withers and Jarvie 2008). The different combination of all these sources of phosphorus plus the climate and hydrologic factors affect the transport and the ultimate amount of phosphorus that reach streams and lakes. For instance, the ratio of impervious surfaces to the total urban area and their corresponding slope grades generally affect the form and the amount of phosphorus transported to surface waters (Novotny 2003).

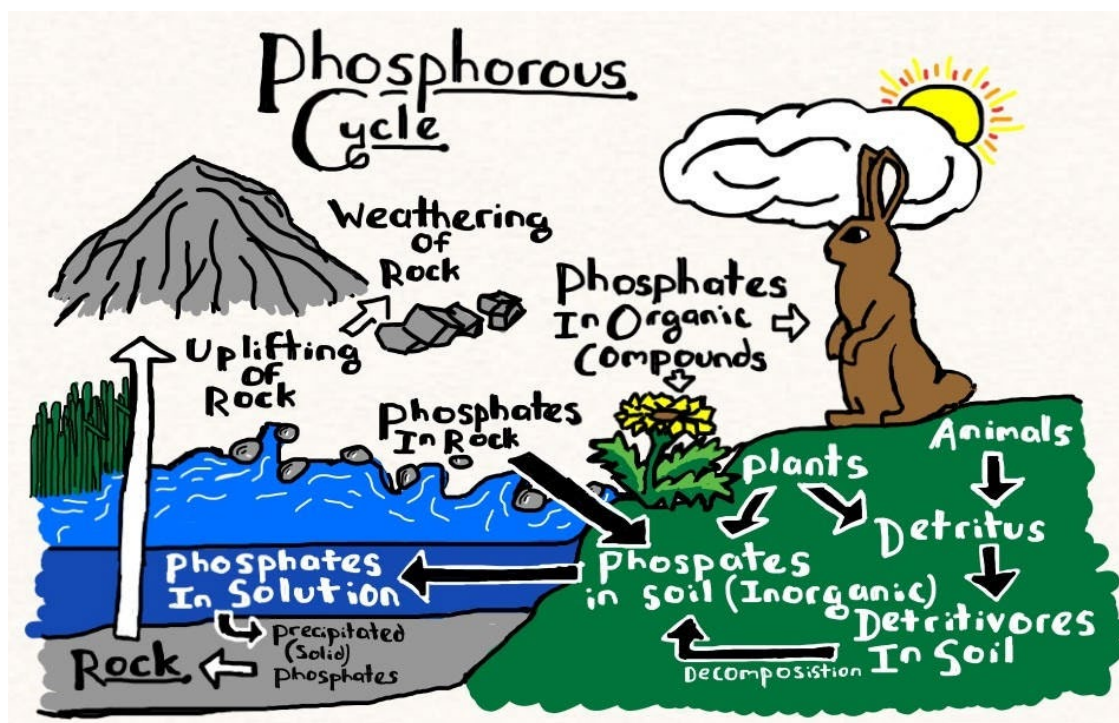


Figure 4. Phosphorus cycle in nature (Source: <https://sssorganicstore.com/>)

Pervious surfaces such as forest, cultivated lands and pasture are also affected by hydrologic factors which enhance the transport of phosphorus within the watershed. During a rain event, the resulting runoff mobilizes and transport inorganic and organic phosphorus from variable areas within the agricultural fields providing a greater degree in both temporal and spatial variability. This variability is caused by the frequency and distribution of storm events, the existing pathways for phosphorus transport and land use and management (Withers and Jarvie 2008). Phosphorus losses from agricultural land use areas including over-fertilized farm lands, concentrated discharge of livestock manure, or contamination during application of fertilizer and manure. The contribution of phosphorus from agricultural sources is a function of the phosphorus loading to soil and from the land management practices which affect the rate at which accumulated phosphorus is lost. Increased phosphorus losses have been measured after the application of fertilizer in surface runoff. Phosphorus losses from fertilizer application depend on the rate, time,

and method of application; form of fertilizer; amount and time of rainfall after application; and vegetative cover (Sharpley and Withers 1994).

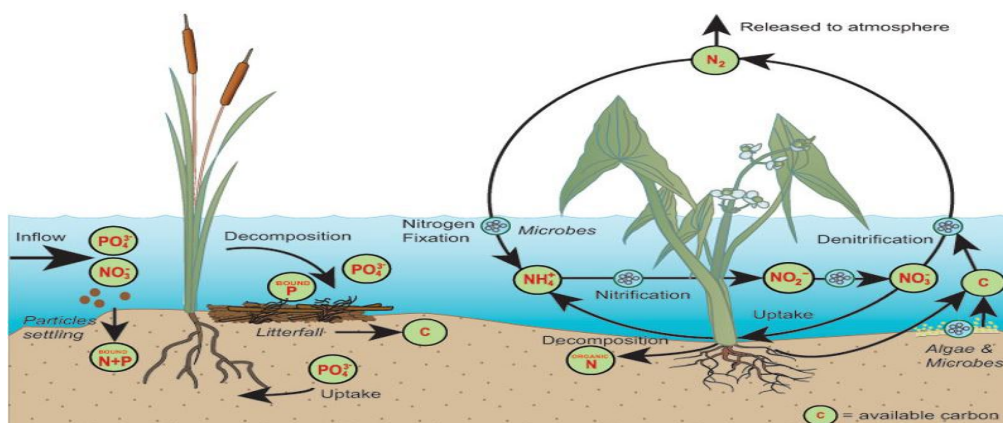


Figure 5. A simplified diagram of Phosphorus and Nitrogen cycle in wetland (Source: Kadlec and Knight, 1996)

Phosphorus derived from agricultural sources circulates in both liquid and particulate forms. The primary sources of dissolved phosphorus are orthophosphates, which are produced from soils, manure, plants, and fertilizers. On the other hand, particulate phosphorus consists of phosphorus that is sorbed by organic matter, including phosphorus, mineral forms of phosphorus, and soil particles, (Sharpley and Withers 1994). Dissolved P is readily available for uptake by aquatic biota while particulate phosphorus may provide a long term source of phosphorus to aquatic biota. When phosphorus reach the stream flow, transformations between the dissolved and the particulate forms may occur depending on their corresponding concentrations. The transformation is affected by the type capacity of the material to sorb or desorb phosphorus and also on the rate of transport. Therefore, temporal variations in rainfall intensity, time length between runoff events, and type of vegetative cover will influence the processes determining phosphorus bioavailability in runoff (Withers and Jarvie 2008).

2.2 Phosphorus cycling in aquatic systems

Phosphorus enters aquatic systems in both dissolved and particulate forms, and both forms consist of a diverse combination of pentavalent phosphorus molecules. Examples of this are: orthophosphate, pyrophosphate, organic phosphates esters, phosphodiester, and long-chain polyphosphates (Correll 1998). Particulate phosphorus that enters streams and lakes can release phosphate and organic phosphates into solution. Some of these forms of phosphorus can undergo chemical or enzymatic hydrolysis to generate orthophosphate. Among the various kinds of phosphorus, only orthophosphate can be absorbed by bacteria and algae. Consequently, the presence of extracellular enzymes is necessary to break down organic forms of phosphorus into phosphate (Correll 1998). A significant quantity of particles can also accumulate and be preserved in bottom sediments, where bacteria can slowly metabolize organic phosphorus into minerals and release substantial amounts of orthophosphate into the water column (Gabriel et al., 2006).

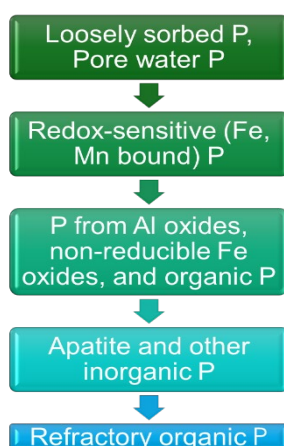


Figure 6. Various forms of Phosphorus available in nature

Phosphorus mobility in aquatic systems is influenced by many factors such as hydrological and geohydraulic conditions, microbial activity, complexation chemistry and redox conditions.

Within these factors the most important processes affecting phosphorus mobilization are mineralization of organic bound phosphorus and desorption/dissolution of redox sensitive bound phosphorus Gabriel et al. 2006, Schauser et al. 2006. Based on the conventional method of phosphorus cycling in rivers and lakes, phosphate is rapidly consumed by both living and non-living suspended particles upon its entry into the system. Ultimately, these particles settle at the lake's bottom. After bacterial decomposition, PO becomes available from sediments. The released phosphate can be bound to inorganic surfaces, combined with recalcitrant organic substances, or formed as iron sulfide (FeS) or vivianite under specific redox circumstances (Gächter and Meyer 1993). But, considering the second situation, the transport of phosphorus at the interface between water and the oxidized sediment is mainly regulated by ferric iron [Fe(III)]. Phosphate binds to ferric iron to create complexes termed as iron oxyhydroxide (FeOOH-PO₄) (Gächter et al. 1988, Mortimer 1941). The existence of oxidized sediment surface impedes the dissolution of phosphate from the sediments. It establishes a structural barrier that inhibits the transfer of phosphate from deeper anoxic layers to the water column (Gächter and Meyer 1993).

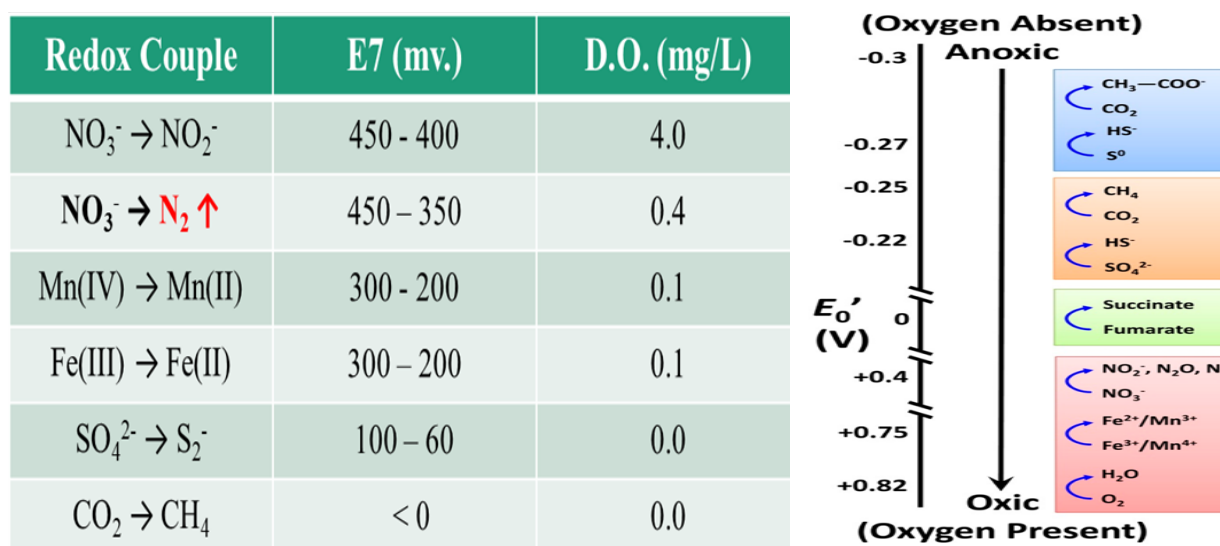


Figure 7. Electron tower for redox condition (Source: Lehman et al., 2015)

