ABSTRACT

The Effect of Suspended Bentonite and Kaolinite Clay
on Phosphorus Uptake and Release by Lotic Periphyton

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Lotic systems act as nutrient buffers to receiving lentic systems. As streams transport allochthonous phosphorus through a watershed, the loads are modified in quantity and quality through biotic and abiotic mechanisms. Lotic systems are frequently dominated by periphyton, the attached benthic community consisting mainly of algae and bacteria. This community exhibits the ability to buffer phosphorus loads to receiving waters through several mechanisms including: biotic uptake, chemical precipitation, and mechanical filtration.

Stream sediments, including clays, influence dissolved phosphorus concentrations primarily through equilibrium-driven sorption/desorption reactions. Additionally, suspended clays in aquatic environments are known to: modify food webs, influence species composition, and affect biotic integrity by altering the physical and chemical conditions. Given the influence that suspended clays exert upon aquatic systems, it was hypothesized that suspended clays would modify, either positively or negatively, phosphorus uptake and/or release by lotic periphyton.

Experiments were conducted using two clay minerals, dissolved reactive phosphorus, and cultivated periphyton communities. Bentonite and kaolinite were selected to represent two clay types commonly found in aquatic systems. Standardized laboratory procedures were utilized to describe the physical characteristics and phosphorus sorption behavior of the clays. An artificial stream system was designed, tested, and operated to control water conditions necessary to cultivate periphyton communities, support suspend clays, and conduct clay-phosphorus-periphyton interaction studies. Periphyton communities were subjected to different clay and phosphorus concentrations under controlled conditions in artificial streams and laboratory microcosms. Phosphorus uptake rates were unaffected by the presence of clays at several different concentrations in artificial stream settings. Periphyton exposure to heavy clay loads in lotic microcosms, under laboratory conditions, had no effect on the ability of the periphyton to uptake or release phosphorus. The results may be helpful to water resource managers working with water quality issues and researchers interested in basic ecosystem function.

The Effect of Suspended Bentonite and Kaolinite Clay
on Phosphorus Uptake and Release by Lotic Periphyton

by

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A Dissertation

Approved by the Department of Biology

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Submitted to the Graduate Faculty of
Baylor University in Partial Fulfillment of the
Requirements for the Degree
of
Doctor of Philosophy

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December 2009

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LIST OF ABBREVIATIONS

AEC................Anion Exchange Capacity
AFDM............Ash Free Dry Mass
ANOVA.........Analysis of Variance
ARS................Agricultural Research Service
ATS................Algal Turf Scrubber
BREC.............Blackland Research and Extension Center
CEC................Cation Exchange Capacity
CPM...............Counts per Minute
CV..................Coefficient of Variation
P ....Phosphorus
PVC................Poly Vinyl Chloride
RFM...............Reconstituted Freshwater Media
SRP ....Soluble Reactive Phosphorus

ACKNOWLEDGMENTS

Thanks to my family to whom this volume belongs. Thanks to my wife Cynthia Ann, I am forever grateful. Matthew Christian and Grant Aaron, you are my inspiration. Thanks to my parents Dorothy Wolfe and June Wolfe, Jr. for my being, and thanks collectively to the families Wolfe, Matthews, Macias, and Sanchez for your love and encouragement. Thanks to my mentors without whom I would not have learned or grown nearly as much. Thank you to Dr. Owen Lind for opportunity, and knowledge, Dr. Robert Doyle for direction, Dr. Kenneth Wilkins for navigation, Dr. Stephen Dworkin for definition, Dr. Stephen Trumble for short notice, Dr. Kevin McInnes for mathematics, Dr. Armen Kemanian for statistics, Dr. Diane Wycuff for technique, and Dr. Arthur Stewart for the written word. Thanks to my classmates, and friends in the Baylor Biology Department. Thanks to Dr. Mikhail Umorin for tolerance, Dr. Christopher Filstrup for accuracy, Dr. Brad Christian for standard, and Dr. Rodrigo Moncayo-Estrada for interest. Special thanks to Dr. Dennis Hoffman for support, and friendship, Dr. Allen Jones for recommendation, Dr. Allen Torbert for materials, Dr. Ken Potter for review, Dr. Hiroaki Somura for translation, and The People of Blackland Research Center for nurture. Thanks to all not mentioned and to God for purpose.

DEDICATION

To my sons Matthew Christian and Grant Aaron

CHAPTER ONE

Phosphorus, Suspended Clay, and Periphyton in the Lotic Environment

Terrestrial-Aquatic Linkages in Watershed Processes

Let us begin with a wide view and narrow the field until we reach the scale at which the following investigations were conducted, and then conclude by working our way back up to the beginning, having gained some insight. Aquatic ecosystems have been historically treated as isolated units, perhaps, due to scale issues; however, cultural eutrophication issues drive current research as the world's population grows and exerts more and more pressure on limited water resources. This creates problems of ever increasing complexity. Howard Odum suggested that all scales of size and time operate with common designs and principles that can be explained using scientific methods. He also noted that scientific progress tends to be faster at scales where overview models are applied because the complexity appears less visible (Odum 1996). Mathematical modeling of ecosystems began with plankton ecosystems where the complexity was seen to be smaller and more manageable as compared to forest ecosystems where the complexity is visibly higher and overwhelming. Early limnologists like F. A. Forel, S. A. Forbes, R. L. Lindeman, and others, took this approach by concentrating their work on small lakes, ponds, or streams while ignoring the surrounding watershed (Forbes 1887; Lindeman et al. 1942). While this is a natural approach, at the landscape level, streams, rivers, and wetlands form an important interface between terrestrial uplands and their adjacent water bodies. They must be considered together. Watershed ecosystems are linked hydrologically since water transports constituents from terrestrial sources to receiving waters through surface and subsurface flow (Reddy et al. 1999). Lake Okeechobee in Florida for example is centered in the middle of a large aquatic ecosystem where lotic-lentic interactions play a critical role the lake's biological, chemical, and physical makeup. Other linkages, such as lentic-lotic, lentic-wetland, and lentic-estuary, also exist in this landscape and influence lake function. Human modification of the area has affected its natural state causing pronounced eutrophication, mainly through reduced nutrient retention in its shrinking streams and wetlands (Steinman and Rosen 2000).

Retention is the capacity of a stream, or wetland, to remove water born matter through physical, chemical, and biological processes and hold it in a form not readily released under normal conditions. Nutrient retention in streams and wetlands reduces the nutrient load to downstream systems such as lakes, rivers, and the sea (Reddy et al. 1999). This process has a positive effect upon preventing or reducing eutrophication, the increase in production due to addition of nutrients. Channelizing rivers and streams for urban or agricultural development reduces the floodplain and negatively impacts the nutrient and sediment filtering capabilities of natural marshes and wetlands (Steinman and Rosen 2000). This results in increased nutrient loading to receiving water bodies as reduced water retention on these landscapes lowers nutrient assimilation from the flows. Agricultural impact is cited as a serious problem. Lake Okeechobee has experienced a doubling of phosphorus loads, from ~ 40 µg to ~100 µg per liter, since the 1970's due to the building of numerous canals and draining of wetlands to provide farmland. The streams and wetlands that were lost previously acted as natural nutrient buffers. In the Chesapeake Bay drainage basin, agricultural activity has contributed to the degradation of surface waters by increasing nutrient and sediment loads from animal production operations. Researchers have suggested that remaining natural wetland systems be protected through legislation and that constructed wetlands be created for water quality improvement (Schaafsma et al. 2000). Cultural eutrophication is a global problem.

Since at least the 1980's investigators have expanded their views regarding lake structure and function to include the surrounding watershed. A watershed landscape may be defined as the entire stream network, including the groundwater flow, from the highest elevation in a catchment to the point of confluence of another catchment or the ocean (Hauer and Lamberti 1996). The water quality and productivity of these systems is ultimately controlled by the quality and quantity of external nutrient loadings (Kennedy and Walker 1990). The specific nutrient inputs of a particular watershed are determined by the local climate, topography, soils, and land use. As rivers and streams transport materials, including sediments and nutrients, from terrestrial sources to reservoirs, reactions, biotic and abiotic, occurring en route, selectively process the quality and quantity of the materials reaching a lake or reservoir (Klarer and Millie 1989; Doyle 1991; Froelich 1988; Koetsier and McArthur 2000; Peterson et al. 2001). This in turn has a significant effect upon lake and reservoir function, specifically upon production and biomass which in turn influence water quality. Reddy concluded (1999) that phosphorus loading from terrestrial sources reaching lentic systems depended upon the assimilating capacity of wetlands and streams protecting downstream waters. The fact that environmental conditions present at any point in a stream system are continuously influenced by the conditions at points upstream should always be considered when working in lotic systems (Hauer and Lamberti 1996).

In lotic ecosystems the nutrient buffering phenomenon is attributable to both biotic and abiotic processes. Streams especially, facilitate physical, biological, and chemical processes that retain phosphorus in biologically unavailable forms. The biota present in lotic systems acts as "biological filters" that temporarily sequester nutrient loads received by lentic waters. Periphyton assemblages are cited as transformers of phosphorus from biologically available and unavailable forms through mechanisms that affect water chemistry and influence the phosphorus chemistry of the system (Dodds 2003). Benthic sediments have been shown to play a major role in regulating the inorganic phosphorus concentration of streams (Froelich 1988). These micro-scale processes, when applied over the landscape-scale, have significant impact on the system as a whole and must be considered.

Lake and reservoir systems are now recognized as being closely coupled to their surroundings, making up only one component of a "watershed landscape" (Vannote et al. 1980; Minshall et al. 1985; Thornton et al. 1990; Squires and Leasak 2001). The development of this idea can be seen in current stream ecosystem theory which has concentrated on four major areas of thought including; holistic approaches, material cycling in open systems, biotic interactions with integration of community ecology principles, and linkages between streams and their terrestrial settings (Minshall et al. 1985). With good reason, watershed-scale linkage studies have focused primarily on cultural eutrophication of lake systems with the goal of determining appropriate mitigation strategies. Researchers are testing whole watershed systems and developing complex mathematical models in an attempt to address and compare the long term impacts of various management strategies on freshwater eutrophication (Smith et al.

1996; Reed-Andersen et al. 2000; Peterson et al. 2001). Balancing the benefits gained from modifying watershed ecosystems with the negative water quality impacts is the ultimate goal.

The Nature of Phosphorus

Phosphorus continually flows through our environment changing form and participating in both organic and inorganic cycles. It is somewhat unique in that it has only one stable isotope, displays only one oxidation state in solids and solutions, and has no significant atmospheric flux (Froelich 1988). The resulting phosphorus exchanges take place between solids and solutions participating in both biotic and abiotic processes.

Phosphorus makes up about 0.1% of the earth's crust and is found in certain igneous rocks called apatites. The most common, fluorapatite ($Ca_5(PO_4)_3F$), releases orthophosphate, primarily as $H_2PO_4^-$, through slow weathering action by water (Foth 1978). The resulting orthophosphoric acid is triprotic and exhibits three ionization stages (H_3PO_4, $H_2PO_4^-$, HPO_4^{--}) when disassociated in water (Nebergall 1980). Phosphorus is most biologically available as the $H_2PO_4^-$ ion and, to a lesser degree, $H_PO_4^{--}$ (Tisdale, 1967). The concentration of these ions is determined by the pH, which, in turn, influences their behavior in the environment. Below pH 5.5, soluble iron and aluminum ions increase and orthophosphate reacts with them forming insoluble iron and aluminum phosphates. Between pH 6 and 7, orthophosphate will react with dissolved calcium forming insoluble calcium phosphates that may precipitate. Calcium phosphate precipitates are sometimes observed in lakes and on aquatic vegetation, which can influence pH through photosynthetic activity, in a condition known as "whitings" (Kalff

2002). Above pH 7, increased hydroxide ions cause $H_2PO_4^-$ to shift toward to the lesser biologically available form HPO_4^{--} (Tisdale 1967).

Many fates await free phosphate ions in the environment. The major phosphorus cycle components include: dissolution-precipitation, sorption-desorption, and mineralization-immobilization processes. Mineral equilibrium is a dissolution-precipitation process in which free phosphate ions are reincorporated into rock material if other ions such as calcium, iron, or aluminum are present. This results in the formation of complexes like tricalcium phosphate or other minerals such as strengite and variscite. Sorption-desorption are chemical reactions in which phosphate ions physically adsorb onto soil or clay particles and end up in sediments (Tisdale 1967). When phosphate is assimilated by living organisms in a mineralization-immobilization process and incorporated into various organic molecules, it is a biologically-mediated conversion from inorganic to organic form.

The various ways in which phosphorus is described depends upon its form and availability. It is generally considered to be either inorganic or organic. Total phosphorus refers to the total amount in both dissolved and particulate phases. Soluble reactive phosphorus (SRP) refers to dissolved orthophosphate which is the form most readily available to biological uptake. Particulate phosphorus is inorganic and organic phosphorus that is associated with eroded sediment or plant detritus. Dissolved organic phosphorous includes polyphosphates and hydrolysable phosphates (Foth 1978; Pierzynski 2000).

Phosphorus, an element critical to all life, is present in many biological molecules including the energy transferring molecules adenosine triphosphate and nicotinamide adenine dinucleotide phosphate, the genetic molecules ribonucleic acid and deoxyribonucleic acid formed from nucleic acids, and in phospholipids found in cell membranes. It also makes up a large portion of vertebrate teeth and bones (Audesirk and Audesirk 1999). When it is in the orthophosphate form, living organisms readily and rapidly absorb it. Because phosphorus is in great demand biologically and present in low concentrations in the natural environment, it is often the limiting nutrient in aquatic systems (Kalff 2002).

In un-polluted systems, phosphorus is high in demand relative to supply and retention is high with little released to receiving waters in well-vegetated drainage basins. That which is released is primarily in the dissolved organic form (Correll 1998) with most contained in particles and sorbed to soils which are entrained in the flow during storm runoff. Since orthophosphate dissolves slowly from parent rock material and the biological demand for it is great, orthophosphate concentrations in natural waters are usually less than 100 µg L^{-1} (Lind 1985).

Phosphorus and Freshwater Eutrophication

Enrichment of waters with biologically active nutrients leading to greater production is known as eutrophication. Phosphorus and nitrogen are those most commonly in shortest supply relative to need for algal growth and in most temperate freshwater systems phosphorus is typically the more limiting of the two (Wetzel 1983). Eutrophication is frequently the result of elevated phosphates (Correll 1998), which in turn often leads to water quality problems. In 1946, Tide, the world's first phosphate-containing detergent, was introduced in the USA. It contained sodium tripolyphosphate which boosted the cleaning power of soap powders by softening the wash water. Within ten short years, phosphate-containing detergents were blamed as the world's number one pollutant causing perpetual algal blooms (Emsley 2000). The constant blooms produced green, smelly, anoxic waters (from decaying algae, fish, and other organisms) that were not fit for drinking or recreation. Between 1970 and 1972, a fierce battle over regulation of phosphorus amounts that could be discharged into rivers and lakes was fought between environmental groups and detergent manufacturers (Emsley 2000). The resulting legislation lead to a patchwork of laws, enacted at the state level, which determine acceptable discharge limits. As recently as 2000, the USEPA have been directed to revise and set national nutrient criteria for rivers, lakes, and estuaries based on current scientific understanding and interpretation of the problem (Dodds and Welch 2000).

Phosphorus Movement in the Lotic Environment

Phosphorus is transported through streams in both soluble and particulate forms. The processes at work during transport affect the availability of phosphorus for assimilation by biota and retention by sediments. These processes include advection, diffusion, seepage, resuspension, sedimentation, and bioturbation (Reddy et al. 1999). Nutrient cycling in flowing waters is often described as a spiral (Newbold et al. 1981; Mulholland et al. 1983). The idea couples nutrient uptake, transformation, and release with downstream transport as nutrient exchange between various compartments in a stream does not take place in one location. Chemical, physical, and biological reactions occur during a continuous movement downstream. A nutrient spiral is defined as the distance required for a nutrient ion to complete one cycle from dissolved form to particulate form and back to the dissolved form (Newbold et al. 1981). The distance it travels in dissolved form before being removed from solution is the uptake length.

Phosphorus uptake rates are a function of stream flow velocity, discharge, biological and chemical characteristics, and underlying sediment composition (Reddy et al. 1999). Turnover length is the distance a nutrient ion travels in particulate form before being released back to the water in a dissolved form. Spiral length is a combination of the two. Streams with short spiraling lengths are more efficient at retaining nutrients and vice versa (D'Angelo et al. 1991). The nutrient spiraling process includes both biotic and abiotic mechanisms. Newbold et al. (1981) determined that a small woodland stream in Tennessee had a P spiraling length of 193 meters. Spiraling distances vary with water velocity, biota present, nutrient loads, and physical conditions in each system (Mulholland et al. 1983; Paul and Duthie 1989; Fisher et al. 1998; Essington and Carpenter 2000). In artificial, recirculating systems, the nutrient spiral comes to equilibrium when nutrient uptake equals release (Bothwell 1993).

The Nature of Clay

The term clay has several meanings. In common usage it refers to a soil that tends to retain water and is soft and malleable when wet. A more precise definition denotes it as a certain fraction of soil particles, that is, anything smaller than 2 μm. In the mineralogical sense, clay refers to a large group of minerals, the phylosilicates (Hillel 1980). This group of soils includes silicate clays and micas. They are formed from layered materials that are strongly bonded internally and weakly bonded between the layers.

Clay Mineral Structure and Form

There are two clay types important in aquatic systems: 1:1 non-expanding (example kaolinite), and 2:1 expanding (example bentonite) (Lind 2003). These are silicate clays formed from primary minerals through weathering processes. Kaolinites are common in tropical areas with intense weathering and contain high concentrations of iron and aluminum hydroxides. Bentonites (also known as Montmorillonite) tend to form in areas where there is an abundant supply of magnesium, a more temperate environment, and less weathering (Foth 1978). Silicate clays consist of two basic components, a silicone-oxygen tetrahedron sheet and an aluminum oxide octahedron sheet. In some clays, such as kaolinites, the tetrahedral and octahedral sheets occur in equal numbers and are referred to as 1:1 type clays. Others, including the smectites, which includes bentonites, have two tetrahedral sheets with an octahedral sheet sandwiched in between. These are known as 2:1 clays due to this configuration. Kaolinites are the simplest with layers of silicate sheets (Si_2O_5) bonded to aluminum oxide/hydroxide layers ($Al_2(OH)_4$). The bonding between the layers is strong enough to keep them together while excluding water and free ions. It is, therefore, a non-expanding clay with little tendency to shrink or swell with differing moisture contents (Thompson and Troeh 1973). Bentonites are also made up of layers of silica and aluminum oxide/hydroxide but are held together with oxygen molecules instead of hydroxyl groups as in kaolinite. These clays are able to absorb a variable amount of water causing them to expand and contract depending upon the water content. The theoretical formula is $Al_2Si_4O_{10}(OH)_2 \cdot H_2O$. Bentonite structure varies slightly from material to material as different ions may replace the aluminum ion. The resulting clay may carry a charge on its surfaces. Both water and dissolved ions may enter between the layers as they expand and bind with the charged surface (Thompson and Troeh 1973).

