A PILOT SCALE STUDY OF DENITRIFYING BIOREACTORS PAIRED WITH PHOSPHATE SORBENTS

By

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Abstract

Denitrifying bioreactors are commonly utilized as a best management practice in agricultural systems to reduce nitrate in drainage waters. The USDA recommends the utilization of woodchips as a carbonaceous substrate to enable denitrification. This study compared the nitrate reducing capabilities of pilot-scale in-stream bioreactors comprised of locally sourced woodchips, pine bark, and peanut hulls (a regional agricultural waste product) operating under hydraulic loading rates (HLRs) between 0.1-0.3 m/day. The phosphate adsorption capacity of expanded slate was also explored. This experiment took place in Greenville, North Carolina, from July-October 2021 (the warm season) and from January-March 2022 (the cold season). Samples were collected on a weekly basis, and the duration of flow and frequency of sample collection varied based on the influent flow rates being tested. The bioreactors were dosed with target concentrations of 20 mg nitrate-N/L and 1 mg phosphate-P/L. Overall, nitrate was reduced more effectively in pine bark (50.4% and 2.6 g N/m^3/day median removal) than woodchips (31.4% and 1.1 g N/m^3/day median removal) and peanut hulls (38.4% and 2.0 g N/m^3/day median removal). Hydraulic loading rate (HLR) was found to significantly impact nitrate reduction. Woodchips and peanut hulls both exhibited negative correlations between nitrate-N percent reduction and HLR, while pine bark exhibited a positive correlation between nitrate-N percent reduction and HLR. Though these correlations were significant, they were not very strong (ρ values between -0.30 – 0.34). This may

be attributed to a poor representation of data across the flow regime, as data for this analysis was limited to HLRs ranging from 0.1 – 0.3 m/s. Temperature was also found to significantly impact nitrate reduction. As expected, pine bark exhibited a positive correlation between temperature and nitrate reduction. Contrary to what has been reported in the literature, woodchips and peanut hulls exhibited negative correlations between temperature and nitrate reduction.

Although effective at reducing nitrate, peanut hulls released significant amounts of ammonium-N, organic N, organic P, and dissolved organic carbon (DOC). Expanded slate was found to be effective at reducing phosphate when paired with woodchips (64.2% and 0.90 g P/m^3/day median removal), pine bark (46.5% and 0.11 g P/m^3/day median removal), and peanut hulls (50.7% and 0.12 g P/m^3/day median removal). These data suggest that in-stream denitrifying bioreactors paired with expanded slate as a phosphate adsorbent can be effective tools for reducing nitrate and phosphate. Given that most studies use woodchips as the carbonaceous substrate to promote denitrification, the increased denitrifying abilities exhibited by pine bark and peanut hulls in this study are of significance.

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CHAPTER I – INTRODUCTION

1.1 Eutrophication

Nitrogen (N) and phosphorus (P) are limiting nutrients for primary producers, such as the algal blooms that are associated with eutrophication (Ågren et al., 2012). Anthropogenic activities that produce high levels of N and P are numerous. The fertilizer used in agriculture that is not taken up by crops may leach into the groundwater or run off into nearby streams. The manure produced in concentrated animal feeding operations (CAFOs) contains bioavailable phosphates and ammonia (Natural Resources Conservation Service, 2007). Wastewater treatment plants and on-site wastewater treatment systems (OWTS) produce nutrient-laden effluents. Human sewage is estimated to contribute 12% of riverine N input in the US (World Resources Institute, 2014). Household septic tanks leach an average of 14 kg of N per system per year into soil, much of which reaches groundwater and surface water systems (World Resources Institute, 2014). Hoghooghi et al. (2016) reported that septic system density is generally correlative with concentrations of nitrates in local streams in the Piedmont region of Georgia. In the Piedmont region of North Carolina, streams in the Lick Creek sub-watershed (of the Falls Lake watershed), a positive correlation between nutrient concentrations (total dissolved nitrogen and phosphate) and septic system density was observed in sub-watersheds. This suggests that septic effluent leaching is the most likely primary nutrient loading mechanism (Iverson et al., 2018). O'Driscoll et al. (2020) reported a positive correlation between OWTS density and nutrient concentrations in Falls Lake watershed streams, supporting the idea that septic effluent leaching is a nutrient loading mechanism to many streams in the Falls Lake watershed.

Eutrophication is excessive plant growth and decay that results from the nutrient enrichment of surface waters. Though a natural process, eutrophication is intensified by human

land-use practices (Smith and Schindler, 2009). It can threaten wildlife, recreational opportunities, and overall water quality (Chislock et al., 2013). Eutrophication ensues when nutrients, such as labile species of N and P, accumulate in a body of water, potentially causing the growth of dense algal blooms on the surface. The algae can affect aquatic vegetation by blocking sunlight, causing other aquatic plants to die. The resulting decay of algae by decomposing bacteria consumes dissolved oxygen (DO), which may eventually cause hypoxic or anoxic conditions (Smith and Schindler, 2009). The algae resulting from excess nutrients can also produce harmful toxins, strong odors and increased turbidity.

The eutrophication of waterways is an environmental issue that affects aquatic ecosystems around the world. The US Environmental Protection Agency (EPA) Drinking Water Standards limit nitrates at 10 mg/L (nitrate-N) (EPA, 2020). Nitrates in drinking water pose numerous health threats including methemoglobinemia, known as blue baby syndrome, when ingested by infants (Chislock et al., 2013; Ward et al., 2018). The EPA reports that an acceptable range of total phosphorus in surface water is between 0.04 to 1.0 mg/L (EPA, 2015). Recent data shows that nitrate-N concentrations in some tributaries to Falls Lake may exceed 10 mg/L, suggesting that anthropogenic nutrient inputs from fertilizer and wastewater sources can be transported to streams through baseflow (O'Driscoll et al., 2020).

1.2 Best Management Practices

Point sources of nutrients, such as wastewater treatment plants, are typically treated onsite, prior to discharge to surface waters. Non-point sources of nutrients, such as OWTS (septic system) leachate and agricultural operations, typically receive less treatment, as they are diffuse and temporally variable. Both point and non-point sources of nutrients can be effectively managed with various best management practices (BMPs). BMPs are recommended methods,

structures, or practices designed to reduce water pollution (Waskom, 1994; North Carolina Forestry Service, 2017). Denitrifying bioreactors are a relatively modern BMP that use a carbonaceous substrate to promote the growth of denitrifying bacteria that, under saturated, anoxic conditions, respire nitrate (NO_3) into inert dinitrogen gas (N_2). Bioreactors are inexpensive to install relative to the cost of damages caused by eutrophication, which is approximately \$2.2 billion annually in the US (Dodds et al., 2009). The cost of installing a bioreactor can range from less than \$5,000 to \$27,000, with cost efficiencies ranging from less than \$2.50 kg^{-1} N $year^{-1}$ to roughly \$20 kg^{-1} N $year^{-1}$ (Christianson et al., 2021). Bioreactors are also easy to maintain, making them a viable nutrient management tool.

One major control on bioreactor effectiveness is the carbon substrate being utilized by the denitrifying bacteria. To optimize nutrient attenuation, it is necessary to evaluate the most effective substrate for nutrient treatment. Woodchips and sawdust are most often utilized in bioreactors (Christianson et al., 2010), but few studies have investigated the denitrifying efficacy of alternative substrates, such as pine bark or peanut hulls. Bioreactors are commonly used to facilitate denitrification of waters receiving nitrate, but the efficacy of phosphate treatments are less understood. Bioreactors can utilize phosphate sorbents that can reduce phosphorus loading to downgradient waters. The use of phosphate sorbents can be incorporated into BMPs such as bioreactors to further reduce nutrient loading in water. Phosphate sorbents consist of metal oxidizing materials (typically those high in Al, Fe, or Ca) that provide the cation to bond with dissolved phosphorus to form insoluble compounds (Weng et al., 2012). Steel byproducts such as chips, slag, and turnings are commonly used as phosphate sorbents (Hua et al., 2016; Christianson et al., 2017), but few studies (Iverson, 2019; Wu et al., 2013) have investigated the phosphate sorbing capacity of expanded slate.

The objective of this project was to quantify nitrate and phosphate reduction in pilot-scale denitrifying bioreactors as a potential strategy to reduce excess non-point source nutrient loading in the low-order streams that feed the city of Raleigh's drinking water supply, Falls Lake. Falls of the Neuse, or Falls Lake (Figure 1.1), is the primary drinking water supply for the City of Raleigh, North Carolina, and has experienced local eutrophication for the past decade (North Carolina Department of Environmental Quality, 2020). Because of this, it has been classified as impaired on North Carolina's 303(d) list for impaired waters (North Carolina Department of Environmental Quality, 2020). Recent studies conducted in the area suggest that OWTS could be a significant source of nutrient inputs to the Falls Lake, especially in first-order streams that drain sub-watersheds dominated by residences served by OWTS (Iverson et al., 2018; O'Driscoll et al., 2020).
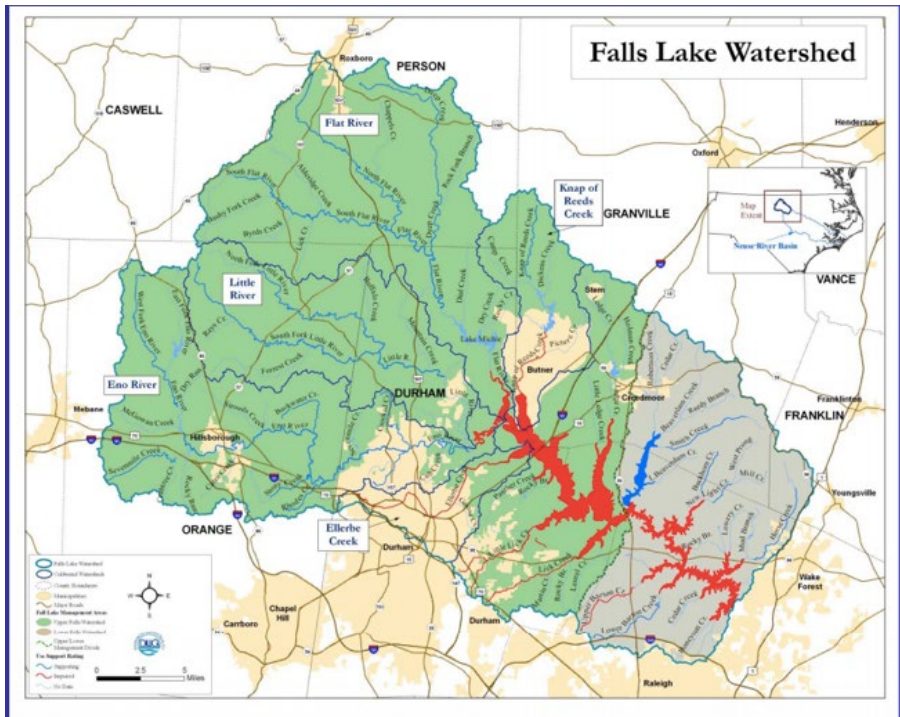
Figure 1.1 Map of Falls Lake watershed (North Carolina Department of Environmental Quality, 2020).

This research also contributes to the understanding of managing the nitrogen cycle, which has been identified as one of the 14 Grand Challenges for Engineering in the 21st century by the U.S. National Academy of Engineering (National Academy of Engineering, 2019). Broadening our understanding of denitrifying bioreactors is important to maximize their effectiveness so that they can be implemented in a variety of settings to reduce nutrient inputs to nutrient sensitive water bodies.

CHAPTER II – LITERATURE REVIEW

2.1 Types of Denitrifying Bioreactors

Denitrifying bioreactors can be utilized in the subsurface as permeable reactive barriers (PRB), adjacent to streams or ditches as denitrifying trenches or beds, or in streambeds (or drainage ditches) as in-stream bioreactors (Schipper et al., 2010). Permeable reactive barriers filled with carbonaceous material are subsurface trenches that intercept shallow groundwater flow that is contaminated with nitrates. Denitrifying beds are contained trenches that can take the form of trench-style bioreactors, up-flow bioreactors, or stream-bed bioreactors (Schipper et al., 2010). Denitrifying trenches are often installed adjacent to an agricultural tile drain; water is routed from the tile and into the bioreactor before being released into a drainage ditch. Stream bed, or in-stream, bioreactors are installed in the beds of streams and drainage ditches to remove nitrates from surface waters (Schipper et al., 2010). Up-flow bioreactors are a type of in-stream bioreactor that are installed in the hyporheic zone of a stream to reduce nitrates in the groundwater prior to being discharged to the surface.

Robertson and Blowes (2000) investigated the denitrifying performance of PRBs in the form of a denitrifying wall designed to intercept a horizontally flowing, down-gradient plume of nitrates in the groundwater operating under a mean hydraulic loading rate (HLR) of 0.06 m/day with a mean hydraulic retention time (HRT), or the amount of time the water spends in the bioreactor, of 10 days and a mean influent concentration of 33.9 mg nitrate-N/L. They reported a mean nitrate percent reduction of 91% and nitrate removal rates between $5.0 – 30$ g N/m^3/day. In a trench-style bioreactor that received tile drainage water from corn fields operating under HRTs between $2 – 8$ hours with influent concentrations ranging from <0.1 to 17 mg nitrate-N/L, Bell et al. (2015) reported a mean nitrate reduction of 63% and a mean nitrate removal rate of 11.6 g

N/m^2/day. In an up-flow bioreactor installed in the riparian zone of an agricultural drainage ditch operating under a mean HLR of 0.42 m/day with a mean HRT of 24 hours and a mean influent concentration of 11.5 mg nitrate-N/L, van Driel et al. (2006) reported a mean nitrate percent reduction of 33% with a mean nitrate removal rate of 0.7 g N/m^2/day.

In an in-stream bioreactor installed in an agricultural drainage ditch operating under a mean HLR of 0.7 m/day with a mean influent concentration of 4.8 mg nitrate-N/L, Robertson and Merkley (2009) reported a mean nitrate percent reduction of 78% with a mean nitrate removal rate of 5.8 g N/m^2/day. In another in-stream bioreactor study installed in a residential stream operating under a median HLR of 0.61 m/day with a median influent concentration of 0.89 mg nitrate-N/L, Iverson (2019) reported a median nitrate percent reduction of 78%.

2.2 Treatment Factors of Denitrifying Bioreactors

The primary treatment factors that affect the efficacy of denitrifying bioreactors are water temperature, HRT, and carbon substrate (Christianson et al., 2012). When analyzing for nitrate reducing efficiency (nitrate % reduction), it is also important to consider influent concentrations. Bioreactors operating under higher influent concentrations can often yield higher nitrate percent reductions.

In an in-stream bioreactor study conducted in Canada with influent concentrations of 4.8 mg nitrate-N/L, Robertson and Merkley (2009) reported higher nitrate removal rates during warm-season operation (effluent water >10°C) than during the cold-season operation (effluent water <10°C). In a field-scale, woodchip bioreactor study with influent concentrations between <0.1 – 17 mg nitrate-N/L and influent water temperatures ranging between 5° and 30° C, Bell (2015) found that nitrate percent reduction and removal rates increased with influent water temperature.

In a column-scale woodchip bioreactor study with influent concentrations between 11.5 – 35.1 mg nitrate-N/L and temperatures of 10°, 15° and 20° C, Hoover er al. (2016) also found that nitrate removal rates and load reduction increased with influent water temperature.

HRT (Equation 2.1) is a function of flow rate and the volume (the size of the bioreactor): the faster water moves through a system (high flows) and the smaller the bioreactor, the less time it spends in the system thereby lowering the HRT. The HLR of a bioreactor is defined as its flow rate normalized over its area (Equation 2.2). This is important to consider because while a bioreactor may have a high flow rate, it may also have a high surface area, thereby decreasing the HLR.

HRTs that are too low may not allow for a sufficient reduction of DO to promote the denitrifying microbial processes, but HRTs that are too high may a variety of negative effects: sulfate reduction can occur when sulfate, which is naturally present in many drainage waters, is converted into hydrogen sulfide gas. The same bacteria responsible for this process are also responsible for the transformation of mercury in the water or in the woodchips into methyl mercury though the process of mercury methylation (Christianson et al., 2011c; Christianson et al., 2012). Methane production can also occur through the degradation of the carbonaceous substrate (Lepine et al., 2015). The USDA (2016) recommends HRTs ranging from 4 – 8 hours.

Equation 2.1 Hydraulic Retention Time

$$HRT = \frac{V \times n}{Q}$$

Where V denotes volume (m³), n denotes porosity, and Q denotes flow rate (m³/day) to determine HRT (days).

Equation 2.2 Hydraulic Loading Rate

$$HLR = \frac{Q}{A}$$

Where Q denotes flow rate (m^3/day) and A denotes surface area (m^2) to determine HLR (m/day).

Robertson and Blowes (2000) conducted a field-scale study of a denitrifying wall operating under HLRs of 0.06 m/day with HRTs of 10 days and a mean influent concentration of 64.5 mg nitrate-N/L. They reported a mean nitrate percent reduction of 91% and a mean nitrate removal rate of 5.0 – 30 g N/m^3/day. Van Driel et al. (2006) conducted a field-scale study of up-flow bioreactors operating under HLRs of 0.42 m/day with HRTs of 24 hours and a mean influent concentration of 11.5 mg nitrate-N/L. They reported a mean nitrate percent reduction of 67% and a mean nitrate removal rate of 0.7 g N/m^2/day. Chun et al. (2009) conducted a column-scale study operating under HLRs of 6.2 – 7.1 m/day with a mean HRT of 15.6 hours and mean influent concentrations of 25.7 mg nitrate-N/L and reported 100% nitrate reduction. Greenan et al. (2009) conducted a column scale study with a mean influent concentration of 50 mg nitrate-N/L and found that 100% nitrate removal was achieved at a HLR of 0.02 m/day (HRT of 9.8 days), but at a HLR of 0.14 m/day (HRT of 2.1 days), only around 30% nitrate reduction was achieved. Christianson et al. (2011a) conducted a pilot scale study of channel style bioreactors operating under HLRs of 4.8 m/day with HRTs of 0.14 – 1.8 hours and influent concentrations of 10.1 mg nitrate-N/L, reported between 30 – 70% nitrate reduction.

Another treatment factor controlling bioreactor performance is media type. Christianson et al. (2012) recommends that substrate be chosen based on C:N ratio, porosity, cost and longevity, as these physical properties influence bioreactor hydraulics and how quickly a substrate will

degrade over time. High C:N ratios are important because materials with low C:N ratios experience higher rates of mass degradation and flushing losses (Christianson et al., 2012). Woodchips tend to have C:N ratios ranging in the several hundred (Greenan et al., 2006). Porosities of woodchips used in bioreactors typically range between 0.6 – 0.86 (Chun et al., 2009), but increased moisture content and packing density can decrease porosity (Christianson et al., 2010). High porosities and hydraulic conductivities are important to allow the flow of water through the bioreactor. Over time, the carbon media will degrade, and the hydraulic conductivity will decrease (Christianson et al., 2012). The longevity of carbon media is largely dependent upon the type of carbon source and flow characteristics (Christianson, 2011b). Carbon media with higher C:N ratios (such as oak and pine, which typically have C:N ratios >100) (Greenan et al., 2006) are typically more sustainable, as they will take longer to degrade. Bioreactors operating under higher flow rates and with less consistent periods of saturation are also less susceptible to degradation (Christianson, 2011b).