When the oxygen levels in the lower layers of a lake or stream decrease, resulting in anaerobic conditions, iron(III) undergoes reduction to iron(II), leading to the dissociation of ferric hydroxide complexes in the sediment surface. The solid Fe(III) oxyhydroxide complexes undergo reductive breakdown, leading to the release of both bound phosphorus and iron (II) into the water column. In addition, the solid ferric hydroxide serves as a physical barrier, effectively blocking the movement of phosphate from the anaerobic sediments below into the water column. However, when this barrier is removed, phosphate is able to freely diffuse into the water column. When at the sediment-water interface there are reducing conditions, sulfate is converted to the form of sulfide. This sulfide then combines with reduced iron to form iron sulfide precipitates. These precipitates deposit at the bottom of the water system under anaerobic conditions. (Stumm and Morgan 1996, Petzoldt and Uhlmann 2006) The deposition of sediment may lead to the burial of FeS, resulting in a reduction of the iron available for binding to soluble phosphate. This phenomenon occurs when bottom water layers in a river or lake change from a state of low oxygen to a state of aerobic condition (Schauser et al. 2006). In thermally stratified lakes, if specific conditions like adequate wind mixing and vertical entrainment are present, or if the phosphorus concentration gradient is too steep for efficient dissemination across the metalimnion, then a certain amount of the phosphorus can escape to the epilimnion and promote the growth of cyanobacteria, resulting in blooms.

In the classical theory outlined above, microorganisms are believed to have an indirect impact on phosphorus mobilization. They achieve this by consuming dissolved oxygen (DO) and nitrate while decomposing organic matter. This creates the necessary conditions for iron reduction and the subsequent release of phosphate. In this scenario, bacteria are treated only as a 'catalyst' that accelerates solubilization of phosphates. Unfortunately, the role of bacteria on phosphorus

mobilization is not solely of that affecting the redox conditions. Sediment bacteria in aquatic systems are also responsible for: (i) catalyzing the mineralization of PO, (ii) assimilate and store phosphorus in their biomass, (iii) store and release phosphorus depending on the stress resulting from different redox conditions, (iv) release phosphorus when they die (lysis), and (v) convert bioavailable phosphorus into particulate refractory phosphorus (Gächter and Meyer 1993).

While a number of field and laboratory investigations provide evidence for the traditional phosphorus mobilization hypothesis, it has not yet been conclusively proven that a significant portion of the phosphate released in anaerobic circumstances was previously attached to iron hydroxides. In some cases, simultaneous iron and phosphate release has not been observed (Gächter and Müller 2003). Therefore, observations of simultaneous iron and phosphate release under anoxic conditions do not provide enough evidence to conclude that the two elements were completely associated before the onset of anoxia. There are other mechanism to consider when quantifying and exploring the phosphorus cycle in lakes and streams. For example, the process of converting organic matter containing phosphorus into minerals and the retention and release of phosphorus by bacteria may play a significant role.

In eutrophic lakes, a considerable amount of organic phosphorus entering directly from the watershed or originating from algal detritus settles to the bottom. Once deposited in the sediments, vigorous aerobic or anaerobic decomposition processes might occur, leading to the mobilization of particular PO and the subsequent release of phosphates into the water column via dephosphorylation reactions. There is some evidence suggesting that aerobic conditions may favor mineralization of organic phosphorus more than anaerobic environments (Hupfer and Lewandowski 2008). If phosphate measured in the water column is a result of organic matter mineralization, then significant amounts of phosphorus may be released from aerobic sediments.

If this is the case then several physical and chemical factors that stimulate microbial activity may also enhance the release of phosphorus from the sediments. For instance, an increase in temperature, organic matter or DO content may result in more phosphorus released from the sediments (Jensen and Andersen 1992). Studies on freshwater (Hino 1989, Jansson et al. 1988) and marine environments (Suzumura and Kamatani 1995) have concluded that phosphatases, including cyanobacterial phosphatases, efficiently hydrolyze organic phosphates and the surrounding biota depends on this process (Whitton et al. 1991). When aerobic mineralization takes place, microorganisms consume oxygen while liberate excess phosphorus from organic matter. Therefore, it is difficult to distinguish whether phosphorus release is the cause or a consequence of DO depletion in the sediments.

Certain bacteria have the ability to store phosphate in the form of polyphosphates (poly-P) when there is an excess of phosphate when suitable conditions are present. For instance, when an adequate amount of organic matter and phosphate is supplied in an oxygen-rich environment, these organisms combine surplus phosphate molecules and store energy in the highly energetic poly-P compound. When there is no oxygen present, poly-P can be broken down and the resulting phosphate with a lot of energy can be used to create ATP from ADP. During this process, inorganic phosphorus is released inside the cell and then diffuses into the surrounding medium along the concentration gradient (Gächter and Meyer 1993 Hupfer et al. 2004). In the absence of oxygen, the ATP: ADP ratio decreases as a result of the absence of oxidative phosphorylation. This reduction triggers the generation of ATP by the breakdown of stored poly-P via hydrolysis (Gachter et al., 1988). The microorganisms present in the sediments can serve as a repository for phosphate by incorporating it into their biomass. Some of this phosphate becomes refractory, making it difficult for other microbes to break down and mineralize (Cheesman et al., 2014).

Another important process to consider when analyzing phosphorus release from sediments is the dissolution of manganese (Mn-P) and calcium (Ca-P) bound phosphorus as well as the aluminum bound phosphorus. The dissolution of these phosphorus complexes can occur in both aerobic and anaerobic environments and are affected more by the pH level of the surrounding environment. Therefore, in some cases dissolution of Ca-P may be more important than the redox driven Fe-coupled phosphorus cycle (Stumm and Morgan 1996, Wetzel 2001). While it is often acknowledged that oxic lake sediments retain phosphorus more effectively than anoxic sediments, it is crucial to recognize that the magnitude of other phosphorus mobilization mechanisms typically surpasses the significance of oxygen-regulated phosphorus release in conjunction with iron. Only an interdisciplinary approach incorporating hydrochemical and biogeochemical factors can help us obtain reliable information which can be useful for a better understanding of the phosphorus cycle in aquatic systems.

CHAPTER 3

A COMPREHENSIVE ANALYSIS

3.1 Introduction

The world's freshwaters are currently experiencing a significant issue with water quality resulting from eutrophication. As we succeed in controlling the external inputs of phosphorus, the internal recycling and subsequent loading of phosphorus is becoming the most important source for primary production, maintaining an undesirable trophic status, and causing algal blooms. In recent years, there has been a growing focus on PO as a unique and potential internal phosphorus pool in freshwater sediments, due to its importance in the contribution to soluble reactive phosphorus and to the development and utilization of various analytical techniques that can be used to quantify and characterize organic phosphorus (Ma et al., 2019, Zhu et al., 2013b). In addition, the presence of bioavailable PO forms in sediments and their involvement in the biogeochemical cycling of sediments are important factors for the self-regulation of phosphorus (P) levels in freshwaters as well as for maintaining their eutrophic condition (Feng et al., 2018). When studying the impact of P on eutrophication, generally conventional sampling methods mostly involve detecting SRP and TP in water samples, with occasional measurements in sediments. However, there is not much data on the generation rates of OP from PO in freshwaters (Diaz et al., 2018).

The causes of eutrophication are complex and differ depending on the specifics of aquatic systems. However, it is widely recognized that the process is exacerbated by nutrient enrichment and the decomposition of excessive organic matter (Wetzel, 2001). During the thermal stratification period in lakes, when there are no other inorganic electron acceptors present, the

depletion of oxygen through respiration is quickly followed by the development of anaerobic conditions. The prevailing conditions have been widely recognized for their ability to facilitate the release of undesirable substances, including phosphorus, iron, manganese, ammonia, and other pollutants, from sediments that have accumulated in lakes and reservoirs. This knowledge has been documented in various studies conducted by Moore and Reddy (1994), Mortimer (1941, 1971), Stumm and Morgan (1996), Weston et al. (2005), and Zhou et al. (2021). The release of nutrients, referred to as internal loading, can contribute significantly to the overall loading of nutrients in streams and lakes (Penn et al., 2000; Welch and Cooke, 2005). This is especially evident for phosphorous, as its release from sediments can significantly impact its concentration in any given system (Xu et al., 2015; Paerl et al., 2014). Many researchers have additionally observed that the internal phosphorus (P) loading in reservoirs and other freshwater systems, which have low external contributions, impedes the enhancement of water quality (Moura et al., 2020). This could be because it delays the regeneration of waterbodies by around 10-15 years (Paytan et al., 2017).

The factors contributing to the release of phosphorus from sediments are numerous and intricate (Corzo et al., 2018, Zhou et al., 2021). Phosphorus is released due to several dynamic abiotic and biotic processes in the water column and sediments. Common processes in aquatic systems include dissolution, desorption, and phosphorus diffusion due to variations in redox conditions and pH, as well as anaerobic and aerobic mineralization of organic matter containing phosphorus. These processes have been documented by Haggard et al. (2005) and Withers and Jarvie (2008). The release of phosphorus from sediments is mainly caused by the microbial reduction of iron (II) (III) oxide complexes that contain phosphates. This process leads to the release of both microbially available inorganic phosphate and ferrous iron into solution (Betel et al., 2008b; Hupfer and Lewandowski, 2008, Smith et al., 2011; Pearce et al., 2013; Wang et al.,

2016). The term "classical explanation" is hence used to refer to the widespread use of this procedure to explain the release of OP into the water column.

Numerous organophosphorus compounds serve as soluble reactive phosphorus (SRP) sources in aquatic environments. Orthophosphate monoesters, diesters, and phosphonates are among the common types that may be found. These compounds may either be readily available or refractory to degradation for any type of biological use. The absence of precise measurement instruments for quantifying the release of orthophosphate (OP) from these organic phosphorus (PO) forms (Bai et al., 2017), together with the high concentration of OP in eutrophic waters, may be considered a key factor contributing to its long-term neglect (O'Neil et al., 2012). The objective of this study is to measure the contribution of organic phosphorus to the bioavailable SRP pool by quantifying the amount of soluble reactive phosphorus (SRP) derived from refractory forms of organic phosphorus (PO) that initiates and sustains harmful algal blooms (HABs) in freshwater ecosystems. Specifically, this study is the initial part of a more comprehensive study that aims to quantify the amount of OP produced through microbial degradation of Inositol Phosphate (IP) (a recalcitrant Organic Phosphorus form) in particulate forms in freshwater. It also intends to evaluate the nutritive status of PO by establishing the functional relationship between the biodegradability of IP and its role in initiating and sustaining harmful algal blooms (HABs) through the measurement of nutrient uptake rates by algae. Additionally, the study strives to identify the specific environmental conditions, such as OP and TP ratios, pH and redox levels, and IP mobility, in which IP is preferentially mineralized other than different forms of phosphorus. Comprehending the kinetics of IP mineralization will aid in determining how recalcitrant organic forms of phosphorus generate harmful algal blooms in settings with low oxygen levels. Thus, this research may enable policymakers to comprehend the mechanisms that control the mineralization of IP for

microbial orthophosphate (OP) acquisition. This understanding can be obtained through a quantitative assessment of OP production from a specific form of phosphorus known as particulate organic (PO) phosphorus. Therefore, this progress in comprehending the P cycle will establish the foundation for enhanced nutrient management, elevated surface water quality, and the formulation of forthcoming research endeavors such as P recovery from sediments.