Silicate clay minerals possess an important characteristic known as cation exchange capacity. Numerous locations on the clay molecule carry negative charges allowing them to attract cations. These sites are exchangeable. Water may deliver cations to the sites where they may replace an existing cation of a lesser ionic charge. Cation substitution occurs within all 2:1 layer silicate clays. 1:1 clays also have exchangeable sites on their edges where the silicon-oxygen tetrahedron and aluminum oxide octahedron sheets are broken. These broken edge bonds can be positively or negatively charged and therefore accept cations or anions (Thompson and Troeh 1973).

Clay Effects on Aquatic Ecosystems

Clays affect biological activity through several mechanisms: primarily through chemical adsorption on to clay particles (Lind et al. 1997) and light attenuation (Lind et al. 1992; Squires and Lesack 2001). In lakes containing high amounts of suspended clay, light is attenuated due to turbidity and phytoplankton production is subsequently reduced (Squires and Lesack 2001). Concurrently, clay particles may form aggregates with dissolved organic carbon and support increased bacterial production (Lind et al. 1997; Squires and Lesack 2001). In this case light may be limiting to production rather than nutrients. An algal assay of water from Lake Chapala, Mexico indicated that nitrogen was the limiting factor for phytoplankton production. In the lake, however, light rather than nitrogen was the limiting factor. This was attributable to clay turbidity and associated low light levels (Davalos et al. 1989).

Some clay types may compete with biota for available nutrients through strong ion exchange capacity. Kaolinite clays, with their strong ionic bonding on their edges, hold ions such as $H_2PO_4^-$ so strongly that they may affect bioavailability (Thompson and Troeh 1973). Physical clay effects include: abrasive scouring and biomass removal at high water velocities, sedimentation and subsequent smothering of attached benthic communities at low water velocities. Both mechanisms have significant impacts upon benthic community structure (Power and Stewart 1986). Burkholder and Cuker (1991) determined that phosphorus loads coupled with clay loading impacted lotic periphyton communities in a number of ways. Clay-only treatments had a negative (lower production) effect upon periphyton while a clay + phosphorus treatment resulted in a positive effect (higher production than controls). It is reasonable to predict that phosphorus-rich clay particles might stimulate benthic algal growth and compensate for periodically reduced light conditions due to sediment turbidity. Squires and Lesack (2001) however found the opposite to be true. They hypothesized, but had no evidence to support, a stimulatory effect of sediment bound nutrients settling on suitable substrates in a study conducted on a river-lake delta in Canada. It is certain however, that both physical and chemical conditions affect clay action upon aquatic biota.

The Nature of Periphyton

General Description

Lotic organisms must be good swimmers or attached to the stream bottom in order to deal with the forces imposed by flowing water. For this reason, the dominant communities in streams are benthic and attached to the substrate (Lampert, 1997). This attached benthic micro-biota, or periphyton, is responsible for significant in-stream processing of allochthonous materials. The term periphyton most commonly refers to the micro-community consisting of algae, fungi, bacterial, protozoa, and other invertebrates inhabiting the mat living upon the surface of submersed objects in water (Wetzel 1983). This is the definition of the German word "aufwuchs" which is not as popularly utilized as it once was, perhaps as a result of the shift toward scientific publication in English and less in German. Periphyton has replaced aufwuchs in most of the literature produced since the mid 1970's. Periphyton can be loosely divided into types based on its association with different inorganic and organic substrates upon which it grows. The most common terms include: epilithon - upon rock substrates, epipelon - upon mud or silt substrates, episammon - upon sand substrates, and epiphyton - upon submerged aquatic macrophytes (Aloi 1990).

Environmental Roles of Periphyton

Periphyton response to environmental conditions in freshwater lotic systems has been well studied. Familiar topics include; the effect of nutrient concentration upon periphyton growth and or community structure (Horner and Welsh 1981; Horner et al. 1990; Humphrey and Stevenson 1992; Steinman and Rosen 2000; Stelzer and Lamberti 2001), the effect of various hydraulic conditions upon periphyton growth and or community structure (Lindstrom and Traaen 1984; Paul and Duthie 1989; Horner et al. 1990; Mulholland et al. 1994), the effect of sediment loads and scouring upon lotic periphyton growth and or community structure (Horner et al. 1990; Humphrey and Stevenson 1992), and the effects of various physical parameters such as light, temperature, and pH on the growth and or community structure of periphyton (Grobbelaar 1983; Hill et al. 1995). In numerous applied studies, periphyton species diversity or composition has proven to be a useful environmental impact indicator (Toetz et al. 1999; Winter and Duthie 2000).

Most periphyton studies focus on response to some variable but a few consider the biota's regulatory effect upon nutrient concentration (e.g. water quality). Periphyton assemblages influence water-borne nutrient concentrations through several methods. They can remove nutrients from the water column by uptake, slow nutrient exchange across the sediment/water boundary and decrease diffusion of phosphorus from the sediments, intercept nutrients diffusing from the sediments or dead plant material, cause biochemical conditions that favor phosphorus precipitation and deposition from solution, and mechanically trap particulate material from the water column (Dodds 2003). Many researchers now promote the use of periphyton culturing techniques as a biological remediation tool useful for cleaning or removing nutrients from aquatic systems (Drenner et al. 1997; Marinelarena and Di Giorgi 2001; Wilkey and Mulbry 2002).

Nutrient Buffering in Aquatic Systems

Freshwater estuaries, wetlands, and streams have been described to "buffer" lentic systems from various constituents entrained in their waters (Klarer and Millie 1989; Doyle 1991; Froelich 1988; Koetsier and McArthur 2000; Peterson et al. 2001). The term "buffer" has several definitions but generally refers to a means or device that cushions or shields against shock or fluxuation in a system. For example, a chemical buffer is a mixture of weak acids, bases, and their salts which resist small changes in the hydrogen-ion concentration with small additions of acids or bases. Ecologically, a "buffer" system can act in either the general or more specific chemical fashion. Stream sediments chemically buffer receiving waters by adsorbing and releasing nutrients thereby maintaining an equilibrium concentration (Froelich 1988). Stream biota protects or "buffers" lentic systems from terrestrial nutrient pulses through uptake and temporary sequestering of soluble reactive nutrients (Dodds 1993). Retention is the capacity of a stream to remove a constituent, such as phosphorus, through physical, chemical, or biological processes and detain it in a form not easily released under normal conditions (Reddy et al. 1999). The amount is not reduced as it is not actually removed from the system, rather, it is biologically converted from an inorganic to organic form, and temporarily detained in the biomass. The load is eventually released through physical scouring, grazing by herbivores, or death and decay. The material is then transported downstream to receiving waters but in a different form and concentration than the original. The timing, magnitude, and form of nutrient loads reaching a receiving lake or reservoir ultimately affects the biota and subsequently the water quality of those systems (Reed-Andersen et al. 2000). Lentic systems should not be regarded as isolated due to the hydrologic linkage to their watersheds through the rivers and steams which feed them.

Abiotic Phosphorus Buffering in Streams

The phosphorus buffer mechanism was described by Froelich (1988) who defined the equilibrium phosphorus concentration (EPC) of a stream as the ambient concentration at which P is neither taken up nor released by the sediments, either entrained or deposited. The ability of a system to assimilate elevated inputs of phosphorus depends upon of the ability of its sediments to adsorb phosphorus (Haggard et al. 1999). Several researchers have confirmed and quantified the sediment buffer mechanism for phosphorus in different stream systems (Martinova 1993; Haggard et al. 1999; Dubus and Becquer 2001). Thornton et al. (1990) noted that stream sediments have greater sorption capacities than the soils in their associated watershed. This was attributed to the presence of chemically altered clays, from stream sediments, which have an enhanced affinity for sorbing metals, nutrients, and dissolved organic compounds. While some studies show the phosphorus buffer mechanism to be dominated by physical rather than biological processes (Klotz 1988), others have found biological processes dominating.

Biotic Phosphorus Buffering in Streams

Several studies attribute significant phosphorus removal to biotic processes in lotic systems. Macrophytes, algae, and the microbial community seem to be most active in phosphorus removal (Elwood and Nelson 1972; Haggard et al. 1999). Dodds (2003) noted that periphytic organisms are not the only ones involved in phosphorus retention but that major phosphorus fluxes can be altered by algal biofilms and so they should be carefully examined. Lotic systems tend to be biologically dominated by periphyton (Biggs and Kilroy 2000). Elwood and Nelson (1972) utilized a ^{32}P balance method to examine periphyton production and grazing rates in a natural stream determining that periphyton was a major player in SRP uptake. Garder and Skulbero (1966) concluded that SRP sorption onto inorganic sediments was minor compared to the quantities uptaken into the periphyton compartment of a small Tennessee stream. Periphyton, both epibenthic and epiphytic, affects phosphorus water concentrations through various mechanisms including uptake and deposition, filtering of particulate phosphorus, attenuation of flow and decreasing advective transport of particulate and dissolved phosphorus from sediments. It may also facilitate precipitation of calcium phosphate complexes through its influence on water pH (Dodds 2003). The importance of periphyton in this process has been demonstrated experimentally numerous times (Bays et al. 2001).

Lotic Periphyton as a Nutrient Buffer

Periphyton often plays a major role in regulating phosphorus concentrations in a stream environment (Reddy et al. 1999). Under optimum conditions, periphyton is capable of uptake rates approaching 160 mg P m^2 d^{-1} and 1900 mg N m^2 d^{-1} and has been noted as having the potential for use as biological wastewater treatment organisms (Drenner et al. 1997). Vymazal (1988) using continuous flow-through channels determined that at a flow rate of 312.5 l hr^{-1}, periphyton could remove 70% of phosphorus from flow-through waters containing concentrations of 0.46 mg l^{-1}. It was determined that nutrient uptake considerably changed periphyton community structure but did not significantly affect phosphorus uptake performance. Periphyton species composition is less important in phosphorus uptake than physical variables such as temperature and flow (Adey et al. 1993).

Algae and bacteria (i.e. periphyton assemblages) are very efficient at removing nutrients from water and numerous environmentally friendly, or “green”, technologies have been developed to exploit this talent. Florida currently uses man-made periphyton ponds to treat phosphorus contaminated water with the goal of obtaining concentrations <0.1 mg P l^{-1} (Bays et al. 2001). Algal turf scrubbers, or periphyton scrubbers are a further refinement. These are cultured communities of attached benthic organisms, including algae and bacteria, that are utilized to remove or “scrub” nutrients, or other contaminates, from water. These types of systems have been developed and used to maintain high water quality for research and aquarium systems. They have been shown to effectively remove phosphorus and nitrogen, as well as many other contaminants, from natural waters (Adey et al. 1993). Culturing and managing freshwater ecosystems provides an environmentally friendly approach to reducing human generated eutrophication. In the past shallow ponds have been utilized to house suspended microalgae cultures for tertiary sewage treatment. More recently algal cultures have been grown as a biofilm (i.e. periphyton) in shallow artificial streams fitted with some sort of growth surface for microorganism attachment (Hoffman 1998). Various growth surfaces are utilized but plastic screens are a favorite. This arrangement is utilized primarily because of the ease of harvesting biomass from the screens. Harvested biomass can be as high as 30% protein and useful as a high-value livestock feed product. It also contains high concentrations of nitrogen and phosphorus and is therefore useful as an organic fertilizer amendment (Wilkie and Mulbry 2002). When dried, the material loses up to ten times its volume and wet weight and can be economically transported. Modified algal turf scrubber designs offer an ideal method for culturing periphyton communities that can be used to experimentally evaluate their performance under differing conditions.

Suspended Clay, Phosphorus, and Periphyton

The physical and biological interactions within a stream undoubtedly affect its ability to buffer phosphorus loads to downstream waters. Clays carried by currents are common inorganic causes of turbidity and can result in sedimentation, erosion, abrasion, and light reduction (Wilbur 1983). Stream periphyton temporally sequesters phosphorus loads within its biomass through several methods including: sorption, assimilation, filtration, and precipitation (Dodds 2003). Various environmental factors affect the health and composition of periphyton communities and hence their ability to process materials (Horner et al. 1990; Stelzer and Lamberti 2001). Sediment loads, nutrient loads, current speed, and light climate all influence lotic periphyton (Horner et al. 1990; Mulholland 1990; Napolitano 1994; Hill et al. 1995). It is my supposition that suspended clays will affect the uptake of SRP by lotic periphyton.

Summary of Research Objectives

We now arrive at the narrow field of view encompassing the research described hereafter. The fundamental question posed by this investigation is: what influence does suspended clay exert upon phosphorus uptake and release by lotic periphyton? The basic hypothesis is H_o: *suspended clays have no effect upon phosphorus uptake or release by stream periphyton.* Suspended clays, nutrients, and periphyton are common components of rivers and streams. Physical, chemical, and biological processes in these lotic systems buffer nutrient loads to downstream receiving waters. Suspended clays exert a strong influence upon the physical and chemical conditions in a stream, a situation which may modify the phosphorus buffering capacity attributable to the periphyton community (Figure 1.1). Laboratory and greenhouse scale artificial streams were employed to measure micro-scale processes that may have significant influence at the watershed scale. The results may be helpful to water resource managers interested in water quality issues and to researchers interested in basic ecosystem function.

General Methodology

Managing Environmental Conditions and Biotic Variation with Artificial Streams

Chapter two describes the construction, operation, and assessment of a constant-volume artificial stream designed to control water quality and reduce environmental

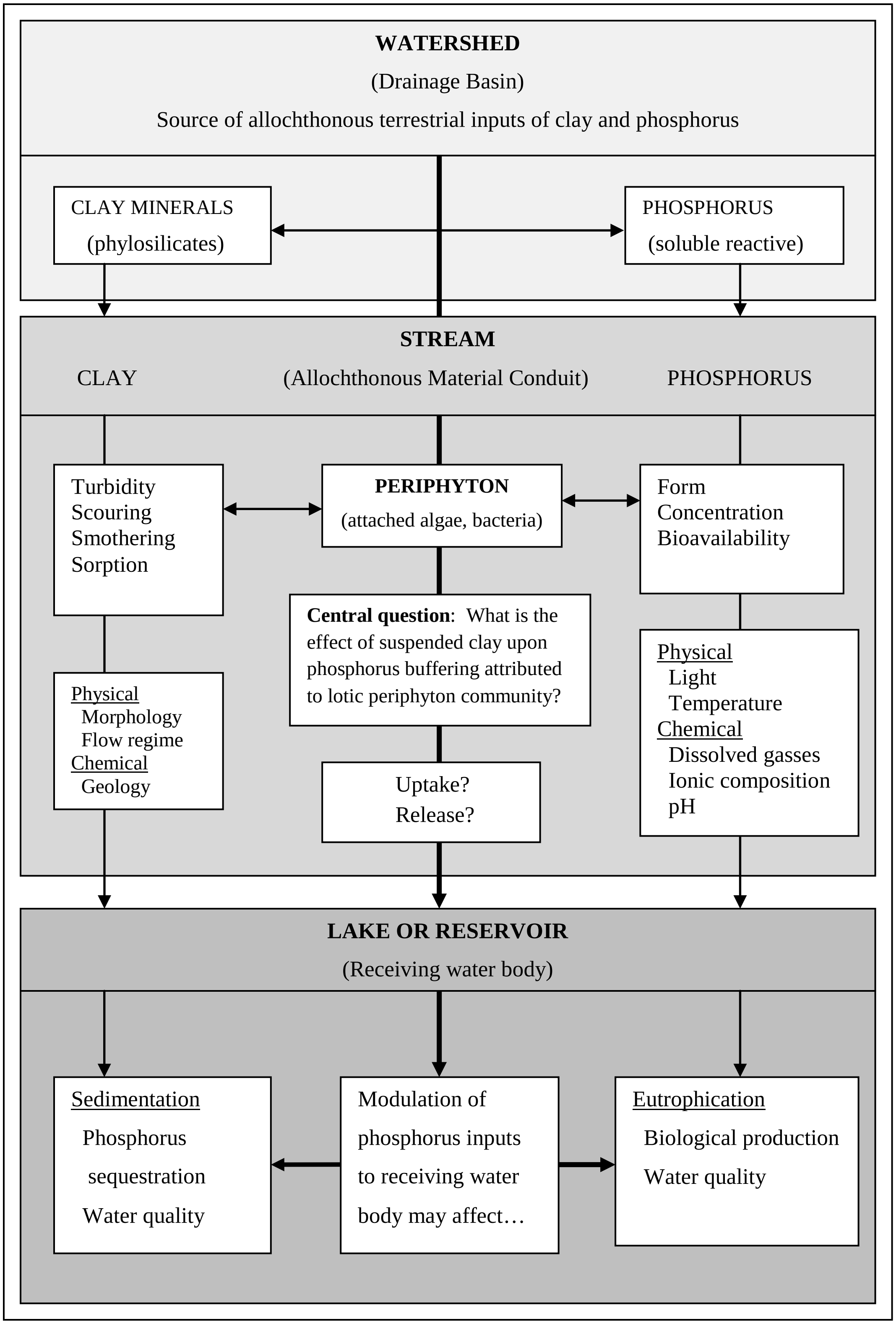

Figure 1.1. Watershed scale periphyton, clay, and phosphorus effects upon receiving waters

variation in order to improve aquatic process measurements. The system was designed and used to investigate suspended clay influence on phosphorus acquisition (load buffering) by lotic periphyton. Spatial and temporal heterogeneity represent the greatest sources of variation in the distribution and activity of aquatic biota in natural systems. Imposing experimental treatments simultaneously addresses temporal variation. Spatial variability is managed by controlling physical and chemical variables such as temperature, light, substrate, flow, and water quality. Controlling environmental conditions leads to reduced variation in biotic state, which improves the measurement of biotic processes. This artificial stream design provides spatial homogeneity and minimizes water volume fluctuation, which facilitates solution manipulation and measurement.

Characterizing Test Clays

Chapter three describes the use of laboratory batch experiments to characterize test clays for their aqueous suspension behavior and affinities for dissolved phosphorus. Commercially available kaolinite and bentonite clays were selected for their low parent phosphorus and availability. The effect of supporting solution ionic strength on clay suspension was determined by settling experiments measured with nephalometry. Phosphorus adsorption "isotherms" were created from laboratory equilibrium experiments. The amount of phosphorus sorbed per amount of clay was plotted as a function of equilibrium concentration, and a Langmuir fit of the data was used to predict the maximum sorption potential for each type of clay. This methodology is also useful for predicting sediment influence on equilibrium driven stream phosphorus concentrations (Froelich, 1988). The rate of SRP adsorption was determined by employing an Elovich fit to the laboratory equilibrium data. The Elovich model describes heterogeneous chemisorption kinetics onto solid surfaces and is frequently used to explain SRP sorption and desorption on soils and soil minerals (Sparks, 1989).