The USDA (2016) recommends that bioreactors consist of woodchips with hydraulic conductivities of at least 0.05 m/s and effective porosities of 0.7. Woodchips should range from 2.5 – 5 cm in size and be free of dirt and debris (USDA, 2016). Woodchips are commonly used as a carbon substrate (Christianson et al., 2010), but some of the earliest denitrifying bioreactors (Blowes et al., 1994) consisted of packing media (sand) mixed with tree bark, woodchips, and compost in barrels buried in an agricultural drainage ditch. Operating under HRTs between 1 – 6 days with influent concentrations between 2 – 6 mg nitrate-N/L, Blowes et al. (1994) reported a nitrate percent reduction of nearly 100%. Robertson and Blowes (2000) tested the denitrifying abilities of sand mixed with sawdust, wood mulch and leaf compost in a subsurface denitrifying wall and reported a mean nitrate percent reduction of 91% and nitrate removal rates between 5.0

– 30 g N/m^3/day. Diaz et al. (2003) compared the nitrate reducing efficacy of pine bark, almond shells and walnut shells in batch reactors operating under HRTs between 16 – 72 hours with influent nitrate concentrations between 18.5 – 35 mg nitrate-N/L. They found walnut shells to be the most effective at reducing nitrate, with nitrate removal rates between 9.6 – 18.4 nitrate-N/m^3/day compared to pine bark, with nitrate removal rates between 4.6 – 8.5 g nitrate-N/m^3/day and almond shells with nitrate removal rates between 4.7 – 7.3 g nitrate-N/m^3/day.

Van Driel et al. (2006) compared the nitrate reducing efficacy of bioreactors packed with coarse woodchips to those packed with fine woodchips and found that reaction rates in bioreactors packed with coarse woodchips were not significantly different than those packed with fine woodchips. Greenan et al. (2006) compared the denitrifying efficacy of woodchips, woodchips mixed with soybean oil, those mixed with cornstalks, and those mixed with cardboard in column-scale bioreactors operating under HRTs between 15 – 180 days with influent concentrations of 15 mg nitrate-N/L. They found the most effective substrates to be (from highest nitrate removal to lowest): woodchips mixed with cornstalks, those mixed with cardboard, those mixed with soybean oil, and 100% woodchips, with all treatments resulting in over 98% nitrate reduction, except the woodchips mixed with cardboard, which resulted in 96% nitrate reduction. Trois et al (2010) conducted a lab-scale batch experiment to compare how well pine bark and lightly composted garden refuse removed nitrate from synthetic landfill leachate water with concentrations of 500 mg nitrate-N/L. Substrates were mixed with the synthetic leachate in air-tight bottles to allow for an anaerobic environment and allowed to saturate for 11 days. They reported complete denitrification in the composted garden refuse batch tests within 8 days, but a final concentration of 150 mg nitrate-N/L in the pine bark batch (30% nitrate reduction) after 11 days. Christianson et al (2011b) conducted a pilot-scale study on denitrifying

bioreactors comprised of pine woodchips operating under HLRs of 0.77 m/day with HRTs between 4 – 15 hours and influent concentrations between 7.7 – 35.6 mg nitrate-N/L. They reported nitrate percent reductions between 14 – 37% and nitrate removal rates between 2.1 – 6.7 g N/m^3/d and found that nitrate reductions were directly correlated to flow rate. Bell et al. (2015) conducted a field scale study of a denitrifying bioreactor comprised of woodchips exposed to the atmosphere and reported an average nitrate reduction of 63%.

There have been very few studies that examined the efficacy of peanut hulls as a carbon-substrate for denitrifying bioreactors. While this media has a lower C:N ratio (31:1) compared to other substrates, research has suggested that they could be effective. Xing et al. (2020) compared the denitrifying efficacy of three different pulverized and sieved substrates – peanut hulls, walnut hulls, and corn cobs – of three different sizes (i.e., particle diameters) in lab-scale batch-reactors operating under HRTs of 12 hours with an influent concentration of 25 mg nitrate-N/L. They reported that the substrates of particle sizes between 0.12 – 0.30 mm were the most effective at attenuating nitrates; at this range, corn cobs were reported to be the most effective at denitrification. Xing et al. (2020) reported that at particle sizes less than 0.125 mm, peanut hull nitrate-N percent reductions (74.86%) exceeded those of corn cobs (12.26%), but pulverized peanut hulls less than 0.215 mm in size may lower hydraulic conductivity and prevent adequate flow in a field-scale bioreactor. Ramirez-Godinez et al. (2015) compared the amount of organic matter leached from woodchips, barley grains, and peanut hulls by soaking each substrate in influent water purged of N_2 and sealed to avoid N contamination. They reported that, though peanut hulls produced the lowest concentrations of nitrogenous species, they produced higher levels of TSS and turbidity than woodchips or barley grains. In addition, the organic matter leached from peanut hulls had a ratio of biological oxygen demand (BOD) to chemical oxygen

demand (COD) of 0.49 while the organic matter leached from woodchips had a BOD:COD of 0.15, which means that the organic matter leached from peanut hulls is more easily biodegradable than that leached from woodchips, implying peanut hulls will degrade faster than woodchips and may be less sustainable to use as a substrate for denitrifying bioreactors.

Table 2.1 Review of denitrifying bioreactors.

Publication	Setting	Substrate	Flow Rate (L/min)	HLR (m/day)	HRT (hours)	Influent Concentration (mg nitrate-N /L)	Nitrate % Reduction	Nitrate Removal Rate (g N/m²/d)
Blowes, 1994	Field-scale; barrels containing reactive carbon buried in agricultural drainage ditch	Sand mixed with tree bark, woodchips and leaf compost	0.007 – 0.04	N/A	24 –144	2 – 6	Nearly 100%	N/A
Robertson and Blowes, 2000	Field-scale PRB	Mix of sand and sawdust	0.01	0.02*	240	33.9	91%	5.0 – 30
Diaz, 2003	Lab-scale open-air reactors	Pine bark, almond shells and walnut shells	N/A	N/A	16 and 24	18.5 and 35	N/A	4.6 – 18.4
Van Driel, 2006	Field-scale up-flow bioreactor	Compared coarse vs fine wood particles	0.35	0.42*	24	11.5	33%	0.7

Table 2.1 Continued

Publication	Setting	Substrate	Flow Rate (L/min)	HLR (m/day)	HRT (hours)	Influent Concentration (mg nitrate-N /L)	Nitrate % Reduction	Nitrate Removal Rate (g N/m²/d)
Greenan, 2006	Lab-scale; subsurface bioreactor in jars	100% woodchips, woodchips mixed with: soybean oil, cornstalks, and cardboard	0 (no flow, left to saturate)	N/A	360 – 4320	15	98%	N/A
Greenan, 2009	Lab-scale; subsurface bioreactor	Woodchips (Oak)	N/A	0.03 – 0.14*	50.4 – 235.2	50	30 – 100%	N/A
Chun, 2009	Lab-scale subsurface bioreactors	Woodchips (mixed species)	0.228 – 0.268	6.2 – 7.1*	15.6	25.7	100%	N/A
Robertson and Merkley, 2009	Field-scale; In-stream bioreactor	Woodchips (species not reported)	24	0.70*	N/A	4.8	78%	0.264 – 4.8 *

Table 2.1 Continued

Publication	Setting	Substrate	Flow Rate (L/min)	HLR (m/day)	HRT (hours)	Influent Concentration (mg nitrate-N /L)	Nitrate % Reduction	Nitrate Removal Rate (g N/m²/d)
Trois, 2010	Lab-scale batch bioreactors	Pine bark and composted garden refuse	0 (no flow, left to saturate)	N/A	264	500	30 – 100%	N/A
Christianson, 2011a	Pilot-scale; bioreactor	Woodchips (mixed species of hardwood)	3.8	4.8*	0.14 – 1.8	10.1	30 – 70%	3.8 – 5.6*
Christianson, 2011b	Pilot-scale bioreactors	Woodchips (pine)	0.33	0.77*	4 – 15	7.7 – 35.6	14 – 37%	2.1 – 6.7
Bell, 2015	Field-scale; bioreactor intercepting tile drainage	Woodchips (mixed species)	N/A	N/A	2 – 8	Range: <0.1 – 17	20 – 98%	11.6
Hoover, 2016	Lab-scale; bioreactor	Woodchips (mixed species of hardwood)	N/A	N/A	2 – 24	11.5 – 35.1	39%	15.6

Table 2.1 Continued

Publication	Setting	Substrate	Flow Rate (L/min)	HLR (m/day)	HRT (hours)	Influent Concentration (mg nitrate-N /L)	Nitrate % Reduction	Nitrate Removal Rate (g N/m²/d)
Iverson, 2019	In-stream affected by residential septic effluent; paired with phosphate adsorbent	Woodchips (mixed species)	25.5 L/min	0.61 m/day*	N/A	0.89	78%	N/A
Xing, 2020	Lab-scale bioreactor	Peanut hulls (<0.125 mm)	0 L/min (no flow, left to saturate)	N/A	12	25	74.86%	N/A

Notes

    * = Value was not provided by the source but calculated based on the variables provided.

    N/A = Value and/or variables needed to calculate value was not provided by the source.

2.3 Phosphate Sorption

Phosphate adsorption occurs when the electrons of a phosphate molecule bond to the positive cations of metal hydroxides (Wilhelm et al., 1994). Metal oxidizing materials high in aluminum (Al), iron (Fe), magnesium (Mg), or calcium (Ca) provide the cations to bond with dissolved phosphorus to form insoluble compounds (Weng et al., 2012). Under aerobic conditions, phosphate is adsorbed by iron and aluminum oxyhydroxides in acidic environments (Bohn et al., 1985) and by calcium carbonates in basic environments (Doner and Lynn, 1989). Phosphate adsorption is generally higher in acidic environments than basic environments (Tofflemire and Chen, 1977). Under anaerobic conditions, phosphate has been observed to desorb from the metal cations (Fillos and Molof, 1972) in response to the reduction of the oxyhydroxides that it bonds with. Adsorbed phosphate can also mineralize into various minerals (variscite, strengite, vivianite, etc.) depending on the concentration of phosphate and the soil pH (Wilhelm et al., 1994).

Weng et al. (2012) studied the control factors on phosphate sorbents in lab-scale experiments and reported that the pH, concentration of Ca ions, and the presence of natural organic matter are the most important factors that control phosphate adsorption to Fe oxides. The presence of Ca ions in the water was reported to enhance the amount of phosphate adsorbed. Weng (2012) reported that the presence of Ca ions decreased the pH dependency of phosphate adsorption as well. Though Tofflemire and Chen (1977) reported increased phosphate adsorption in acidic environments, Weng (2012) reported that in the presence of Ca ions, phosphate adsorption increased with pH between 4 and 7. Weng (2012) also reported that the presence of DOC can

lead to a decrease in phosphate adsorption as it reduces the binding capacity of the reactive

barrier.

2.4 Phosphate Sorbents Paired with Denitrifying Bioreactors

Phosphate sorbents have been paired with denitrifying bioreactors in a variety of studies.

Goodwin et al. (2015) compared nitrate and phosphate reductions in lab-scale bioreactors with

phosphate sorbents (steel chips and turnings) placed upstream vs downstream of the denitrifying

media (woodchips). This study operated under HLRs of 0.24 m/day with HRTs between 1 and 3

hours and a mean influent concentration of 4.36 mg P/L. Goodwin et al. (2015) reported that

higher nitrate load reduction was associated with woodchips placed downstream, while higher

phosphate load reduction was associated with woodchips placed upstream, with an average

phosphate reduction of 95.6%. Similarly, Thapa (2017) quantified the phosphate reduction in a

phosphate adsorption bed installed downstream of a denitrifying bioreactor operating under

HLRs between 4500 and 9000 m/day and an HRT of 40 minutes. With mean influent

concentrations between 0.0011 and 0.0712 mg/L dissolved reactive phosphorus (DRP), Thapa

(2017) reported an average DRP reduction of 45% and an average DRP removal rate of 49 g

DRP/m^3/day. Hua et al. (2016) investigated the phosphate sorption capacity of steel byproducts

(steel slag and turnings) placed downstream of woodchips in column-scale up-flow bioreactors

operating under HLRs of 0.006 – 0.024 m/day and HRTs between 2.4 – 9.5 hours with a mean

influent concentration of 30 mg P/L. Hua et al. (2016) reported that this configuration was

successful in reducing nitrates and phosphates in the water, the steel shavings removed 100% of

the phosphate with an average removal rate of 12.4 g P/m^3/day. Hua et al. (2016) reported the

phosphate sorbing capacity of the steel byproducts to be 3.70 mg P/g under continuous flow conditions.

Christianson et al. (2017) used column-scale bioreactors to compare the nitrate and phosphate removal efficacy of placing acid mine drainage treatment residual (MDR) and steel slag downstream vs upstream of denitrifying material (woodchips). The iron rich MDR was sieved to between 0.6 and 4.0 mm and the steel slag, containing aluminum, iron and calcium was sieved to between 0.21 and 0.60 mm. This experiment operated under an average HLR of 58.7 m/day and HRT ranging between 7.2 and 51 hours, with an average influent concentration of 1.39 mg DRP/L. Christianson et al. (2017) reported DRP reductions between 56 and 98% for the MDR and between 23 and 89% for the steel slag, with the percent reduction generally increasing with HRT. DRP removal rates were reported between 31 and 133 g DRP/m^3/day for the MDR and between 8.8 and 48 g DRP/m^3/day for the steel slag, with removal rate generally decreasing with HRT. Christianson et al. (2017) concluded that MDR was more effective at reducing nitrates than steel slag. This study also concluded that nitrate removal was independent of phosphate sorbent configuration, but phosphate sorption was optimized by the denitrification occurring upstream of phosphate sorbents.

Steel byproducts such as chips, slag, and turnings are commonly used as phosphate sorbents, but few studies have investigated the phosphate sorbing capacity of rotary-kiln expanded slate. Iverson (2019) documented the installment of a field-scale in stream denitrifying bioreactor comprised of woodchips overlain by a locally-produced phosphate-sorbing expanded-slate aggregate (Stalite brand) operating under a median HLR of 0.61 m/day with an average influent concentration of 0.23 mg P/L. Iverson (2019) reported that using expanded slate for phosphate sorption reduced phosphate concentrations by 74%. In a lab-scale batch study operating under

saturated conditions with HRTs of 0.5 hours and an influent concentration of 3.33 mg phosphate-P/L, Wu (2013) tested the phosphate sorbing capacity of expanded slate aggregate (Stalite) paired with a woodchip bioreactor to investigate their compatibility. The bioreactor was comprised of 80% expanded slate, 20% pine bark and removed an average of 0.6 mg/L of phosphate-P.

Numerous studies have evaluated the phosphate adsorbing capabilities of steel byproducts, acid mine drainage residual, and expanded slate when paired with woodchips. But there is a gap in knowledge when it comes to understanding the phosphate adsorbing capabilities of expanded slate when paired with other substrates such as pine bark or peanut hulls. This study will quantify the phosphate sorption capacity of expanded slate in pilot-scale bioreactors comprised of 65% denitrifying media and 35% expanded slate.

Table 2.2 Review of phosphate sorbents paired with denitrifying bioreactors.

Publication	Setting	Material	Flow Rate (L/min)	HLR (m/day)	HRT (hours)	Influent Concentration (mg P/L)[1]	Phosphate % Reduction	Phosphate Removal (g PO$_4$-P/m^3/d)[1]
Wu, 2013	Column-scale paired with denitrifying bioreactor	20% pine bark, 80% expanded slate (Stalite)	N/A	N/A	0.5	3.33 mg PO$_4$-P/L	N/A	Attained an average of 0.6 mg/L of phosphate
Goodwin, 2015	Column-scale paired with denitrifying bioreactor	Steel turnings	0.00185 – 0.00882	0.24*	1 – 3	4.36	95.6%	N/A
Thapa, 2017	Field scale paired with denitrifying bioreactor	Steel chips and turnings	500 – 1000	4500 – 9000*	0.66	0.0011 – 0.0712 mg DRP/L	45%	49 g DRP/m^3/day
Hua, 2016	Column-scale; paired with denitrifying bioreactor	Steel shavings and turnings	0.0025 – 0.01	0.006 – 0.024*	2.4 – 9.5	30	53.5 – 100%	12.4

Table 2.2 Continued

Publication	Setting	Material	Flow Rate (L/min)	HLR (m/day)	HRT (hours)	Influent Concentration (mg P/L)[1]	Phosphate % Reduction	Phosphate Removal (g PO$_4$-P/m^3/d)[1]
Christianson, 2017	Field-scale paired with denitrifying bioreactor (agricultural drainage ditch)	Acid-mine drainage residuals	0.02	58.7*	0.13	1.39	56 – 98%	25 – 133
Christianson, 2017	Field-scale paired with denitrifying bioreactor (agricultural drainage ditch)	Steel slag	0.02	58.7*	0.13	1.39	23 – 89%	8.8 – 48
Iverson, 2019	In-stream affected by residential septic effluent; paired with denitrifying bioreactor	Expanded slate aggregate	25.5	0.61*	N/A	0.23	74%	N/A

Notes

* = Value was not provided by the source but calculated based on the variables provided.

N/A = Value and/or variables needed to calculate value was not provided by the source.

1 = Unless otherwise noted.

2.5 Objectives

As discussed above, many studies have investigated the nitrate removal efficacy of numerous species of woodchips and bark, but few studies have investigated how well peanut hulls may promote nitrate reduction. In addition, while many studies have investigated the phosphate sorbing capabilities of steel shavings, steel turnings, and steel slag, few studies have examined the phosphate sorbing capabilities of expanded slate.

The overall goal of this study was to contribute to the knowledge of which woody substrates and phosphate adsorbents can effectively reduce nitrates and phosphates, respectively, when paired. The objectives of this study were:

- To compare the nitrate removal efficacy of three substrates: roasted peanut hulls (a local agricultural waste product), pine bark, and wood chips of mixed species (i.e., "waste-wood mix") at a range of HLRs and temperatures.
- To investigate the phosphate sorbing capabilities of an expanded slate aggregate (Stalite brand)

All three substrates tested in this study are regionally available and cost effective. The woodchips and pine bark were chosen because they have a high C:N ratios (Table 3.3) and thus, should promote microbial denitrification (Christianson, 2011b). The woodchips were also chosen because they were a local waste product, so they represent a cost-effective option. Pine bark is particularly useful to this study because *Pinus taeda*, or Loblolly Pine, is the most common tree in the piedmont of North Carolina (Holmes, 2012), so it would be easy and cost effective to use as a carbon media source for a denitrifying bioreactor in a North Carolina stream. Peanut, or *Arachis hypogaea,* hulls, were chosen to use as an experimental substrate for this experiment

because they are a regional agricultural waste product and there have been few studies (Xing et al., 2020) quantifying their denitrifying efficacy.

CHAPTER III – METHODOLOGY AND MATERIALS

3.1 Experimental Design

Denitrifying bioreactors were tested in a pilot-scale experiment consisting of nine (9) troughs (Figure 3.1) at the East Carolina University West Research Campus in Greenville, NC (35.6127° N, 77.3664° W). The sampling period started in July of 2021 and ended in March of 2022. Three types of denitrifying substrates were compared – roasted peanut hulls, pine bark, and wood chips of mixed species. The upstream 70% of the troughs (245 L) were filled with the denitrifying media and the downstream 30% (105 L) were filled with a phosphate-sorbing, expanded slate aggregate encased in a geotextile material (Figures 3.2). The ratios of expanded slate to denitrifying media are different than in the Wu (2013) study because the primary focus of their study was phosphate sorption whereas the primary focus of this study was nitrate removal. For this reason, there was a higher volume of woodchips than of expanded slate in the bioreactors in this study.