3.2 Study Area

The Ogeechee River, Occoquan Reservoir and RAC pond were chosen purposefully to collect inflows from sub-basins with varying land uses and different nutrient loads. This decision was made because both Ogeechee and Occoquan systems already have an established water quality monitoring system in operation. The Ogeechee River Basin has a vast range of marshes, streams, and recreational areas spanning 5,540 square miles. The Ogeechee River covers around 290 miles with various tributaries, such as the Canoochee River, which alone drains an expansive area of 1,400 square miles. Other contributing rivers include the Bear River and Big Creek, among others. This stream has been defined as a blackwater river (Meyer et al., 1997) and is one of the few rivers in Georgia that still flows freely. The Ogeechee River Basin relies heavily on forestry as a significant contributor to its economy (Figure 8). Agriculture is also a major land use activity in the region, providing support for various animal operations and manufacturing of goods. Bulloch and Tattnall Counties are among the top 10 counties in terms of swine production. Conversely, the RAC pond (Figure 9) is a reclamation pond which collects water from the wastewater treatment plant from the city of Statesboro and also storm run-offs from the RAC parking lot. As it is a reclamation pond, the OP load is really high in the water which makes it completely different from the other two river basins that we have selected for sampling. On the other hand, the Occoquan Reservoir is a component of the Occoquan Watershed in Northern Virginia, encompassing a land

area of 1,515 square kilometers (Figure 10). The distribution of land use in the watershed is as follows: approximately 54% of the area is covered by forests, 28% is urbanized, 11% is used for agriculture, and 7% is designated for pasture use (Cubas et al. 2014a). This man-made freshwater system, spanning from the northern to the southern regions, functions both as a recreational space and a water source for a population of 2 million residing in Fairfax County and Alexandria.

Although water and sediment samples were initially collected from four sampling points along the Ogeechee River, only two sites in the southeastern part of the Ogeechee River Basin in Southeast Georgia, specifically Rocky Ford landing and Ogeechee Outpost landing, were selected for the experimental investigation because of consistency.

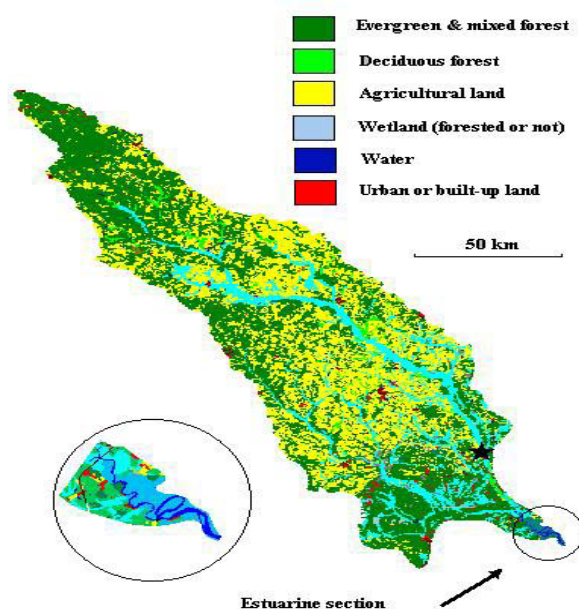


Figure 8. Ogeechee (top) watershed land use map (Source: <https://www.georgiaencyclopedia.org/>)



Figure 9. RAC pond (Reclamation Pond at GSU campus)

Ogeechee Outpost collects runoff from a mixture of pasture and forest land, whereas the station at Rocky Ford primarily collects runoff from forested areas. Conversely, the reclamation pond at Georgia Southern University (RAC) has a higher concentration of urban discharge. A single sampling station was chosen from the Occoquan Reservoir in Northern VA. The sampling station located at Occoquan is situated in the main drainage area of the reservoir, near the dam (RE10). The samples obtained at the sampling site were processed and preserved before being sent to GSU in Statesboro, GA, where all samples were analyzed.

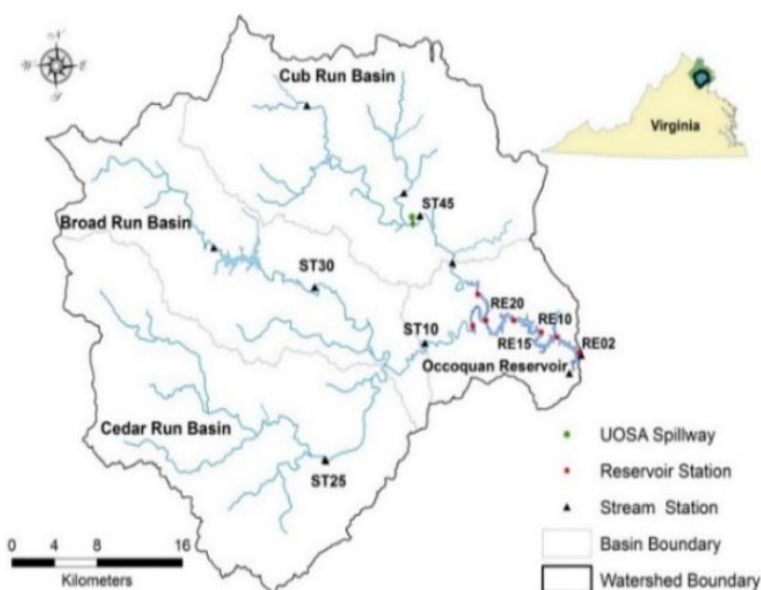


Figure 10. Occoquan (bottom) Watershed (Source: F. Cubas 2012)

3.3 Working Methods

A two-part study designed to understand and quantify the contribution of legacy recalcitrant P forms to the total pool of soluble reactive phosphorus (SRP) is underway. The operational methodology was designed to achieve the study's objectives and goals effectively and systematically. The strategy included a predetermined order of working steps that are as followed:

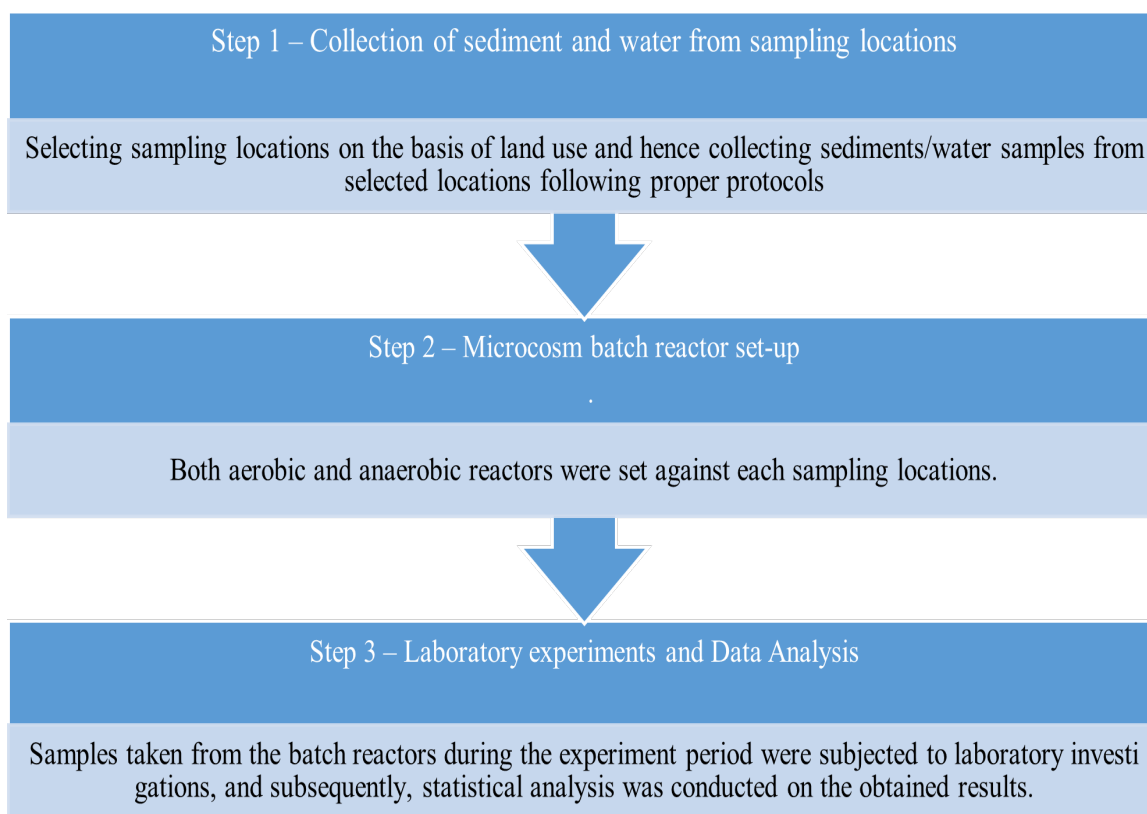


Figure 11. Tasks distribution per working step

3.3.1 Collection of sediment and water from selected sampling locations

Initially, it was important to ascertain the availability of P and IP in the waters during the investigation. To ensure the presence of IP in the samples, two distinct water bodies that receive runoff from forest, agricultural, and urban areas were selected (Cooke et al., 2005, Carpenter et al., 1998). IP is a small P-rich compound found in cereals, legumes, and oil seed crops and functions as major P storage in seeds (Doolette et al., 2010). Pollen, in a wide range of plant species (e.g., pine trees) and poultry manure are important sources of IP (Turner et al., 2002). During the initial year of the study, water samples were collected from each water system between March and September to assess the impact of seasonal variations on the levels of phosphorus (P) and total phosphorus (TP). The summer season was chosen because of the anticipated increase in enzymatic

activity and the change of the Occoquan Reservoir into a thermally stratified water body. This allows for the measurement of P in both aerobic and anaerobic hypolimnetic conditions. The sediments from all sampling stations in the Ogeechee watershed were collected and stored in glass jars at a temperature of 4°C. The water sourced from the Rocky Ford site was utilized to set up both the Rocky Ford reactors and the RAC reactors because of its initial low nutrient concentrations, limiting initial nutrient impact on the experiments. Water was collected in airtight plastic containers and subsequently stored at room temperature for all purposes. The initial phosphorus concentration in the water samples collected from Ogeechee Outpost and Rocky Ford were measured to be 0.01 mg/l as P and 0.06 mg/l as P, respectively.