Phosphorus Uptake by Periphyton in the Presence of Suspended Clay

Chapter three describes the effect varying amounts of suspended clay exert upon the phosphorus uptake rate exhibited by lotic periphyton communities growing in replicated artificial stream channels. Periphyton was grown in a recirculating stream system and subjected to simultaneous suspended clay and phosphorus additions. Phosphorus removal from solution, both in the presence and absence of suspended clays, was used to quantify phosphorus uptake parameters by periphyton. Periphyton phosphorus uptake parameters describing first-order uptake kinetics were obtained by non-linear, least-squares regression. Differences in phosphorus uptake parameter estimates were evaluated with one-way ANOVA.

Phosphorus Uptake and Release by Periphyton in the Presence of Suspended Clay

Chapter four expands upon earlier experiments and includes the effect suspended bentonite and kaolinite clays exert on both phosphorus uptake and turnover by lotic periphyton in laboratory microcosms. In separate experiments, clay loads were suspended in radiolabeled (^{32}P) soluble reactive phosphorus solutions to which periphyton cultures were added. The phosphorus uptake rates were determined by measuring radiolabeled phosphorus disappearance from solution. Phosphorus turnover to solution was evaluated over a 10 day period utilizing recirculating laboratory microcosms. Radiolabeled phosphorus remaining in periphyton tissue was measured to determine the phosphorus turnover rate. Periphyton phosphorus uptake and release parameters, describing first-order uptake and release kinetics, were determined by non-linear, least squares regression. Parameter coefficients were evaluated for significant differences using repeated measures ANOVA.

Conclusion

The concluding Chapter Five restates the individual problems addressed in Chapters Two through Four and summarizes their results. A brief comment on future research directions and the application of the findings finishes the dissertation.

CHAPTER TWO

A Constant-Volume Artificial Stream for Reducing Variation in Aquatic Process Measurements

Introduction

This article describes the construction, operational assessment, and improved experimental resolution gained from a compact, constant volume, recirculating, artificial stream system used to investigate the influence of clay loads on phosphorus buffering by periphyton. Spatial and temporal heterogeneity generate the greatest sources of variation in the distribution and activity of aquatic biota in natural systems (Stewart and Loar 1994; Palmer and Poff 1997). Dodds (2003) noted that periphyton-mediated phosphorus uptake rates varied over five orders of magnitude in natural systems as a result of both temporal and spatial variation. Investigating ecological problems under the extremely variable conditions found in flowing waters often requires a tool that reduces environmental variation so that stream processes can be effectively measured. Artificial streams are ideal for this purpose.

The "artificial stream," sometimes called a "laboratory stream," provides varying degrees of control over the physical and chemical environment of flowing water systems in which aquatic processes may be studied (Warren and Davis 1971). Differences in time or season (Marti and Sabater 1996; Francoeur 2001), temperature, light, ionic composition (Romani and Sabater 2000), substrate materials (Boyero 2003), trophic interactions (Hillbrand 2008), nutrient concentration (Van Nieuwenhuyse and Jones 1996; Carr et al. 2005), water flow (Saravia et al. 1998), and environmental scale (Cooper et al. 1997) all contribute to the extreme variation found in lotic systems. Artificial streams can be employed to effectively manage temporal variability and reduce spatial variability, thereby improving the observation and measurement of aquatic processes. They have long been used by engineers investigating hydrologic problems (McIntire 1993) and more recently utilized by biologists and environmental scientists investigating biological and water quality issues (Swift et al. 1993; Birkette et al. 2007). In addition to reducing environmental variation and improving experimental control, artificial streams offer additional benefits, including cost management, ease of replication, and experimental design flexibility (Smith and Mercante 1989; McIntire 1993). The comprehensive Lamberti and Steinman (1993) review outlined the basic types, uses, advantages, and limitations in relation to biological research.

Most studies utilizing artificial streams describe their construction and operation briefly, secondary to the research, leaving design and operational details to the imagination of the researcher. We present detailed instructions for constructing a recirculating artificial stream system that manages water quality conditions by maintaining a steady water volume. Recirculating designs such as this have inherent problems with the propulsion mechanisms used to generate water flow that should be considered before adopting their use. Our closed "trough-cum-waterfall" design uses pumps to lift water to the working section from where it falls back into the holding tank. The energy required to drive the pumps can heat the water and require cooling if the lift is great or the channel volume is very small. Others have circumvented this problem through the use of flumes combined with propellers (Vogel 1994), horizontal ellipsoidal channels combined with a paddle wheel (Nowell and Jumars 1987), and axial impeller chambers utilizing large return line diameter (Dodds and Brock 1998). These schemes provide a large range of water flow velocities without inducing excessive heat into the system.

Several reasons exist for maintaining constant operating water volumes within artificial streams, including accurate replication, quantitative determination (of water born constituents), and, as in our case, maintenance of specific physical and chemical requirements. Stoichiometric manipulations are facilitated as dissolved constituent measurements are determined by volumetric analysis, which is complicated by volumetric errors. Sampling variability has been shown to greatly affect the accurate determination of dissolved nutrient concentrations in natural waterbodies (Donohue and Irvine 2008). This effect can be reduced under the controlled conditions found in artificial streams. Maintaining constant water volumes lends confidence that the observed results are attributable to experimental treatments rather than variation in water volume and dissolved constituent concentrations.

The artificial stream design described in this article was used to investigate suspended clay influence on phosphorus acquisition (load buffering) by lotic periphyton (Wolfe and Lind 2008). An artificial stream was required for these investigations because the turbidity conditions required by the experiments presented a set of problems more easily managed in a controlled environment, mainly creating and maintaining water quality conditions favorable to suspending clays and supporting periphyton communities for experimental manipulation.

Our objectives with this article are to (1) provide those contemplating the use of an artificial stream with information that will speed design, construction, and operation, and (2) demonstrate the improved experimental control and reduced biotic variation obtained when applying this design.

Materials and Methods

Construction

This artificial stream was designed to maintain working water volumes and water quality conditions within 5% of beginning values while providing a continuous supply of flowing water to experimental channels over a one-week time frame. Figure 2.1 illustrates the overall recirculating plumbing scheme and mechanism for volume maintenance. Table 2.1 lists construction materials and is referenced to Figure 2.1. Construction of the system in a laboratory or greenhouse provides for control of temperature, light, and water inputs. Artificial streams may be constructed from a variety of materials to suit the researcher's goals and resources; however, polyvinyl chloride (PVC) plastic is highly recommended because of its availability, cost, ease of use, low chemical activity, and low biotic toxicity (Stelzer et al. 2003).

The stream channels are constructed from (PVC) rain gutter sections, which are typically 0.1 m wide by 0.08 m deep by 3.0 m long (Figure 2.2a). This material is convenient for small-scale systems not requiring large, deep channels, as the straight channel construction simplifies assembly. Sections are easily spliced to any required length by overlapping and gluing pieces together with a 100% silicone rubber caulking compound. The sumps are constructed from 0.25 m diameter Schedule 40 PVC sewer pipe cut to a 0.65 m length and sealed at one end with a PVC cap (Figure 2.2a). The sump houses a submersible impeller pump (model PE-1, Little Giant Pump Co.,

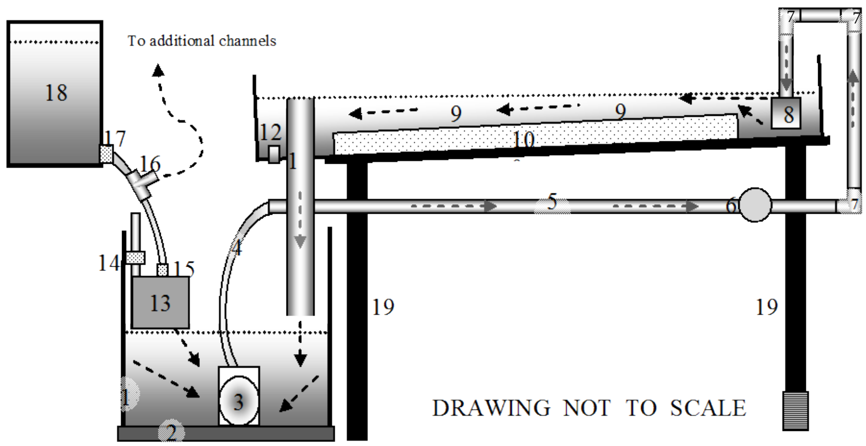

Figure 2.1. Recirculating artificial stream design. Dotted lines indicate water level, arrows indicate flow direction, and numbers correspond to part number in table 2.1. Note that drawing is not to scale.

Oklahoma City, Okla.) that provides lift for the circulating solution (Figure 2.2b). Typical delivery for this pump, at 1 m height, is approximately 1230 l h^{-1}. The sump also houses a float valve (Figure 2.2c; model TM825, Miller Manufacturing Co., Eagan, Minn.), which replaces evaporative water loss with deionized water from a 100 l fiberglass reservoir (Figure 2.2a). Replacement water flows into the system when the level in the sump drops enough to allow the float valve to open. The valve body is fastened to a 1.3 cm diameter by 30 cm long piece of PVC pipe fixed to the inside wall of the sump with an electrical conduit hanger clamp (Figure 2.2b). This arrangement allows vertical adjustment of the float valve's opening and closing position by sliding the PVC pipe up or down through the clamp. The valve's height in the sump controls the sump's water level and overall system water volume. The float valve's connection to the deionized water reservoir is made using flexible PVC tubing and nylon fittings. With operating equipment (pump, valve, hoses, etc.) placed in the sump, the stream's working volume ranges from ~10 to 30 l. The ends are closed by cutting a wedge- shaped piece

Table 2.1. List of artificial stream construction materials, uses, quantities, and associated costs. Cost estimates are rounded to the nearest dollar and based on 2005 material prices. Part numbers correspond with parts labeled in figure 1.

Part No.	Item and Material	Function	Quantity	Approx. Cost
1	SC40 PVC pipe, 45.7 cm × 66 cm (18 in. × 26 in.)	Sump sidewall	1	$27
2	SC40 PVC end cap, 45.7 cm (18 in.)	Sump bottom	1	$14
3	Impeller pump (Little Giant model PE-1)	Solution flow	1	$60
4	Flexible PVC tubing, 1.3 cm × 7.6 m (1/2 in. i.d. × 25 ft length)	Delivery/reservoir	1	$25
5	SC40 PVC pipe, 1.3 cm × 3 m (1/2 in. × 10 ft)	Solution delivery conduit	1	$3
6	SC40 PVC ball valve, 1.3 cm × 1.3 cm (slp 1/2 in. × slp 1/2 in.)	Solution delivery conduit	1	$6
7	SC40 PVC elbow fitting, 1.3 cm × 1.3 cm (slp 1/2 in. × slp 1/2 in.)	Solution delivery conduit	3	$1
8	SC40 PVC pipe, 2.5 cm × 3 cm (1 in. dia. × 1.5 in. length)	Solution delivery (diffuser)	1	$1
9	Substrate material (various, can be natural or artificial)	Substrate	NA	NA
10	SC40 PVC rain gutter, 3 m (10 ft) section	Channel conduit	1	$12
11	SC40 PVC pipe, 1.3 cm × 61 cm (1.25 in. × 24 in.)	Stand-pipe drain	1	$4
12	Rubber stopper, No. 6	Channel	1	$1
13	Float valve (Miller Manufacturing model TM825)	Volume maintenance	1	$8
14	Electrical conduit hanger clamp, 1.3 cm (1/2 in.)	Valve positioning	1	$1
15	Nylon adapter fitting, 1.3 cm × 1.3 cm (slp 1/2 in. × mpt 1/2 in.)	Volume maintenance	1	$2
16	Nylon tee adapter fitting, 1.3 cm × 1.3 cm (slp 1/2 in. × slp 1/2 in.)	Volume maintenance	1	$2
17	Nylon 90° elbow fitting, 1.3 cm × 1.3 cm (slp 1/2 in. × slp 1/2 in.)	Volume maintenance	1	$2
	Total (per channel)			$169
18	Fiberglass tank	Deionized water reservoir	1	$100
19	Steel support frame with expanded steel top (supports five channels)	Channel support (five channels)	1	$150
20	100% silicone rubber (GE Silicone 1), not shown in drawing	Channel sealant (five channels)	1	$20
	Total (one reservoir supply all channels, each frame supports five)			$255

from the corner and folding the end up (Figures 2.2d, 2.2e, and 2.2f). Holes are punched near the top and the pieces fastened together with a plastic wire tie. The joint is sealed with silicone caulking compound, forming a watertight trough. Very long reach lengths and riffles-pools scenarios can be simulated by using multiple sections, placing blocks under the channel, and arranging substrate materials within the channel. A standpipe drain on the channel's downstream end returns flow to the sump (Figure 2.2e). Eight cm from the end, a 3.2 cm hole is cut in the channel bottom with an electric drill fitted with a hole-saw bit. This size hole, if prepared carefully, provides a tight, friction fit for the standpipe made of 3.2 cm schedule 40 PVC pipe, 61 cm in length. The friction fit of the standpipe allows easy vertical adjustment, which controls the water depth and volume within the channel. Temporary channel solution storage can be accommodated during brief periods by adjusting the standpipe drain's height enough to retain some depth within the channel. Stream contents then remain hydrated, without flow, until the problem is corrected or adjustment made. A 1.3 cm secondary drain, made by drilling a 1.3 cm hole between the standpipe drain and the end of the channel, provides a means of completely draining the channel without disturbing operational volume settings. This drain is sealed during operation with a No. 6 laboratory stopper (Figure 2.2e).

Steel frames, 1 m in height by 1 m in width by 2.8 m in length, are covered with an expanded-steel mesh top and provide the elevation necessary for gravity return flow in the channels (Figure 2.2a). Each frame supports five PVC channels, which cantilever over the edge of the support frame so that the standing drain pipes extend directly into the sumps. A configuration of five channels was found to be convenient for working. Tables

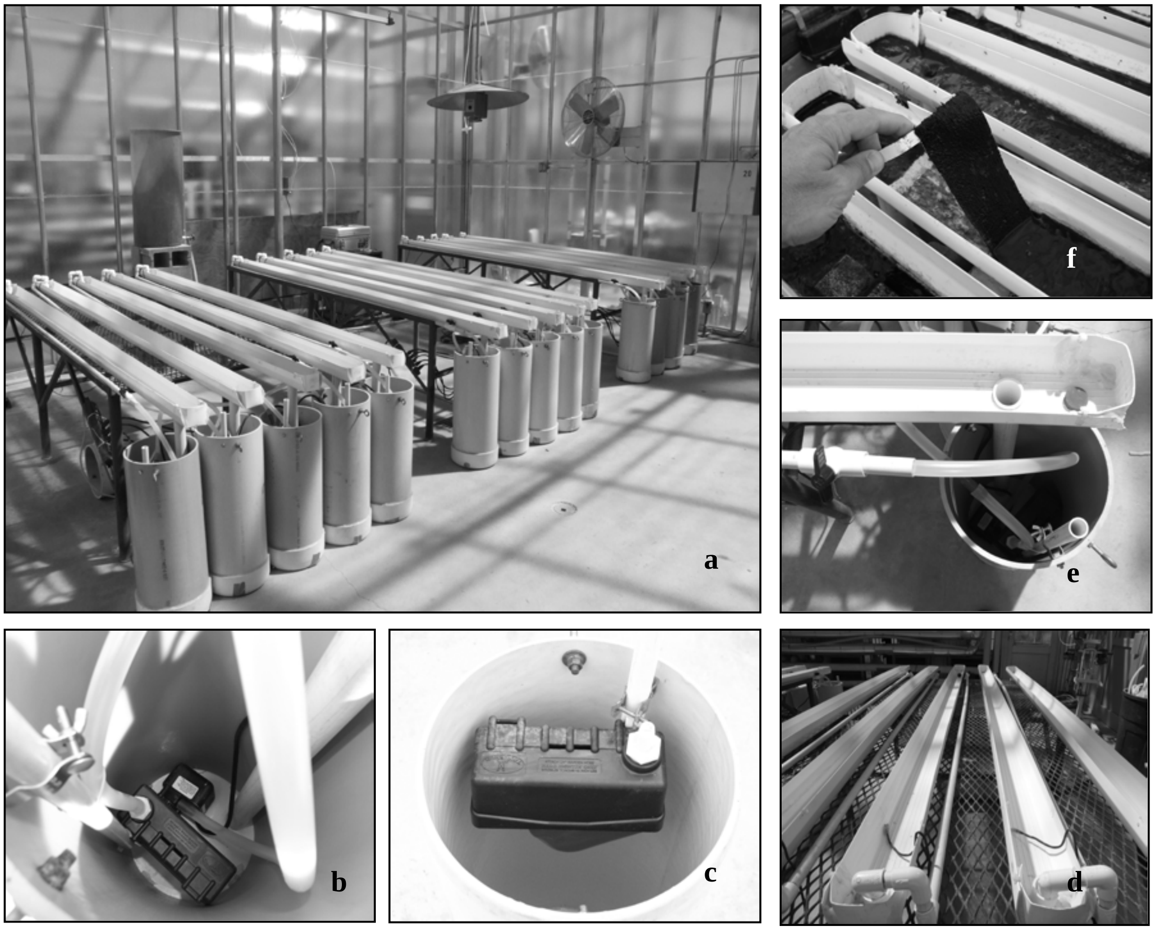

Figure 2.2. Photographs of artificial stream system in a greenhouse. (a) Overall system showing individual channels, sump arrangement, and support platforms. Refill reservoir is visible left rear. (b) Inside view of sump. Float valve shown near lower center and the adjustment clamp at left. Pump is on the bottom of the sump and identified by white sticker on pump body. Delivery line is attached to pump. Return flow standpipe is visible at right. (c) Close-up of float valve in highest position. Refill line has been disconnected, and float is hanging in open position (d) Close-up of individual channels near channel head. PVC diffusers are visible below delivery pipes in middle channels. (e) Close-up of channel end showing standpipe and alternate drain. Deliver line and in-line flow restriction valve is parallel and below channel. f) Periphyton grown on nylon screen substrate.

supporting more than five channels should be avoided because access to the center channels becomes difficult when the operator must lean over a large number of outer channels.

A delivery pipe directs flow from the sump to the head of the stream channel through a 1.3 cm PVC pipe routed alongside the channel on the support frame (Figure

2.2d). A 1 m length of flexible plastic hose is used to connect the submersible pump to the rigid PVC delivery pipe. Three 45° angle PVC fittings are placed at the delivery end to direct the water into the channel head. A 1.3 cm PVC ball valve is plumbed in the delivery line to provide a means of adjusting flow and compensating for variation among individual pump outputs (Figure 2.2d). A flow diffuser is constructed from a 3.0 cm length of 2.54 cm diameter PVC pipe, slightly larger than the delivery pipe. This piece is open at either end and simply placed under the end of the 1.3 cm deliver pipe (Figure 2.2d). Without this piece, water exiting the delivery pipe may have enough energy to displace substrate materials near the channel head, flow over the channel edges, or splash out. As water leaves the delivery pipe, it flows into the larger diameter diffuser and exits though the open top and bottom, thus providing a pressure drop and reducing exit energy at the head of the channel. The delivery pipe can be used to empty the system by diverting the pump flow from the channel head to any convenient drain.