The bioreactors were comprised of a higher percentage of woody substrate than expanded slate because nitrate removal was the primary objective of this study. Each experimental substrate had three replicates (for a total of nine experimental units). The effluent samples were collected from each effluent pipe at the downstream end of each replicate. The remaining effluent water flowed into a shared gutter leading into a composite effluent bucket where the effluent from all three substrate replicates was continuously monitored (Figures 3.3 and 3.4). This experiment was designed to simulate an in-stream bioreactor in which only a small percentage of water would infiltrate as the rest of it continues to flow downstream. To best simulate these conditions, overflow holes were drilled into the top, downstream ends of each trough (Figure 3.4) to allow the water that did not have time to infiltrate to flow directly over and out of the bioreactor.
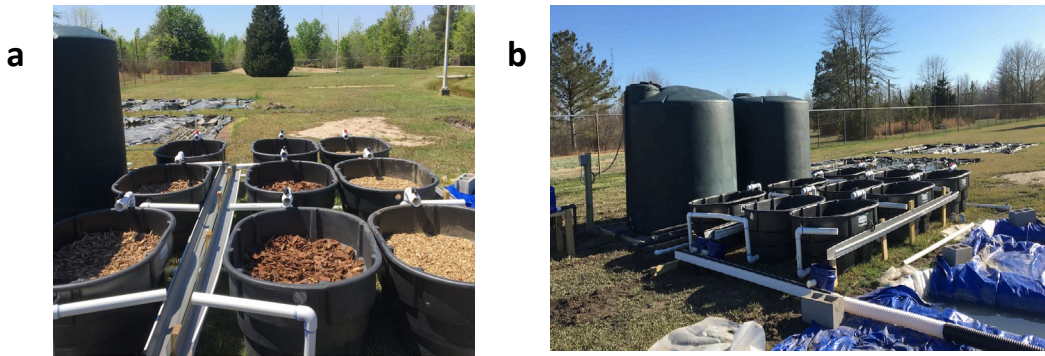
Figure 3.1 Images of final experimental set up in Greenville, North Carolina at the East Carolina University West Research Campus. There were three replicates of each substrate.

Figure 3.2 Images of uncovered bioreactors demonstrating the various denitrifying media (a) woodchips, b) pine bark, c) peanut hulls). The denitrifying substrate comprises the upstream end of the bioreactor (closest to the influent pipe), and the expanded slate (encased in a geotextile sack) comprises the downstream end.
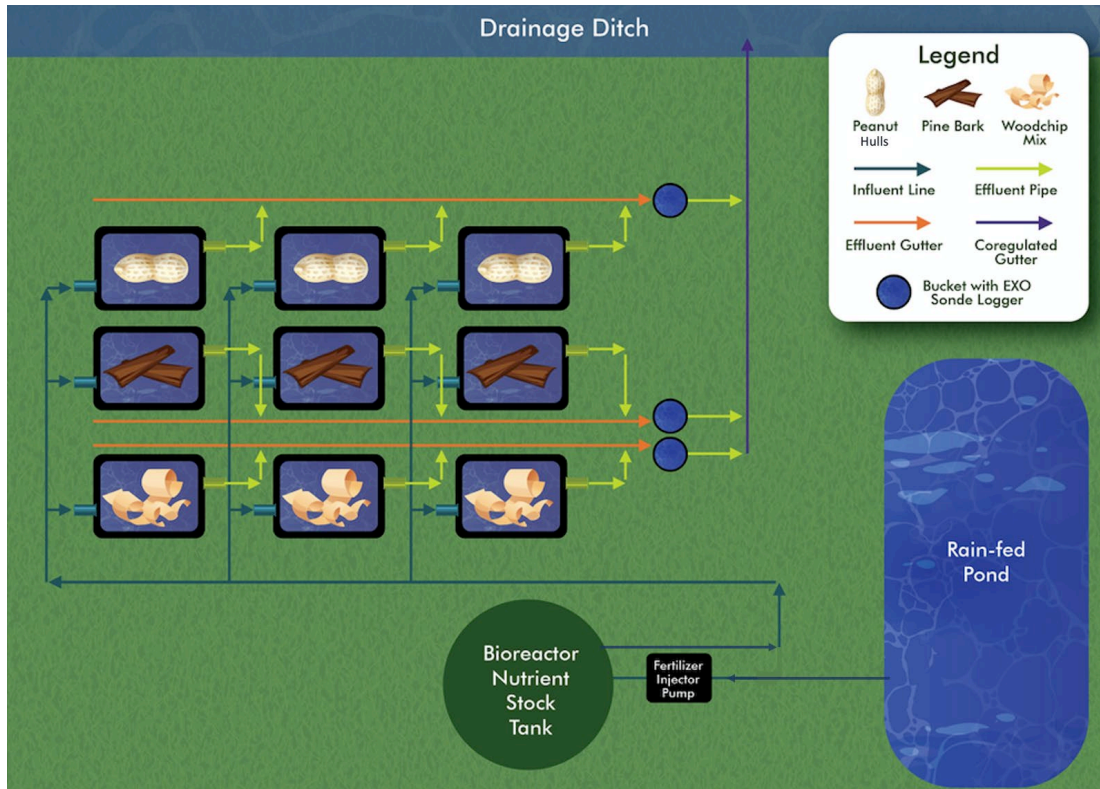
Figure 3.3 Aerial diagram of experimental set up.
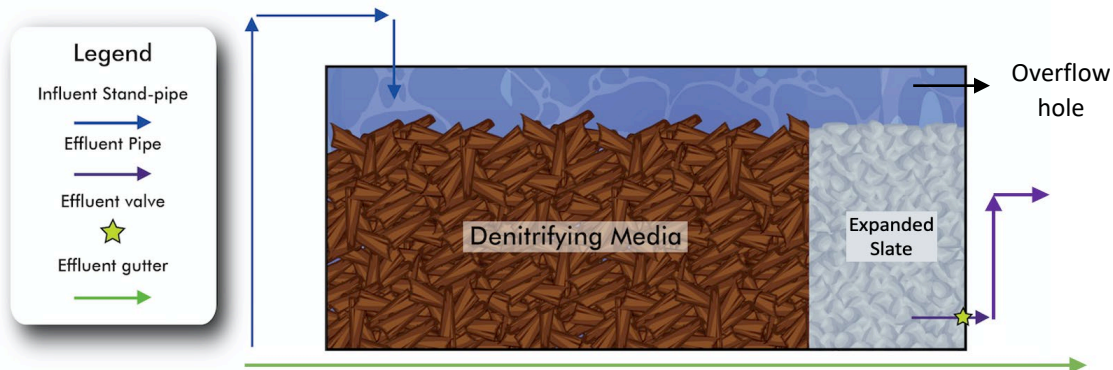
Figure 3.4 Cross sectional diagram of bioreactor.

3.2 Structure of Experiment

The sampling period consisted of 24 sampling events over the course of two seasons: warm (15 sampling events between July – October) and cold (9 sampling events between January – March). The bioreactors were left saturated in between experimental runs, as well as in

between the warm period and the cold period to simulate conditions of a first order stream in between storm events. Samples were collected over the course of one day on a weekly basis, the duration of flow and frequency of sample collection varied based on the influent flow rates being tested (Table 3.1). Water was only turned on to flow through the bioreactors on sampling days, it was turned off at the end of a sampling day and was left to saturate the bioreactors in between sampling days. On sampling days, six samples were collected from the influent and from the effluent of each of the nine bioreactors. The first sample was collected immediately after the flow had been turned on (Sample 0), to capture conditions after bioreactors had been saturated for a week. The collection frequency of the next five samples varied based on the influent flow rate grouping of that sampling day (Table 3.1).

Table 3.1 Influent Flow Rate groupings with duration of flow and frequency of sample collection.

Group Name	Influent Flow Rates (L/day)		Duration of Flow (hours)	Frequency of Sample Collection (hours)
	Pine Bark and Peanut Hulls	Woodchips	All Substrates	All Substrates
Low Flow	2600	3000	10	2
Medium Flow	5200	6000	5	1
High Flow	10400	12100	2.5	0.5

3.3 Flow Rate

Over the course of the experiment, three influent flow rate groupings were tested (Table 3.1). Due to differences in substrate porosity (Table 3.2), influent flow rates for woodchip bioreactors (porosity of 0.88) were slightly different than those of pine bark and peanut hull bioreactors (porosities of 0.75 and 0.76, respectively). The more porous woodchips operated under slightly higher influent flow rates than the pine bark and peanut hulls (Table 3.1).

Influent flow rates were calibrated at the beginning of and throughout each sampling day to ensure consistent flow rates throughout the sampling period. Influent flow rates were determined by measuring the volume of water flowing into the bioreactors from each influent pipe during a 5 second period. The actual rates of flow through the bioreactors were determined by measuring the volume of water flowing out of the bioreactors from each effluent pipe during a 5 second period. Hydraulic loading rate (Equation 2.2 and Table 3.3) is defined as the flow rate through a system normalized by surface area, which is important to consider when quantifying how much water is being treated and understanding how long it spends in the system. As this experiment was designed to simulate an in-stream bioreactor, much of the influent water flowed over the bioreactor and out of the overflow holes (Figure 3.4). At the highest range of HLRs, only 1% of water was treated in woodchip bioreactors, 3% in pine bark bioreactors, and 6% in peanut hull bioreactors. At the middle range of HLRs, 4% of water was treated in woodchip bioreactors, 6% in pine bark bioreactors, and 11% in peanut hull bioreactors. At the lowest range of HLRs, 5% of water was treated in woodchip bioreactors, 10% in pine bark bioreactors, and 23% in peanut hull bioreactors (Table 3.4). Woodchips consistently treated less water than peanut hulls and pine bark due to the high variability in particle sizes, which inhibited the hydraulic conductivity of the bioreactor. Another important thing to note when considering the implementation of these substrates in a field scale is that pine bark and peanut hulls floated in their troughs due to their low bulk density, allowing more water to infiltrate for treatment.

Table 3.2 Substrate characterization including carbon content, nitrogen content, C:N ratios, volumes, porosities, and bulk densities.

Substrate	C Content (ppm)	N Content (ppm)	C:N	Volume (L)	Porosity	Bulk Density (g/cm^3)
Woodchips	490000	4280	114	245	0.88	0.20
Pine Bark	539000	2710	199	245	0.75	0.17
Peanut Hulls	518000	16100	31	245	0.76	0.09

Table 3.3 Ranges of infiltration rates and HLRs of each experimental substrate and each experimental HLR (surface area = 1.395 m^2). *Denotes the omission of one or more reps so that only the replicates operating under the specified range of flow rates (0.1 – 0.3 m/day) were compared. n denotes the number of samples that were considered during statistical analysis.

As Assigned	Actual rate of flow through bioreactor (L/day)			HLR (m/day)		
Substrate Replicate	Low Influent	Medium Influent	High Influent	Low Influent	Medium Influent	High Influent
Woodchips n = 8	121 – 155	173 – 277	121 – 225*	0.10 – 0.11	0.12 – 0.20	0.12 – 0.16*
Pine Bark n = 9	138 – 328	173 – 380	277 – 397	0.10 – 0.24	0.12 – 0.27	0.20 – 0.28
Peanut Hulls n = 3	277*	294 *	328*	0.20 *	0.21*	0.24*

Table 3.4 Percent of water treated in each substate at each range of HLRs.

Substrate	HLR		
	Low	Medium	High
Woodchips	5%	4%	1%
Pine Bark	10%	6%	3%
Peanut Hulls	23%	11%	6%

3.4 Substrate Characterization

The woodchips were donated by ECU Facilities Services. They were sourced from a waste pile of assorted species, but most likely consist of common trees in the region: *Pinus* sp. (pine), *Quercus* sp. (oak), and *Liquidambar* sp. (sweetgum) (Brown, 2018). The woodchips ranged from 4 – 8 cm in length. The pine bark was purchased from Lowes ($3.18 per 56 L bag) and ranged from 6 – 17 cm in length. The peanut hulls were donated by Hampton Farms peanut processing facility in Severn, NC. They were roasted, un-pulverized, un-sieved and ranged from 2 mm – 4 cm in length.

Substrates were analyzed for C:N ratios, porosities, and bulk densities. The C:N ratios were determined by the NC Agronomics Lab (Table 3.2) by analyzing the substrates for Total N and C (AOAC Method 972.43) using the Dumas oxygen combustion method on the Vario MAX Cube (Elementar Americas, Inc.; Ronkonkoma, NY, USA). Porosity was determined using the methods described by Hoover (2016). Bulk density was determined using the methods described by the US Pharmacopeia (2015).

3.5 Influent Water Preparation

Influent water was pumped from a rain-fed pond onsite through a fertilizer injector and into a 2,100-gallon storage tank. The fertilizer injector pumped concentrated nutrient solution from a 100-gallon stock tank into the storage tank at a 2% injection rate. The stock tank was prepared with KNO_3 and KH_2PO_4 so that the storage tank would contain target concentrations of ~20 mg nitrate-N/L and ~1 mg phosphate-P/L.

As discussed, this experiment utilized water from a rain-fed pond onsite. It should be noted that during the entirety of the sampling period, the pond contained algae. Water was filtered

through a 200-mesh screen filter upstream of the fertilizer injector before being pumped into the tank.

3.6 Substrate Inoculation

To encourage growth of denitrifying bacteria on the substrate, the bioreactors underwent a 14-day start up period. During this start up period, influent water was dosed with micronutrients to the following concentrations: 4.0 mM $CaCl_2$, 2.0 mM KH_2PO_4, 1.0 mM K_2SO_4, 1.0 mM $MgSO_4$, 25 µM H_3BO_3, 2.0 µM $MnSO_4$, 2.0 µM $ZnSO_4$, 0.5 µM Na_2MoO_4, and 0.5 µM $CuSO_4$ (Nadelhoffer, 1990) and 20 mg nitrate-N/L at an influent flow rate of 0.038 L/min to achieve an HRT of approximately 4 days.

During start up, the influent water was also dosed with 26 mg phosphate-P/L. This resulted in the expanded slate reaching phosphate adsorption capacity before the sampling period began. During the sampling period, influent phosphate-P concentrations were reduced to 1 mg phosphate-P/L to simulate maximum concentrations expected in first order streams that may be impacted by septic leachate and other non-point sources.

3.7 Field and Lab Analyses

The influent tank was monitored for temperature, dissolved oxygen (DO), pH, oxidation-reduction potential (ORP), and specific conductivity (SpC) at the start of each sampling day with the ProDSS multiparameter water quality probe (YSI Inc.; Yellow Springs, OH, USA). Each composite effluent bucket was monitored throughout the entire sampling day for temperature, DO, pH, ORP, SpC, turbidity, and DOM at an interval of 15 minutes on EXO-2 Sondes (YSI Inc.; Yellow Springs, OH, USA).The ProDSS and Sondes were calibrated on a bi-weekly basis.

All samples were filtered through 0.45 μm syringe filters and frozen within five days until the day of analysis. All samples were analyzed for nitrite + nitrate-N (NO_2+NO_3-N) (QuikChem Method 10-107-04-1-R), phosphate-P (PO_4-P) (QuikChem Method 10-115-01-1-V) and ammonia-N (NH_3-N) (QuikChem Method 10-107-06-1-M) using Flow Injection Analysis on the Lachat QuikChem 8500 (Hach; Loveland, CO, USA). Samples collected at the start and at the end of each sampling day were analyzed for dissolved organic carbon (DOC) (USEPA Direct Method 10267) using Hach test kits (Hach; Loveland, CO, USA), and for total Kjeldahl nitrogen (TKN) (10-107-06-2-M) and total Kjeldahl phosphorus (TKP) (10-115-01-2-B) using Flow Injection Analysis on the Lachat QuikChem 8500 (Hach; Loveland, CO, USA). For the analysis of DOC, samples were digested at 100° C for two hours. For the analysis of TKN and TKP, samples were digested at 200° C for one hour and then at 390° C for 30 minutes. QA/QC methods included check standards every 10 samples for all analyses with a 10% acceptable range of error.

Nitrite-N is typically oxidized into nitrate-N in aerobic surface waters (EPA, 1990), so it is deemed negligible in surface waters (such as the rain-fed pond that was the source of water for the bioreactors). For this reason, nitrite + nitrate-N will be referred to as nitrate-N. Ammonia-N (NH_3-N) is the gaseous form of the ammonium-N (NH_4-N) dissolved ion, for this reason it will be referred to as ammonium-N. TKN is comprised of dissolved organic nitrogen (DON) and ammonium-N, so DON was determined by subtracting ammonium-N from TKN, and total dissolved nitrogen (TDN) was determined by calculating the sum of nitrate-N and TKN. TKP, or total dissolved phosphorus (TDP), is comprised of dissolved organic phosphorus (DOP) and phosphate-P, so DOP was determined by subtracting phosphate-P from TKP.

Nutrient percent reductions were calculated by dividing the difference in influent and effluent concentrations by the influent concentration and multiplying by 100 (Equation 3.1). Nutrient removal rates (g/m³/day) were calculated using the method described by Manca et al. (2021), adapted from Warneke et al. (2011) (Equation 3.2). TDN and TDP reductions were calculated in kg/year by multiplying the amount of nitrogen or phosphorus reduced in mg/L by the flow rate in L/day and converting to kg/year (Equation 3.3).

Equation 3.1 Nutrient percent reduction.

$$\% \ Reduction = \left(\frac{In - Ef}{In}\right) \times 100$$

Where *In* denotes the average influent concentration of that sampling day (mg/L), and *Ef* denotes the concentration of the effluent from a particular bioreactor (mg/L).

Equation 3.2 Nutrient removal rate.

$$Removal \ Rate = \frac{In - Ef}{Vol_{sat}} \times Q$$

Where *In* denotes the average influent concentration of that sampling day (g/m³), *Ef* denotes the concentration of the effluent from a given bioreactor at a given time (g/m³), *Vol*$_{sat}$ denotes the saturated volume of the bioreactor (m³), and *Q* denotes flow rate (m³/day) (Equation 3.1).

Equation 3.3 Total dissolved nutrient reduction (TDN and TDP).

$$Total \ dissolved \ nutrient \ reduction = \frac{mg}{L} \times \frac{L}{day} = \frac{mg}{day} \to \frac{kg}{year}$$

Where *mg/L* denotes the concentration of TDN or TDP reduced, *L/day* denotes the flow rate within the bioreactors, and *mg/day* denotes the mass of TDN or TDP removed per day.

3.8 Statistical Analysis

Statistical analysis of the data was conducted using the R statistical software. After performing Shapiro-Wilk tests on the nitrate percent reduction, the nitrate removal rate, the phosphate percent reduction and phosphate removal rate data, it was determined that the data were not normally distributed, and thus failed to satisfy parametric assumptions. Kruskal-Wallis rank sum tests were performed with a Bonferroni p-adjustment factor to determine if there were statistically significant differences between treatment groups (substrates, HLRs, seasons). Pair-wise comparisons were used to determine level of significance in differences between treatment groups. Spearman's rank order correlation was used to determine if nitrate and phosphate reduction was correlated to HLR and temperature. A 95% confidence interval ($\alpha = 0.05$) was used for all statistical tests. The significance of a correlation is determined by the p value, if $p < 0.05$, then the correlation is significant. The strength of correlation is determined by the rho (r) value, the closer to +1, the stronger the positive correlation, the closer to -1 the stronger the negative correlation. Correlation coefficients (e.g., r values) near 0 suggest that the data were not correlated or only had a weak association. Data figures were developed with the ggplot2 package using the R statistical software. Some figures were developed using the ggbreaks package (Xu et al., 2021).