Figure 12. Sediment sample collection from Ogeechee watershed



Figure 13. Water sample collection at Rocky Ford



Figure 14. Collected sediment condition of RAC pond

On the other hand, the laboratory staff members of the Occoquan Watershed were in charge of collecting and sending the sediment to the Department of Civil Engineering and Construction at Georgia Southern University. The sediment was stored in glass jars at a temperature of 4°C

before they were shipped to the GSU main campus. Sediment samples were shipped on ice containing coolers to preserve the sediments during transport and the whole duration before being used. The sediments that were received were stored in a refrigerator at an identical temperature. The water utilized to start the reactors was obtained from the Ogeechee River in Georgia (Rocky Ford station), serving as a substitute for the water from the Ocoquan River. Both water sources exhibited comparable water quality traits in terms of nutrients, with the exception of nitrate concentration, which were not part of the main focus of the study. In order to simulate the natural surroundings conditions of the Reservoir, lab-based studies were undertaken by closely reproducing them in a laboratory configuration.

3.3.2 Microcosm batch reactor set-up

To replicate oxidized and reduced circumstances, the laboratory experiments were studied in two sediment environments: aerobic (oxidized environment) and anaerobic (reduced conditions). As part of the experimental setup for all three aerobic reactors and both the aerobic and anaerobic reactors for the Ocoquan samples, five continuously stirred tank batch reactors (2000 mL flasks) and three 10L were operated within the range of 40-52 days. Three batch reactors were used in a controlled laboratory setting to evaluate the anoxic state, with all of these reactors being 10 L plexiglass containers. To preserve a natural volume-area ratio, the exposed sediment area and water volume in the microcosms were adjusted and downscaled based on the laboratory configuration. Within the setup of the microcosm experiment with 10L plexiglass cylinders, six 5 mL beakers were employed to contain the sediments, while the remaining cylinders were filled with water. To replicate the natural watershed system, approximately 4 cm of sediment was added at the bottom of the 2L volumetric flasks and rest of the flask volume was filled out with water. All the reactors were first infused with a 100% concentration of dissolved oxygen (DO) with use

of an air diffuser. The aerobic condition was achieved through the use of an air diffuser, while the anaerobic reactors were sealed to the environment and purged with nitrogen gas. To ensure the accurate dissolved oxygen (DO) concentration in all the aerobic reactors, an air pump called Whisper 40 was used to supply air when needed. The aerobic dissolved oxygen (DO) concentration was maintained by operating these reactors in an open system, allowing oxygen to enter the water column by air supply, which also facilitated the mixing.



Figure 15. Initial stage of batch reactors preparation



Figure 16. Batch reactors in operation

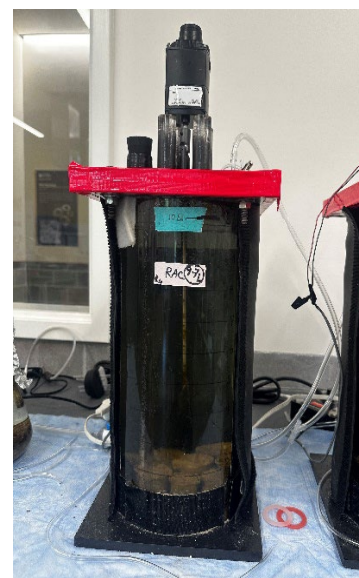


Figure 17. 10L Plexiglass anaerobic reactor

3.3.3 Laboratory experiments and Data Analysis

As a part of the research, initially, field and laboratory studies were conducted to assess the characteristics of P at each sampling location. This was done by measuring both OP and TP in water inflows and outflows, as well as in the deposited sediments. The laboratory investigations were focused on evaluating the mineralization of TP to OP in samples that were initially assumed to have significant PO levels. The evaluation of the degraded TP in a series of microcosms,

consisting of water and sediments from each sampling location, had therefore established the extent to which organic P acts as a reservoir for SRP (Golterman 2006). Dissolved oxygen (DO), Temperature, pH, Oxidation Reduction Potential (ORP) were done periodically to sustain the required experimental environments. In order to achieve the anoxic condition, sucrose ($C_{12}H_{22}O_{11}$) was introduced at a dosage of 1 mg/L to accelerate the drop in level of oxygen in the anaerobic reactors. The ORP measurements were adjusted and standardized to a standard hydrogen electrode (SHE) by adding the potential difference between the reference electrode and SHE. The ORP data were also adjusted using pH readings and temperature. The concentration of dissolved oxygen (DO), oxidation-reduction potential (ORP), and temperature levels were measured every 2 days using an HQ40d portable multimeter which was equipped with appropriate DO and ORP probes.



Figure 18. Measuring DO with HQ40d Portable Multimeter



Figure 19. DO measuring probe & ORP measuring probe used with HQ40d Portable Multimeter

Water samples were taken at regular intervals of 3-5 days to consistently monitor the nutrient levels throughout the experiment. By following standard procedures (Rice 2017), the concentration of nutrients was determined using a Thermo Scientific™ 32 Gallery™ Discrete Analyzer. Water samples were obtained from the upper and lower levels of the reactors utilizing a custom-made probe consisting of a syringe and a connected pipe. The lower sampling was conducted at a depth of 2cm above the sediment layer in order to investigate the highest levels of enzymatic activity. The phosphorus water samples were subsequently evaluated using the Thermo Scientific™ Gallery™ Discrete Analyzer following standard methods (insert the reference). To prevent the mixing of sediments with water during the collection process, a gentle suction was applied to create a little pressure. For each instance, a quantity of 20-25 milliliters of water was collected in distinct sample tubes for obtaining both OP (Orthophosphate) and TP (Total Phosphorous) results and the volume extracted was replaced by new water. The study would be considered successful if the majority of samples collected from both water bodies include substantial levels of PO in comparison to the available OP. This is important since the presence of PO can serve as a repository for SRP. In the second run of the nutrient analyzer, in order to determine the TP level of the collected sample water, digestion has been done to convert all P form to OP by using standard operating procedure for the determination of TP by Alkaline Persulfate Digestion (Patton et al., 2003). After that, the processed samples were passed through a filter with a pore size of 0.45 μm and subsequently analyzed with the Thermo Scientific™ Gallery™ Discrete Analyzer. After collecting and analyzing all the data, *the criteria for success, in this case, would be the recognition of support suggesting that organic phosphorus, which has been overlooked for a significant period, has the ability to serve as a source of soluble reactive phosphorus (SRP) under*

favorable conditions in the environment, such as low orthophosphate (OP) levels, extended aerobic and anaerobic conditions, and so on.

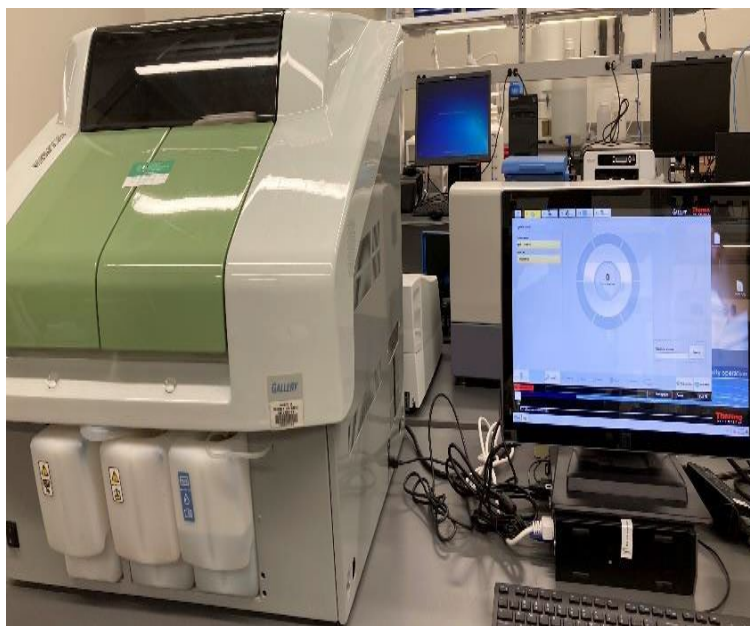


Figure 20. Thermo Scientific Gallery Discrete Analyzer used for nutrient concentration

3.4 Results and Analyses

3.4.1 Field Data and Preliminary Assessment in the Occoquan Reservoir

The role of inorganic P on eutrophication and subsequent HABs in freshwater is well understood. Nonetheless, there is still a significant knowledge gap regarding the bioavailability of organic P and its role in promoting HABs (Feng. Et al, 2018). From a qualitative perspective, it is known that soil-borne organic P entering freshwater ecosystems may serve as a continuous source of nutrients, perpetuating HABs (Heisler. Et Al, 2008). However, the amount of organic P that becomes readily bioavailable, the rate at which it is mineralized, and its ability to initiate and sustain HABs are poorly understood (Vilmin. Et al, 2018).

Data from field observations made during the summer months in the Occoquan Reservoir showed that not all the phosphorus being consumed was OP released from the sediments. Reservoir field data revealed that P was released from the sediments after the onset of reduced conditions at the SWI (Figure. 21). Under reduced conditions, most of the TP measured in the water column was OP. However, by early August, OP decreased while TP concentrations kept increasing until late September. Initial OP accumulation was the result of iron-bound OP that was released as iron (III) was reduced. As OP became abundant it began to decrease as microorganisms consumed it. By the end of the summer, it is very possible that the increase in OP concentrations was the result of organic P mineralization because other sources, such as iron-bound OP, were already depleted earlier in the summer. The OP released towards the end of the summer was also consumed.

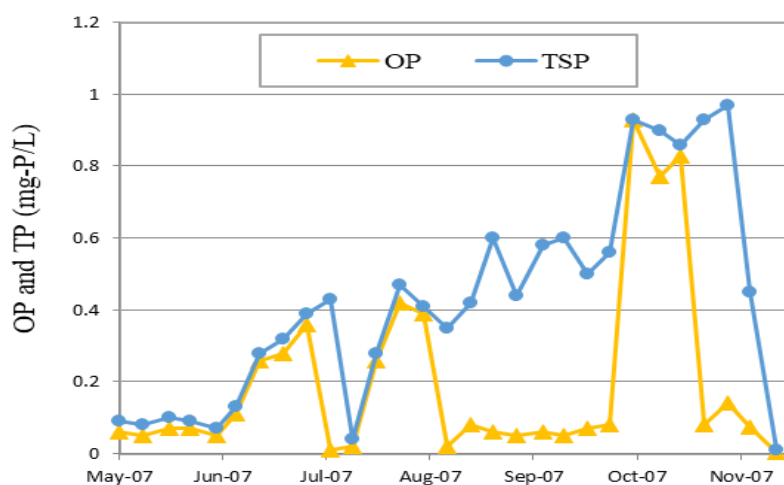


Figure 21. OP and TP bottom waters Occoquan reservoir

To demonstrate that not all OP observed in the reservoir was the result of iron bound phosphorus controlled by redox reactions, lab-controlled experiments using sediments from the reservoir were done as a preliminary assessment. Microcosm studies revealed that soluble OP accumulation was initially the result of OP bound to iron that was released when sediment Fe^{3+}

was reduced to Fe^{2+} confirming the classical explanation of sediment OP release (Figure. 22). However, a high correlation between OP and the total soluble iron ($R^2 = 0.85$) existed only at the early stage (day 22-45), but after day 45 OP continued to accumulate although the Fe concentrations decreased. It is possible that the accumulation of OP at the end of the experiment was the result of organic P mineralization. Continuous changes in the OP:TP ratio at the sediment water interface (SWI) prompted the need to quantify organic P dephosphorylation in subsequent studies.