Operation

Both water quality and quantity determine what kind of artificial stream system can be assembled and effectively operated (Lamberti and Steinman 1993). The pH, alkalinity, and hardness of water influence the nutrient availability, toxicity of some materials (especially metals), and turbidity. Small-scale systems such as this one offer the significant advantage of a small water volume requirement. Small water volumes make it possible to easily and economically create specific water conditions through the addition of salts to deionized water. This allows the researcher to control water chemistry specific to experimental needs. Standard Method 8010E (APHA, 2005) provides chemicals and quantities recommended for a reconstituted freshwater media (RFM) capable of emulating various specific freshwater mineral hardness conditions and buffering capacities, as required by the researcher.

The operating volume within individual stream units is determined and controlled by manipulating the water level in the sump. First, the adjustable float valve is placed in its lowest position, and a measured water volume is added to the sump. The pump is then turned on, and the delivery pipe and channel are allowed to fill. After the system comes to "flow equilibrium," the sump refill level is set by careful adjustment of the float valve position. The valve's opening point is determined by moving the valve assembly vertically up until water begins to flow into the sump from the reservoir. Fine adjustment is accomplished by observing bubbles trapped in the reservoir's delivery tubing. This operation should be done slowly and carefully, as valve position affects the final operating volume of the stream unit. In order to maintain volumetric accuracy with this design, care must be taken when working in and or around the sumps and channels. Any changes to the equipment placement in the sump (pump, hoses, etc.), the position of the standpipe drain, or substrate within the channel can induce volumetric error.

Different stream discharges and flow velocities can be obtained in the artificial streams by adjusting the pump flow using the delivery line restriction valve or by manipulating the channel slope. Any mechanical differences in the pumps can be compensated for by measuring the time necessary to fill a standard volume. The inline restriction valve on each stream should be opened or closed to the point that the delivered volumes are equal. Placing blocks under the upstream end of the frame provides simple adjustment of the channel slope to produce different flow velocities within the channels.

These are easily determined with timed floats. This adjustment must be made for each set of five channels per support table.

Substrates placed within the channel provide the living and research areas for aquatic organisms and fall into two general categories: natural and artificial. Natural substrates such as rocks, gravel, and sand can provide a base for biota to colonize and add realism to the artificial environment, but they are difficult to sample and analyze due to a lack of uniformity (Aloi 1990). We have used natural quartz gravel, glass slides, and plastic screen materials with success for different experiments within our artificial streams. Before carrying out research studies, all substrates and system construction materials should be assessed for their toxicity and sorption characteristics of the constituents under investigation.

Power and Safety

The combination of water and electricity are inherently dangerous. Since the impeller pumps operate on 120 VAC line current and water is present, it is important to employ ground-fault circuit breakers in line with the power delivery. Our arrangement consisted of a metal frame supporting five channels with five sumps at the draining end. The impeller pumps were powered through a multiple-outlet power strip with a built-in circuit breaker. The power strips were connected to extension cords leading to the wall mains, which were equipped with ground-fault circuit breakers. The extension cords and power strips were supported with PVC pipe to prevent direct contact with the greenhouse floor. While servicing the artificial streams, we found it convenient to wear rubber "mud boots." They are relatively comfortable, provide good traction, and keep one's feet dry when water is present on the greenhouse floor. Most important, they offer some degree of shock hazard protection due to their insulating construction.

Assessment

Prior to experimental use, the system's operating water volume and water quality maintenance were monitored over a seven-day period in five separate stream units using electronic datalogging equipment. Volume fluctuation was determined by tracking changes in stream sumps and reservoir water levels with bubble flowmeters (model 4230, ISCO, Inc, Lincoln, Neb.). These instruments measure and record changes in water depth (1 mm operating resolution) using a pressure transducer. Daily evaporative loss was calculated by dividing the reservoir water volume loss by 5, as the deionized water reservoir was shared between five individual stream units. Individual stream units were filled and calibrated with a 10 l operating volume. Converting water depth to volume was facilitated by the cylindrical sump design. Individual sump water depth was converted to volume by multiplying sump depth times the sump surface area (i.e., 1 mm depth change = 5 ml volume change).

Our experimental objectives required that we emulate streams with high clay turbidity. The necessary conditions were created and monitored over a one-week period to determine the system's ability to maintain those conditions. Ten liters of very soft RFM were added to each of five artificial streams along with a 200 mg l^{-1} clay load consisting of a 1:1 non-expanding kaolinite (Kaolin P, U.S. Silica, Kosse, Tex.). The salts present in water heavily influence the behavior of suspended materials, so we used RFM as per Standard Method 8010E (APHA 2005) to minimize clay flocculation and settling. In general, most clays will remain suspended in poorly buffered waters of low hardness (Hargreaves 1999), typically <20 mg $CaCO_3$ l^{-1}. To track changes in the total concentration of dissolved ionic matter (dissolved salts), a rating curve was developed to estimate total dissolved salt (TDS) concentration from specific conductance, measured daily using a portable probe (model Quanta, Hach Co., Loveland, Colo.). Conductivity provides an accurate, quick check of the total concentration of ionic matter in water (Lind 1985; APHA 2005). A rating curve was developed to determine suspended clay concentration from turbidity, measured daily as nephalometric turbidity units (NTU), using a nephalometer (model 2100, Hach Co., Loveland, Colo.). Individual stream turbidity and conductivity was measured daily for seven days. The results were converted to clay and salt concentrations and plotted as functions of time.

Periphyton biomass and phosphorus uptake was measured within the 15 artificial stream channels. Nylon screen material with a harvestable area of 0.45 m^2 served as a growth substrate, and RFM at <20 mg l^{-1} $CaCO_3$ hardness was used to support periphyton growth. Periphyton seed material was collected from Salado Creek, Salado, Texas (30° 56' 38" N, 97° 32' 11" W). Periphyton communities were subjected to simultaneous suspended clay and phosphorus loads following a two-week establishment period. Periphyton ash-free dry weigh (AFDW) biomass was determined gravimetrically (APHA 2005), and SRP concentrations were determined using the Murphy and Riley (1962) method. The coefficient of variation (CV) was used to express the variability of periphyton AFDW biomass and SRP uptake because it adjusts the sample variance relative to the mean and is a better comparative measure of variability than individual sample variance when comparing across scales (Palmer et al. 1997).

Results and Discussion

Low material and energy costs allowed us to construct and operate numerous replications without exceeding our experimental budget. Our 15-channel artificial stream facility at the Texas AgriLife Research, Blackland Research and Extension Center in Temple, Texas, was built for less than \$3000 in materials. Construction cost for a single channel is estimated at ~\$169. A one-time cost of ~\$100 is required to supply the fiberglass reservoir, which may be shared between multiple channels. A channel support table costing ~\$100 is required for a single channel and can be shared by up to five channels. Cost totals, based on the desired number of channels, can thus be calculated from the list given in table 2.1. Operational energy cost of the Little Giant PE-1 pump is also quite low (36 W requires ~\$0.12 per day at \$0.14 per kWh). Our 15-channel system was operated for less than \$2 per day. Water and reagent requirements were also manageable. The system used less than 100 l of deionized water per week to replace evaporative loss, and since the solution was recirculated, replacement salts and clay minerals were not needed to maintain experimental water quality conditions.

The system's performance in maintaining accurate water volumes and supporting specific water quality conditions over time was assessed prior to experimental use. Evaporative water loss over a one-week evaluation period totaled 2448 ml, or 24.5%, per stream. Figure 2.3 illustrates the relative change in reservoir water volume and in one stream unit. Evaporative rate ranged from 5 to 41 ml h^{-1}. Despite a 25% loss in stream volume over the evaluation period, the maximum observed stream volume fluctuation, measured by changes in sump level, was 29 mL over a 1 h period, reflecting a 0.29% fluctuation in the total 10 l operating water volume. The maintenance of a constant operating water volume helped keep dissolved and suspended constituent concentrations constant.

Total dissolved salts, measured as specific conductance, and suspended clay concentrations, measured as turbidity, were tracked over a one-week period to determine the effectiveness of volume maintenance to hold these water constituents constant (Figure 2.4). Suspended clay concentrations at initial addition ranged from 206 to 199 mg l^{-1}, within 3.5% of the targeted 200 mg l^{-1} load. Settling of large clay particles, about 30% of the total addition, occurred within the first 24 h. Low water turbulence within the sump was the probable cause and could be prevented by adding an agitator or bypass flow

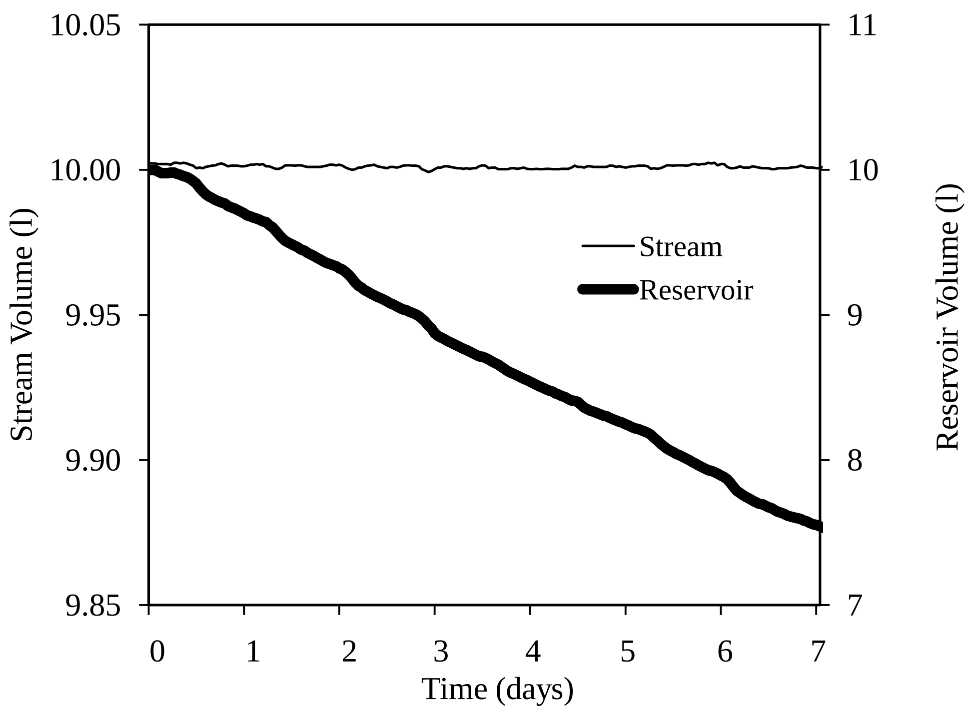

Figure 2.3. Changes in stream and reservoir volumes over a one-week period. Individual stream volume fluctuated less than 0.5% from initial volume as the reservoir replaces ~2.5 l or 25% of the stream's operating volume lost through evaporation.

circulator to the sump. After initial settling, the mean suspended clay concentration dropped to 139 mg l^{-1} where it remained within 4.3% until the end of the evaluation.

Mean TDS daily ranged from 23.8 to 26.3 mg l^{-1}, a relative change of 9%; however, the final mean conductivity was within 3% of the initial value. Constant, steady replacement of evaporative loss with deionized water prevented the volumetric reduction of the recirculating solution and subsequent concentration of the dissolved salts. Maintaining the solution's ionic character prevented "domino effects" that change water alkalinity, hardness, and pH, leading to additional clay flocculation and settling. As long as water

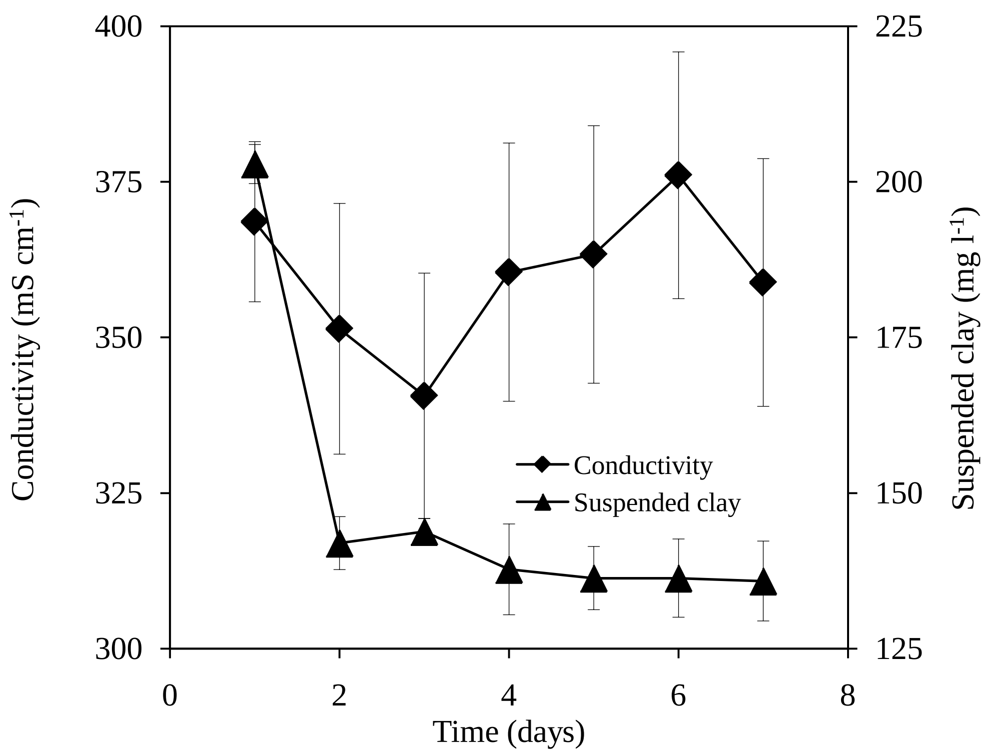

Figure 2.4. Total dissolved salts and suspended clay concentration over a one-week period for five replicated streams. Dashed line associated with suspended clay indicates settling of larger particles during first 24 h after addition. Each point represents five replications with one standard deviation.

depth is not a critical factor to the community under investigation, increasing channel slope can be used to increase water velocity. With the support platform level and the standpipe drain of the channel set to maintain a depth of 3 cm**,** the average flow velocity in this system, measured with timed floats, was found to be 4.9 cm s^{-1}. Lowering the standpipe drain to its lowest level and increasing the slope to 0.025% doubled the flow velocity to 10.9 cm s^{-1}. Additional increases in slope, to 2%, rapidly increased the velocity to 57 cm s^{-1}. It should be noted that increases in water velocity are accompanied by a reduction in water depth. Creating level and sloped sections within an artificial stream can be used to emulate the riffle-pool configuration of natural stream channels (Matthews et al. 2006). Excessive heating of the working water volume by the recirculating pumps was not found to be a problem. Temperature differences between the recirculating water in the channels and the greenhouse air were less than 1°C over a one-week timeframe.

Periphyton biomass and SRP uptake CVs were lower in the artificial streams than in several natural streams, which was probably due to reduced environmental homogeneity gained by controlled physical and chemical conditions. This is speculation and was not specifically tested. Mean periphyton AFDW biomass produced in the artificial stream system ranged from 5.2 to 9.8 g m^{-2} and had corresponding CVs ranging from 9% to 22%. By comparison, periphyton AFDW biomass measured in several natural streams studies ranged from 13 to 873 g m^{-2}, with CVs ranging from 48% to 185% (Sipfil et al. 1998; Tank and Dodds 2003; Carr et al. 2005; Alvarez and Pardo 2007). Mean SRP uptake by periphyton ranged from 2% to 5% within experiments conducted in the artificial streams. Several reported CVs for mean SRP uptake rates measured in natural streams ranged from 39% to 93% (D'Angelo and Webster 1991; Marti and Sabater 1996; Simon et al. 2005). The higher CVs observed in natural streams were due to a combination of high temporal and spatial heterogeneity.

Conclusions

Accurate water volume management maintained the measured water quality characteristics within 5% of their original values. This design was able to hold operating water volumes within 1% while keeping dissolved and suspended materials within 3% and 4% of target values over a one-week period. The system was used to study the effect of suspended clays on phosphorus uptake by periphyton because creating stream turbidity conditions necessary for the research and measuring biotic processes under those conditions presented a set of problems that was more easily managed in a controlled environment. Variation, as CV, in periphyton biomass among 15 replicated artificial stream channels was <20% and SRP uptake variation was <5%. Spatial variation cannot be totally eliminated due to the inherent nature of biological variation itself. However, by providing environmental homogeneity in the form of stream area, substrate, water flow, nutrient concentration, light and temperature levels, thereby reducing the most common sources of variation, biotic variation can be reduced, allowing a more accurate measurement of the process of interest. Periphyton biomass and SRP uptake in our artificial streams was less variable than that reported in several published natural stream studies. The low cost, low water volume requirement, controllable physical-chemical water conditions, and minimized natural fluctuation make closed systems like this ideal for studying lotic processes. Many experimental scenarios may be accommodated by using our design as a starting point.

Acknowledgments

The authors would like to thank the Texas Water Resources Institute for funding, and the USDA-ARS Grassland Soil and Water Research Laboratory in Temple, Texas, for resources used to construct and operate the Artificial Stream System described in this article. Suggestions and reviews by Arthur Stewart and Steve Potter are gratefully acknowledged. The comments and suggestions of three anonymous reviewers greatly improved the original manuscript and were much appreciated.

CHAPTER THREE

Influence of Suspended Clay on Phosphorus Uptake by Periphyton

Introduction

Streams and rivers hydrologically link terrestrial uplands to downstream water bodies (Hauer and Lamberti 1996) and act as reactive conduits for dissolved nutrients (D'Angelo et al. 1991). Inorganic clays frequent aquatic ecosystems and can influence many physical, chemical, and biological conditions (Wilber 1983; Davies-Colley et al. 1992; Lind et al. 1992; Doyle and Smart 2001; Lind 2003). When the sources, characteristics, and impacts of fine sediments in lotic environments were reviewed by Wood and Armitage (1997), they summarized that reduced light and photosynthetic potential, reduced organic cell content, abrasive damage, and reduced attachment due to smothering were the major mechanisms by which suspended sediments affect stream primary productivity. Much of this productivity is associated with stream periphyton, the attached benthic community consisting mainly of algae and bacteria, which can play a major role in determining water column phosphorus concentrations (Reddy et al. 1999). Runoff carrying eroded clays creates turbid conditions that reduce light intensities and smother benthic substrates in the aquatic habitat (Parkhill and Gulliver 2002). These physical changes subsequently affect nutrient–biota processes, at least in part through lowered photosynthetic potential but also through diverse sorption–desorption reactions (Cuker 1993; Lind 2003). Major changes in the structure and function of stream benthic and periphytic communities can occur in response to increased inputs of clays and fine sediments (Power and Stewart 1986; Graham 1990; Horner et al. 1990).