CHAPTER IV – RESULTS AND DISCUSSION

4.1 Effect of Substrate on Nitrate-N Removal

Comparisons of nitrate and phosphate removal between substrates and between seasons focuses on a range of HLRs between 0.1 – 0.3 m/day. Since nitrate percent reduction decreased over the course of a sampling day (Figure 4.1), analyses for this study will only include the final sample collected each sampling day (Sample 5) to make the most conservative estimates of treatment efficacy within the limitations of this study.
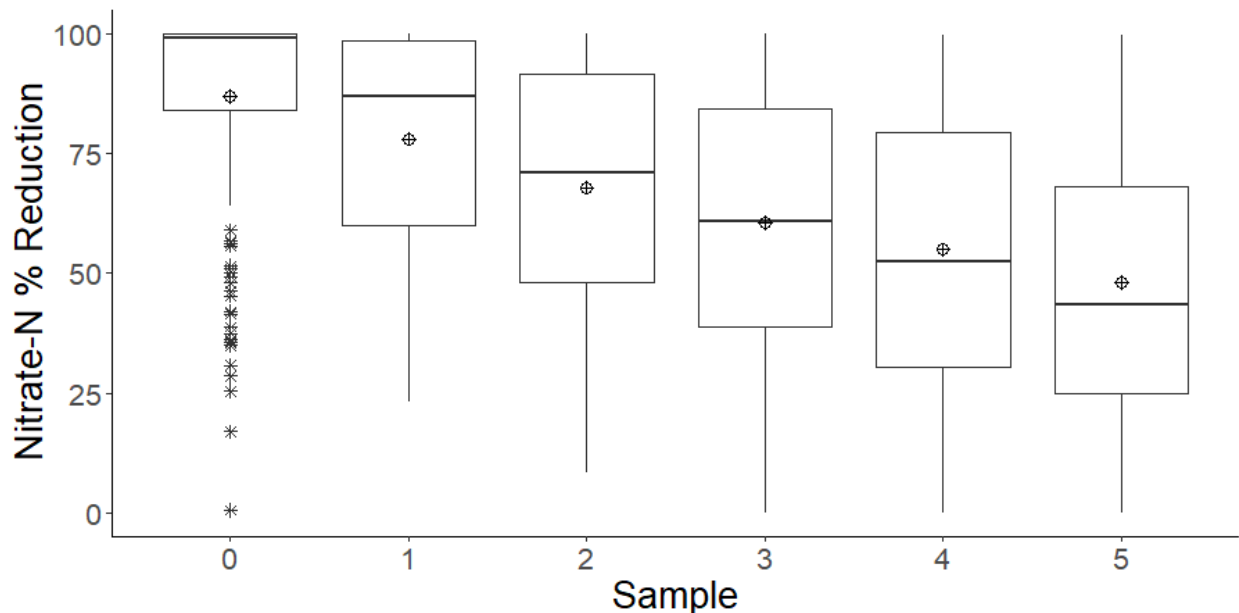
Figure 4.1 Change in nitrate-N percent reduction over the course of a sampling event across all substrates and all sampling events. Sample 0 is the first sample collected per sampling event and sample 5 is the last sample collected per sampling event. The middle bars indicate the medians, the crosshairs (⊕) indicate the means, and the asterisks (*) indicate the outliers. n = 24.

Throughout the sampling period, influent nitrate concentrations ranged from 15.3 to 21.6 mg nitrate-N/L with a median influent concentration of 17.5 mg nitrate-N/L (Figure 4.1). There were significant differences between influent and effluent nitrate concentrations for all substrates ($p = 4.53e\text{-}11$). The median effluent concentrations of woodchips, pine bark, and

peanut hulls were 11.0, 8.4, and 10.8 mg nitrate-N/L, respectively. There were not significant differences in final effluent concentrations between substrates when aggregating all data from every sampling event; however, as discussed later, note that effluent concentrations varied substantially by season. Pairwise comparisons between influent and substrate concentrations are presented in Appendix A, Table A1.
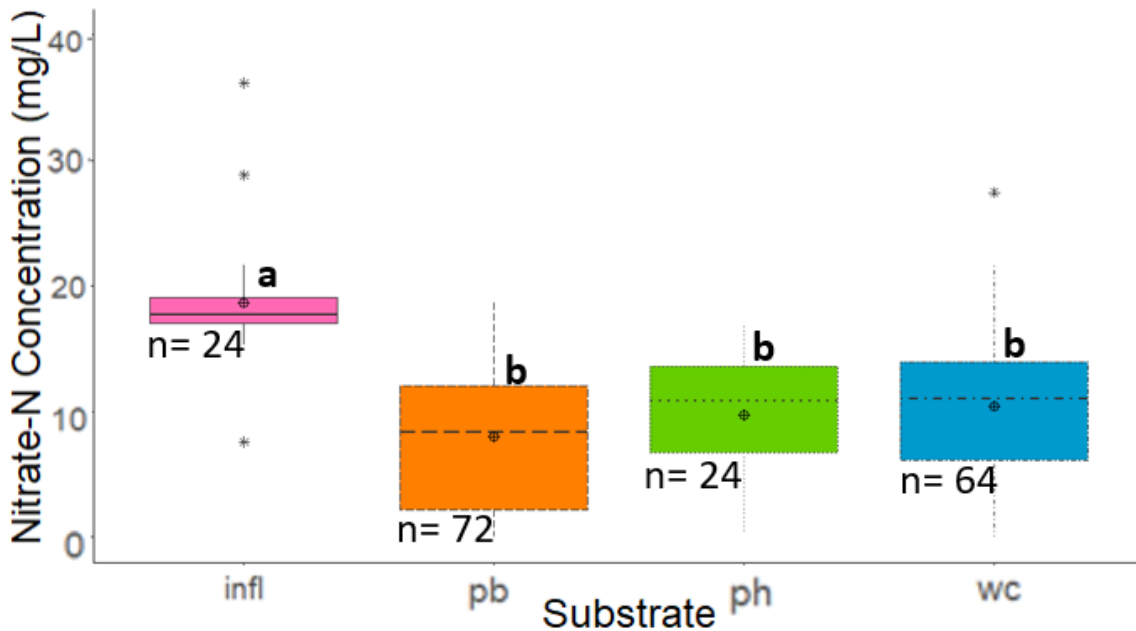
Figure 4.2 Influent and effluent nitrate-N concentrations at HLRs ranging between 0.1 – 0.3 m/day. Infl denotes influent , pb denotes pine bark, ph denotes peanut hulls, and wc denotes woodchips. Significant differences are denoted by the letter above the box (substrates that share a letter were not significantly different). The middle bars indicate the medians, the crosshairs (⊕) indicate the means, and the asterisks (*) indicate the outliers, n denotes the number of samples considered in statistical analysis.

Pine bark (median nitrate reduction of 50.4%) reduced a higher percentage of nitrate (p = 0.0093) than woodchips (median nitrate reduction of 31.4%), which suggests that pine bark was better at reducing nitrate than the more commonly used woodchips under HLRs between 0.1 – 0.3 m/day (Figure 4.2). Woodchips and peanut hulls reduced similar amounts of nitrate (p =

1.00), as did pine bark and peanut hulls (p = 0.2009). Pairwise comparisons between the nitrate

percent reductions of each substrate are presented in Appendix A, Table A2.

Nitrate removal rates of pine bark and peanut hull bioreactors were greater than that of

woodchip bioreactors (p = 1.5e-06 and p = 0.009, respectively). Removal rates ranged from 0 –

4.35 g N/m^3/day, 0 – 7.37 g N/m^3/day, and 0.75 – 4.93 g N/m^3/day in woodchip, pine bark, and

peanut hull bioreactors, respectively. Pine bark had a median removal rate of 2.6 g N/m^3/day,

peanut hulls of 2.0 g N/m^3/day, and woodchips of 1.1 g N/m^3/day. This data suggests that pine

bark and peanut hulls had higher nitrate removal rates than woodchips under HLRs between 0.1

– 0.3 m/day (Figure 4.2). Pairwise comparisons between the nitrate removal rates of each

substrate are presented in Appendix A, Table A3.

a)
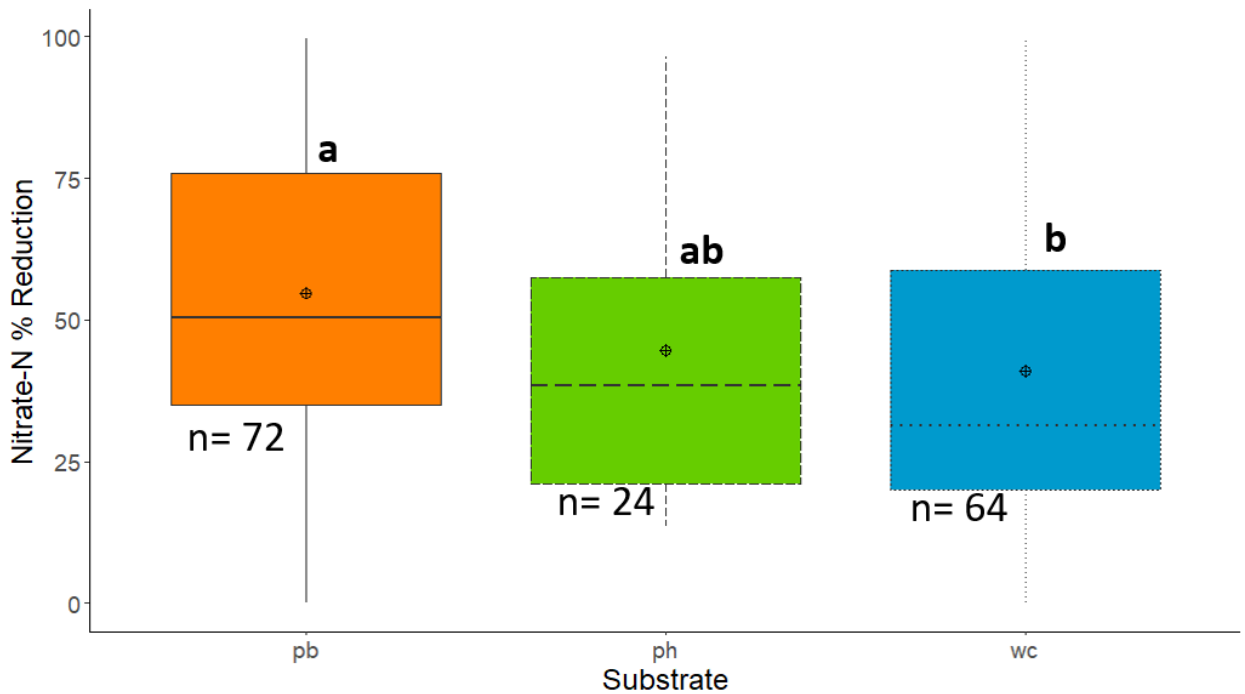
b)
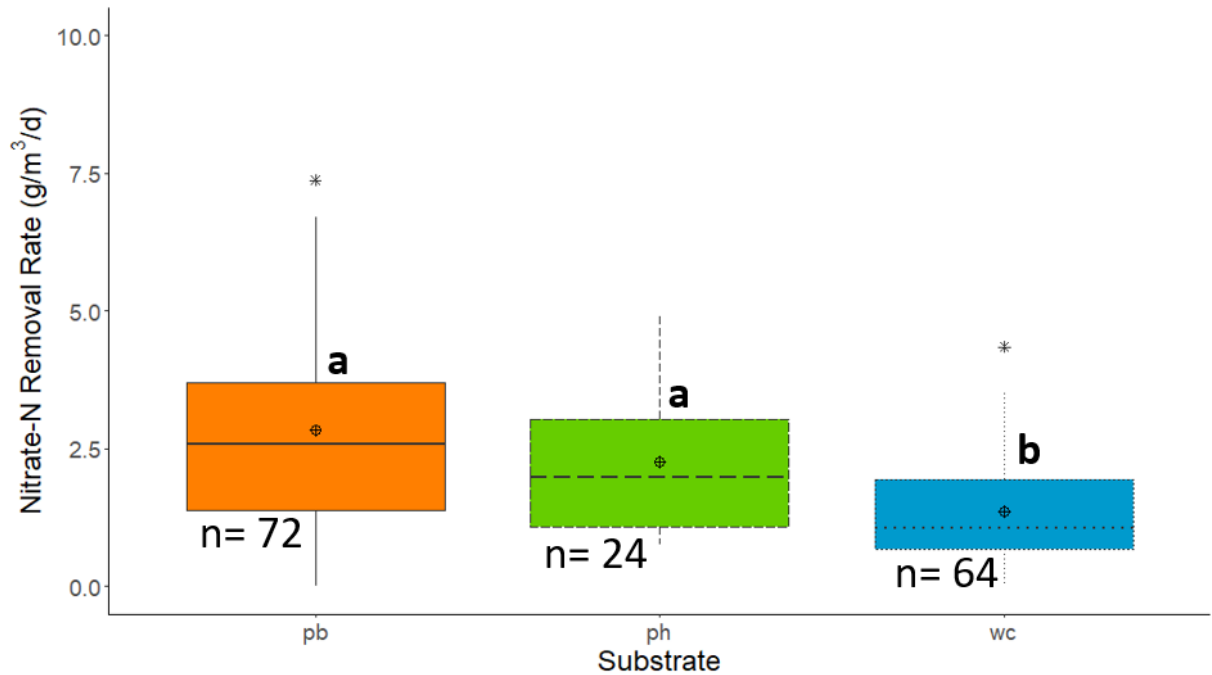
Figure 4.3 a) Nitrate-N percent reductions and **b)** nitrate-N removal rates (bottom) of each substrate at HLRs between 0.1 – 0.3 m/day. The middle bars indicate the medians, the crosshairs (⊕) indicate the means, and the asterisks (*) indicate the outliers, n denotes the number of samples considered in statistical analysis. Significant differences are denoted by the letter above the box (substrates that share a letter were not significantly different).

These results are similar to those reported in the literature. Christianson (2011a) reported a nitrate percent reduction between 30 – 70% and a nitrate removal rate between 3.8 – 5.6 g N/m^3/day in pilot-scale, woodchip bioreactors. In field-scale woodchips bioreactors, Robertson and Merkley (2009) reported an average nitrate percent reduction of 78% and nitrate removal rates ranging from 0.264 – 4.8 g N/m^3/day, with nitrate removal rates reported to be generally correlated with flow rate. Both studies operated under very different flow conditions than the current study. In the pilot scale experiment conducted by Christianson (2011a), flow rates were sharply increased to 2 L/min and then consistently decreased over the course of three days down to 0.3 L/min to simulate a storm event. The field scale experiment conducted by Robertson and

Merkley (2009) operated under flow rates ranging from 1 – 48 L/min, rather than periods of flow and periods of no flow, with flow through the bioreactor reportedly declining over time due to the deterioration of the woodchips. These differences in flow conditions may have contributed to the differences in nitrate percent reduction and nitrate removal rates as compared to the current study. Past studies researching nitrate treatment by pine bark bioreactors reported nitrate mass removal ranged from 4.6 – 8.5 g N/m^3/day (Diaz et al. 2003) and concentration reductions ranged from 30 – 100% (Trois et al. 2010). This is congruent with results reported by the current study that found pine bark bioreactors reduced nitrate masses by up to 7.37 g N/m^3/day and nitrate concentrations by up to 99%. In a lab-scale experiment conducted by Xing et al. (2020), bioreactors comprised of pulverized peanut hulls were reported to reduce a maximum of 74.86% nitrate. This concentration reduction was more than double what the current study observed (median nitrate reduction: 38.4%). This was likely due to differences in experimental design and substrate characteristics. Xing et al. (2020) utilized pulverized (<0.125 mm) peanut hulls and operated bioreactors under completely saturated conditions for periods of 50 hours. Utilizing a smaller substrate reduces the hydraulic conductivity, thereby increasing the HRT and provides higher accessibility of substrate to denitrifiers.

4.2 Effect of Hydraulic Loading Rate on Nitrate-N Removal

Loading rate is a function of flow rate and area, which impacts the HRT of a bioreactor – an important treatment factor for nitrate reduction. Since the bioreactors in this study operated under a range of HLRs (Table 3.2), it is important to consider how HLR affected the nitrate reducing capabilities of each substrate. The woodchips operated under HLRs ranging from 0.09 – 0.20 m/day (Figure 4.5), the pine bark operated under HLRs ranging from 0.10 – 0.28 m/day, and the peanut hulls operated under HLRs ranging from 0.20 – 0.63 m/day.

Since HLR is a function of flow rate, a negative correlation between nitrate percent reduction and HLR and a positive correlation between nitrate removal rate and HLR would be expected. In other words, though nitrate has been shown to be more efficiently removed during low loading conditions (higher percent reduction), the mass removal (removal rate) has been shown to be lower during low loading conditions than for higher loading conditions (Christianson et al., 2011b; Iverson et al., 2019; Robertson and Merkley, 2009).

All substrates showed a significant correlation between nitrate percent reduction and HLR. As expected, woodchips (ρ = -0.3, p = 0.01) and peanut hulls (ρ = -0.25, p = 0.036) exhibited significant negative correlations (Figure 4.4), but pine bark (ρ = 0.34, p = 0.0034) exhibited a significant positive correlation between nitrate percent reduction and HLR (Figure 4.4). Woodchips and peanut hulls appear to reduce nitrate more efficiently under lower HLRs, but pine bark appears to reduce nitrate more efficiently under higher HLRs, which is not expected or in agreement with what has been reported in the literature.

Pine bark (ρ = 0.62, p = 6.9e-09) and peanut hulls (ρ = 0.28, p = 0.016) both exhibited significant positive correlations between nitrate removal rate and HLR, while woodchips (ρ = 0.085, p = 0.48) did not exhibit any significant correlation (Figure 4.4). Pine bark and peanut hulls appear to reduce more nitrate under higher HLRs, which is to be expected and is in agreement with what has been reported in the literature.

Though correlations between nitrate reduction and HLR are significant (p < 0.05), they are not all very strong (ρ < 0.50). This may be attributed to the fact that the ranges of HLRs are relatively small for each substrate (Table 3.3) so there is poor representation of data across the flow regime.
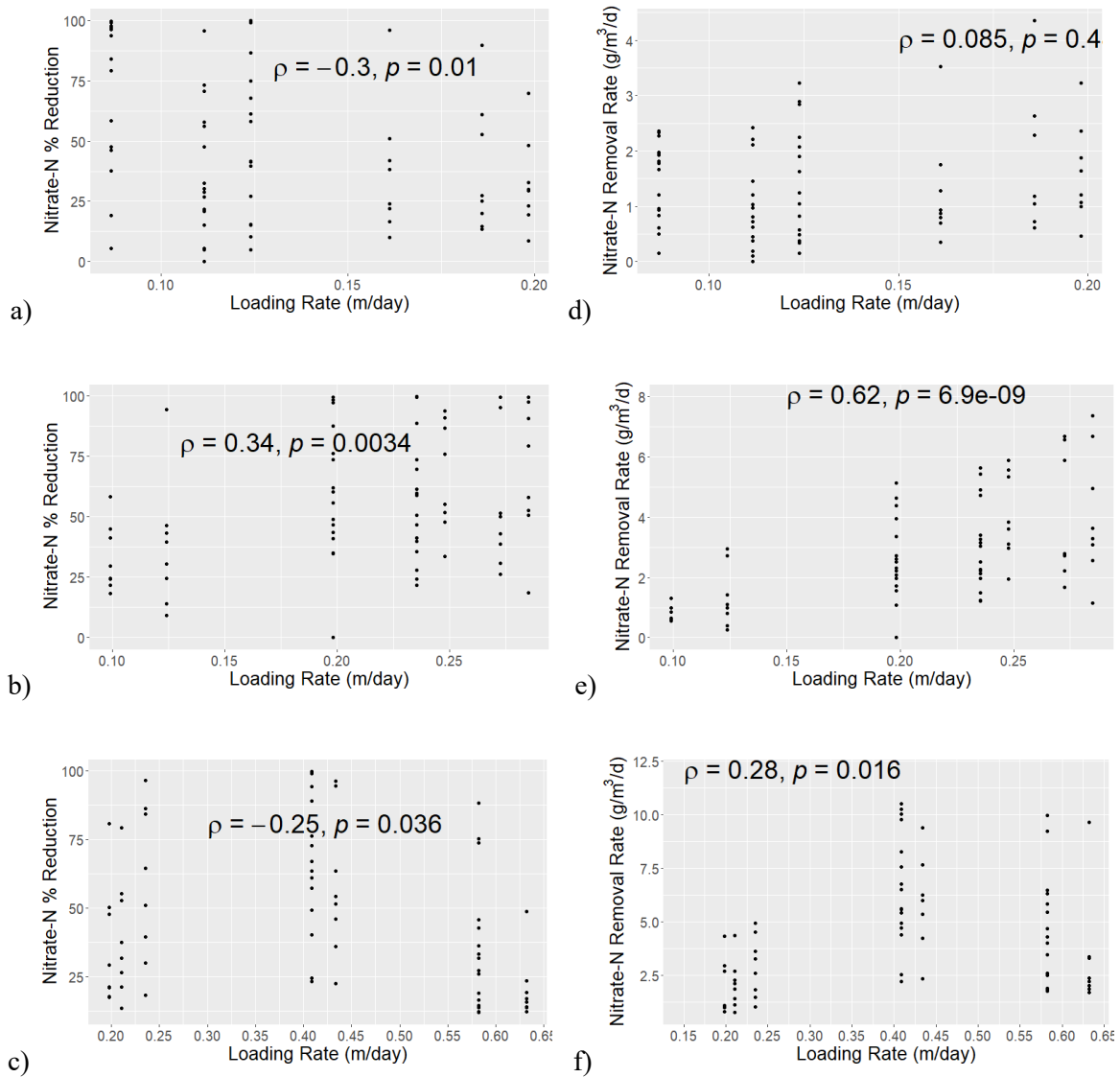
Figure 4.4 Correlation analysis of HLR vs nitrate-N percent reduction (a-c) and nitrate-N removal rate by substrate type: woodchips (a &d), pine bark (b & e), and peanut hull (c & f) bioreactors.