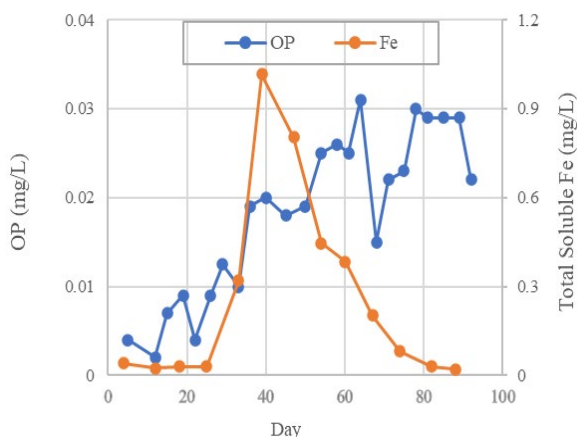


Figure 22. Time series for OP and total soluble Fe microcosms with Occoquan sediments

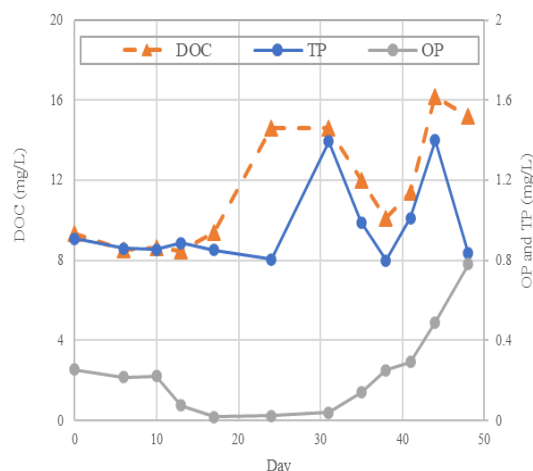


Figure 23. OP, TP & DOC bottom Occoquan reservoir

Results also showed that when OP concentrations were low in a reduced environment, and that organic matter (OM) and organic P were released from the sediments (Figure. 23). Initially, TP concentrations decreased as OP decreased. However, as the reduced environment continued within the reactor, OM and TP release increased while the OP levels remained low suggesting the mineralization of organic P in the absence of SRP. As organic P was mineralized, OP increased supporting a second TP spike. The availability of phosphorus promoted microbial activity and OM mineralization supporting the OM increase at the end of the experiment. It is also possible that the

second Dissolved Organic Carbon (DOC) peak was more closely related to the presence of algae as the chlorophyll-a concentration increased from less than 0.1 to more than 3 $\mu\text{g/L}$ at the end of the experiment (data not shown), suggesting that OP from the mineralization of organic P promoted algal growth in the absence of initial SRP.

3.4.2 Laboratory Data Analyses

Eight reactors were set up to conduct lab-based research investigations using sediment and water from four sampling locations, replicating natural environment conditions. The experiments had a length of 40-52 days. The reactors were first aerated until they reached a dissolved oxygen concentration near 100% saturation, roughly 8.8 – 8.9 mg/L. This was considered the start of the experimental run. The table below presents the reactor numbers that will be referenced based on sample locations and experimental setting.

Table 1. Assigning reactor numbers to corresponding sample sites

Reactor Number	R2	R3	R4	R5	R6	R7	R11	R12
Location & Sediment Condition	RAC Pond, Aerobic	Ogeechee Outpost, Aerobic	Rocky Ford, Aerobic	RAC Pond, Anaerobic	Ogeechee Outpost, Anaerobic	Rocky Ford, Anaerobic	Occoquan reservoir, Aerobic	Occoquan reservoir, Anaerobic

The DO levels of reactors R2, R3, R4, and R11 were monitored every other day and kept within the range of 8.7-8.9 mg/l by aerating as needed to maintain complete aerobic environment for the sediment all through the experiment. Reactors R5, R6, R7, and R12 were operated to achieve shifting DO concentrations (transition from aerobic to anaerobic) in the water column. This was done to create a fully anaerobic sediment environment by maintaining DO concentrations as near to 0 mg/L as possible. Following the initial loss of oxygen, it took approximately 10-12

days to transition into the anoxic stage with negative ORP values. Water sampling was conducted on a biweekly basis, with the frequency determined by the DO, ORP, and pH levels. In both the aerobic and anaerobic reactors, water samples were collected and then replenished to maintain the initial volume throughout the experiment.

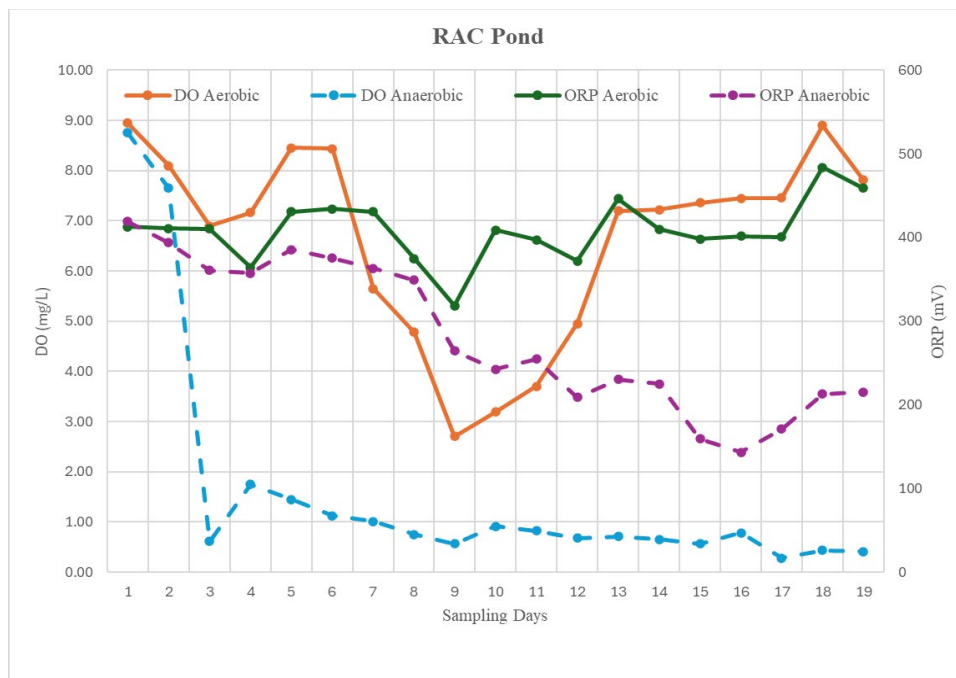


Figure 24. DO and ORP graph of RAC pond (Aerobic & Anaerobic)

At our first sampling station, RAC pond (reactors 2 and 5), which acts as a reclamation pond and collects run-offs from nearby parking lots and a wastewater treatment facility (secondary treatment only), the DO levels for the aerobic condition (R2) were consistently maintained within the range of 2.70-8.95 mg/l, with an average of 6.65 mg/l (Figure. 24). Upon careful review of the readings, it was observed that there was a decrease in oxygen levels in the middle of the run. This decrease can be attributed to the increasing microbial activity in the sediments. However, the DO levels were later improved through the implementation of sufficient aeration. The oxidation-

reduction potential (ORP) curve exhibited a similar pattern to the DO curve and remained within the range of 318.53 mV to 484.07 mV, with an average value of 408.72 mV during the whole duration of the experiment. ORP results for R2 demonstrated that oxidized conditions prevailed in this reactor throughout the experimental run. The pH remained within the normal range of 6.9-7.8, with an average value of 7.3. The temperature during the whole experimental period lied within the range between 19.2 to 23.1°C. Conversely, in reactor R5 under anaerobic conditions, the average DO concentration was 0.79 mg/l, with a minimum value of 0.28 mg/l after the initial oxygen depletion. When the reactor was in a completely anaerobic state, the average ORP value was 280.7 mV, with the lowest recorded value being 143.31 mV showing a brief period of slight reduced conditions above the sediment water interface between days 7 and 13 (Figure. 24). The temperature and pH were controlled within the range of 19.1 to 23.4°C and 6.7-7.4, respectively. The average OP/TP ratio for reactor R2 and R5 were maintained 0.333 for both the aerobic and anaerobic sediment conditions (Figure. 25). Throughout the duration of the aerobic run, there were no instances where the OP/TP ratio deviated significantly from the average. However, in the RAC anaerobic reactor, there was a significant decrease to 0.3 and 0.25 on sampling day 6 and 38 respectively, but it quickly returned to its usual levels afterwards. On the sixth day of sampling, it is possible that the presence of loosely bound organic pollutants (OP) in the water caused the observed phenomenon. However, on the 38th day, it is expected that the phenomenon is a result of the mineralization of organic P.

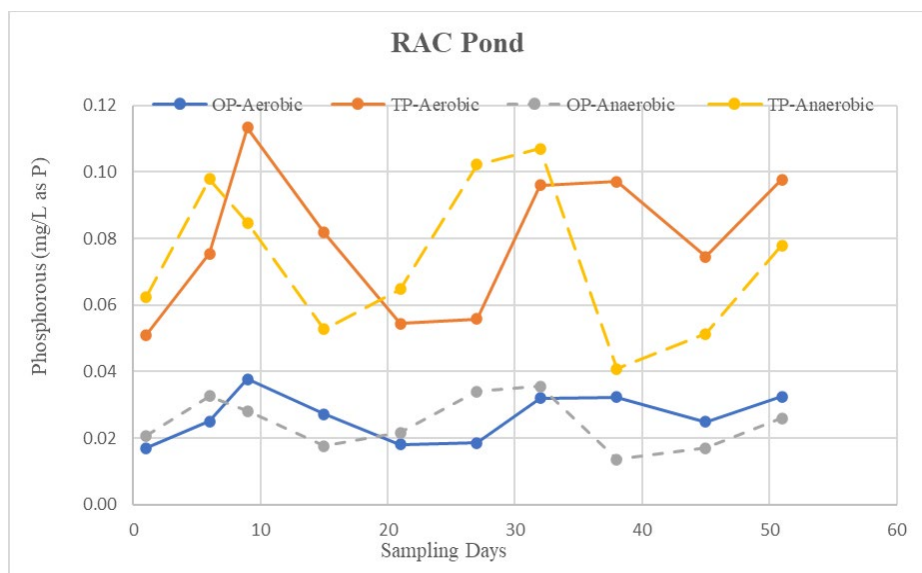


Figure 25. OP and TP graph of RAC pond (Aerobic & Anaerobic)