Uptake of soluble reactive phosphorus (SRP) in flowing waters is rapid and due to both biotic and abiotic processes (Newbold et al. 1981; Dodds 1993; Peterson et al. 2001). Periphyton, often the dominant primary producer in small streams, lowers SRP concentrations in the water column through several mechanisms, including biotic sorption and uptake (Dodds 2003). Its ability and efficiency to uptake and sequester phosphorus loads have been explored as a water treatment method (Wilkie and Mulbry 2002). Stream sediments affect water column SRP concentrations through abiotic, equilibrium-driven sorption/desorption reactions (Froelich 1988; Klotz 1988). Sediment bound phosphorus, in dynamic equilibrium with the water column concentration, is released when biotic uptake shifts equilibrium potential (Haggard et al. 1999; Webster et al. 2001).

In natural stream systems, suspended clays are coated with layers of ions, organic substances, and bacteria (Lind and Davalos 1990; Lind 2003). These surface characteristics may be of more importance than the underlying mineral to biotic processes. Clay minerals affect nutrient–biota relationships in aquatic ecosystems mainly through ion exchange reactions (Lind and Davalos-Lind 1999). The ability to adsorb and release sorbed ions in an exchangeable state with other ions is known as cation or anion exchange capacity (CEC or AEC, respectively). Numerous locations on the clay particle carry charges because clay particles are comprised largely of aluminum and ferric oxides. These charged sites are exchangeable when water delivers dissolved ions to the sites where they may replace an existing ion of a lesser charge (Lind et al. 1992). CEC occurs within all 2:1 layer silicate clays; whereas 1:1 clays have exchangeable sites on their edges, where broken-edge bonds can be positively or negatively charged and accept cations or anions (Thompson and Troeh, 1973). AEC is generally low compared to CEC for most clay sediments (Grim 1953).

Suspended sediment effects upon aquatic communities have been investigated by numerous researchers. Table 3.1 lists several studies involving suspended sediment along with the type and concentration used in natural and experimental systems. Suspended clays shifted phytoplankton and zooplankton species in responses to SRP, montmorillonite, and kaolinite clay loadings in lentic systems (Cuker et al. 1990; Cuker and Hudson 1992). Burkholder and Cuker (1991) found increases in periphyton growth when suspended clay was combined with SRP amendments in a lake mesocosm. The investigators speculated that clay particles may have facilitated SRP delivery to periphyton through settling, a positive effect not usually expected with turbidity issues. Suspended kaolinite clay concentrations were shown to lower stream respiration and periphyton biomass in artificial streams (Parkhill and Gulliver 2002). Large aggregates of clay, organic materials, and bacteria may form in turbid lake environments, and these aggregates can induce alternate heterotrophic based food webs, due to lowered production by light-limited phytoplankton (Lind and Davalos-Lind 1999). Bacterial production increases due to the formation of clay-organic carbon aggregates which enhance bacterial growth by concentrating nutrients (Lind et al. 1997).

Table 3.1. Studies investigating effects of suspended sediments, at varying concentrations, upon the biota of streams, lakes, and wetlands

Source	System	Material	Concentration ($mg\ l^{-1}$)	Effect on:
Burkholder and Cuker (1991)	Limnocorral	Kaolinite, Montmorillonite	25	Periphyton
Cuker (1987)	Limnocorral	Kaolinite, Montmorillonite	33	Plankton
Cuker et al. (1990)	Limnocorral	Kaolinite, Montmorillonite	33	Phytoplankton
Davies-Colley et al. (1992)	Natural stream	Smectite	15 to 100	Benthic algae
Horner et al. (1990)	Artificial stream	Glacial flour	25	Periphyton
Lind et al. (1997)	Natural lake	Mineral clay	20–200	Phytoplankton
Parkhill and Gulliver (2002)	Artificial stream	Kaolinite	50–300	Macrophytes, periphyton
Schallenberg and Burns (2004)	Laboratory study	Lake sediments	150–300	Phytoplankton
Squires and Lesack (2001)	Limnocorral	Lake sediments	6–18	Benthic algae

Given the rapid assimilation of SRP by stream biota and the influence that suspended clays exert on SRP activity in aquatic environments, we hypothesized that suspended clays would reduce SRP uptake rates exhibited by periphyton. To explore this idea, we (1) selected and characterized the ionic suspension behavior and SRP sorption capacity of two clays, (2) constructed a recirculating artificial stream system appropriate for maintaining conditions needed to suspend clays and house lotic periphyton, and (3) measured the SRP uptake rates of lotic periphyton in the presence and absence of suspended clays.

Methods

Clay Description

Two commercially available clay minerals with low parent phosphorus were chosen to represent those commonly found in rivers, streams, and reservoirs. A kaolinite (Kaolin P, U.S. Silica, Berkley Springs, West Virginia) was selected to represent 1:1 nonexpanding kaolinite clays, and a bentonite (Bentolite L-10, Southern Clay, Gonzalez, Texas) was selected to represent the 2:1 Montmorillonite family of expanding clays. The Kaolin P material contains 90–94% pure mineral kaolin; various surface oxides make up less than 2.4% of the total surface area (personal communication, S. Geho, U. S. Silica, Berkeley Springs, WV). The Bentolite L-10 material contains 90% pure mineral bentonite with about 0.6% iron oxides (personal communication, B. Knudson, Southern Clay, Gonzales, TX). The physical characteristics of each material are listed in Table 3.2.

Table 3.2. Physical characterization of the bentonite and kaolinite clay minerals used in this study

Property	Bentolite L-10	Kaolin P
Mean particle size (lm)	<1.0	1.0
Bulk density (kg m-3)	0.39–0.58	0.35–0.44
Total surface oxides (%)	0.6	2.3
pH (as a 10% slurry)	7.5	6.0
Maximum SRP sorption, 24 h (mg P kg^{-1} clay)	125	58
Phosphorus present on virgin clay (mg)	<0.006	<0.006
Percent SRP sorption, 1 h	75	68
Percent SRP sorption, 2 h	84	74

Clay Suspension in Aqueous Solutions

The effect of supporting solution ionic strength on clay suspension was determined by settling experiments measured with nephalometry. A HACH model 2100 nephalometer was used to develop a curve relating turbidity to suspended clay concentration. The clay settling behavior was then assessed by preparing reconstituted freshwater media (RFM) solutions, as per standard method 1080E.4b, at hardness levels of 20, 40, 80, and 160 mg l^{-1} (as $CaCO_3$) (APHA 2005). One gram of clay was added to duplicate 300 ml aliquots of each RFM solution. The resulting clay suspensions were placed in glass bottles (5 cm in diameter by 30 cm tall). The bottles were capped and allowed to stand undisturbed for 3 days at 22°C. At 24-h intervals, a 20-ml sub-sample was taken from each bottle and assayed for turbidity. The concentration/turbidity rating curves were used to determine suspended clay concentration of the subsample. Suspended clay concentration was plotted over time for very soft and moderately hard RFM solutions.

Clay Affinity for Dissolved Phosphorus

SRP adsorption by Bentolite L-10 and Kaolin P was quantified using laboratory batch experiments. A series of solutions were prepared by diluting a KH_2PO^4 stock in very soft (<20 mg l^{-1} $CaCO_3$) RFM. Duplicated 30 ml aliquots containing 0.05, 0.1, 0.5, 1.0, and 3.0 mg SRP l^{-1} were added to 50 ml polypropylene centrifuge tubes, each contained 0.3 g clay. Treatments were equilibrated for 24 h at 22°C using an end-over-end shaker operating at 15 revolutions per minute. After equilibration, the solutions were centrifuged at 6,000 relative gravitational fields for 15 min to remove the clay from suspension. The supernatant was analyzed for SRP concentration using the Murphy–Riley (1962) ascorbic acid reduction method. A Genesys-5 spectrophotometer (Thermo Fisher Scientific, Waltham, MA) with a 50-mm path length was used to measure absorbance at 880 nm. The results were compared to known standards carried through the experiment to ensure good quality control. The amount of SRP sorbed per amount of clay was plotted as a function of equilibrium concentration, and a Langmuir fit of the data was used to predict the maximum sorption potential (S_{max}) for each type of clay. The Langmuir equation has been useful in predicting sediment influence on stream SRP concentrations (Froelich, 1988) and has the linearized form:

$$C/S = 1/kS_{max} + 1/S_{max}$$

where S is the SRP retained by the solid phase, or clay in our case (mg kg^{-1}), C is the concentration of SRP in solution after 24 h equilibration (mg l^{-1}), S_{max} is the SRP sorption maximum (mg kg^{-1}), and k is a constant related to the bonding energy. Values for S_{max} and k can be determined from empirical measurements of C and S (Graetz & Nair, 2000).

The rate of SRP adsorption was determined for Bentolite L-10 and Kaolin P. A 0.1 mg SRP l^{-1} solution was prepared, as per preceding sorption experiments, and 30 ml aliquots were placed in 50 ml polypropylene centrifuge tubes. Duplicated 0.3 g samples of clay were allowed to equilibrate in the SRP solution for 0.01, 0.25, 0.5, 1, 2, 4, 8, and 12 h. SRP remaining in solution then was measured as described previously, and the amount SRP sorbed was plotted as a function of equilibration time. A simple Elovich fit of the data was used to describe the relationship. The Elovich equation is one of the most widely used models describing the kinetics of heterogeneous chemisorption onto solid surfaces and is frequently used to describe the kinetics of SRP sorption and desorption on soils and soil minerals (Sparks, 1989). This equation has the form:

$$S = a + b \ln^{t}$$

where S is the amount of SRP sorbed (mg), a is the initial SRP concentration (mg l^{-1}), b is a rate constant, and t is time (h). The rate constant b can be determined by empirical measurements of s and a over varying times (t).

Artificial Stream and Periphyton Cultivation

We conducted our studies between 15 October and 5 December 2005 at the Texas AgriLife Research/Blackland Research and Extension Center (BREC) in Temple, Texas, USA. An artificial stream system was constructed within a greenhouse at the facility. Fifteen individual stream units were assembled to provide adequate treatment replication. Plastic gutter flumes, each 10 m in length, 10 cm in width, and 5 cm deep, were used as stream channels and 12,000g of natural quartz gravel, , screened to ~1.0 cm diameter particle size, was added to each channel as a nonreactive growth substrate. Prior to experimental manipulations, all system materials, including the gravel substrate, were tested for SRP sorption to insure that any SRP disappearance from solution could be attributed to periphyton uptake or clay sorption rather than sorption onto artificial stream construction materials. The system utilized a recirculating solution of RFM made by adding salts to demineralized water to produce soft water at <20 mg l^{-1} $CaCO_3$ hardness. During periphyton establishment, all stream channels drained to a common sump from which a single pump delivered the operating solution to a header pipe and delivered the flow back into the 15 individual channels. Water flowed by gravity though each channel before being returned to the sump. PVC ball valves were installed at the delivery of each channel allowing water to be supplied in an equitable, reproducible, and continuous flow. The channels were re-plumbed to an individual stream unit configuration during experimental manipulations. In this configuration, 15 separate sumps and pumps, each associated with an individual channel, were used to provide statistical replication. Each artificial stream had a recirculating volume of 50 l during experimental periods.

Artificial stream seed material was prepared by brushing the surfaces of periphyton encrusted rocks collected from Salado Creek in Salado, Texas (Lat: 30° 56' 38" N, Lon: 97° 32' 11" W). The material removed was suspended in approximately 1 liter of source water and added to the common sump of the artificial stream system. Weekly additions of nitrogen (as KNO_3) and SRP (as KH_2PO_4), at 0.1:1.5 mg N:P l^{-1}, were made during the pre-experimental period to promote periphyton growth. Once periphyton communities were established, the stream channels were re-plumbed to form individual stream units, and nutrients were withheld for 7–10 days. Preliminary experiments indicated this period was sufficient to prevent phosphorus saturation of the biota. Experimental additions of SRP and clay followed. A rest period of 10 days was allowed between SRP and clay addition experiments. Settled clay was left within the channel.

Phosphorus Uptake by Periphyton in the Presence of Suspended Clays

The periphyton communities were subjected to SRP loads with and without suspended clay loads present. Treatments consisted of no clay (control), Kaolin P, or Bentolite L-10, each with five replications; the treatments were randomly assigned to individual streams. The effects of three clay concentrations on uptake of SRP by periphyton were examined in three separate experiments which included additions of clay at concentrations of 20, 80, and 200 mg l^{-1}. Clay levels were chosen based upon values reported in natural systems and utilized in similar studies (see Table 1). SRP additions to the 20 and 80 mg l^{-1} clay experiments were made at 0.1 mg l^{-1}, while the 200 mg l^{-1} clay experiment received a 0.5 mg l^{-1} SRP addition. SRP concentrations were initially selected to represent double the natural concentration common to unpolluted waters (Lind 1985). This was increased in the final experiment due to the rapid uptake observed in the first two experiments. SRP uptake by periphyton and sorption to suspended clays was determined by measuring SRP concentration of effluent sub-samples collected from each stream through time. Water samples were collected before experimental amendments were started to determine pre-addition SRP concentrations. The appropriate amount of suspended clay was added to each channel and allowed to circulate for approximately 5 min to disperse. The SRP amendments were then added, and water samples were collected at 1, 15, 30, 60, and 90 min after addition to determine SRP uptake. Samples were handled and analyzed for SRP as previously described. A 10-day rest period was imposed between experiments to allow depletion of uptaken phosphorus through growth and prevent carry over effects. Preliminary experiments indicated that after an initial SRP addition, wet weight periphyton biomass increased up to the seventh day after which it became steady or declined.

Periphyton biomass was determined gravimetrically after each experiment and is reported as ash free dry weight (AFDW). A stream substrate sub-sample (~50 g) was collected from several locations in each channel, dried overnight at 85_C, weighed, ashed at 500°C for 1 h, re-wet, re-dried overnight at 85°C, and re-weighed. The pre- and post-combustion weights were compared, and AFDW biomass was determined by difference. The substrate material in each channel was pre-weighed so that sub-samples could be used to determine whole stream biomass.

To account for variation, all SRP uptake values were standardized on the basis of periphyton biomass and expressed as unit mass of SRP uptake per unit mass of periphyton AFDW (mg g^{-1}). SRP remaining in solution was plotted against time. SRP lost from the recirculating stream water (mg SRP g^{-1} AFDW min^{-1}) was described by the first-order equation:

$$C = C_i \, \mathrm{Exp}^{-kt} + b$$

where C is phosphorus mass (mg) in solution, standardized by periphyton biomass (g^{-1} AFDW) at any time (t), C_i is the initial phosphorus mass (mg SRP g^{-1} AFDW), k is the first-order rate constant (min^{-1}), and b is the asymptote or AFWD standardized mass in solution at which SRP uptake by periphyton equals SRP efflux. SRP loss from solution was assumed to indicate SRP sorption onto clay or uptake by periphyton.

Statistical Procedures

Variations in periphyton biomass within and among three separate experiments were evaluated using oneway ANOVA. Periphyton SRP uptake parameters describing first-order uptake kinetics were obtained by non-linear, least-squares regression and are presented as means ± standard error (SE). Differences in SRP uptake parameter estimates were evaluated with one-way ANOVA. All statistics and model fits were performed using the SAS (version 8.0) for Windows statistical package (Statistical Analysis System, Cary, NC).

Results

Clay Description and Suspension Behavior

Particle size, bulk density, and surface oxides differ only slightly between the two clays (Table 2). The Bentolite L-10 clay has a smaller mean particle size and greater surface area, compared to the Kaolin P material, yet produces one-third the turbidity, per unit mass, than the Kaolin P. Turbidity measurements were used to determine suspended clay concentration in settling studies. Turbidity versus concentration rating curves developed for Bentolite L-10 and Kaolin P are shown in Figure 3.1. RFM with mineral hardness values above 40 mg l^{-1} $CaCO_3$ hardness caused visible flocculation and settling of both clays. Hardness levels greater than 80 mg l^{-1} $CaCO_3$ flocculated and settled both clays in less than 1 day and values over 160 mg l^{-1} $CaCO_3$ in less than 1 h. RFM containing less than 20 mg l^{-1} $CaCO_3$, however, supported 25–50% of the original quantity of clay added after 3 days. Figure 3.2 illustrates the suspended sediment concentration for both clays over a 3-day period at 20 and 80 mg l^{-1} $CaCO_3$ hardness.

Because of its ability to maintain clay suspensions, RFM containing 20 mg l^{-1} of $CaCO_3$ hardness was selected as the supporting solution for subsequent experiments involving SRP uptake by periphyton.