Christianson (2011b) reported a positive correlation between nitrate percent reduction and HRT, similar to what was reported in the current study for woodchips and peanut hulls. Robertson and Merkley (2009) and Iverson (2019) reported a positive correlation between nitrate mass removal rate and flow rate. As expected, all substrates in the current study achieved higher

nitrate mass removal rates under higher HLRs. A higher HLR means more water and increased influent loading of nitrate, thereby yielding a higher reduction of nitrate.

While woodchips and peanut hulls demonstrated higher nitrate percent removal under lower flow rates, pine bark had higher nitrate percent removal under higher flow rates. One possible explanation for this may be that pine bark had the highest initial content of carbon and C:N ratio (Table 3.3) but appeared to release the lowest amount of DOC (Figure 4.1). So, after the initial flush of DOC experienced by all the substrates, pine bark may have retained more of its carbon. Since carbon is a limiting factor to the microbial communities responsible for respiring nitrate into N_2 gas (Wilhelm et al. 1994), it may be possible that the microbial communities in the pine bark were stronger due to their access to carbon, and could not only withstand higher HLRs but thrived on them. So, during higher HLRs with higher masses of nitrate entering into the system, nitrate was reduced more efficiently (higher % reduction) but during lower HLRs with lower masses of nitrate entering into the system, nitrate was reduced less efficiently (lower % reduction). This potential explanation has not been reported anywhere else in the literature and should be further explored in future studies.

Table 4.1 Carbon content and release of each substrate substrates.

Substrate	C content (ppm)	C:N ratios	Median DOC release (mg/L)	Mean DOC release (mg/L)	Max DOC release (mg/L)
Woodchips	490,000	114:1	11.82	25.40	323.4
Pine Bark	539,000	199:1	8.53	13.24	95.4
Peanut Hulls	518,000	32:1	9.67	41.21	344.7

During the startup period (Sampling Event 0), the woodchip bioreactors released between 140 –

323 mg DOC/L, pine bark bioreactors released between 47 – 95 mg DOC/L, and peanut hull

bioreactors released between 330 – 344 mg DOC/L. DOC steadily decreased in the effluent of all

substrates before reaching a plateau. This is commonly reported in bioreactor studies due to the

initial flushing of labile carbon from the substrate. Robertson and Blowes (2000) reported an

initial flush up to 250 mg DOC/L in woodchip bioreactors. Hoover (2012) reported an initial

flush up to 230 mg DOC/L, stabilizing below 20 mg/L around day 50. In the current study, DOC

release stabilized to below 20 mg/L by week 19 for the woodchip bioreactors, by week 6 in the

pine bark bioreactors, and by week 13 for peanut hull bioreactors (Figure 4.5).

a)

b)

c)
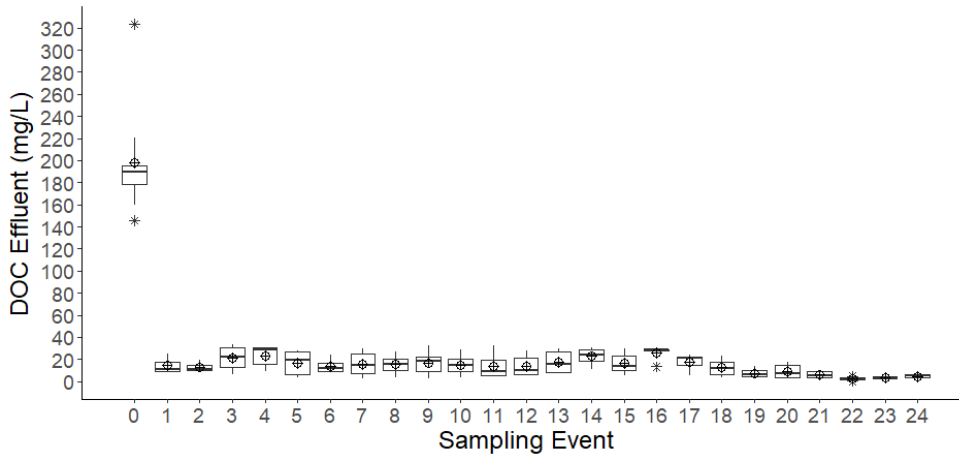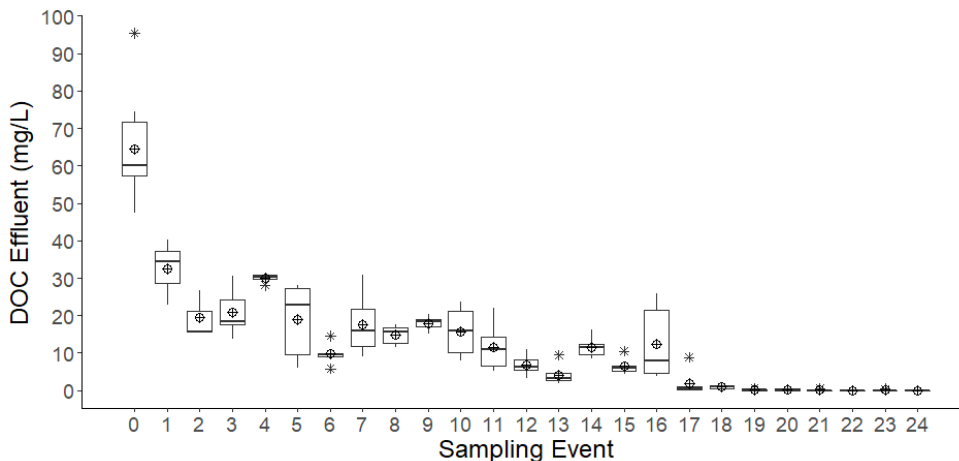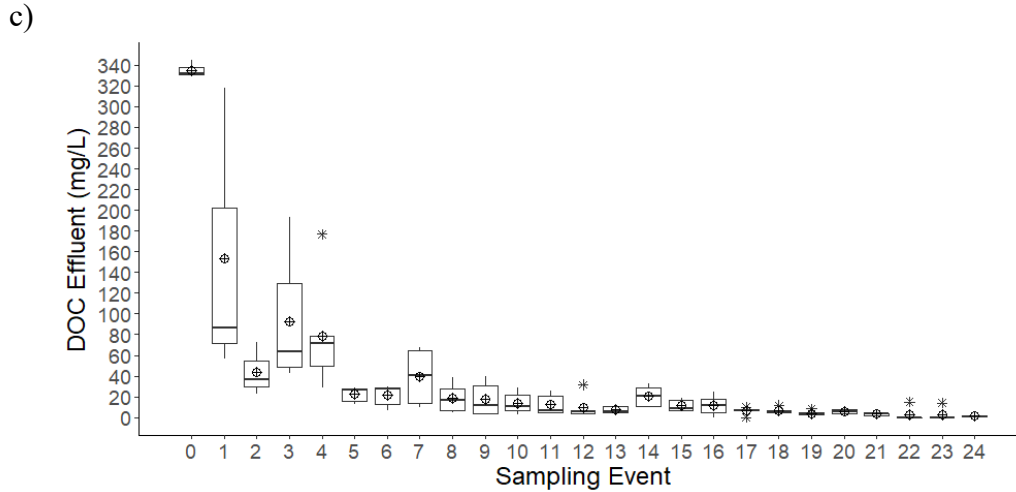
Figure 4.5 DOC release over time in woodchips (A), pine bark (B), and peanut hulls (C). Sampling Event 0 was the entire 14-day start-up period, Sampling Event 1 marks the beginning of the warm period. Sampling Event 16 marks the beginning of the cold period.

4.3 Effect of Temperature on Nitrate-N Removal

Temperature is an important treatment factor for nitrate reduction in denitrifying bioreactors. Since the bioreactors in this study operated under a range of temperatures between July 2021 and March 2022, it is important to consider how temperature affected the nitrate reducing capabilities of each substrate. Overall, pine bark was found to be the most effective at reducing nitrate, followed by peanut hulls and then by woodchips. However, there were seasonal differences in how effectively the substrates reduced nitrate. As previously stated, the mean influent water temperature of the warm season was 27.4°C and the mean influent water temperature of the cold season was 13.2°C.

Table 4.2 Average monthly atmospheric, influent, and effluent temperatures collected from Pitt-Greenville Airport Station, daily monitoring of influent tank, and continuous monitoring of composite effluent buckets, respectively.

Temperatures (° Fahrenheit)	July 2021	August 2021	September 2021	October 2021	January 2022	February 2022	March 2022
Average Atmospheric	79	79	73	67	40	48	56
Average Influent	81	83	77	72	40	54	60
Average Effluent	83	83	77	72	40	54	59

While pine bark reduced nitrate more effectively than the other substrates during the warmer months, it was found to be least effective at reducing nitrates during the colder months. Significant differences in nitrate percent reduction between substrates were found during both the warm (p= 5.608e-09) and the cold (p= 0.003544) season (Table 4.3). During the warm season, pine bark had a median nitrate percent reduction of 61.5%, peanut hulls of 29.3%, and woodchips of 27.2%. The differences between pine bark and peanut hulls (p= 0.00014) and between pine bark and woodchips (p= 1.4e-09) were statistically significant, whereas the difference between peanut hulls and woodchips was not (p= 1.00000) (Appendix A, Table A4). During the cold season, peanut hulls had a median nitrate percent reduction of 64.5%, woodchips of 61.5%, and pine bark of 35.7% (Figure 4.6). Similar to the warm season, the differences between pine bark and peanut hulls (p= 0.020) and between pine bark and woodchips (p= 0.011) were statistically significant, whereas the difference between peanut hulls and woodchips was not (p= 1.00000) (Appendix A, Table A4). Significant differences in nitrate percent reduction were found between seasons for woodchips (p= 0.0001552), pine bark (p= 1.324e-05), and peanut hulls (p= 0.01851) (Figure 4.6).
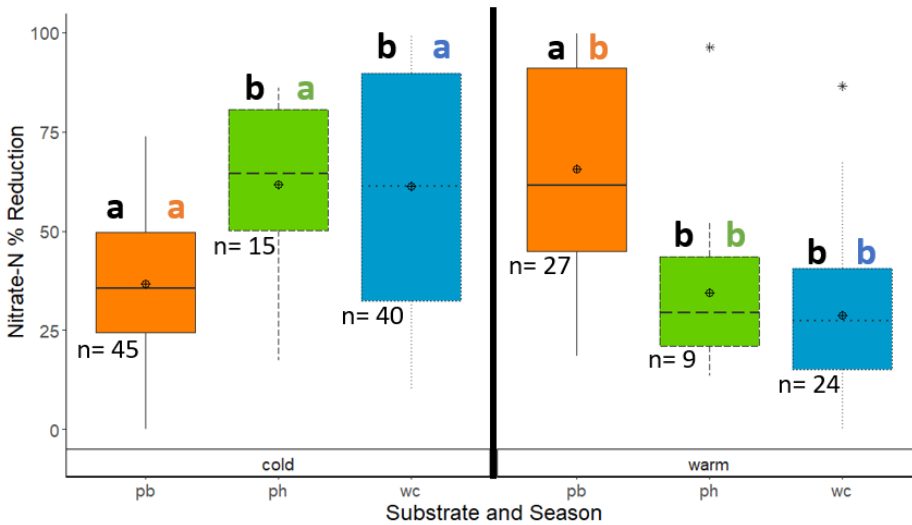
Figure 4.6 Nitrate-N percent reduction of each substrate during the cold (left) and warm (right) months. The middle bars indicate the medians, the crosshairs (⊕) indicate the means, and the asterisks (*) indicate the outliers, n denotes the number of samples considered in statistical analysis. Significant differences between substrates within seasons are denoted by the black letter to the top left of the box, significant differences between seasons within the substrates are denoted by the colored letter to the top right of the box. Substrates that share a letter were not significantly different.

Significant differences in nitrate removal rates between substrates were found during the warm (p= 9.499e-10) season, but not during the cold (p= 0.05026) season (Table 4.4). During the warm season, pine bark had a median nitrate removal rate of 2.96 g N/m^3/day, peanut hulls of 1.41 g N/m^3/day, and woodchips of 0.81 g N/m^3/day. The differences between pine bark and peanut hulls (p= 0.0065), between pine bark and woodchips (p= 2.8e-09), and between woodchips and peanut hulls (p= 0.0128), were all statistically significant (Appendix A, Table A5). During the cold season, peanut hulls had a median nitrate removal rate of 3.26 g N/m^3/day, woodchips of 2.21 g N/m^3/day, and pine bark of 1.98 g N/m^3/day (Figure 4.7). None of the differences between woodchips and peanut hulls (p= 0.095), between woodchips and pine bark (p= 1.00000), or pine bark and peanut hulls (p= 0.058), were statistically significant (Appendix A, Table A5). Significant differences in nitrate removal rates were found between seasons for

woodchips (p= 7.205e-05), pine bark (p= 0.006266), and peanut hulls (p= 0.009491) (Figure 4.7).
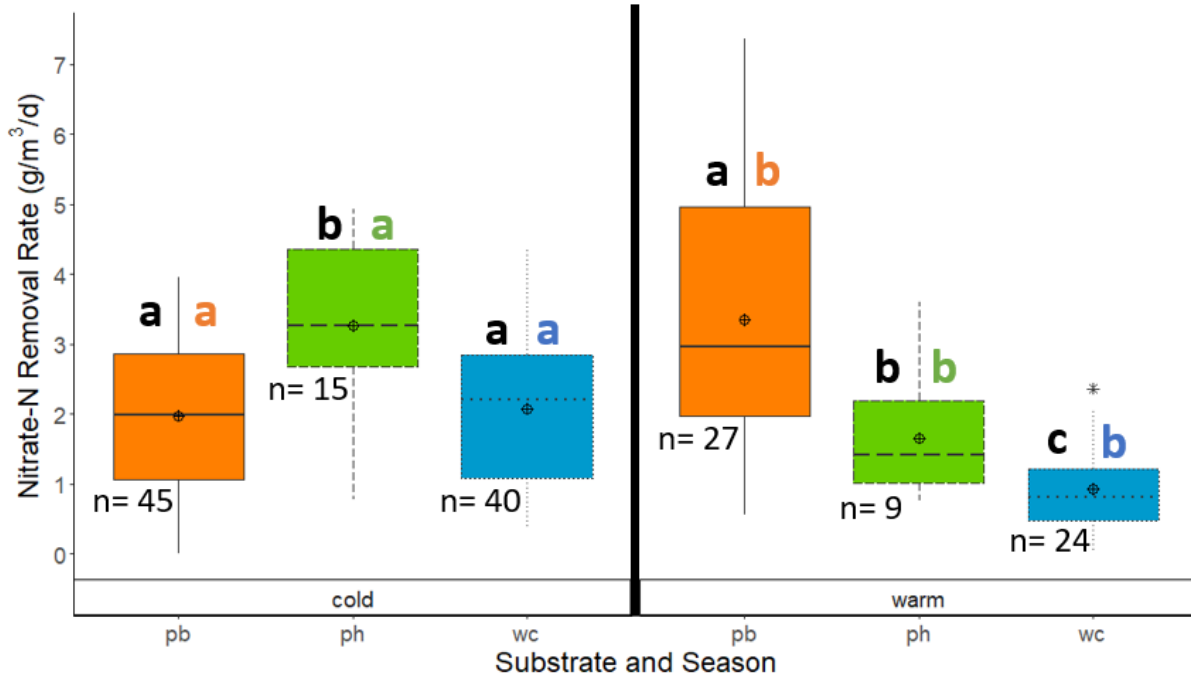
Figure 4.7 Nitrate-N removal rate of each substrate during the cold (left) and warm (right) months. The middle bars indicate the medians, the crosshairs (⊕) indicate the means, and the asterisks (*) indicate the outliers, n denotes the number of samples considered in statistical analysis. Significant differences between substrates within seasons are denoted by the black letter to the top left the box, significant differences between seasons within the substrates are denoted by the colored letter to the top right of the box. Substrates that share a letter were not significantly different.

During warmer months, pine bark consistently had the greatest concentration reduction percentages and mass removal relative to woodchips and peanut hulls. However, both woodchips and peanut hulls performed better than pine bark during colder months. The fact that woodchips and peanut hulls reduced nitrate more effectively during the colder months is not expected and does not agree with what has been reported in the literature (Robertson and Merkley, 2009; Bell, 2015; Hoover, 2016). So, to further explore these observations, correlation analyses were performed to investigate the strength of the relationships between nitrate reduction and

temperature (Figures 4.6). Woodchip (r = -0.49, p= 0.00015) and pine bark (r= 0.57, p= 3.4e-7)

bioreactors suggested moderate, significant correlations between nitrate percent reduction and

temperature, but peanut hulls (r= -0.26, p= 0.24) did not. Similarly, woodchip (r= -0.48, p=

0.00022) and pine bark (r= 0.44, p= 0.00015) bioreactors exhibited moderate, significant

correlations between nitrate removal rate and temperature, but peanut hulls (r= -0.21, p= 0.32)

did not (Figure 4.8). As observed in the pairwise comparisons, woodchips and peanut hulls

reduced nitrate more effectively during the colder months, while pine bark reduced nitrate more

effectively during the warmer months.
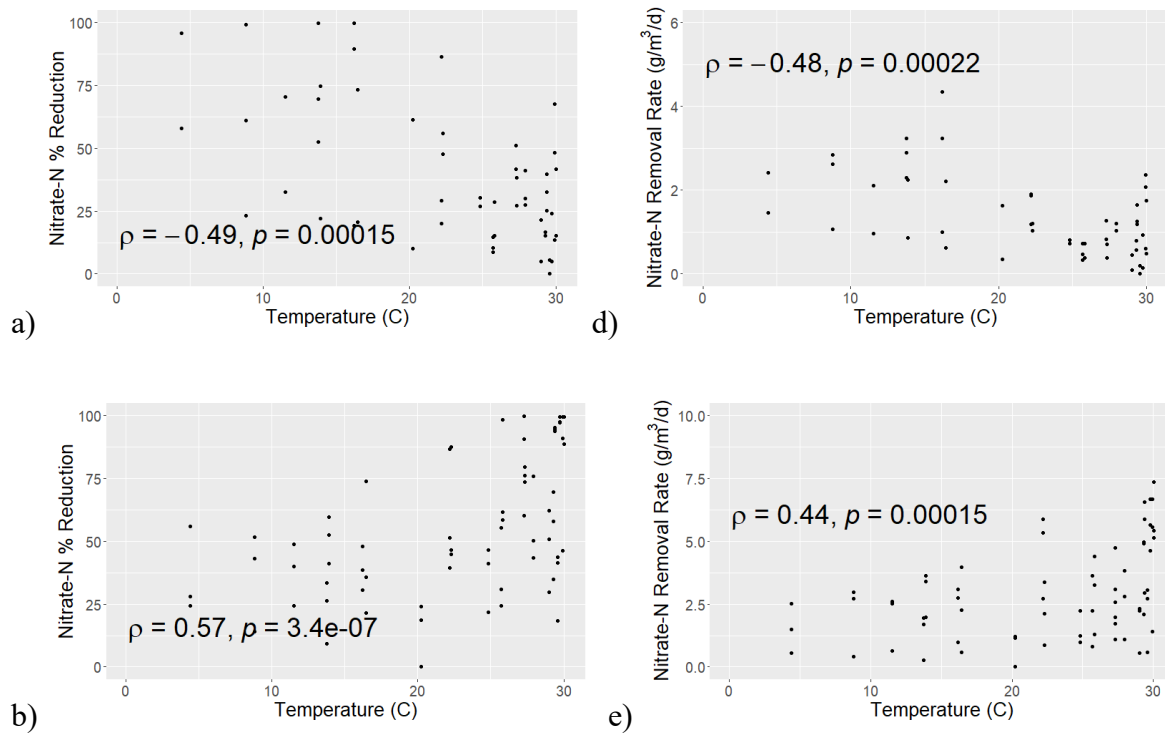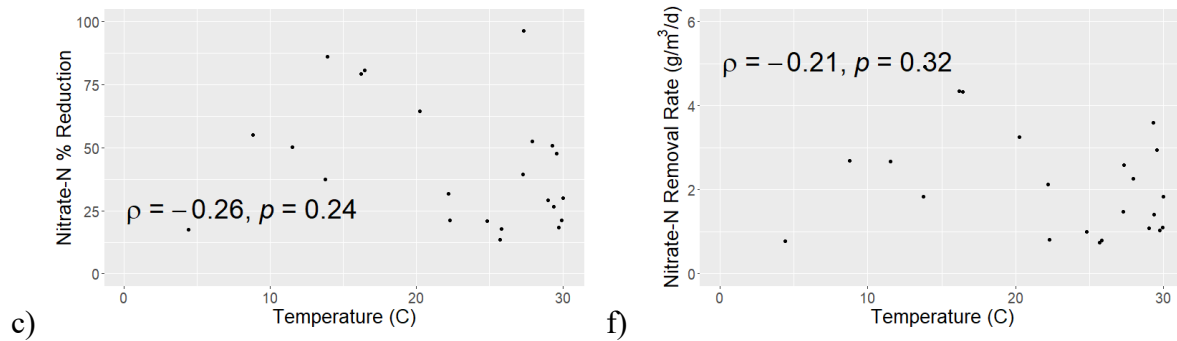
Figure 4.8 Correlation analysis of temperature vs nitrate-N percent reduction (a-c) and nitrate-N removal rate by substrate type: woodchips (a & d), pine bark (b & e), and peanut hull (c & f) bioreactors.