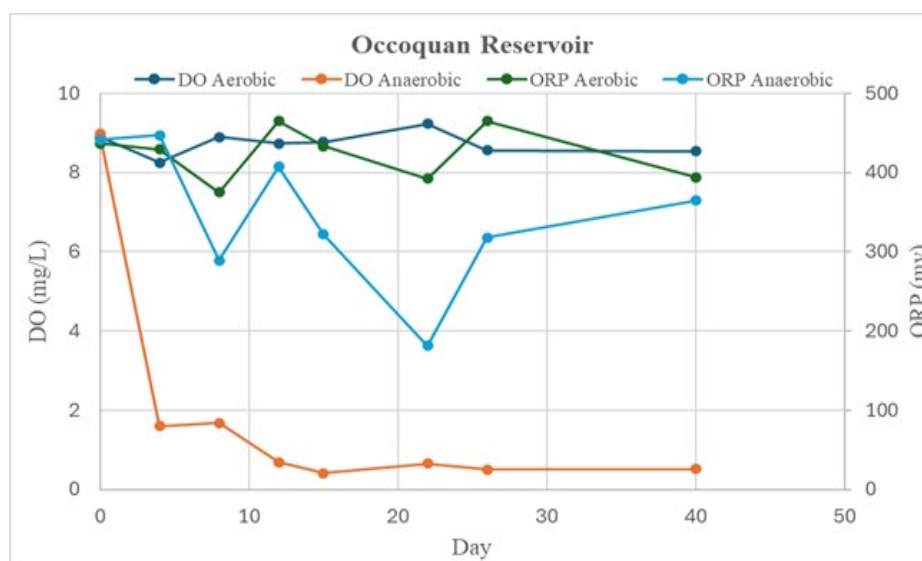


Figure 26. DO and ORP graph of Occoquan reservoir (Aerobic & Anaerobic)

Observation into the Occoquan Reservoir sediments revealed that these sediments were very productive and contained a significant amount of organic matter as seen in both R11 and R12. As a result, the DO level occasionally dropped below 4 mg/l in R11 (aerobic reactor). During those

instances, the reactor was supplied with air, which restored dissolved oxygen levels to the aerobic condition. The dissolved oxygen (DO) level, following aeration, remained consistently within the range of 8.25 to 9.23 mg/l, with an average of 8.74 mg/l (Figure. 26). The pH ranged from 7 to 7.6 while the temperature varied between 19.0 and 22.0 °C. Similarly, the ORP values varied from 375.1 to 465.1 mV in response to changes in oxygen, with a few exceptions at certain sampling dates. DO and ORP results showed that oxidized conditions prevailed in R11. In the anaerobic reactor, the DO value decreased to below 2 mg/L in the initial days of the experiment, and consistently remained between 0.41-1.61 mg/L sustaining anaerobic sediment conditions, indicating sustained anaerobic productivity in R12. The pH level remained consistently low at 6.7 during the anaerobic phase, occasionally experiencing a jump to 7. The lower pH was probably due to higher CO₂ levels because of high productivity and anaerobic conditions that developed in this reactor. The temperature ranged between 19.10 and 22.10 °C, while the ORP values ranged from 181.25 to 447.27 mV, with a average alue of 340 mV.

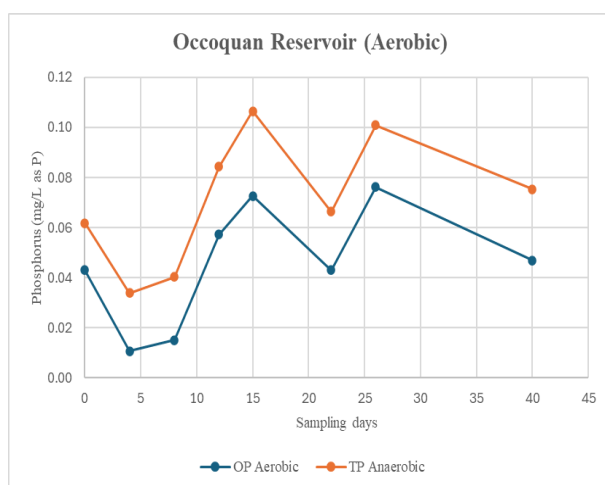


Figure 27. OP and TP graph of Occoquan reservoir (Aerobic)

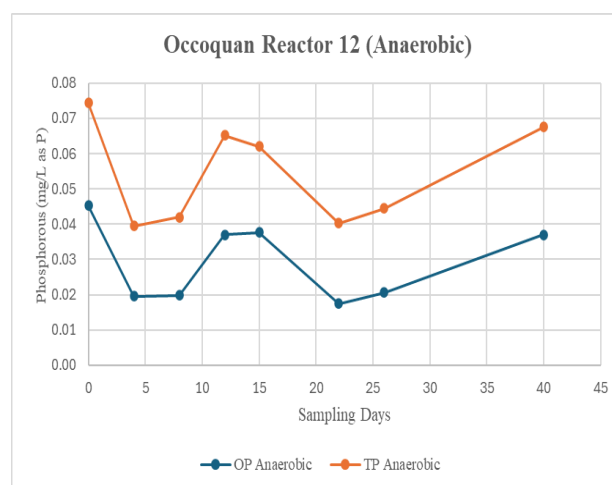


Figure 28. OP and TP graph of Occoquan reservoir (Anaerobic)

OP and TP concentrations varied following a similar trend throughout the experiment (Figure. 27 & 28). In the aerobic reactor both OP and TP concentrations decreased at the beginning of the experiment, due to some initial OP consumption while the sediments acclimated to the high oxygen levels. After day 5, OP and TP levels increased possibly due to the mineralization of liable organic P within the sediments as redox levels were not low enough to promote OP release from the reduction of iron in the sediments. Previous experiments have shown that sediments from the Occoquan are rich in iron and susceptible to iron reduction and subsequent OP release under anaerobic conditions. From day 15 until the end of the experiment, OP variation was not excessive suggesting a consistent OP level for most of the experiment. In the anaerobic reactor, phosphorus values ranged from 0.02 to 0.05 mg/l for OP and 0.04 to 0.07 mg/l for TP, resulting in a consistent OP/TP ratio of 0.64 for both sediment conditions (Figure. 28). At the beginning of the experiment, OP and TP concentrations changed following a similar trend to the aerobic reactor. Initially, phosphorus levels decreased as DO was used to mineralize organic matter. A slight increase in phosphorus levels was observed between day 7 and 15 probably due to the mineralization of liable organic P similar to what happened in the aerobic reactor. From days 15 to 22 phosphorus levels decreased as DO was still being consumed within the reactors. However, after day 23, an increase in OP and TP levels was observed due to the release of OP under reduced conditions as the system became anaerobic and reduced. It is probable that the OP levels in this reactor would have increased more if the experiment duration was increased. It is important to highlight that both OP and TP concentration changes followed a similar trend in both reactors suggesting that changes in TP values were predominantly driven by changes in OP and not by significant changes in organic P concentrations.

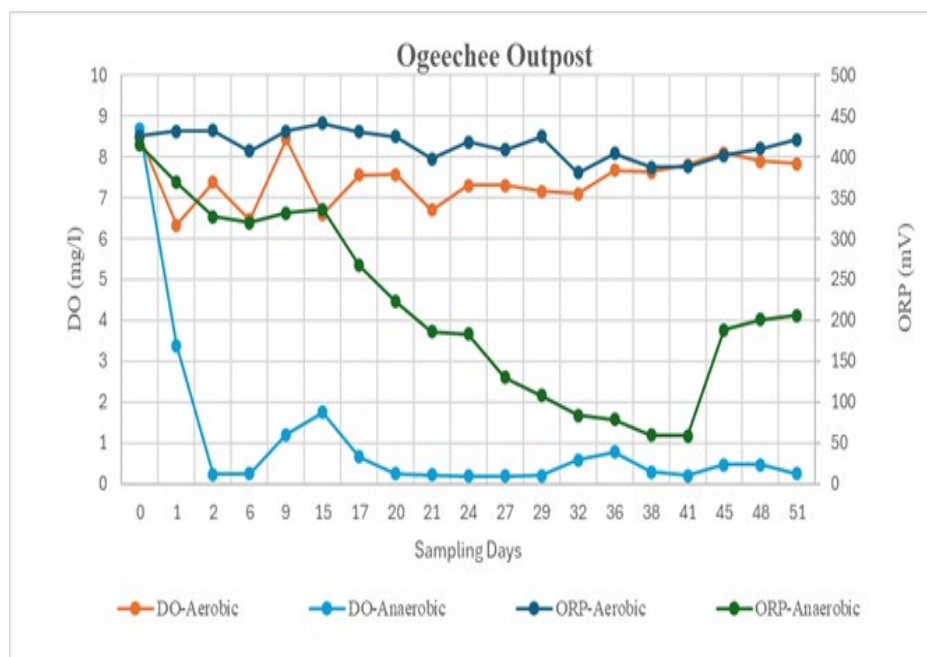


Figure 29. DO and ORP graph of Ogeechee outpost (Aerobic & Anaerobic)

In the case of reactor R3 (aerobic) and R6 (anaerobic) using samples from Ogeechee Outpost, the sediment's low productivity made it relatively simple to sustain aerobic conditions at R3. On the other hand, achieving complete anaerobic conditions for long period of time in R6 was quite challenging. R3 maintained a consistent pH range of 6.9-8 throughout the experiment, whereas the temperature ranged from 19.3-23.1 °C. The DO and ORP graphs exhibited a highly symmetrical trend, with median values of 7.44 mg/l and 414.04 mV, respectively (Figure 29). In R6, the pH value decreased to a minimum of 6 as the system became anaerobic, with an average value of 6.5. The temperature stayed within the same range as R3. The minimum dissolved oxygen (DO) level recorded was 0.19 mg/l for the anaerobic reactor, while the average DO level was 0.48 mg/l. Conversely, an extended anaerobic condition resulted in a decrease in oxidation-reduction potential (ORP) of up to 58.58 mV, with a median value of 214.23 mV for the entire anaerobic period.