Affinity of Clays for SRP

Initial phosphorus, maximum SRP sorption potential, and percent SRP sorbed over 1- and 2-h equilibrations, for both clay types, are listed in Table 2. Standard isotherm methods used for the analysis of soils suggest using a 0.1 M CaCl solution

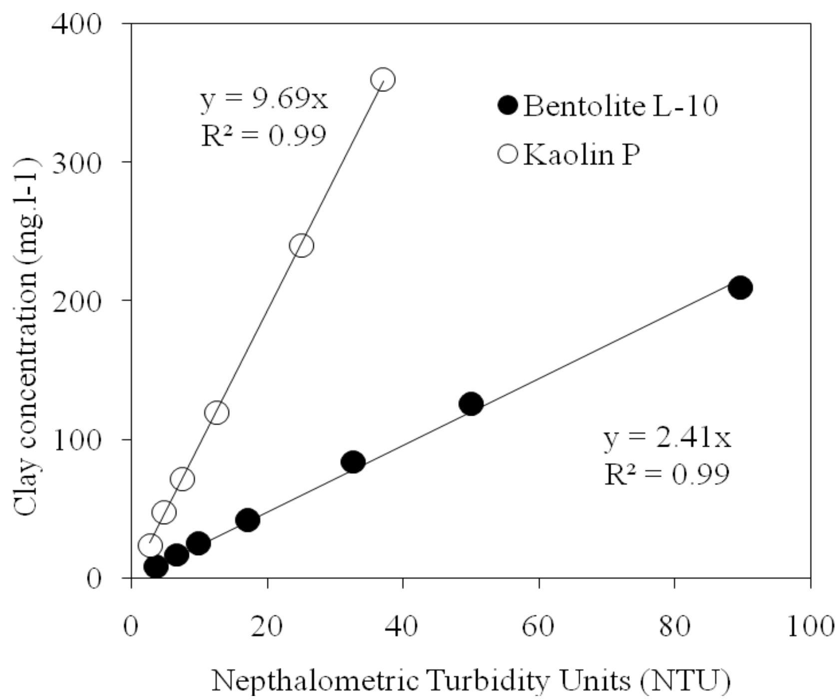

Figure 3.1. Relationship between turbidity, as nepthalometric turbidity units (NTU), and suspended clay concentration (mg l-1) for Bentolite L-10 and Kaolin P

for the aqueous matrix, so as to allow a semi-standardized comparison of soils from various sources. Very soft RFM was substituted for the standard CaCl solution because very soft RFM yielded the most stable aqueous clay suspensions, and periphyton growth

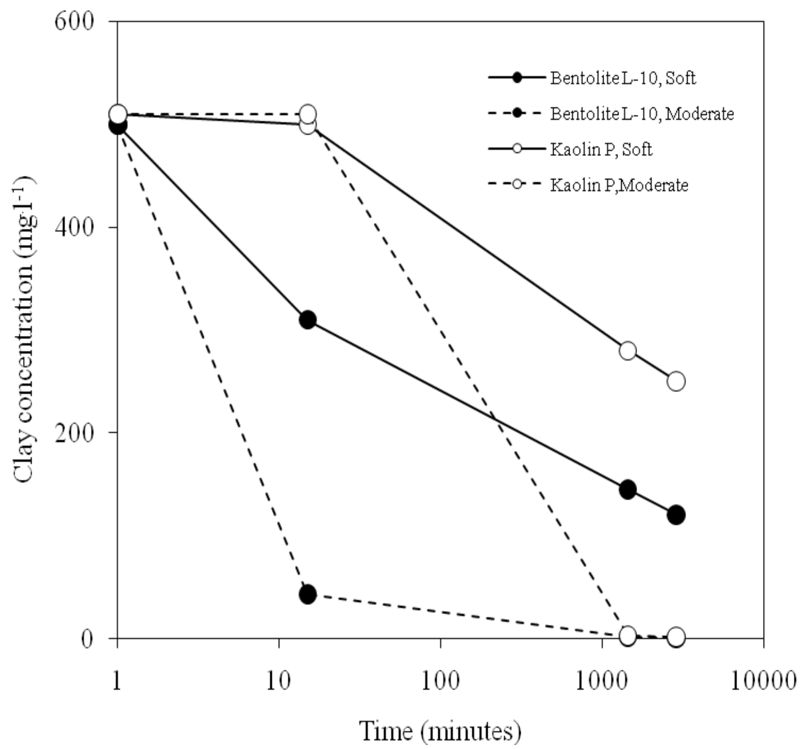

Figure 3.2. Clay settling measured over 3 days as reduction of Bentolite L-10 and Kaolin P clay concentration in reconstituted freshwater media (RFM) prepared at very soft (20 mg l^{-1}) and moderately hard (80 mg l^{-1}) $CaCO_3$ hardness levels. Log scale for time was used due to rapid initial settling in moderately hard solutions.

was found to be satisfactory in this media. The adsorption isotherms of the two clays, the relationship between the amount of SRP adsorbed per unit mass of clay and its equilibrium concentration, and a Langmuir fit for each clay type are shown in Figure 3.3. Maximum SRP adsorption by Bentolite L-10, as predicted by the Langmuir asymptote, was 125 mg of SRP per kg clay. Maximum SRP adsorption by Kaolin P was 58 mg SRP per kg of clay, about half the value found for Bentolite L-10. The results of the SRP adsorption kinetic experiments are shown in Fig. 3.4. Bentolite L-10 sorbed 73–77% of capacity within 60 min. Kaolin P sorption of P was slightly lower, reaching 60–71% of capacity within 60 min. The amounts of P sorbed increased to 74% and 84% for Kaolin P and Bentolite L-10, respectively, within 2 h.

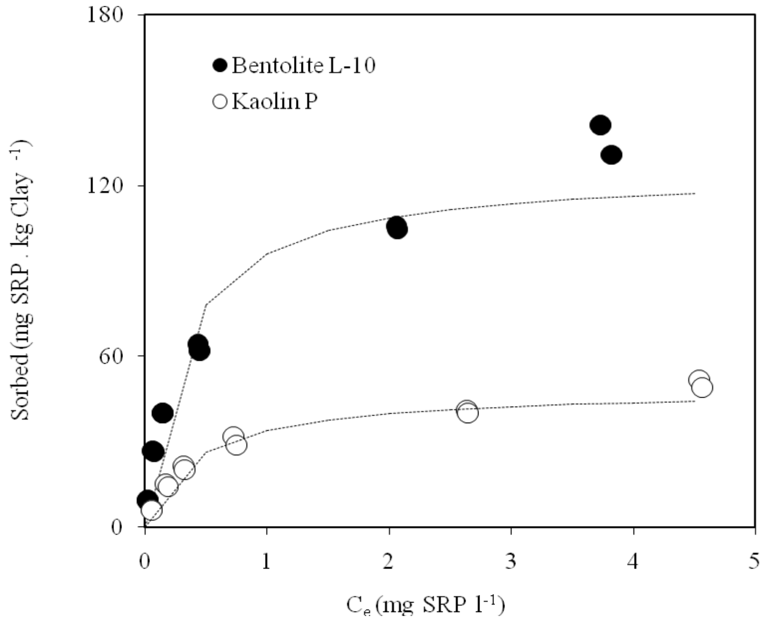

Figure 3.3. Adsorption isotherms for soluble reactive phosphorus (SRP) to Bentolite L-10 and Kaolin P (Note: SRP sorption onto the sorbate is determined by disappearance from solution). The concentration at equilibrium (C_e) values determined after 24 h at 22°C. Dashed lines represent a Langmuir fit of the data.

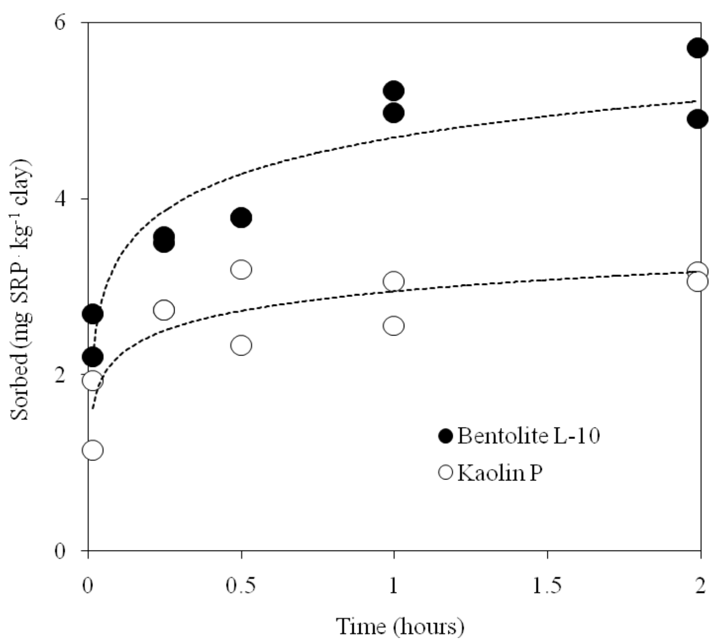

Figure 3.4. Soluble reactive phosphorus (SRP) sorption rate onto Bentolite L-10 and Kaolin P clays with a 0.3 mg l^{-1} initial SRP concentration (Note: SRP sorption onto the sorbate is determined by disappearance from solution). Dashed lines represent an Elovich fit of the data.

Periphyton Growth in Artificial Streams

Physical conditions present in the artificial stream system were similar to those measured outside the greenhouse and favored a periphyton community dominated by canopy-forming green filamentous algae. Periphyton growth in very soft RFM was robust and reached an average of 18.5 g m^{-2} within the channels after four weeks. Biota samples were collected and examined with a light microscope to determine species present. The streams were dominated by a filamentous green alga (*Rhizoclonium* spp.). Two unidentified species of unicellular green algae and two diatoms (both *Pinnularia* spp.) were also present, but in very low numbers.

One-way ANOVA showed that the effect of artificial stream channel assignment (i.e., treatment) upon AFDW biomass was not significantly different within any of the three experiments performed (Exp 1: $F_{2,12}$ = 0.24, P = 0.79; Exp 2: $F_{2,12}$ = 3.30, P = 0.07; Exp 3: $F_{2,12}$ = 0.24, P = 0.17). However, the combined AFDW biomass among the three experiments conducted was different ($F_{2,42}$ = 24.86, P=0.001). Post hoc analysis, using the least significant difference (LSD) post hoc criterion for significance, showed combined AFDW biomass to be different among all experiments (Exp 1: M = 18.57, SD = 5.03; Exp 2: M = 31.45, 10.61; Exp 3: M = 40.23, SD = 8.77). Figure 3.5 shows the mean AFDW biomass and one standard deviation for each experiment.

Phosphorus Uptake by Periphyton

SRP added to individual channels rapidly disappeared from solution for all treatments in all three experiments. Comparison of the nonlinear regression model coefficient k using one-way ANOVA found no differences between SRP uptake in the

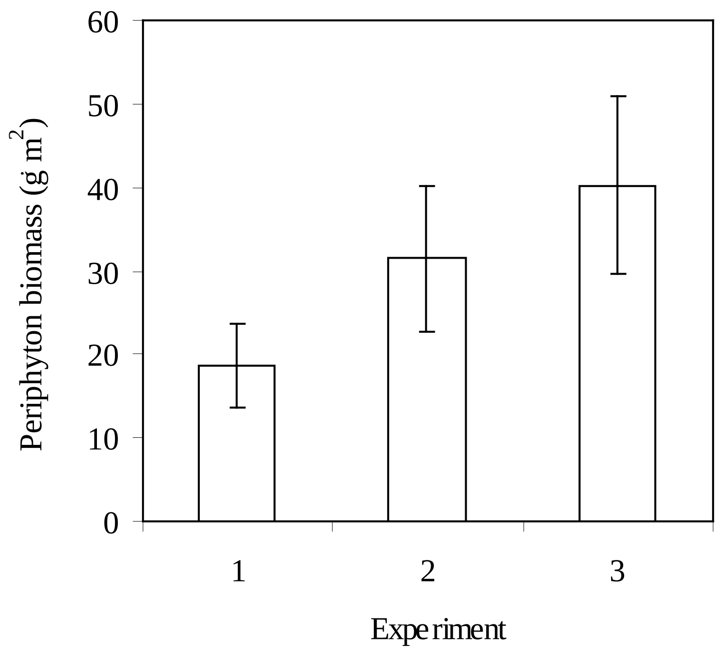

Figure 3.5. Mean ± one standard deviation of periphyton biomass, as ash free dry weight, for each experiment

presence or absence of the two clay at three different concentrations (Exp 1: $F_{2,12} = 0.17$, $P = 0.85$; Exp 2: $F_{2,12} = 2.17$, $P = 0.16$; Exp 3: $F_{2,12} = 0.53$, $P = 0.60$). SRP disappearance from solution can be attributed to either uptake by periphyton, sorption by suspended clays, or sorption by artificial stream construction and substrate materials. Calculations based on clay isotherm data indicated that less than 0.5% of SRP sorption could be attributed to sorption by the suspended clay load. Less than 0.5% was attributable to other materials leaving the majority of sorption due to biotic uptake.

Under the 20 mg l^{-1} clay load, SRP uptake rate constants ranged from 0.06 to 0.07 min^{-1} (Table 3.3), with 55–68% of SRP uptake occurring within 15 min of phosphorus additions, for all treatments. After 90 min, 90–95% of the added SRP had been removed from solution. Control streams, which received no clay additions, had SRP uptake rates similar to those noted for the clay treatments. While the SRP uptake amounts were

Table 3. 3. Solution initial SRP concentration, standardized by periphyton ash free dry weight (AFDW), (mean ± SE) and estimated curve parameters (mean ± SE), number of observations, and r^2 of the exponential SRP removal from solution curves.

Experiment Treatment	Initial SRP concentration (Ci, mg g^{-1} AFDW)	SRP removal rate (k, min^{-1})	Asymptotic intercept (b, mg g^{-1} AFDW)	Obs	r2
Experiment 1					
No clay	0.63 ± 0.04	0.06 ± 0.02	0.07 ± 0.04	5	0.83
Bentolite L-10 (20 mg l-1)	0.47 ± 0.02	0.07 ± 0.01	0.04 ± 0.02	5	0.91
Kaolin P (20 mg l-1)	0.53 ± 0.03	0.07 ± 0.01	0.05 ± 0.02	5	0.94
Experiment 2					
No clay	0.26 ± 0.01	0.09 ± 0.01	0.00 ± 0.01	5	0.97
Bentolite L-10 (80 mg l-1)	0.25 ± 0.02	0.08 ± 0.02	0.00 ± 0.01	5	0.90
Kaolin P (80 mg l-1)	0.31 ± 0.02	0.08 ± 0.01	0.00 ± 0.01	5	0.86
Experiment 3					
No clay	0.36 ± 0.03	0.03 ± 0.01	0.01 ± 0.04	5	0.81
Bentolite L-10 (200 mg l-1)	0.25 ± 0.02	0.03 ± 0.01	0.00 ± 0.02	5	0.63
Kaolin P (200 mg l-1)	0.26 ± 0.03	0.02 ± 0.02	0.04 ± 0.02	5	0.88

Fitted curves are shown in Fig. 4 and have the form $C = Ci\ Exp^{-kt} + b$

slightly less for the control channels, they did not differ statistically from SRP uptake amounts measured for the clay-amended channels (Fig. 6, experiment 1). SRP uptake rates under the 80 mg l^{-1} clay concentration were slightly greater than SRP uptake rates in the 20 mg l^{-1} treatment; they ranged from 0.08 to 0.09 min^{-1} (Table 3.3), with between 83 and 88% of the added SRP being removed from solution within the first 15 min. More than 95% of the SRP was removed within the first 30 min, and no significant differences were observed between experimental treatments (Fig. 6, experiment 2). As the ratio of periphyton biomass to SRP concentration increased between experiments 1 and 2, SRP uptake rate also increased (Fig. 6, experiments 1 and 2). We compensated for the continued biomass accumulation in experiment 3 by increasing the SRP concentration. The 200 mg l^{-1} experiment yielded results similar to those obtained in the 20 mg l^{-1} experiment, with SRP removal rate constants ranging from 0.02 to 0.03 min^{-1} (Table 3.3), and with 66–76% SRP removal within the first 15 min after SRP addition. Between 88% and 92% of the added SRP disappeared from solution by 60 min (Fig. 6, experiment 3). In all cases for all treatments, SRP disappeared rapidly from solution. The rate of SRP removal from solution increased with periphyton biomass.

Discussion

Clay Behavior in Aqueous Phosphorus Solutions

Characterization of the test clay materials (Table 3.2 and Figures 3.1-4) was useful for planning and interpreting experimental results. Minimal SRP sorption could be attributed to the test clays because of their relatively low affinity for SRP. We found that aqueous solutions containing greater than 40 mg l^{-1} $CaCO_3$ hardness promoted rapid flocculation and settling of both experimental clays (Figure 3.2), which is a general behavior exhibited by most clays (Hargreaves 1999). Water hardness, alkalinity, and pH are closely related and affect not only the flocculation and settling of suspended materials but also the biotic availability of many nutrients and metals (APHA 2005).

Constructing adsorption isotherms is a common method for determining sorption capacity of a material for a particular ion, and the importance of soluble reactive nutrients to plants and animals makes this a very useful technique for studying nutrient availability. The adsorption of nutrients onto substrates is frequently described mathematically by the Langmuir model, which can be used to predict the maximum sorption potential of a given material (Graetz and Nair 2000). In our study, the isotherm data indicate that SRP adsorption for both clays increased with SRP concentration until saturation at about 125 mg P kg^{-1} of Bentolite L-10 and about 48mg P kg^{-1} of Kaolin P (Figures 3.2 and 3.3). This is probably due to the structural differences of the clays. The 1:1 type, Kaolin P, adsorbs anions only on its broken edges, whereas Bentolite L-10, a 2:1 type clay, has more surface area per unit mass and a correspondingly larger number of ion exchange sites. Amorphous silica clays, such as Bentolite L-10 and those high in iron and aluminum oxides, have relatively high AEC, while kaolinites are typically lower

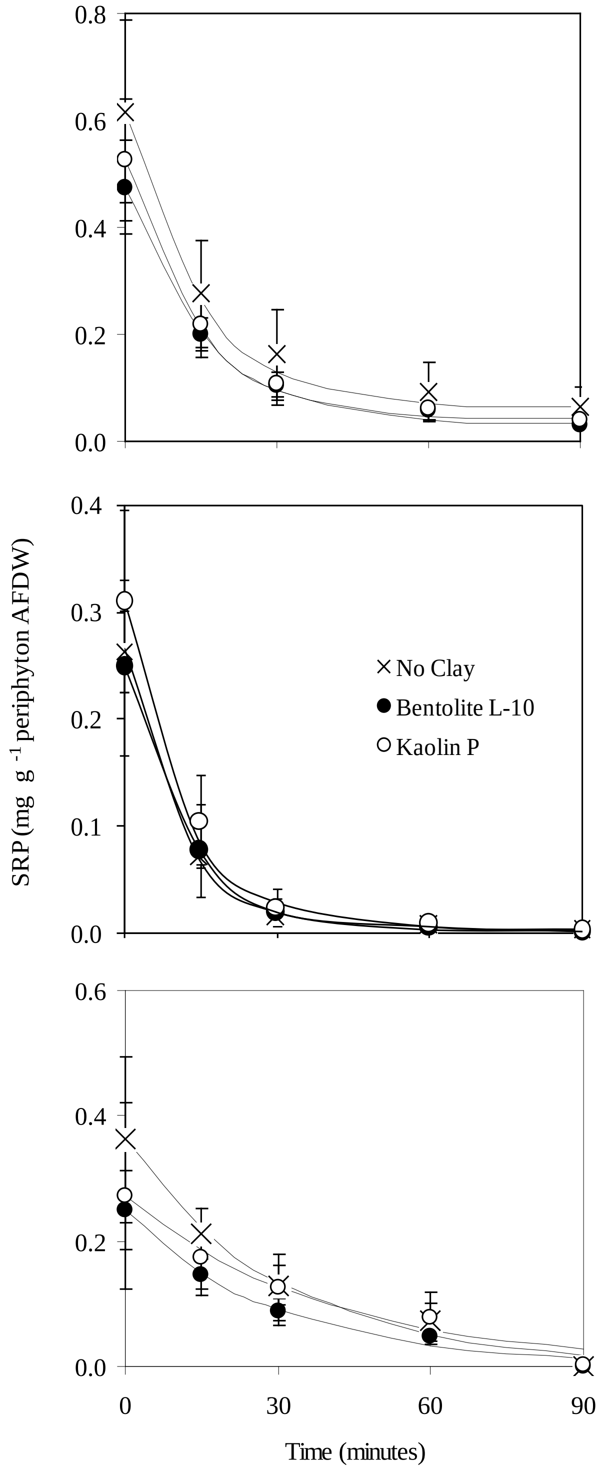

Figure 3.6. Removal of SRP from solution by periphyton, standardized by periphyton ash free dry weight (AFDW) biomass. Curves show first-order kinetic fit of five replications and one standard error. Experiment 1: SRP loss through time for experiments containing clays at 20 mg l^{-1}, or no clay, when SRP was added at an initial concentration of 0.1 mg l^{-1}. Experiment 2: SRP loss through time for experiments containing clays at 80 mg l^{-1}, or no clay, when SRP was added at an initial concentration of 0.1 mg l^{-1}. Experiment 3: SRP loss through time for experiments containing clays at 200 mg l^{-1}, or no clay, when SRP was added at an initial concentration of 0.5 mg l^{-1}.