These data imply that woodchips and peanut hull bioreactors operated more effectively (higher removal rate and higher percent reduction) during the cold season while the pine bark bioreactors operated more effectively during the warm season. The fact that woodchips and peanut hulls reduced nitrate more effectively during the colder months is unexpected, as most of the literature reports a positive relationship between nitrate reduction and water temperature. Bell et al. (2015) reported a positive correlation between temperature and nitrate removal rate and percent reduction. Hoover er al. (2016) found that nitrate removal rates and load reduction increased with water temperature. Addy et al. (2016) reported that denitrifying beds with temperatures below 42° F had significantly lower nitrate removal than those at temperatures higher than 42° F.

In the current study, all of the substrates experienced the highest flush of DOC in the first few weeks of the sampling period. As expected, pine bark reduced the highest mass of nitrates (removal rate) the most efficiently (percent reduction) during the warm season in the beginning of the sampling period. However, woodchips and peanut hulls reduced the highest mass of nitrates (removal rate) the most efficiently (percent reduction) during the cold season towards the

end of the sampling period. One possible explanation for this is that the microbial communities in the woodchips and peanut hull bioreactors took longer to fully develop than those in the pine bark bioreactors and thus, the nitrate percent removal and removal rates increased later in the sampling period. This potential explanation has not been reported anywhere else in the literature and should be further explored in future studies.

Another factor that may have affected the nitrate reducing capabilities of the substrates is the concentration of DO in the systems. Apart from a few outliers, daily averages of DO range from 1.5 – 4.0 mg/L during the warm period and from 3.0 – 8.7 mg/L during the cold period. The seasonal difference in DO is to be expected, as water absorbs lower quantities of DO at warmer temperatures (Veraart, 2011), and may be the reason why pine bark reduced nitrate more effectively (higher removal rate and higher percent reduction) during the warmer months than in the colder months. Though peanut hulls did not exhibit a strong or significant relationship between nitrate percent reduction and DO (ρ= 0.19, p= 0.38), pine bark exhibited a significant negative correlation (ρ= -0.53, p= 0.01) between nitrate percent reduction and DO, as expected, while woodchips exhibited a significant positive correlation (ρ= 0.57, p= 0.0055) between nitrate percent reduction and DO.

a)
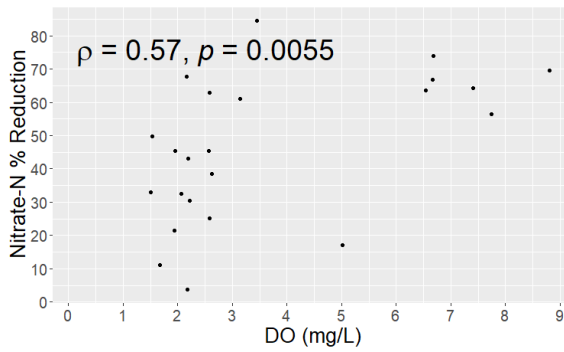
b)
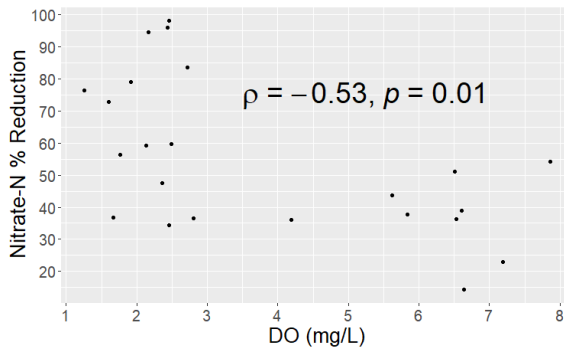
c)
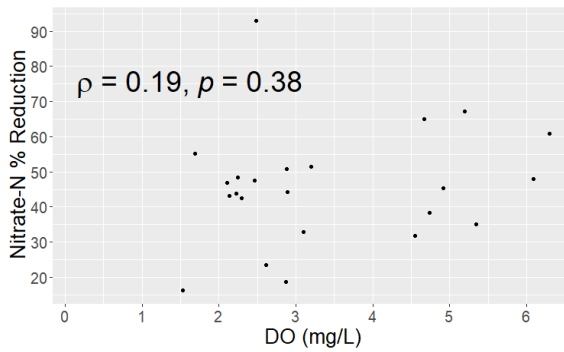
Figure 4.9 Correlation analysis of DO vs nitrate-N percent reduction in woodchip (a), pine bark (b) and peanut hull (c) bioreactors.

Positive relationships between temperature and nitrate reduction have been reported in the literature (Robertson, 2009; Bell, 2015; Hoover, 2016). While pine bark exhibited a positive relationship between temperature and nitrate reduction (in terms of percent reduction and

removal rate) both woodchips and peanut hulls exhibited a negative relationship between the two variables. As denitrifying bacteria require anaerobic environments to respire nitrate, a negative relationship between DO and nitrate reduction would be expected. While pine bark demonstrated this negative relationship, woodchips exhibited a positive relationship and nitrate percent reduction was observed to increase with DO. This may imply that factors influencing the nitrate reducing capabilities of a substrate, and a microbial community's sensitivity to those factors, can vary by the substrate they are growing on. The nitrate reducing efficacy of pine bark may be more dependent on DOC availability, temperature, and DO than woodchips and peanut hulls, while woodchips and peanut hulls may be more sensitive to higher HLRs than pine bark. Another possible explanation is that the nitrate reducing efficacy of woodchips and peanut hulls had less to do with the temperature of the water and more to do with the sustainability of the microbial communities in the bioreactors. In other words, the microbial communities in the woodchips and peanut hull bioreactors may have taken longer to fully develop and respire as effectively (high nitrate percent removal and high mass removal rates) as those in the pine bark bioreactors. This potential explanation has not been reported anywhere else in the literature and should be further explored in future studies.

4.4 Effect of Substrate Pairing on Phosphate-P Removal

As previously discussed, the bioreactors were dosed with 26 mg phosphate-P/L during the 14-day start up period, resulting in the expanded slate likely reaching phosphate adsorbing capacity. Influent concentrations were then reduced to a median concentration of 0.85 mg phosphate-P/L at the start of the sampling period. Effluent phosphate-P concentrations for all substrates were higher than influent concentrations and consistently decreased over the first nine weeks of the study, likely indicating that desorption was occurring (Figure 4.10). To better

understand the rate at which the expanded slate released phosphate after the start-up period, correlation analysis between phosphate-P concentration and sampling event was performed on all three substrates combined (Figure 4.10) The expanded slate effectively desorbed phosphate when paired with all substrates over the course of nine (9) weeks (r= -0.71, p < $2.2e^{-16}$). All analyses hereon will focus on sampling events 10 – 24 of the study, following the desorption period, and will be referred to as the post-desorption period.

a)
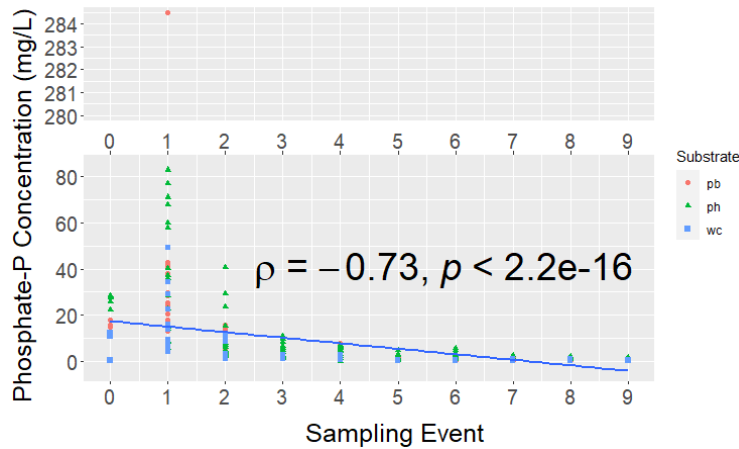
b)
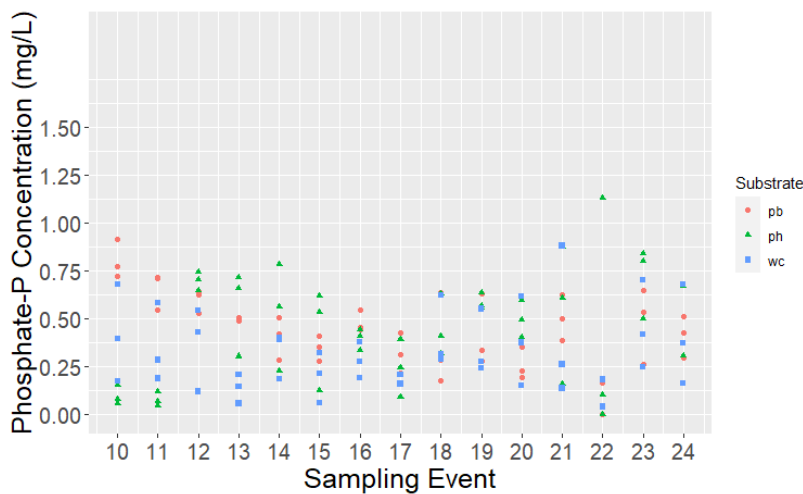
Figure 4.10 Phosphate-P effluent concentrations during each sampling event of the desorption period (a) and phosphate-P effluent concentrations of each sampling event of the post-desorption period (b). Sampling Event 0 was the entire 14-day start-up period, Sampling Event 1 marks the beginning of the warm period. Sampling Event 16 marks the beginning of the cold period. Note the difference in y-axis scales between the desorption period and the post-desorption period.

Throughout the post-desorption period, influent phosphate-P concentrations ranged from 0.2 to 1.3 mg/L with a median influent concentration of 0.85 mg/L (Figure 4.9). There were significant differences between influent and effluent phosphate-P concentrations for all substrate pairings (p= 6.038e-07). The median effluent concentrations of woodchips, pine bark, and peanut hulls were 0.29 mg phosphate-P/L, 0.44 mg phosphate-P/L, and 0.41 mg phosphate-P/L,

57

respectively. There were not significant differences in final effluent concentrations between substrates. Pairwise comparisons between influent and substrate concentrations are presented in Appendix A, Table A8.
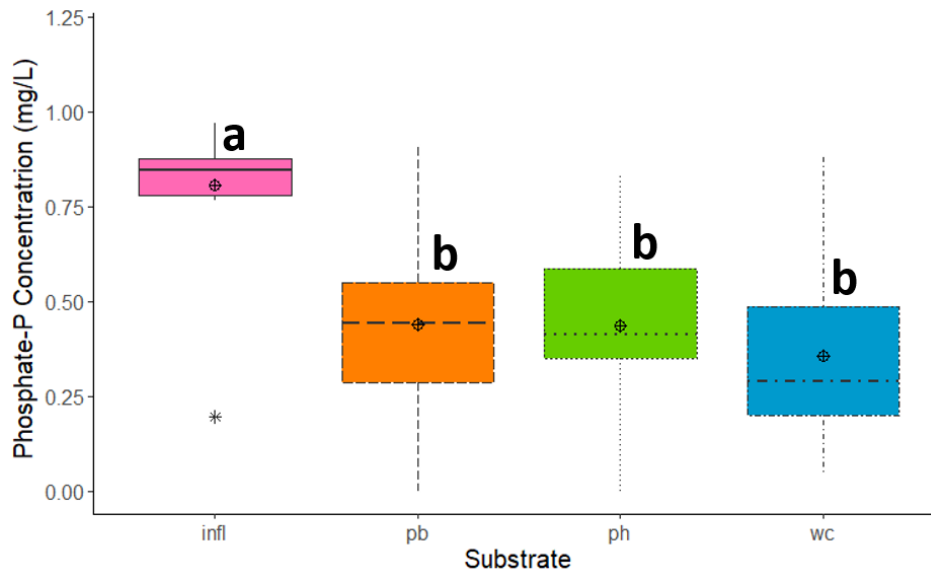
Figure 4.11 Influent and effluent phosphate-P concentrations at HLRs ranging between 0.1 – 0.3 m/day beginning after the first nine weeks of the study period (i.e., during the post-desorption period).

There were no significant differences in the phosphate percent reduction (p= 0.08605) or removal rate (p= 0.3005) between any of the substrate pairings. Woodchips had a median phosphate percent reduction of 64.2%, peanut hulls of 50.7%, and pine bark of 46.5% (Appendix A, Table A9). Peanut hulls had a median phosphate removal rate of 0.12 g P/m^3/day, pine bark of 0.11 g P/m^3/day and woodchips of 0.90 g P/m^3/day (Appendix A, Table A10). These data suggest that expanded slate reduces phosphate effectively when paired with any of the three experimental substrates, and that no substrate pairing works better than another. Though the phosphate reducing capacity of expanded slate paired with peanut hulls has not been widely explored, Wu (2013) reported an average of 15% phosphate reduction in expanded slate paired

with pine bark in a column scale batch style study with influent concentrations of 3.33 mg

phosphate-P/L and Iverson (2019) reported a 74% phosphate reduction in a layer of expanded

slate lain on top of woodchips in a field scale study with a median influent concentration of 0.23

mg P/L. It is important to consider that the difference in desorption rates between expanded slate

paired with woodchips, pine bark, and peanut hulls (Figure 4.8) may have impacted the ability of

each substrate to reduce phosphate during the post-desorption period.

a)

b)
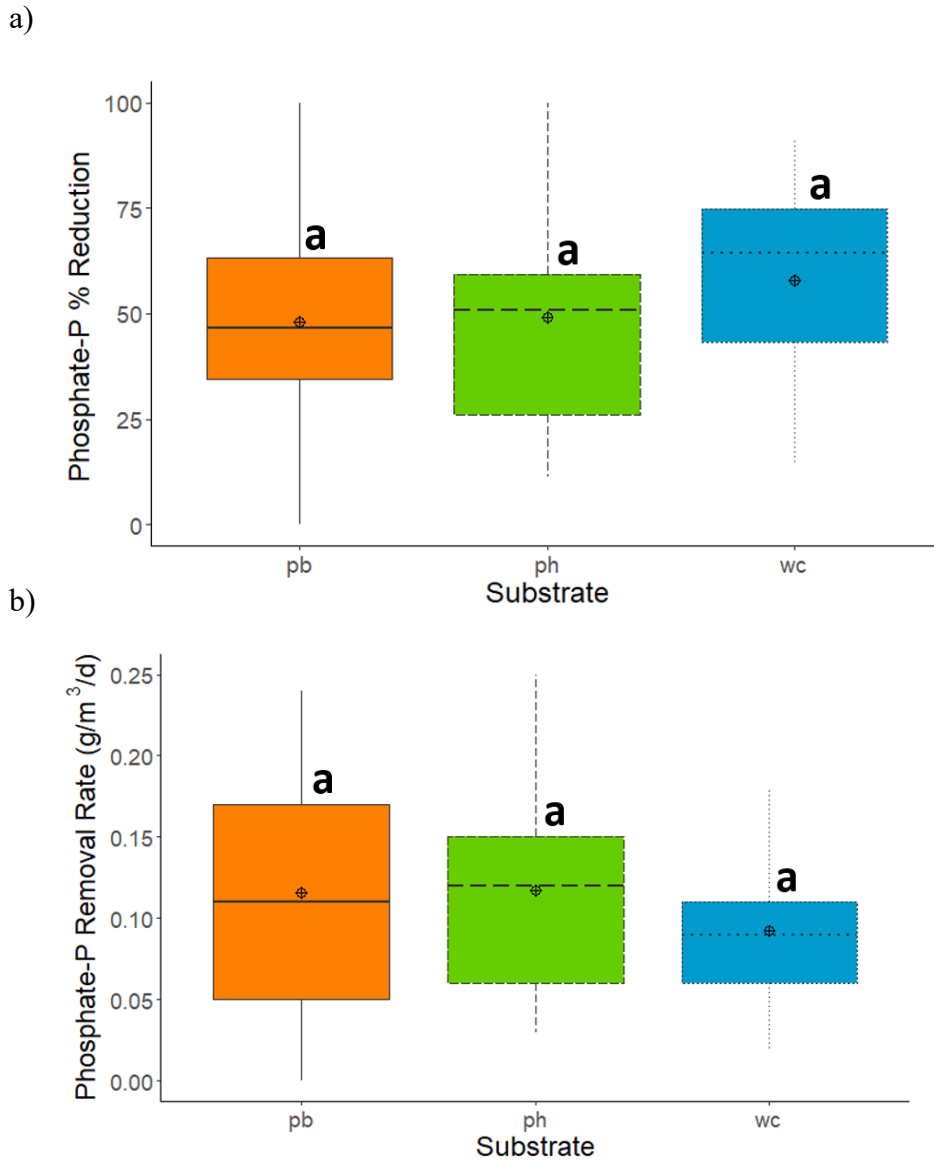
Figure 4.12 Phosphate percent reductions **(a)** and phosphate removal rates **(b)** vs substrates at loading rates between 0.1 – 0.3 m/day.