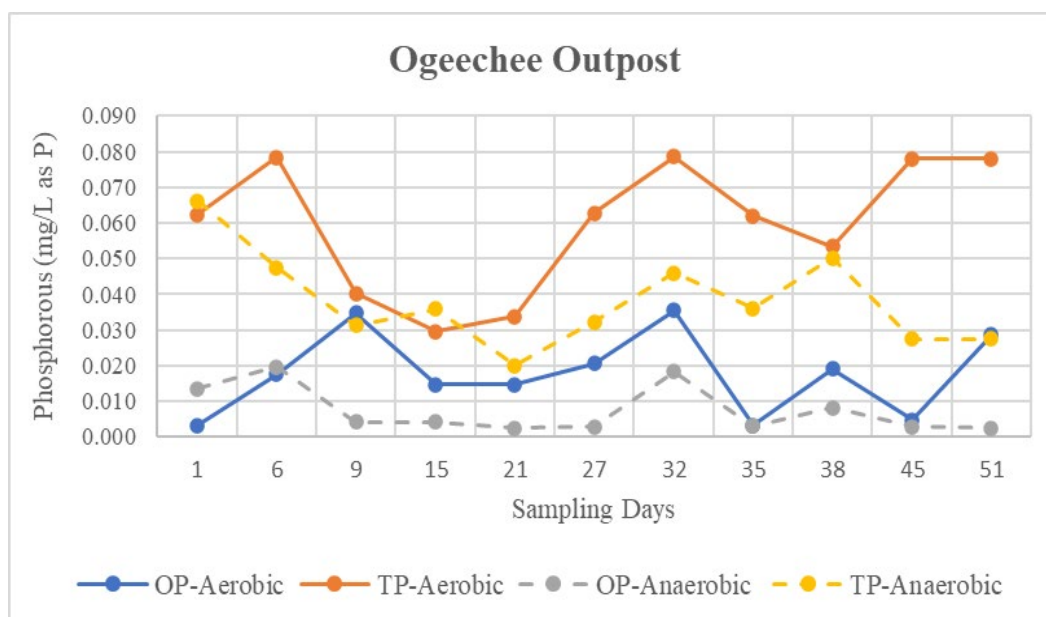


Figure 30. OP and TP graph of Ogeechee Outpost (Aerobic & Anaerobic)

Regarding phosphorus levels, reactor R3 exhibited a minimal OP load in the water. The average values for OP and TP in R3 were measured at 0.018 and 0.06 mg/l, respectively, resulting in an average OP/TP ratio of 0.3. At the beginning of the experiment, both OP and TP increased simultaneously, but after day 6 TP decreased while OP increased. This sudden change in OP and TP trend suggested that TP was not initially controlled solely by OP loads whether external or internal. After day 9, OP and TP began to decrease bringing the TP concentration to its lower observed level of 0.03 mg-P/L (Figure. 30). Through the remainder of the experiment, OP and TP levels exhibited similar trends, but with some changes when comparing their values. In certain occasions, the OP/TP ratio did not exhibit a consistent pattern, especially during Days 6, 35, and 45, where it decreased to 0.22, 0.05, and 0.06 correspondingly. While it is typically anticipated that this amount of OP/TP alteration occurs mostly in anaerobic reactors, it should be noted that the increase in TP is solely attributable to the mineralization of organic P, as the OP load in the

water was very minimal and had no influence on this change. For reactor R6, the average values of organic P and TP were determined to be 0.007 and 0.038 mg/l, respectively. Additionally, there was a significant change in the OP/TP ratio, which shifted to 0.19 compared to the aerobic condition. The transition of the sediment environment to an anaerobic state has led to a progressively more noticeable change in the OP/TP ratio. On day 15, 35, and 45, there was a deviation to 0.11, 0.05, and 0.1, respectively. This can be solely attributed to the mineralization of PO under prolonged anaerobic conditions.

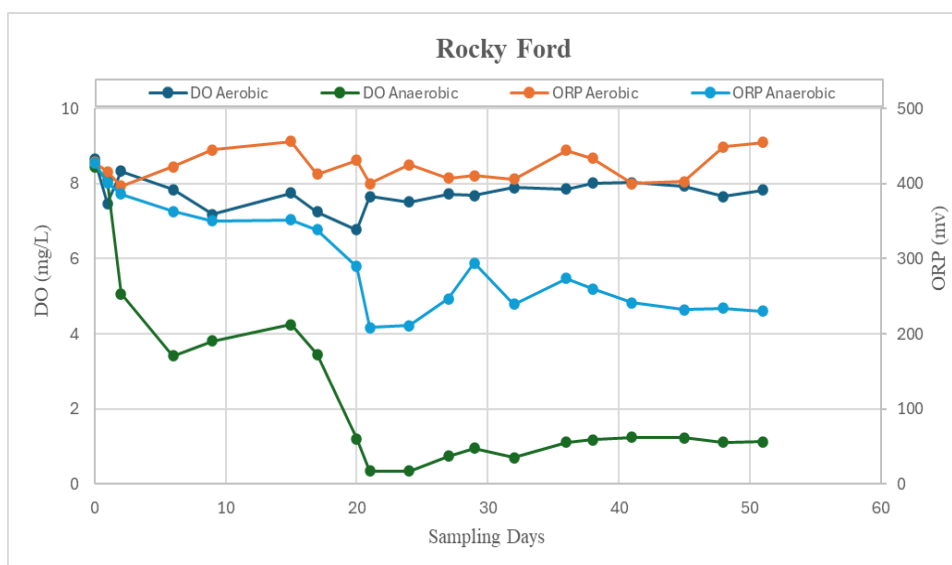


Figure 31. DO and ORP graph of Rocky Ford (Aerobic & Anaerobic)

When analyzing the graphs of Rocky Ford reactors R4 and R7, a consistent trend in both the dissolved oxygen (DO) and oxidation-reduction potential (ORP) curves can definitely be noticed. The range of dissolved oxygen (DO) levels for R4 ranged from 6.77 to 8.66 mg/l, with an average of 7.74 mg/l. The range of ORP values observed was between 396.93 and 456.48 mV, with an average value of 423.23 mV. The pH range observed during the experiment remained

consistently around 7.5, while the temperature remained within the range of 19 to 23.1 °C. In contrast, in R7, the minimum values for DO, ORP, and pH were 0.35 mg/l, 208.51 mV, and 6.8, respectively. The temperature remained consistently within the range of 18.9 to 23.4 °C throughout the entire duration of the experiment.

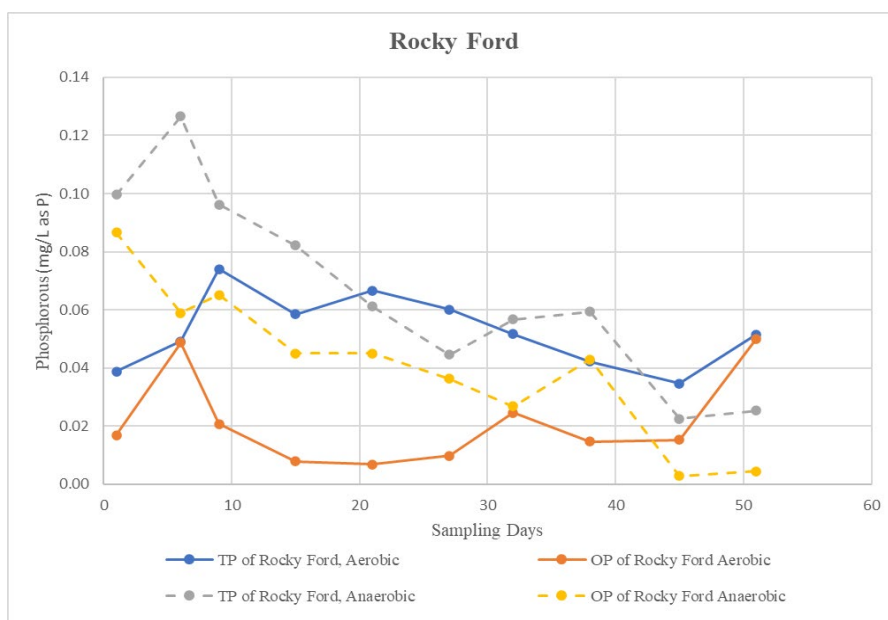


Figure 32. OP and TP graph of Rocky Ford (Aerobic & Anaerobic)

The OP and TP graphs of R4, the minimum OP and TP values were 0.05 and 0.07 mg/l as P respectively with an OP/TP ratio of 0.41. Whereas, in R7, the maximum OP and TP values were 0.087 and 0.13mg/l as P with a shifted OP/TP ratio of 0.61. Throughout the aerobic period, notable variations in the OP/TP ratio were seen on several occasions, such as on sampling days 9, 21, and 27 following the trend of the Ogeechee Outpost. However, it took an extended period for the reactor to become entirely anaerobic for this specific sampling point, as evidenced by the observed levels of OP and TP. A notable alteration in the OP/TP ratio was seen after the 40th day of sampling. The minimum observed OP/TP ratio was 0.13, while the average ratio was 0.61.

3.5 Discussion

Phosphorus, being the key element for life and the most limiting nutrient in water system, contributes to eutrophication and fuels harmful algal blooms (HABs) (Conley et al., 2009). Recent management efforts have been successful in decreasing external OP loads, but eutrophication and HABs still persist (Dai et al., 2023, Smith and Schindler, 2009). Despite an overwhelming amount of knowledge about phosphorus cycling in nature, its application has not been sufficient to prevent harmful algal blooms (HABs) linked to phosphorus (Cade-Menun et al., 2019). The objective of this study is to determine whether organic phosphorus, which can be easily utilized by organisms or is resistant to degradation, can add to the pool of soluble reactive phosphorus (SRP) when the availability of orthophosphate concentration is insufficient to meet the demand in the water column.

Despite the presence of four sampling locations, the gathered data reveals two separate trends that need to be discussed. The RAC Pond, a reclamation pond with a high OP load in water, had a similar pattern to the Occoquan Reservoir, which had low OP load in the water but significant levels of iron-bound OP in the sediment. This phenomenon may be attributed to the process of organic phosphorus mineralization occurring in aerobic settings, as well as the enhanced solubility of mineral-associated phosphorus in anaerobic (reduced) conditions (Hurley et al., 2017). Looking at the aerobic graphs in Figure. 25 for RAC pond, the first impression and the average OP/TP ratio of 0.333 indicate that the concentration of OP dominated the overall TP trend. However, further analysis identified that the OP/TP ratio shifted marginally to 0.30 throughout the 32 and 38-day sampling periods, with TP showing a higher gradient change than OP. Regarding the anaerobic condition with significantly lower ORP values, the ratio between OP and TP remained quite stable, except on day 38 of sampling. On that day, the ratio temporarily decreased from 0.33 to 0.25 but

promptly restored to its original value. It is evident that, even in a water system with a high OP load, there remains a potential for organic mineralization. A recent study using the temporal Bayesian Hierarchical Framework (BHM) also demonstrated similar results that the passage of sediment containing phosphorus is the main factor determining the limitation of phosphorus for harmful algal blooms (HABs) in highly eutrophic systems, rather than the input of phosphorus from external sources (Wu et al., 2017). On the other hand, in Figure 27 & 28, for the Occoquan reservoir, both the aerobic and anaerobic graphs showed a symmetrical trend for OP and TP, except for a deviation on day 12 in the anaerobic reactor. It is observed that, within a short time frame, the OP/TP ratio underwent a rapid transition from an average of 0.64 to 0.57 and then immediately reverted to its original position. During that specific period, there was a rise in the TP value that surely did not originate from the OP load. The phenomenon of P release from freshwater sediments has been acknowledged for a minimum of 80 years, ever since Mortimer (1941) and Einsele (1936) postulated that the reduction of Fe (III) oxy-hydroxides was the underlying cause of sediment P release (Chen et al., 2018, Wang et al., 2016). In that case, it can be explained following the classical explanation of phosphorus cycling as the Occoquan reservoir sediment is undoubtedly rich with OP in mineral-bound forms within the sediments (F. Cubas 2012).