(Gardiner and Miller 2004). The construction of isotherms and determining the maximum sorption capacity of the test clays allowed us to estimate the SRP fraction load potentially sorbed by suspended clays in our stream system. Less than 0.5% of SRP removal from solution in the channels could be attributed to the suspended clay, in any of the experiments. Also, less than 0.5% of SRP removal was attributed to the artificial stream construction materials. The remaining 99% of SRP removal can be attributed to biotic uptake by periphyton.

Artificial Stream Use

The conditions found in flowing waters make streams and rivers some of the most variable places on earth; artificial streams were employed to simplify this complexity to a manageable level for study. Artificial streams provide the environmental control and experimental isolation necessary to observe specific processes with additional benefits including cost management, ease of replication, and experimental design flexibility (Smith and Mercante 1989; McIntire 1993). Our recirculating design was successful in culturing robust periphyton communities using very soft RFM medium and conducting experiments with suspended clay loads.

SRP was rapidly removed from solution in all three experiments, regardless of treatment. Differences in SRP uptake rates between experiments were due to the relative difference between the SRP concentration and SRP sink (i.e., nutrient mass to periphyton biomass). The greater uptake rate in experiment 2, compared to experiment 1, can be attributed to differences in periphyton biomass (Figures 3.3 and 3.4). ANOVA of periphyton biomass (as AFDW) indicated no significant differences among individual streams during each experiment. Significant differences observed between experiments are attributed to growth and biomass accrual.

Suspended Clay Effect Upon Phosphorus Uptake by Periphyton

For our 200 mg l^{-1} clay experiment, the SRP concentration was increased from 0.1 to 0.5 mg phosphorus l^{-1} due to the greater amount of periphyton biomass present. Young periphyton mats with less biomass have been shown to exhibit slower SRP uptake rates compared to older, more mature communities with greater biomass, when subjected to the same concentration of dissolved nutrients (Marinelarena and Di Giorgi 2001). Based on the results of our first two experiments, an SRP load of 0.1 mg l^{-1} would have been assimilated faster than previous experiments, thus causing difficulty in measurement and generation of the SRP uptake curve. As a result, when the SRP load was increased, a lower SRP uptake rate was produced as the ratio of biomass to phosphorus increased. This result was not surprising. Increasing periphyton biomass has been shown to increase phosphorus uptake by about 25% for each twofold increase in biomass (Pizzaro et al. 2002). Craggs (2001), while examining periphyton biofilms used to remove nutrients from wastewater, noted that effective nutrient removal could be achieved by varying water flow rates and frequency of biofilm harvesting. Harvesting periphyton biomass directly affects the amount of periphyton biomass available to sorb or take up nutrients. The flow rates of water in our stream system remained constant throughout the study, but periphyton growth increased the total biomass, which resulted in a steeper SRP uptake slope for experiment 2. When the SRP concentration was increased in experiment 3, the SRP uptake slope decreased (Figure 3.4, experiment 3). This decrease can be explained by the greater relative difference between SRP load and the periphyton sink. Changes in

SRP uptake rate were consistent across all treatments, and no significant differences were found among the clay types and concentrations we tested.

At least two studies have measured short-term phosphorus removal rates from solutions containing various concentrations and forms of dissolved phosphorus but neither focused upon suspended sediment influence factor. Scinto and Reddy (2003) reported phosphorus removal rate constants ranging from 0.02 to 0.17 (min^{-1}) by periphyton harvested from a subtropical freshwater wetland where they were interested in explaining the large periphyton mats present in a sparsely vegetated, phosphorus limited, open water marsh. Pizzaro et al. (2002) reported a greater range of values, from 0.07 to 0.61 (min^{-1}), when examining the effect of different manure effluents upon phosphorus uptake rates by periphyton. Our results are consistent with these studies as we observed uptake rate constants in the range of 0.02–0.09 (min^{-1}).

While long-term exposure to settling of clays with adsorbed SRP has been shown to affect periphyton growth and species composition over long time periods (days), our study focuses upon the short-term effect of suspended clays. Parkhill and Gulliver (2002) found that SRP was rapidly uptaken and sequestered via biotic uptake by periphyton during long periods of clay induced turbidity. Differences were noted in stream respiration and periphyton biomass but rapid adaptation was credited to the steady maintenance of whole stream productivity. Marinelarena and Giorgi (2001) observed rapid uptake of dissolved phosphorus regardless of differences in light, temperature, and periphyton community composition. Long-term exposure to clays with sorbed SRP affected algal growth and species composition (Cuker 1993); however, rates of SRP uptake were unaffected. Similarly, we found no differences between periphyton SRP uptake in lotic systems containing suspended clay and those without.

Conclusions

Laboratory batch experiments showed that 90% of potential SRP adsorption onto the experimental clays occurred within 2 h. Although rapid, the amount of SRP adsorption attributable to clay or artificial stream construction material sorption was low relative to the total amounts added to experimental stream systems (~1%). The remaining 99% SRP disappearance from solution was attributed to periphyton uptake. The artificial stream system assembled at BREC, Temple, supported vigorous periphyton communities dominated by filamentous green algae (*Rhizoclonium* spp.) using a very soft RFM. Total stream biomass was statistically different among experiments, due to growth between successive experiments, but not among treatments within each 90-min experiment. SRP uptake rate increased with increased biomass between experiments 1 and 2. Increasing the initial SRP concentration in experiment 3 decreased the SRP uptake rate demonstrating the effect of SRP concentration to biomass ratio. Suspended clay type and concentrations at 20, 80, and 200 mg l^{-1} ultimately had no effect upon uptake rates of SRP by periphyton (Table 3.3 and Figure 3.6). These results suggest that the presence of suspended clays with low SRP sorption potential has little, if any, effect upon the removal rate of SRP from water column by periphyton. Others noting similar results from increased sediment and turbidity have attributed this to the adaptability of primary producers. They are among the most resistant members of the lotic community to increased turbidity (Parkhill and Gulliver 2002). Our results are consistent with other finding turbidity conditions, caused by suspended sediments, which have little immediate effect upon SRP removal from the water column by periphyton.

Acknowledgments

This work has been graciously supported through grants, time, expertise, and/or facilities of the Texas AgriLife Research/Blackland Research and Extension Center, Texas Water Resource Institute, U.S. Department of Agriculture, and Baylor University. The authors gratefully acknowledge the valuable contributions of time and comments given by Allen Torbert, Ken Potter, Arthur Stewart, Armen Kemanian, and several anonymous reviewers.

CHAPTER FOUR

Phosphorus Uptake and Release by Periphyton in the Presence of Suspended Clays

Introduction

Phosphorus (P) cycles through aquatic environments participating in many biotic and abiotic processes. Streams link terrestrial uplands to downstream water bodies and act as reactive conduits that modify dissolved P loads. Attached algae and bacteria (i.e. periphyton) provide efficient sinks for dissolved P (Reddy et. al. 1999; Dodds 2003) and stream sediments can regulate dissolved P concentrations (Froelich 1988; House 2003). Suspended clays found in sediments are known to influence physical, chemical, and biological processes (Wilber 1983; Davies-Colley et al. 1992; Lind et al. 1997; Doyle and Smart 2001) mainly by creating turbid conditions leading to light reduction and sedimentation of the aquatic habitat (Parkhill and Gulliver 2002). Biotic nutrient utilization can be affected by suspended clays through lowered photosynthetic potential and equilibrium-driven sorption/desorption reactions (Lind 2003). Community structure and function may also be affected. Lind et al. (1992) documented a heterotrophic, bacteria based, food web in a turbid, tropical lake. High turbidity severely limited autotrophic production by phytoplankton, but nutrients sorbed to allochthonous suspended clays supported the bacterial base of the food web (Lind et al. 1997). Cuker (1987) observed shifts in dominant plankton species during controlled turbidity experiments conducted with suspended clays.

P cycling within aquatic systems is attributable to both biotic and abiotic processes (Dodds 1993; Newbold et al. 1981) but it can be difficult to measure. Carrier-free ^{32}P radio-tracer offers a sensitive marker for P activity. It can be used without substantial P additions so it is particularly useful for examining natural systems without disturbing the ambient P conditions (Noe et al. 2003). Periphyton is often abundant in small streams and removes dissolved P from the water column through biotic sorption and uptake (Dodds 2003). P may be returned to the water column directly through cell leakage and death or indirectly through herbivory (Newbold et al. 1981). Burkholder and Cuker (1991) found increases in periphyton growth when suspended clay was combined with P amendments in a lake mesocosm. They speculated that clay particles may have facilitated P delivery to periphyton communities through settling.

We used laboratory microcosms to examine the hypothesis that suspended clays affect P uptake and turnover by stream periphyton. Commercially available kaolinite and bentonite clays with low parent P content were characterized for their P affinity. Stream periphyton grown on glass substrates was used to evaluate radiolabeled P uptake from and turnover to solution alone and in the presence of suspended clays in short-term laboratory experiments

Methods

Clay Characterization

A kaolinite (Kaolin-P, U.S. Silica, Berkley Springs, West Virginia) was selected to represent 1:1 non-expanding clays and a bentonite (Bentolite L-10, Southern Clay, Gonzalez, Texas) was selected to represent 2:1 expanding clays. Clay capacity and rate of soluble reactive phosphorus (SRP) sorption, as potassium phosphate (KH_2PO_4), was determined using laboratory batch methods. Duplicated 30 mL aqueous aliquots of 0.05, 0.1, 0.5, 1.0, and 3.0 mg SRP L^{-1} were placed in 50 mL polypropylene centrifuge tubes and equilibrated with a 0.3 g clay sample for 24 h at 22 C°. During the equilibration period the tubes were maintained in an end-over-end shaker operating at 15 revolutions per min. Following equilibration the solutions were centrifuged at 6000 g for 15 min to remove suspended clay and the supernatant was analyzed for remaining SRP concentration using the Murphy-Riley (1962) ascorbic acid reduction method. A Genesys-5 spectrophotometer (Thermo Fisher Scientific, Waltham, MA) with a 50 mm path length was used to measure absorbance at 880 nm. A reagent blank and two known standards were prepared and carried through the analysis to ensure good quality control. The unit mass of SRP sorbed per unit mass of clay was plotted as a function of equilibrium concentration, creating a sorption isotherm. A Langmuir fit of the data was performed to determine the maximum SRP sorption potential (Froelich 1988). Adsorption rates were determined by preparing a 0.1 mg L^{-1} SRP solution from which 30 mL, duplicated aliquots were equilibrated for 0.01, 0.25, 0.5, 1, 2, 4, 8, and 12 h with 0.3 g clay under the same conditions described for the sorption isotherm experiments and SRP remaining in solution was measured as previously described. The unit mass of SRP sorbed was plotted as a function of equilibration time and a simple Elovich model was used to describe the uptake rate (Sparks 1989).

Periphyton Culture

Periphyton was established on artificial substrates in an artificial stream facility at the Texas AgriLife Research / Blackland Research and Extension Center in Temple, Texas. Plastic rain gutters were used as stream channels and water was recirculated through these channels by pumping. Reconstituted freshwater media (RFM) described in Standard Method 1080E.4b (APHA 2005) was used as a working solution for periphyton cultivation (Wolfe et. al. 2009). Channel slope and pump output was adjusted to provide a current velocity rate of 5 cm s^{-1}. Weekly additions of nitrogen (1.5 mg L^{-1}), as KNO_3, and P (0.1 mg L^{-1}), as KH_2PO_4, were made during the establishment period to promote periphyton growth. Standard 25x75 mm frosted, glass microscope slides were placed perpendicular to the flow within the channel to provide a removable surface for periphyton growth. Periphyton encrusted rocks, collected from Salado Creek, in Salado, Texas (Latitude: 30°56"38.00" North, Longitude: 92°32'11.0" West), were used to inoculate the artificial stream. Three weeks following inoculation, the glass microscope slides were dominated by filamentous green alga (*Rhizoclonium* spp.). Once established, periphyton-covered slides were removed to the laboratory for experimental manipulation with radiolabeled phosphorus (^{32}P) and suspended clays.

Phosphorus Uptake

A one hour P uptake experiment was conducted using individual 50 mL polypropylene centrifuge tubes as microcosms for individual periphyton-covered slides. Rates of P uptake from solution were determined simultaneously in 3 treatments, each consisting of 5 replicate tubes, including; periphyton with no clay (control), periphyton with Bentolite L-10, and periphyton with Kaolin-P. A 0.02 mg L^{-1} SRP RFM solution was labeled with carrier-free ^{32}P (MP Biomedical, Costa Mesa, CA) and placed in individual centrifuge tubes along with a 200 mg L^{-1} clay load. A single, periphyton-covered slide was added to each tube at the initiation of the experiment. Microcosms were placed in racks on a laboratory window sill receiving indirect sunlight. Each microcosm was inverted every 5-10 minutes throughout the experiment to provide solution mixing. Sub-samples (0.1 mL) were removed from each microcosm at 10, 20, 30, 45, and 60 min of incubation. Sub-samples were filtered through a 0.45 μm nitrocellulose filter, followed by a 1.5 mL rinse, and the entire filtered volume was added to a scintillation vial containing 10 mL of scintillation cocktail (EcoLite Plus, MP Biomedical, Costa Mesa, CA). Samples were assayed for one minute by liquid scintillation (Beckman Model LS6500, Beckman Coulter, Inc., Fullerton, CA) and the rate constants for ^{32}P uptake were computed from the concentrations of radiolabeled SRP remaining in solution over time. Each periphyton-covered slide was dried overnight at 85 C°, weighed, combusted in a muffle furnace at 500 C° for 1 h, and reweighed to determine AFDW m^2. Rehydration and re-drying of samples prior to final weight determination was omitted because AFDW overestimation due to water loss from suspended clays settling on periphyton samples was minimal, < 0.5% of total. ^{32}P uptake, expressed as counts per minute (CPM), was standardized by periphyton AFDW biomass per $m^2$and the ^{32}P uptake rate was calculated with the first order rate coefficient of radiotracer depletion from solution. ^{32}P uptake by periphyton was described by the first order reaction:

$$^{32}P = {}^{32}P_i \, Exp^{kt} + b$$

where ^{32}P is the radiolabeled P mass, as counts per minute (CPM), in periphyton tissue standardized by periphyton ash free dry weight (g^{-1} AFDW m^{-2}) at any time (t), $^{32}P_i$ the initial ^{32}P mass (CPM/AFDWg^{-1} m^{-2}), k the first-order rate constant (min^{-1}), and b is the asymptote or ^{32}P in periphyton tissue at equilibrium.

Phosphorus Turnover

A 10 day P turnover experiment was conducted in laboratory microcosms constructed from 15 x 30 x 12 cm plastic containers and filled with 3 L of RFM solution. An aquarium air pump supplied a continuous stream of air to a stone diffuser producing numerous rising bubbles at one end of the microcosm. The operating solution was lifted by the rising bubbles to the upper portion of the microcosm created by a plastic divider and flowed over the periphyton covered glass microscope slides. Air was supplied through a stone diffuser to one end of each container, causing water to be lifted and recirculated across a plastic divider upon which the periphyton-covered slides were placed (Figure 1). Rates of ^{32}P turnover from periphyton tissue were determined simultaneously in three treatments including; periphyton with no clay (control), periphyton with Bentolite L-10 and, periphyton with Kaolin-P. Periphyton-covered slides was placed in a RFM solution containing 0.2 mg L^{-1} carrier-free ^{32}P labeled SRP for 6 h. After ^{32}P exposure, ten slides were placed in each recirculating laboratory microcosm. A 200 mg L^{-1} clay load was added to the clay treatments and all microcosms were placed in a laboratory window where they received indirect, natural sunlight light. Two slides were harvested for analysis from each microcosm after 0.25, 1, 3, 5, and 10 days of ^{32}P exposure. Sample AFDW biomass was determined as already described. Samples were assayed together at the end of the experiment to avoid the need to correct for decay. P turnover to recirculating stream water was determined by regressing log normalized ^{32}P counts (CPM) in periphyton tissue, over time. ^{32}P turnover from periphyton to solution was then described by the first order reaction:

$$^{32}P = {}^{32}P_i \, Exp^{-k*t} + b$$

where ^{32}P is the radiolabeled P mass, expressed as counts per minute (CPM), in periphyton tissue standardized by periphyton ash free dry weight (g^{-1} AFDW m^{-2}) at any time (t), $^{32}P_i$ the initial ^{32}P mass (CPM/AFDWg^{-1} m^{-2}), -k the first-order rate constant ($days^{-1}$), and b is the asymptote or ^{32}P in periphyton tissue at equilibrium.

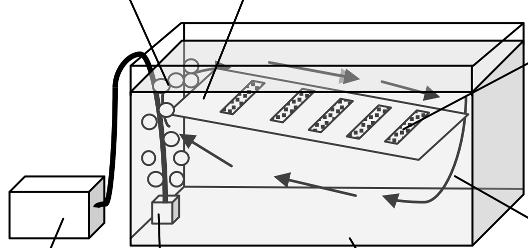

Figure 4.1. Lotic microcosm (3 liter volume) utilized in phosphorus turnover experiment. Rising bubbles generate pneumatic lift and a gentle recirculating flow between the upper and lower portion of the microcosm separated by the plastic divider. Microcosms were placed in a laboratory window receiving indirect sunlight during the 10 day phosphorus turnover experiment.

Samples were assayed together at the end of the experiment to avoid the need to correct for decay. P turnover to recirculating stream water was determined by regressing log normalized ^{32}P counts (CPM) in periphyton tissue, over time. ^{32}P turnover from periphyton to solution was then described by the first order reaction:

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Statistical Procedures

Periphyton P uptake and turnover parameters describing first-order uptake and turnover kinetics were obtained by non-linear, least squares regression. Differences in parameter estimates of *k* were evaluated with repeated measures ANOVA. Biomass-normalized mean Ps uptake and turnover was compared among treatments using one-way ANOVA. All statistics were performed using the SPSS statistical package (SPSS for Windows, Rel. 15.0 2007. Chicago: SPSS Inc.).