4.5 Effect of Hydraulic Loading Rate on Phosphate Removal

The expanded slate paired with woodchips (ρ= -0.4, p= 0.0071) had a significant correlation between phosphate percent reduction and HLR (Figure 4.13). The negative correlation implies that when paired with woodchips, the expanded slate was able to reduce phosphate more

efficiently (higher percent reduction) under lower HLRs. The expanded slate paired with pine

bark (ρ= 0.34, p= 0.023) had a positive correlation between phosphate percent reduction and

HLR (Figure 4.14), suggesting that when paired with pine bark, the expanded slate was able to

reduce phosphate more effectively under higher HLRs. The expanded slate paired with peanut

hulls (ρ= -0.16, p= 0.29) showed no significant correlation between phosphate percent reduction

and HLR (Figure 4.15). The expanded slate paired with woodchips (ρ= 0.22, p= 0.15) showed no

significant correlation between phosphate removal rate and HLR (Figure 4.1). However, the

expanded slate paired with pine bark (ρ= 0.58, p= 3.3e-05) and peanut hulls (ρ= 0.34, p= 0.024)

showed significant positive correlation (Figures 4.14 and 4.15). These results corroborate with

those found in the literature. Christianson (2017) reported a negative correlation in flow rate and

phosphate percent reduction and a positive correlation in flow rate and phosphate removal rate in

acid mine drainage residuals paired with woodchip bioreactors. Iverson (2019) also reported a

positive correlation between phosphate removal rate and flow rate in expanded slate lain on top

of a woodchip bioreactor.

a)

b)

d)
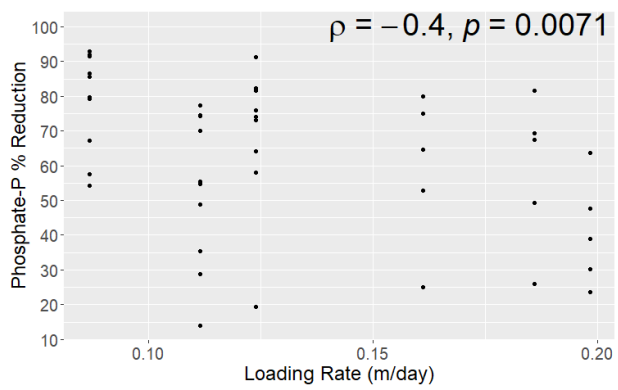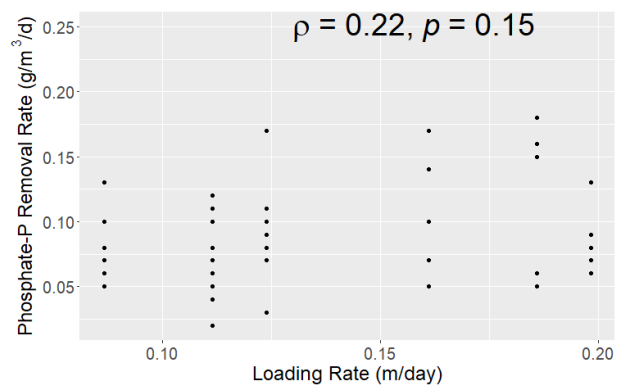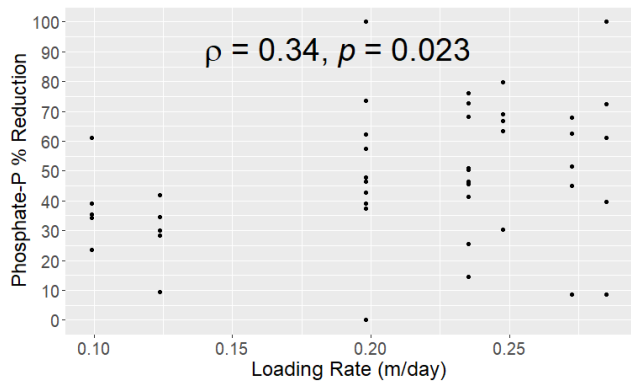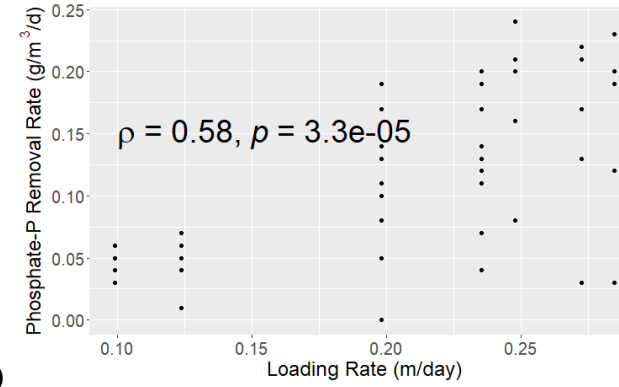
e)
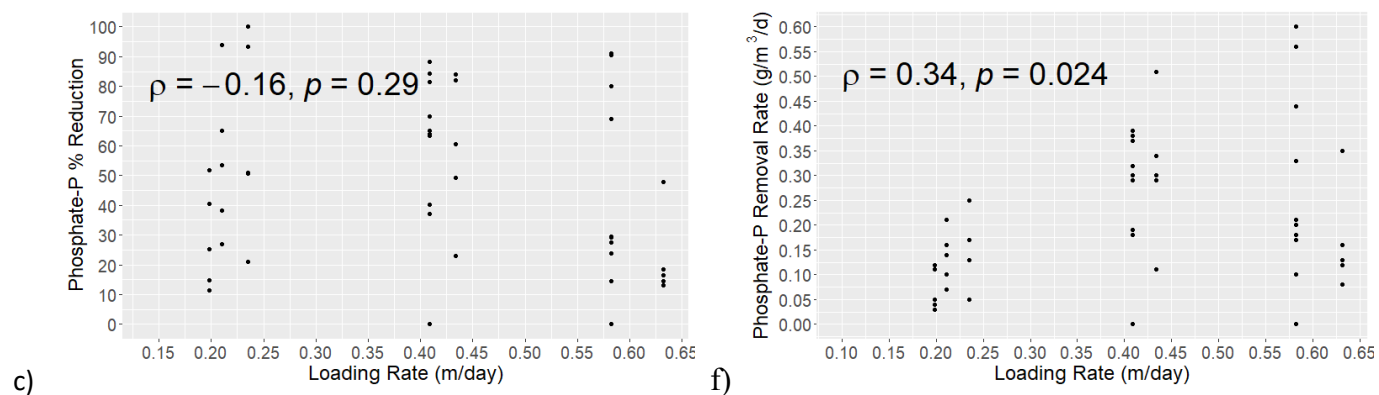
Figure 4.13 Correlation analysis of HLR vs phosphate-P percent reduction (a-c) and phosphate-P removal rate by substrate type: woodchips (a & d), pine bark (b & e), and peanut hull (c & f) bioreactors.

4.6 Byproducts of Substrates

When determining which substrate may be a viable option for a field-scale bioreactor, it is important to consider the byproducts released. Ammonium-N may be oxidized into nitrate through the process of nitrification during periods of flow through. Organic nitrogen may be converted into bioavailable ammonium-N during periods of stagnation (Bernhard, 2010). Organic phosphorus may be converted into phosphate through the process of mineralization (EPA, 2012). If the byproducts of a substrate will be converted back into nitrate or phosphate, that would negate how well the substrate works to remove the nutrients from the water. Pollutant swapping between species of N and P does not reduce the overall total dissolved concentrations of either nutrient. Therefore, analyzing for TDN and TDP is important when evaluating performance of a bioreactor designed to remove N and P (Table 4.4).

Table 4.3 Median concentrations of N species and P species for effluent samples collected over the course of the entire sampling period (all HLRs).

	Woodchips	Pine Bark	Peanut Hulls
Median ammonium-N Release (mg/L)	0.028	0.037	1.178
Median DON Release (mg/L)	0.140	0.000	0.897
Median TDN Reduction (kg/year)	0.00077	0.00127	0.00083
Median DOP Release (mg/L)	0.050	0.003	0.124
Median TDP Reduction (kg/year)	0.628	0.822	0.970
Median DOC Release (mg/L)	11.82	8.53	9.67

All substrates effectively reduced nitrate-N to similar effluent concentrations (Figure 4.14a). While the peanut hull bioreactors were effective at reducing nitrate, they leached significantly

more DON (0.897 mg/L) and ammonium-N (1.178 mg/L), than the woodchips or pine bark (Table 4.14). Pine bark had the lowest median DON release (<0.25 mg/L), and a negligible ammonium-N release (0.037 mg/L). Woodchips also had a low median DON release (0.140 mg/L), a negligible ammonium-N release (0.028 mg/L). Peanut hulls released significantly more ammonium than the other two substrates, suggesting they decompose at a much faster rate than the other two. As a result, peanut hulls (0.00083 kg/year) and woodchips (0.00077 kg/year) reduced significantly less TDN than pine bark (0.00127 kg/year) (Table 4.2).

Though pine bark released more phosphate than peanut hulls or woodchips (Figure 4.14b), it is important to consider that the phosphate desorption period occurring during the first nine weeks of the sampling period may have confounded the actual amount of phosphate released from the woody substrates throughout the sampling period. Much of the phosphate released by the pine bark may have been residual phosphate still desorbing from the expanded slate. Peanut hulls released more DOP (0.124 mg/L) than woodchips (0.050 mg/L) and pine bark (0.003 mg/L), presumably a product of degradation (Table 4.2).

As previously mentioned, pine bark released less DOC (8.53 mg/L) than peanut hulls (9.67 mg/L) or woodchips (11.82 mg/L) (Figure 4.16). This is interesting because pine bark also had the highest carbon content and C:N ratio (Figure 3.2), suggesting pine bark retained more carbon than woodchips or peanut hulls. As previously mentioned, carbon is a limiting factor to the microbial communities responsible for respiring nitrate into N_2 gas (Wilhelm et al. 1994), so it may be possible that the microbial communities in the pine bark were able to reduce more nitrate due to their access to carbon.

a)

b)
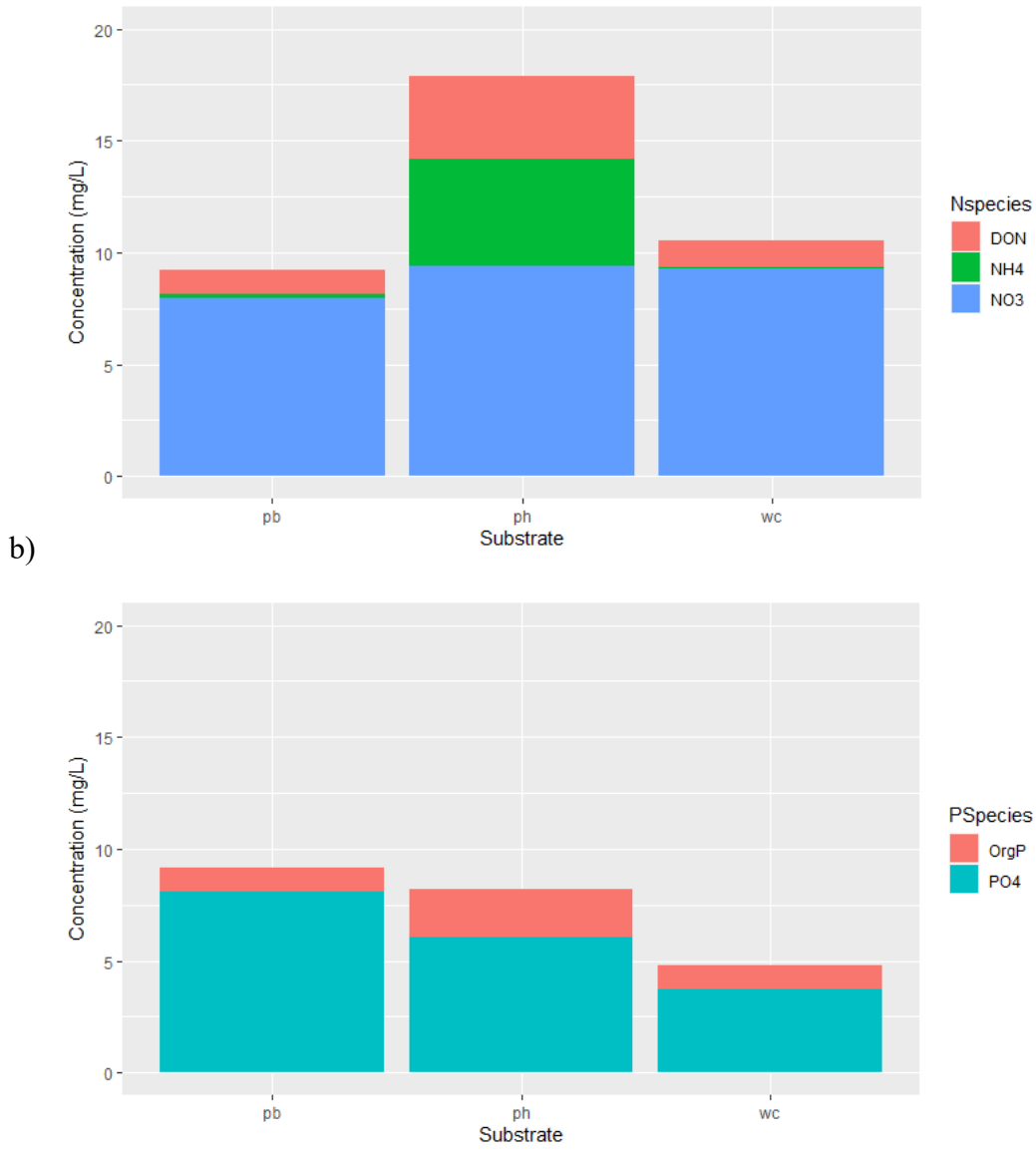
Figure 4.14 Average concentrations of N species (a) and of P species (b) in effluent samples collected over the course of the entire sampling period (all HLRs).

a)

b)

c)

67
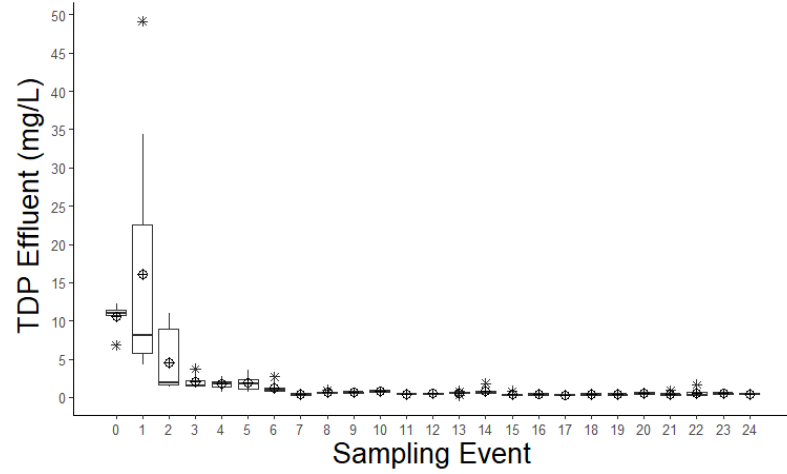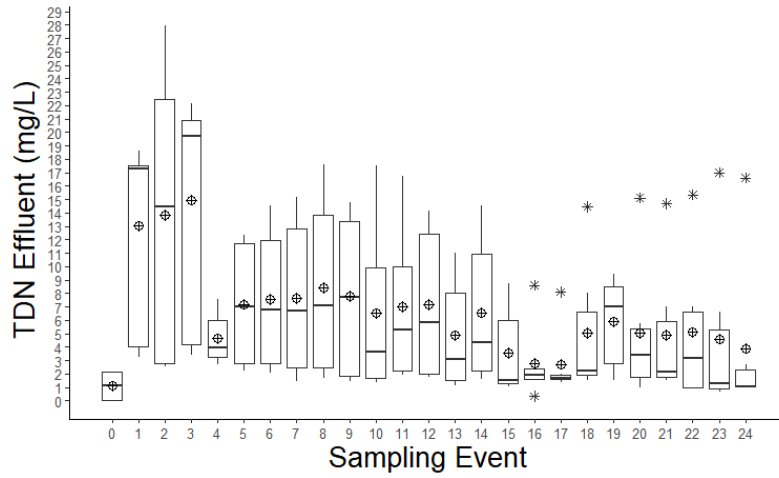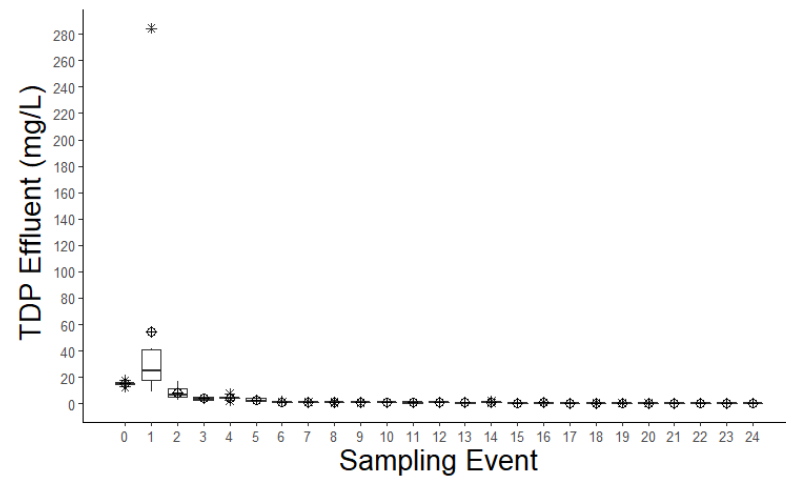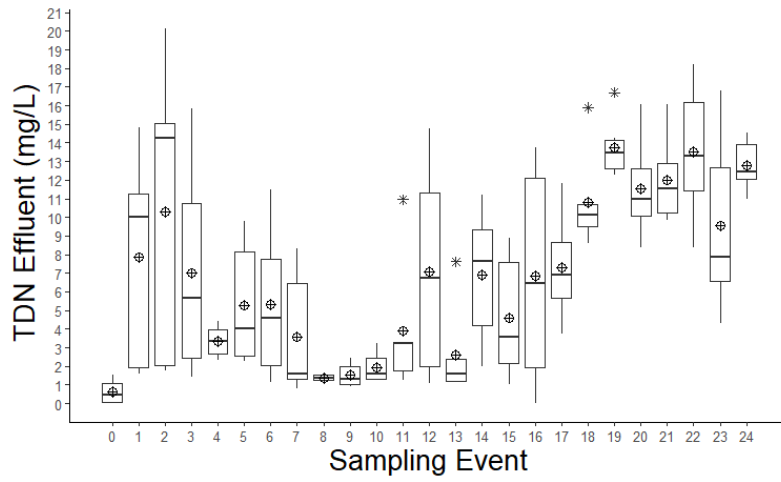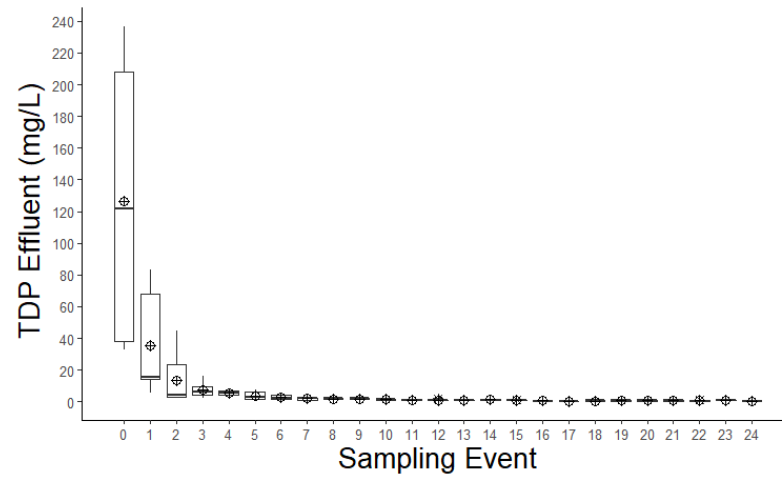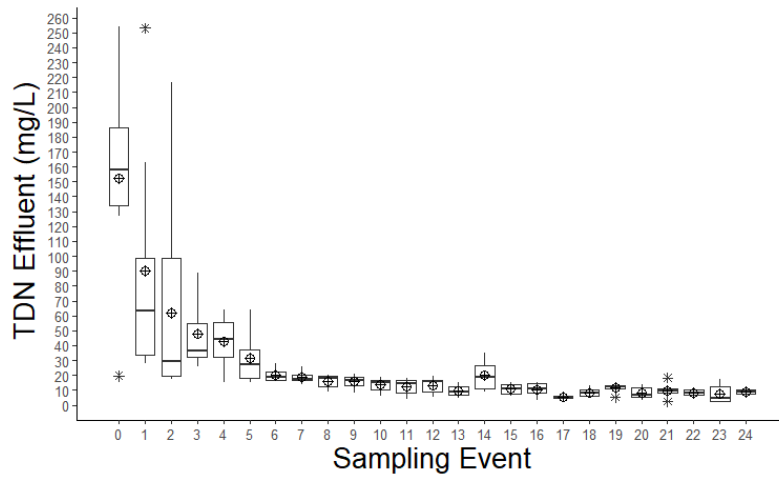
Figure 4.15 TDN (left) and TDP (right) effluent concentrations in all samples collected over the course of the sampling period in bioreactors comprised of woodchips (a), pine bark (b) and peanut hulls (c). Note the difference in y-axis scales between substrates.
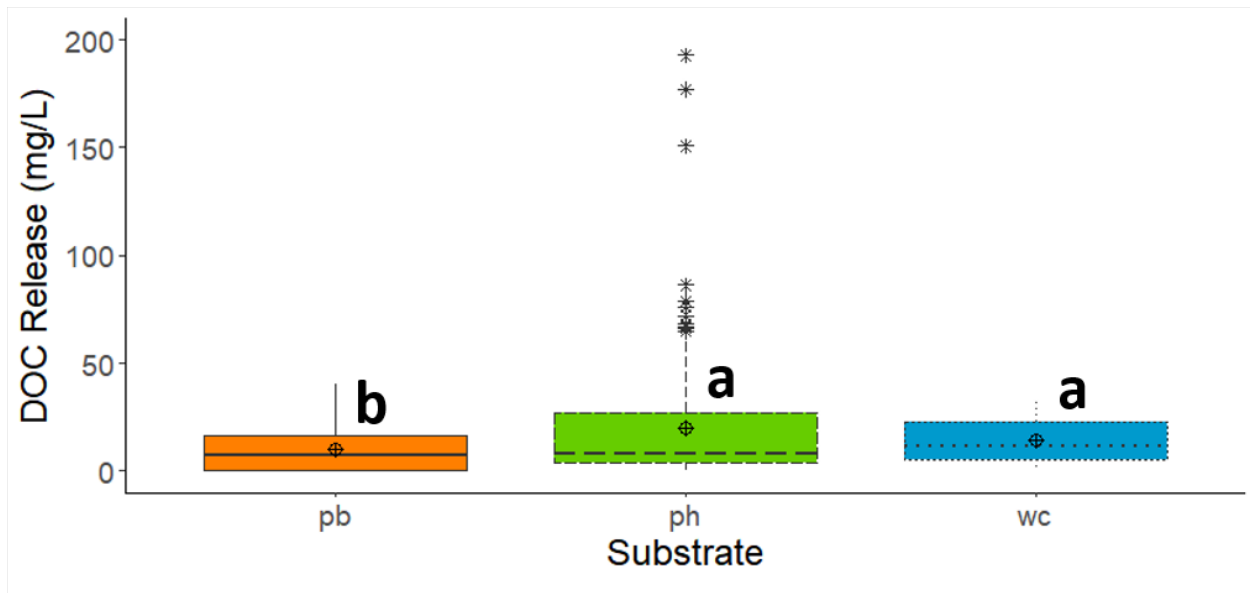
Figure 4.16 DOC release of each substrate across all HLRs and temperatures. The middle bars indicate the medians, the inside circles indicate the means, and the asterisks indicate the outliers.