Both study sites within the Ogeechee watershed exhibit forest and agricultural land-use and have a minimal concentration of OP load in the water column. Under aerobic conditions at Ogeechee Outpost (Figure. 30), the ratio of OP to TP remained predictable, except for the last few days between the 40th and 45th sample dates, when there was a significant increase in TP, but OP remained practically unchanged. It can be referenced here that the governing stage for microbial growth in many processes is the distribution of oxygen, which might impact the progress of different types of biochemical processes (Garcia-Ochoa et al., 2010) and also be concluded saying

that in low OP environments organic P mineralization and subsequent OP release can occur in both aerobic and anaerobic environments. Regarding the anaerobic graphs, there have been few instances where the OP/TP ratio has shifted below the average. Particularly during the last couple of experimental days, when the anaerobic condition persisted for an extended period, despite having ORP values higher than the minimum, there was an evident rise in TP levels. This increase wasn't driven by the existing OP load, as the OP load was very low in the water column. Instead, it was due to the breakdown of organic P forms, as the sediment does not contain mineral-bound orthophosphate forms. Linked with the classical view about P cycling in lakes and streams which says phosphate entering the system is quickly assimilated by living and non-living suspended particles, later settles to the bottom of the lake (Heisler et al., 2008). Once in the sediment, the organic P is released from the sediments through bacterial decomposition when the bottom waters/sediments of a lake or stream becomes anaerobic (Jilbert et al., 2011). Similarly, in Figure. 32, the sampling location of Rocky Ford exhibited comparable circumstances in both the aerobic and anaerobic graphs. However, it is noteworthy that the disparity in the OP/TP ratio increased. This could be attributed to the fact that Rocky Ford had a higher OP load in the water compared to Ogeechee Outpost, or it may be a result of modest variations in land usage. Ultimately, the overall explanation confirms our criteria for success which says both low OP environment and extended aerobic/anaerobic sediment condition can facilitate significantly towards the mineralization of organic phosphorus forms into readily bioavailable forms in the sediment-water interface of a freshwater system.

As we know, historically, external loading generated a surplus of OP in our water systems. The regulations and significant endeavors to control the introduction of organic pollutants into the environment have been successful in past decades. Nevertheless, a significant proportion of the

existing phosphorus from the past has already been accumulated as organic phosphorus in sedimentary deposits. HABs may therefore continue to persist through bacterial mineralization of organic phosphorus happening at the sediment-water interface in our freshwater systems. On this circumstance, a modified P-cycle is suggested that incorporates the breakdown of resistant organic phosphorus compounds into soluble reactive phosphorus in Figure 33. Hence, in the presence of limited external OP loading, certain aquatic microorganisms have the ability to enhance the production of particular enzymes, such as phosphatases and phytase, to get the necessary phosphorus from organic sources.

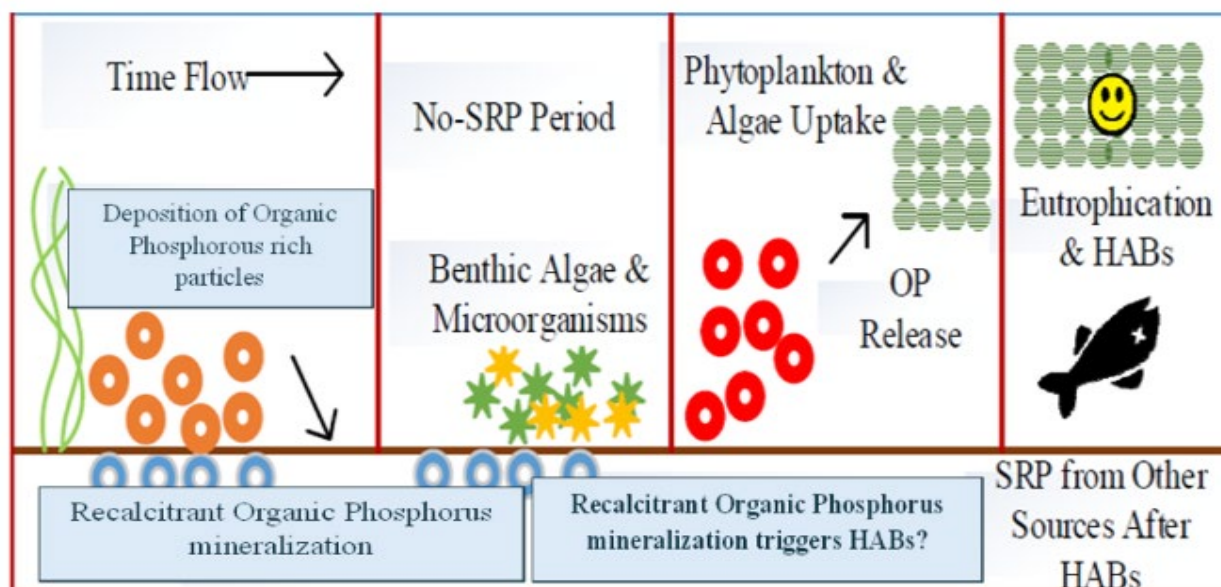


Figure 33. Proposed P-cycle including recalcitrant organic phosphorus mineralization

CHAPTER 4

CONCLUSIONS AND FUTURE WORK SCOPES

Undoubtedly, the globe has made significant progresses in terms of scientific advances and technical developments throughout the past century. However, the massive industrialization and ongoing rise of capitalism have resulted in numerous environmental challenges in recent decades. One significant issue is the widespread occurrence of uncontrollable eutrophication and algal bloom crises in both freshwater and marine environments globally. This research aims to enhance current surface water management practices by integrating our fundamental knowledge of the P cycle and providing quantitative evidence that highlights the vital connection between controlling long-term P sources and preventing Harmful algal blooms and eutrophication in our water system. Validating the hypothesis, it is evident that the mineralization of recalcitrant organic phosphorous has the potential for a significant rise to the SRP pool. Quantifying OP production from refractory PO forms, traditionally considered non-labile, would provide reliable measures of IP dephosphorylation. This will eventually contribute to a better understanding of the P cycle. Therefore, comprehending the process of PO mineralization will also assist in determining how persistent PO forms contribute to the occurrence of harmful algal blooms (HABs) in various aquatic settings.

5.1 Concluding remarks

Over time, surface water management has mostly concentrated on regulating nutrient groups that have an immediate detrimental influence on water quality, while neglecting or downplaying the long-term adverse effects of other species. Regarding P, typical sampling

techniques employed to evaluate the impact of P on eutrophication primarily concentrating on quantifying SRP and TP in water samples, with occasional measurements in sediments. However, there is a lack of comprehensive data on OP production from PO. Furthermore, during the past few decades, significant endeavors have been made to regulate the release of OP discharges from both point and non-point sources. These efforts have successfully reduced the amount of external pollutants entering the ecosystem. However, the consequences of having elevated levels of phosphorus in freshwater bodies are still evident. Large-scale harmful algal blooms, excessive nutrient enrichment (eutrophication), and oxygen depletion in surface water (hypoxia) caused by elevated P concentrations continue to be ongoing issues that impact water quality and have economic implications in the United States and globally. The objective of this study was to further investigate and evaluate the potential of Organic Phosphorus forms that accumulate at the bottom of freshwater systems and may significantly contribute to the SRP pool. Given the challenging nature of measuring PO mineralization, the initial phase of the study primarily aimed to compare the concentrations of TP and OP under aerobic and anaerobic sediment conditions. Various sources and sampling locations were chosen to set up reactors as the mineralization process relies exclusively on the presence of PO in sediment. Fortunately, positive insights have been obtained for all of the sampling locations. While the graphs for both RAC Pond reactors and Occoquan Reservoir reactors were primarily driven by OP concentrations, there were also indications of PO mineralization over certain time periods. However, in the instance of the Ogeechee sampling stations, the regulation of TP was not just affected by OP, as there was a lack of Fe-bound OP, but also by organic P which invariably validate the hypothesis for all the considered situations. The results reflect that although anaerobic conditions boost the process of PO mineralization, it is clear that the OP/TP ratio undergoes changes in the aerobic environment as well. It was also evident

that in low OP environments, organic P mineralization and subsequent OP release occur in both aerobic and anaerobic environments. The classical explanation of the P cycle heavily relies on the redox-driven release of orthophosphate from iron-bound compounds concentrating solely on the anaerobic sediment conditions due to the presence of a specific type of bacteria. However, that explanation tends to overlook the possibility of the mineralization of organic forms of phosphorus in the absence of other P sources and also in the presence of different types of bacteria. On that note, the results obtained from this part of the study certainly cement the knowledge gap regarding the role of the PO fraction to the total pool of SRP and its effect in starting and sustaining HABs.

5.2 Limitations of the study

Assessing the overall contribution of recalcitrant POs to the SRP pool is challenging and time-consuming. Once again, it is important to note that a one-year study may not accurately represent the original situation. Therefore, a comprehensive long-term research strategy is currently being implemented in order to acquire a more thorough understanding of these persistent problems. Uniform sediment layers were employed in all the reactors, following an experimental approach. However, as previously stated, the productivity of the sediment varied. For certain reactors that were constructed with Ogeechee River sediment samples, it took a longer time to achieve the fully anaerobic stage. To speed up the process, more sucrose was added. However, maintaining aerobic conditions in the aerobic reactors for RAC Pond and Occoquan Reservoirs was challenging due to the high productivity of sediments. The dissolved oxygen (DO) levels frequently fell below the necessary threshold, necessitating further aeration during the operation. These additional requirements may have certain impacts on the acquired results. On a different note, the physiological processes involved in all those chemical reactions may vary for any lentic

water body that undergoes a phase of di-mixing. Therefore, it is essential to do additional research that includes the investigation of mixis as well as the total process of freezing and thawing.

5.3 Future works in progress as a continuation of this project

In order to support the presence of mineral bound OP, liable OP, organic P, and resistant organic P forms, fractionation analyses are being conducted on sediments as a continuation to the following portion of this project. The subsequent measure would involve quantifying the contribution of PO to the overall reservoir of SRP that sustains harmful algal blooms. To do this, inositol phosphate, a prevalent and resistant form of organic phosphorus, will be used as a surrogate form of organic phosphorus to quantify its biological mineralization. Phytic acid is the most abundant form (about 90%) of Inositol Phosphate (Turner et al., 2002) and Inositol hexaphosphate (IP₆) constitutes between 2-50% of the riverine sediment. Despite being chemically refractory, phytic acid has been observed to be effectively mineralized to inorganic phosphorus by enzymatic degradation processes (Golterman 2006). Inositol phosphate will be used because of its suitability as a synthetic substrate for an IP probe, which enables precise measurement of dephosphorylation and accurate quantification of OP synthesis. In addition, experiments will be conducted in low OP conditions to quantify the generation of OP resulting from the breakdown of IP. Therefore, this will help evaluate the significance of organic phosphorus forms in promoting eutrophication and harmful algal blooms.

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