Results

Clay Characterization

The physical characteristics reported by the manufacturers and P affinities of each clay determined by laboratory experiments are listed in Table 4.1. SRP adsorption for both clays increased with concentration until saturation. Langmuir isotherm data indicated that maximum sorption was very low and occurred at 125 mg SRP kg^{-1} of Bentolite L-10 and 48 mg SRP kg^{-1} of Kaolin-P. Bentolite L-10 sorbed 73 to 77% of its total SRP sorption capacity within 60 min. Elovich data indicated that Kaolin-P reached between 60 to 71% of maximum sorption capacity within 60 min. Based on these results experiments in which periphyton was exposed to P in the presence of these clays were designed around the sorption time required for the clays to sorb >50% of their maximum potential, about1 hr. For result interpretation, knowing the maximum sorption potentials allowed the calculation of the fraction of the SRP load attributable to uptake by sorption to the clays was <0.01% of the total.

Table 4.1. Physical characterization and soluble reactive phosphorus (SRP) affinity of bentonite and kaolinite clays used in periphyton phosphorus uptake and turnover experiments.

Property	Bentolite L-10	Kaolin-P
Mean particle size (µm)	>1.0	1.0
Bulk density (kg m^{-3})	0.39 - 0.58	0.35 – 0.44
Total surface oxides (%)	0.6	2.3
pH (as a 10% slurry)	7.5	6.0
Native phosphorus present on clays (mg)	< 0.006	< 0.006
Maximum SRP sorption predicted by Langmuir model (mg kg^{-1})	125	58
SRP sorbed within 60 minutes predicted by Elovich model (%)	75	68

Phosphorus Uptake

Radiolabeled SRP was rapidly uptaken from solutions containing periphyton regardless of clay treatment (Figure 4.2). P uptake, expressed as a percent of the total, ranged from 89 to 96% among treatments. Model estimates of uptake rate *k* were similar for both clays at 0.15 and 0.17 min^{-1} for Bentolite L-10 and Kaolin-P, respectively (Table 4.2). There was a slight difference between control (0.14 min^{-1}) and clay treatments but a comparison of the model coefficient *k*, by treatment, over time, using repeated measures ANOVA detected no significant differences ($F_{2,\ 12} = 1.49$, $P = 0.27$). Comparison of treatment means, using one way ANOVA also showed no significant difference in mean uptake among treatments ($F_{12,\ 2} = 0.94$, $P = 0.42$). Treatment means and standard deviations for uptake are shown in Figure 4.3.

Phosphorus Turnover

Radiolabeled P turnover to solution by periphyton was much slower than uptake. Figure 4.2 shows ^{32}P turnover from periphyton over a 10 day period. Turnover, expressed

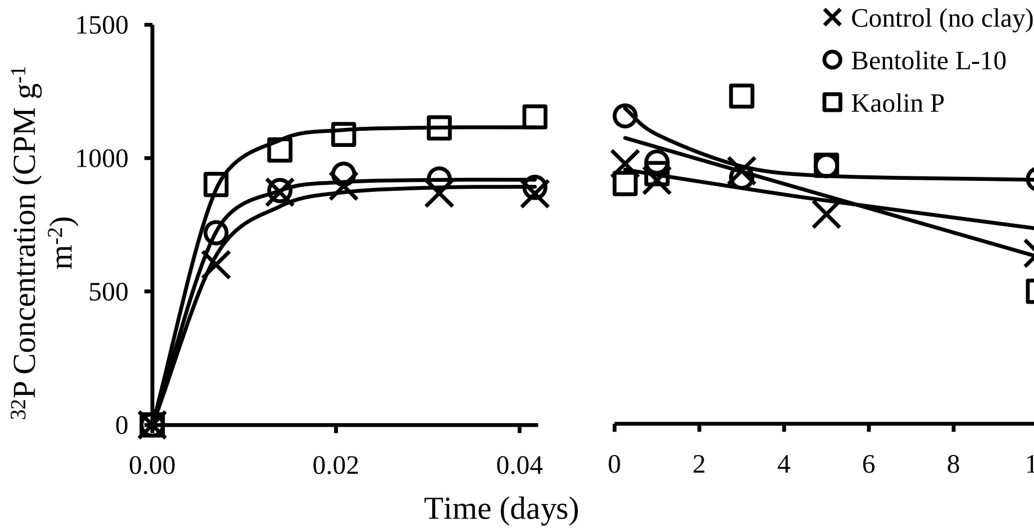

Figure 4.2. Uptake from solution and turnover to solution of radiolabeled phosphorus (32P) as counts per minute (CPM), normalized by ash free dry weight biomass (g^{-1}) per unit area (m^{-2}), by periphyton alone and in the presence of suspended Bentolite L-10 and Kaolin-P clays. Points indicate mean value at time measured (Uptake: n=5, Turnover: n=2) and curves show first order reactant kinetics. Suspended clay concentration was 200 mg clay L^{-1} and initial phosphorus concentration was 0.02 mg L^{-1}.

Table 4.2. Mean ± one standard deviation of initial, final, and flux rate of ^{32}P concentrations as counts per minute (CPM), normalized by periphyton ash free dry weight biomass expressed as grams per square meter, percent of total phosphorus load uptaken or turned over (U/T), number of observations, and r^2 of exponential curves describing ^{32}P uptake from and turnover to solution.

Experiment Treatment	^{32}P Initial (CPM g^{-1} m^{-2})	^{32}P Final (CPM g^{-1} m^{-2})	^{32}P flux rate (*k*, min^{-1})	U/T (%)	Obs	r^2
Uptake						
Control (no clay)	0 (0)	867 (67)	0.14 (0.05)	89	5	0.98
Bentolite L-10	0 (0)	890 (126)	0.15 (0.03)	91	5	0.99
Kaolin-P	0 (0)	1155 (260)	0.17 (0.04)	95	5	0.99
Turnover			(*k*, $days^{-1}$)			
Control (no clay)	939 (51)	616 (56)	0.07 (0.05)	34	2	0.58
Bentolite L-10	1112 (68)	884 (199)	0.04 (0.04)	21	2	0.50
Kaolin-P	868 (496)	478 (304)	0.06 (0.05)	45	2	0.43

as a percent of the total ranged from 21 to 45% among treatments. Model estimates of mean turnover (*k*) ranged from 0.04 to 0.07 days^{-1}, for control and clay treatments (Table 2). There was no significant difference among control and clay treatments, over time, using repeated measures ANOVA ($F_{2, 3}$ = 1.37, P = 0.38). Comparison of treatment means, using one way ANOVA, also showed no significant difference in turnover rate among treatments ($F_{2, 3}$ = 0.34, P = 0.74). Treatment means and standard deviations for turnover are shown in Figure 4.3.

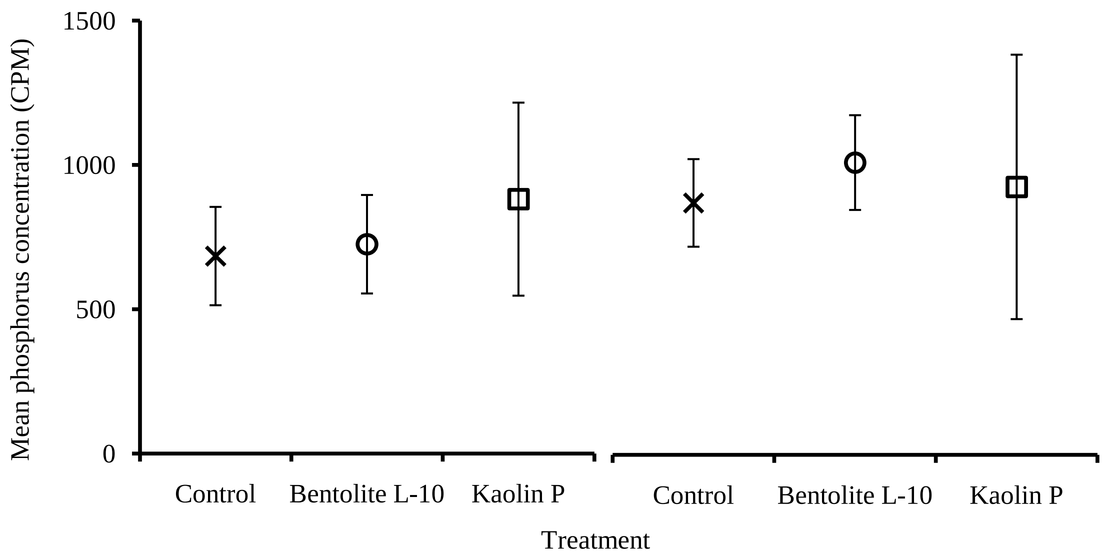

Figure 4.3. Mean ± one standard deviation of phosphorus concentration during uptake and turnover experiments expressed as ^{32}P counts per minute (CPM) normalized by ash free dry weight biomass per square meter for three treatments including: Control (No Clay), Bentolite L-10, and Kaolin P. Suspended clay concentration was 200 mg clay L^{-1} and initial phosphorus concentration was 0.02 mg L^{-1}.

Discussion

Clay Characterization

Development of an adsorption isotherm is useful for determining the sorption capacity of a material for a particular ion and the Langmuir model is commonly used to predict the maximum sorption potential for a given material. This method yields results that are comparable between different systems (Graetz and Nair 2000). For example, Klotz (1985) found phosphate sorption indices differed significantly between stream sediments originating from agriculture and woodland dominated watersheds. Isotherm techniques can be applied to determine the equilibrium phosphate concentration (EPC) existing between sediment-bound and water column P (Froelich 1988; Klotz 1988). The importance of soluble reactive nutrients to biotic organisms makes this technique particularly useful for studying nutrient availability and productivity in natural streams. Haggard et al. (1999) used it to describe the relative importance of biotic sorption to physical and chemical processes controlling stream water P concentration in two Oklahoma streams and Klotz (1985) used it to determine that temporal changes in stream water calcium concentration affected EPC and reactive P concentrations in a New York stream. Webster et al. (2001) used isotherm data to show streams with suspended sediments had higher biologically-available P than similar streams with low turbidity.

The range of sediment-bound P in natural streams varies widely. Algal-available phosphorus sorbed to glaciofluvial sediments collected in northern polar regions ranged 1 to 23 mg P kg^{-1} (Hodson et al. 2004) while biologically-available P in Amazon river sediments was higher at 100 to 300 mg P Kg^{-1} (Grobbellar 1983). In Australian lowland rivers P desorbed from sediments reached 390 mg P Kg^{-1} (Webster et al. 2001). Fluvial sediments containing higher clay portions have a larger capacity to sorb nutrients due to their large surface-to-area ratios and charged surface characteristics (Grobbellar 1983, Lind 2003). The clay portion of river and lake sediments reported in several published studies ranged between 3 and 21% of the total (Grobbellar 1983; Lind et al. 1992; Jin et al. 2005). The experiments described in this study used 100% clay rather than mixtures.

Clays used in this study exhibited relatively low SRP affinity however measurable differences in maximum SRP capacities and sorption rates were observed. These were probably due to their structural differences. The 1:1 Kaolin-P adsorbs anions only on its broken edges while the 2:1 Bentolite L-10, with more surface area and exchange sites, absorbs almost twice as much in the same amount of time. Calculations based on batch experiment results indicated the SRP adsorption attributable to the clay mineral fraction of the experimental systems containing periphyton was minimal. The experimental clay's phosphorus sorption capacity was less than 0.01% of the phosphorus load added indicating that 99.9% of SRP uptake was attributable to biotic activity.

Phosphorus Uptake

The presence of suspended clays had no significant effect on periphyton SRP uptake. Uptake rate constants measured in these experiments were slightly higher than those measured in previous experiments which examined the effect of these clays at concentrations ranging from 20 to 200 mg^{-l} (Wolfe and Lind 2008). This may be due to the utilization of centrifuge tubes as microcosms, a safety requirement of working with the radioactive tracer. Placing lotic periphyton into a static flow environment, with limited circulation simulated through tube inversion at 5-10 minute intervals throughout the experiment may have contributed to the differences. Other studies have measured short term P removal rates from solutions containing various concentrations and forms of dissolved P but they did not focus upon suspended sediments. Scinto and Reddy (2003) reported P uptake rate constants ranging from 0.02 to 0.17 in periphyton collected from a subtropical freshwater wetland. In a study examining the effect of different manure effluents on P uptake by periphyton, uptake rates ranged from 0.07 to 0.61 min^{-1} (Pizzaro et al. 2002). Our results are comparable with these studies as we observed uptake rate constants within these reported ranges.

Phosphorus Turnover

As with uptake, the presence of suspended clays had no measurable effect upon P turnover. A comparison of model coefficients, by treatment over time, using repeated measure ANOVA found no significant difference among control (no clay) and Bentolite L-10 or Kaolin-P. These results should be considered with caution because only 50% or less of the turnover rate variation is explained by the uptake curves and the microcosm used in the experiment is of a recirclulating design, which was necessary due to the restrictions of working with radiolabeled P. As the biota leak cellular products and die, the initially uptaken P moves from the biomass to solution. The P released increases the concentration in solution and some portion of this is available for re-uptake. Turnover rates, indicated by model estimates of k, are thus probably underestimated due to the recirculating nature of the experimental microcosm design. Clay effects upon species composition were not examined due to the relative danger imposed by the ^{32}P radiotracer. However, periphyton species composition has been shown to be less important in P cycling than physical variables such as water temperature and flow (Adey et al. 1993; Craggs 2001).

Conclusions

Our experiments indicate that suspended clay sediments with dissolved P loads have no immediate effect upon the short-term P uptake or turnover by stream periphyton.

These findings refute our hypotheses that suspended clays with low P affinity affect P uptake and turnover by stream periphyton. Long term exposure to settling clays with adsorbed SRP have been shown to affect periphyton growth and species composition (Burkholder and Cuker 1991) but other studies have demonstrated periphyton adaptability and resistance to changes in respiration and overall productivity under turbid conditions (Parkhill and Gulliver 2002). Ultimately the surface characteristics of a clay particle and its ability to sorb nutrients may be more important than the underlying mineral, with respect to periphyton P interactions. These results may be of interest to researchers working with P issues in turbid systems.

Acknowledgments

This work was accomplished through generous support from the Texas AgriLife Research / Blackland Research and Extension Center, the Texas Water Resource Institute, the U. S. Department of Agriculture, and Baylor University. The authors gratefully acknowledge the contributions of time and comments from Arthur Stewart, Hiroaki Somura, and two anonymous reviewers.

CHAPTER FIVE

Conclusions

The nutrient coupling which occurs between terrestrial uplands and receiving waters merits investigation because of the increasingly important role reservoirs are poised to play as resources for population growth (Thornton et al. 1990). It is noted in the River Continuum Concept (Vannote et al, 1980) that rivers and streams are the conduits through which large quantities of allochthonous materials are collected, transported, and transformed. Biological reactions occurring en route selectively process or modify the quality and quantity of these materials before they enter a reservoir (Wetzel, 1983). Nutrients delivered to receiving waters then regulate metabolism and determine productivity. Understanding nutrient controlled eutrophication requires not only the knowledge of sources (quantity, form, etc.) and receiving water response (loading, flushing rates, sedimentation, etc.) but also the in-stream processing of the nutrients (Kimmel et al., 1990). The effects of lotic biota on the quality and quantity of allochthonous loading to receiving waters can be profound (Wetzel, 1983). The purpose of this research was to determine and quantify the effect of suspended clays upon the in-stream phosphorus processing by lotic periphyton in order to improve basic limnological understanding.

An artificial stream was employed to create and maintain stream turbidity conditions necessary for the research. Measuring biotic processes under these specific conditions presented a set of problems that was only manageable in a controlled environment. Accurate water volume management facilitated measurement of nutrient concentrations. During a pre-experiment evaluation period, artificial stream design was able to hold operating water volumes within 1% while keeping dissolved and suspended materials within 3% and 4% of target values. Providing environmental homogeneity allowed an accurate measurement of the process of interest. Periphyton biomass and SRP uptake in this artificial stream system was less variable than that reported in several published natural stream studies. The low cost, low water volume requirement, controllable physical-chemical water conditions, and minimized natural fluctuation aided the experimental process.

Laboratory batch experiments showed that 90% of potential SRP adsorption onto the experimental test clays occurred within 2 hours. Although rapid, the amount of SRP adsorption attributable to clay or artificial stream construction material sorption was low relative to the total amounts added to experimental stream systems. More than 99% of SRP disappearance from solution was attributable to periphyton uptake.

The artificial stream system assembled at BREC, Temple, using a very soft RFM, supported vigorous periphyton communities dominated by filamentous green algae (*Rhizoclonium spp*.). Total stream biomass was statistically different among experiments, due to growth between three successive experiments, but not among the three treatments within each experiment. SRP uptake rates increased with increased biomass between experiments. Increasing the initial SRP concentration decreased the SRP uptake rate demonstrating the effect of SRP concentration to biomass ratio. Suspended clay type and concentrations at 20, 80, and 200 mg l^{-1} ultimately had no effect upon uptake rates of SRP by periphyton suggesting that the presence of suspended clays with low SRP sorption potential has little, if any, effect upon the removal rate of SRP from water column by periphyton. These results are consistent with others investigating clay turbidity effects upon periphyton. Burkholder and Cuker (1991) determined that phosphorus additions reduced deleterious clay effects upon productivity in lake periphyton while Parkhill and Gulliver (2002) found that whole stream productivity was unaffected by sediment loading. These and others (Davies-Collies 1992; Squires and Lesack 2001) have speculated that the adaptability of aquatic autotrophs to suspended sediment turbidity occurs primarily through increased photosynthetic efficiency.

Short term (2 hour uptake and 10 day turnover) experiments utilizing radiolabeled ^{32}P tracers indicated that stream inputs containing suspended clay sediments with dissolved phosphorus loads have no immediate effect upon the phosphorus uptake or phosphorus turnover by stream periphyton. These findings support the null hypotheses that: suspended clays, with low phosphorus affinity, have little effect upon phosphorus uptake or turnover by stream periphyton. Long term exposure to settling clays with adsorbed SRP affects periphyton growth and species composition (Burkholder and Cuker 1991) but periphyton is highly adaptable and resistant to changes in respiration and overall productivity under turbid conditions (Parkhill and Gulliver 2002). A clays surface characteristic ability to sorb nutrients may be more important than the underlying mineral, with respect to periphyton- phosphorus interactions. Further research using clay minerals with different phosphorus affinities should be explored. Understanding these basic processes may ultimately help environmental engineers design and manage aquatic ecosystems.

APPENDIX

APPENDIX

Publications Related to This Research

Chapter Two

Wolfe, III, J. E., O. T. Lind, and D. W. Hoffman. 2009. Technical Note: A Constant-Volume Artificial Stream for Reducing Variation in Aquatic Process Measurements. Transactions of the American Society of Agricultural and Biological Engineers 52:1-7.

Chapter Three

Wolfe, III, J. E. and O. T. Lind. 2008. Influence of suspended clay on phosphorus uptake by periphyton. Hydrobiologia 610:211-222.

Chapter Four

Wolfe, III, J. E. and O. T. Lind. In Press. Phosphorus uptake and turnover by periphyton in the presence of suspended clays. Limnology.

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