The peanut hull bioreactors released significantly more ammonium-N, DON, DOP and DOC than the woodchips or pine bark (Figure 4.12; Table 4.2). Though peanut hulls were able to reduce comparable percentages of nitrate, they reduced significantly less TDN (0.00084 kg/year) than pine bark (0.00127 kg/year) (Table 4.2), making them a less attractive substrate to use in a field-scale bioreactor. Pine bark reduced significantly more TDN than the woodchips and the peanut hulls, making them a more attractive substrate to use in a field-scale bioreactor.

CHAPTER V – CONCLUSIONS AND FUTURE WORK

Overall, nitrate was reduced more effectively (higher percent reduction and removal rates) in pine bark (50.4% and 2.6 g N/m^3/day) than woodchips (31.4% and 1.1 g N/m^3/day) and peanut hulls (38.4% and 2.0 g N/m^3/day). Significant differences were observed between nitrate reduction and temperature and between nitrate reduction and HLR.

Woodchips and peanut hulls appear to reduce nitrate more effectively under lower HLRs, but pine bark appears to reduce nitrate more efficiently under higher HLRs, which is not expected or in agreement with what has been reported in the literature. This could potentially be explained by the fact that pine bark had the highest initial content of carbon and C:N ratio but released the lowest amount of DOC. So, after the initial flush of DOC experienced by all the substrates, pine bark had retained most of its carbon. Since carbon is a limiting factor to the microbial communities responsible for respiring nitrate into N_2 gas (Wilhelm et al. 1994), it may be possible that the microbial communities in the pine bark were better acclimated, due to their access to carbon, and could not only withstand higher HLRs, but thrived on them. So, during higher HLRs with a higher input of nitrate, the denitrifying bacteria in the pine bark were able to reduce more nitrate, but during lower HLRs with a lower input of nitrate, nitrate reduction decreased. This potential explanation has not been reported in the literature and should be further explored in future studies.

Woodchips and peanut hull bioreactors operated more effectively (higher removal rate and higher percent reduction) during the cold season while the pine bark bioreactors operated more effectively during the warm season. The fact that woodchips and peanut hulls reduced nitrate more effectively during the colder months is unexpected, as most of the literature reports

a positive relationship between nitrate-N reduction and water temperature. One possible explanation for this is that the microbial communities in the woodchips and peanut hull bioreactors took longer to fully develop than those in the pine bark bioreactors and thus, the nitrate percent removal and removal rates increased later in the sampling period. Another possible explanation for this is that the seasonal variations of DO (higher in the winter and lower in the summer) played a larger role in the nitrate reducing efficacy of pine bark than it did for peanut hull or woodchip bioreactors. Nitrate reducing efficacy of pine bark may be more dependent on DOC availability, temperature and DO than woodchips and peanut hulls, while woodchips and peanut hulls may be more sensitive to higher HLRs than pine bark. This potential explanation has not been reported in the literature and should be further explored in future studies. More work should be done to quantify the relationship between these variables and nitrate reduction in pine bark and peanut hulls.

There were no significant differences in the phosphate percent reduction or removal rate between any of the substrate pairings over the course of the sampling period. However, correlations were observed within each substrate between phosphate percent reduction and HLR and between phosphate removal rate and HLR. The expanded slate paired with woodchips reduced phosphate more efficiently (higher percent reduction) under lower HLRs, while the expanded slate paired with pine bark, expanded slate reduced phosphate more effectively under higher HLRs. The expanded slate paired with pine bark and with peanut hulls reduced more phosphate (higher removal rates) under higher HLRs. Though these results agree with much of what has been reported in the literature, they may have been affected by the high initial phosphate-P concentration during the start-up period.

Though peanut hulls did effectively reduce nitrate, they also released significantly more ammonium-N, DON, and DOP than the woodchips and pine bark. Because of this release, peanut hulls reduced several orders of magnitude less TDN (0.00084 kg/year) pine bark (0.00127 kg/year). This high ammonium-N release suggests that peanut hulls may degrade faster than woodchips or pine bark. Pine bark released significantly less DOC than woodchips and peanut hulls. The high retention of its carbon may have contributed to why pine bark was able to reduce significantly more nitrate than peanut hulls or woodchips in the early trials of the experiment.

Before implementing a substrate into a field-scale bioreactor, it is important to consider how each substrate behaves across a range of flow conditions compared to static conditions. This affects the longevity of the bioreactor and the percentage of water that can be treated. Factors such as flow rate, bioreactor size, and the hydraulic conductivity of the substrate can influence how the substrate behaves under periods of varying flows. As previously discussed, overflow holes were drilled at the top, downstream ends of each trough so that the water that did not have time to infiltrate could flow directly over and out of the bioreactor, as it would in an in-stream bioreactor. At the highest range of HLRs only 1% of water was treated in woodchip bioreactors, 3% in pine bark bioreactors, and 6% in peanut hull bioreactors. At the middle range of HLRs, 4% of water was treated in woodchip bioreactors, 6% in pine bark bioreactors, and 11% in peanut hull bioreactors. At the lowest range of HLRs, 5% of water was treated in woodchip bioreactors, 10% in pine bark bioreactors, and 23% in peanut hull bioreactors (Table 3.4). Though peanut hull bioreactors promoted the best flow, allowing a higher percentage of water to be treated, they didn't reduce much TDN due to how much organic N and ammonium-N they released. The peanut hulls also released the highest amount of organic P and DOC as compared to the other two substrates.

These data suggest that the lowest range of HLRs would be most effective in maximizing treatment. They also suggest that pine bark and peanut hull bioreactors have the potential to treat more water than woodchip bioreactors. However, pine bark and peanut hulls both floated in their respective troughs, while woodchips did not, so the substrates would need to be held down by some sort of rip rap to prevent them from floating away. Another option would be to put a layer of expanded slate on top of the substrate, instead of at the down-stream end. Iverson (2018) tested this configuration in a field-scale bioreactor and reported 78% nitrate reduction and 74% phosphate reduction.

The overall objective of this project was to quantify nitrate and phosphate reduction in pilot-scale denitrifying bioreactors as a potential strategy to reduce excess non-point source nutrient loading in the first order streams, such as those that feed the city of Raleigh's drinking water supply, Falls Lake. The results of this study suggest that while pine bark may work the best for first order streams with high HLRs, woodchips may work the best for streams with low HLRs, as they may take longer to degrade. Though peanut hulls effectively reduced nitrate, they also released a lot of ammonium, DON, and DOC, so they may not be a feasible option for a field scale bioreactor.

This study shed light on how well pine bark, woodchips, and peanut hulls can reduce nitrate under HLRs between 0.1 – 0.3 m/day and found that, contrary to what has been reported in the literature, pine bark reduces nitrate more efficiently (higher percent removals and higher removal rates) than woodchips at higher HLRs. Further investigation is needed to confirm whether this would still apply in field scale settings. The pine bark in this study operated under a low range of HLRs (between 0.10 – 0.30 m/day) in between periods of saturation. It could be useful to investigate the denitrifying efficacy of pine bark under a larger range of HLRs to see if

this positive correlation between nitrate reduction and HLR still holds true. The peanut hulls in this study appeared to degrade quickly and released high amounts of ammonium-N and DON, but it could be useful to investigate the denitrifying efficacy of peanut hulls under periods of constant flow or allowing for drainage from substrate between flow periods, as periods of saturation can lead to substrate degradation. Expanded slate effectively reduced phosphate when paired with all substrates, but it could be useful to explore the phosphate reducing capabilities of expanded slate when paired with other substrates, or when paired with pine bark under different flow conditions.

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APPENDIX – STATISTICAL ANALYSES

Table A1. Kruskal Wallis rank sums tests determining significant differences between influent and effluent nitrate-N concentrations. Median influent= 17.6855 mg/L; P value= 2.716e-11

Comparisons of each substrate between influent	P value	Significant difference	Median Concentration (mg/L)
Influent	1.7e-07	Yes	17.50
Woodchips			11.03
Influent	9.0e-11	Yes	17.50
Pine bark			8.374
Influent	1.7e-09	Yes	17.50
Peanut hulls			10.82
Woodchips	0.092	No	11.03
Pine bark			8.374
Woodchips	0.506	No	11.03
Peanut hulls			10.82
Pine bark	1.000	No	8.374
Peanut hulls			10.82

Table A2. Results of Kruskal Wallis rank sums tests determining significant differences between influent and effluent nitrate-N percent reductions. P value= 0.007616

Comparisons of each substrate between influent	P value	Significant difference	Median % reduction
Woodchips	0.0093	Yes	31.3486
Pine bark			50.40807
Woodchips	1.00	No	31.3486
Peanut hulls			38.41482
Pine bark	0.2009	No	50.40807
Peanut hulls			38.41482

Table A3. Results of Kruskal Wallis rank sums tests determining significant differences between influent and effluent nitrate-N removal rates. P value= 1.332e-06

Comparisons of each substrate between influent	P value	Significant difference	Median nitrate-N removal rates ($g\ N/m^3/day$)
Woodchips	1.5e-06	Yes	1.055
Pine bark			2.585
Woodchips	0.009	Yes	1.055
Peanut hulls			1.98
Pine bark	0.552	No	2.585
Peanut hulls			1.98

Table A4. Results of Kruskal Wallis rank sums tests determining significant differences between substrate nitrate-N percent reductions during the warm season and during the cold season. Warm P value: 5.608e-09; Cold P value: 0.003544

Season	Comparisons of each substrate between influent	P value	Significant difference	Median % reduction
Warm	Woodchips	1.4e-09	Yes	27.24338
	Pine bark			61.52537
	Woodchips	1.00000	No	27.24338
	Peanut hulls			29.30378
	Pine bark	0.00014	Yes	61.52537
	Peanut hulls			29.30378
Cold	Woodchips	0.011	Yes	61.25626
	Pine bark			35.65646
	Woodchips	1.00000	No	61.25626
	Peanut hulls			64.46857
	Pine bark	0.020	Yes	35.65646
	Peanut hulls			64.46857

Table A5. Results of Kruskal Wallis rank sums tests determining significant differences between substrate nitrate-N removal rates during the warm season and during the cold season. Warm P value: 9.499e-10; Cold P value: 0.05026

Season	Comparisons of each substrate between influent	P value	Significant difference	Median removal rate
Warm	Woodchips	2.8e-09	Yes	0.81
	Pine bark			2.96
	Woodchips	0.0128	Yes	0.81
	Peanut hulls			1.41
	Pine bark	0.0065	Yes	2.96
	Peanut hulls			1.41
Cold	Woodchips	1.00000	No	2.21
	Pine bark			1.98
	Woodchips	0.095	Yes	2.21
	Peanut hulls			3.26
	Pine bark	0.058	Yes	1.98
	Peanut hulls			3.26

Table A6. Results of Kruskal Wallis rank sums tests determining significant differences between nitrate-N percent reductions during the warm season versus during the cold season in woodchips, pine bark and peanut hulls. Woodchips P value: 0.0001552; Pine bark P value: 1.324e-05; Peanut hulls P value: 0.01851

Season	Comparisons of each substrate between influent	P value	Significant difference	Median % reduction
Woodchips	Warm	8.7e-05	Yes	27.24338
	Cold			61.25626
Pine Bark	Warm	5.6e-06	Yes	61.52537
	Cold			35.65646
Peanut Hulls	Warm	0.018	Yes	29.30378
	Cold			64.46857

Table A7. Results of Kruskal Wallis rank sums tests determining significant differences between nitrate-N removal rates during the warm season versus during the cold season in woodchips, pine bark and peanut hulls. Woodchips P value: 7.205e-05; Pine bark P value: 0.006266; Peanut hulls P value: 0.009491

Season	Comparisons of each substrate between influent	P value	Significant difference	Median removal rate
Woodchips	Warm	7.5e-05	Yes	0.81
	Cold			2.21
Pine Bark	Warm	0.0064	Yes	2.96
	Cold			1.98
Peanut Hulls	Warm	0.0083	Yes	1.41
	Cold			3.26

Table A8. Kruskal Wallis rank sums tests determining significant differences between influent and effluent phosphate-P concentrations. Median influent concentration: 0.847; P value= 6.038e-07

Comparisons of each substrate between influent	P value	Significant difference	Median Concentration (mg/L)
Influent	1.0e-05	Yes	0.847
Woodchips			0.289
Influent	6.9e-06	Yes	0.847
Pine bark			0.443
Influent	0.00012	Yes	0.847
Peanut hulls			0.412
Woodchips	0.18334	No	0.289
Pine bark			0.443
Woodchips	1.00000	No	0.289
Peanut hulls			0.412
Pine bark	1.00000	No	0.443
Peanut hulls			0.412

Table A9. Results of Kruskal Wallis rank sums tests determining significant differences between influent and effluent phosphate-P percent reductions at HLRs between 0.1- 0.3 m/day (right). P value= 0.08605

Comparisons of each substrate between influent	P value	Significant difference	Median % reduction
Woodchips	0.085	No	64.21605
Pine bark			46.44959
Woodchips	0.619	No	64.21605
Peanut hulls			50.73812
Pine bark	1.0000	No	46.44959
Peanut hulls			50.73812

Table A10. Results of Kruskal Wallis rank sums tests determining significant differences between influent and effluent phosphate-P removal rates at HLRs between 0.1- 0.3 m/day (right). P value: 0.3005

Comparisons of each substrate between influent	P value	Significant difference	Median Removal Rate
Woodchips	0.51	No	0.09
Pine bark			0.11
Woodchips	0.67	No	0.09
Peanut hulls			0.12
Pine bark	1.00	No	0.11
Peanut hulls			0.12

Table A11. Results of Kruskal Wallis rank sums tests determining significant differences between DOC release (all LR, all PV, including start up). P value: 0.0004593

Comparisons of each substrate between influent	P value	Significant difference	Median DOC Release (mg/L)
Woodchips	0.0005	Yes	11.815
Pine bark			8.53
Woodchips	1.0000	No	11.815
Peanut hulls			9.665
Pine bark	0.0113	Yes	8.53
Peanut hulls			9.665

Table A12. Results of Kruskal Wallis rank sums tests determining significant differences between ammonium-N release (all LR, all PV, including start up). P value: < 2.2e-16

Comparisons of each substrate between influent	P value	Significant difference	Median ammonium - N Release (mg/L)
Woodchips	0.022	Yes	5e-04
Pine bark			0.0375
Woodchips	<2e-16	Yes	5e-04
Peanut hulls			1.3725
Pine bark	<2e-16	Yes	0.0375
Peanut hulls			1.3725

Table A13. Results of Kruskal Wallis rank sums tests determining significant differences between DON release (all LR, all PV, including start up). P value: < 2.2e-16

Comparisons of each substrate between influent	P value	Significant difference	Median DON Release (mg/L)
Woodchips	0.00045	Yes	0.1405343
Pine bark			0
Woodchips	7.9e-11	Yes	0.1405343
Peanut hulls			0.94075
Pine bark	< 2e-16	Yes	0
Peanut hulls			0.94075

Table A13. Results of Kruskal Wallis rank sums tests determining significant differences between DOP release (all LR, all PV, including start up). P value: 0.0008759

Comparisons of each substrate between influent	P value	Significant difference	Median DOP Release (mg/L)
Woodchips	1.0000	No	0.059
Pine bark			0.009
Woodchips	0.0066	Yes	0.059
Peanut hulls			0.114
Pine bark	0.0024	Yes	0.009
Peanut hulls			0.114

Table A14. Results of Kruskal Wallis rank sums tests determining significant differences between TDN reduction in kg/yr (all LR, all PV, not including start up). P value: 2.643e-08

Comparisons of each substrate between influent	P value	Significant difference	Median TDN Reduction (kg/yr)
Woodchips	7.6e-11	Yes	0.0007763532
Pine bark			0.001103271
Woodchips	1.0000	No	0.0007763532
Peanut hulls			0.0008307686
Pine bark	0.0023	Yes	0.001103271
Peanut hulls			0.0008307686

Table A15. Results of Kruskal Wallis rank sums tests determining significant differences between TDP reduction in kg/yr (all LR, all PV, not including start up). P value: 1.658e-06

Comparisons of each substrate between influent	P value	Significant difference	Median TDP Reduction (kg/yr)
Woodchips	0.0011	Yes	0.6385252
Pine bark			0.1310321
Woodchips	1.4e-06	Yes	0.6385252
Peanut hulls			0.01513728
Pine bark	0.8072	No	0.1310321
Peanut hulls			0.01513728