Using unique carbon source combinations to increase nitrate and phosphate removal in bioreactors

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Dedication

Dedicated to all those who have supported me on this journey ï I couldnôt have completed this thesis without that help.

ñIf I have seen further it is only by standing on the shoulders of giantsò

- Isaac Newton

Abstract

Nitrogen (N) and phosphorus (P) losses from croplands contribute to impairment of water bodies. This study was conducted to test candidate denitrifying bioreactor media for nitrate-N and dissolved reactive P (DRP) removal from agricultural effluent in drainage ditches. The nitrate-N and DRP removal performance of carbon materials widely available in the Midwest, wood chips (WC) and corn cobs (CC), were compared to treatments of mixed materials: wood chips and hardwood biochar (WC+BC), wood chips and sodium acetate (WC+A), corn cobs and modified coconut coir (CC+MC), and corn cobs, modified coconut coir, and modified macadamia biochar (CC+MC+MBC). Water with a nitrate-N concentration of 20 mg N L^{-1} and a DRP concentration of 0.3 mg P L^{-1} was pumped through PVC columns packed with treatment media. The flow rate was adjusted to match the rise and decay of a typical drainage hydrograph. Effluent was sampled after hydraulic residence times (HRT) of 1.5, 8, 12, and 24 h. The laboratory experiment was conducted at 15°C for 14 weeks, 5°C for 13 weeks, and 15°C again for 7 weeks in a temperature controlled chamber, designated the warm run, cold run and rewarm run, respectively. Nitrate-N load reductions ranged from 24% to 96% in the warm and rewarm runs and from 4% to 80% in the cold run. Nitrate-N load reduction performance at all temperatures was in the order of: WC+A > CC+MC > CC > CC+MC+MBC > WC > WC+BC. The nitrate removal rate (NRR) was highest at the 1.5h HRT for the WC+A treatment at all temperatures. Cumulative DRP load reductions in the warm and rewarm runs were statistically higher in the CC, CC+MC, and

CC+MC+MBC treatments, with DRP load reductions of 74%, 81%, and 67%, respectively. The WC+A treatment had the highest DRP load reduction in the cold run, with a 45% reduction. The CC, CC+MC, and CC+MC+MBC treatments had both high NRR and high DRP percent concentration removal in the warm and rewarm runs, but the WC+A treatment had higher removal of both nutrients in the cold run and specifically at lower HRTs. For both nitrate-N and DRP load reductions during high flows and cold temperatures, WC+A would be the recommended treatment. Future work should focus on the addition of carbon such as sodium acetate to enhance bioreactor performance during high drainage and cold temperature conditions.

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Introduction

Excess nitrogen and phosphorus delivered to the Mississippi River Delta causes overgrowth of algae and subsequent depletion of oxygen known as hypoxia (Rabalais et al., 2002). In the United States, these conditions contribute to the Gulf of Mexico hypoxic zone (a.k.a. ñDead Zoneò), an annual phenomenon that negatively affects aquatic organisms (Rabalais et al., 2002). The area of this zone can vary by year; in 2014 and 2015 the Dead Zone was approximately 5,052 and 6,474 square miles, respectively (NOAA, 2015). Excess nutrients do not only harm oceans, however; these detrimental conditions can occur in any water body. Minnesota is the ñLand of 10,000 Lakesò and hypoxic conditions have occurred in both lakes and rivers (MPCA, 2014). A significant portion of Minnesotaôs state income comes from tourism; between the years 2010 and 2013 tourism has remained steady at about 70 million visitors annually, and spending has increased from $9 billion to $10.3 billion during this time frame (Tourism Economics, 2013). Lakes are enjoyed by both tourists and residents; states like Minnesota have over 70% of their own citizens utilize the lakes (Anderson et al., 1999). Besides affecting recreational enjoyment, excess nitrate in local water sources can be expensive to remediate to the EPA standard of 10 mg L^{-1}. Des Moines Water Works in the state of Iowa is currently suing three Iowa counties (Buena Vista, Calhoun, and Sac) because the company is straining to remove nitrate-N concentrations as high as 60 mg L^{-1} from drinking water, which can cost $4,000 a day (Meinch, 2015a). The company alleges that the excess nitrate is specifically from agricultural subsurface drainage and the counties have not done all they could to prevent or mediate it (Meinch, 2015b).

The Minnesota Nutrient Strategy, published in 2014, calls for a 45% reduction of both phosphorus and nitrogen to the Mississippi River by 2040 (MPCA, 2014). Since 50% of nitrogen and 25% of phosphorus delivered to the Mississippi River has been estimated to be originating from corn and soybean fields (Alexander et al., 2008), mitigation of excess nutrients from agricultural drainage is vital. Strategies can include in-field changes in nutrient management such as adjustments to fertilizer type and timing. However, even when no fertilizer is used in a field, there can still be nutrient loss due to the properties inherent to the soil and drainage (Dinnes et al., 2002; Kovacic et al., 2006). Cover crops are another in-field strategy used to reduce erosion and prevent nutrient losses. In the Midwest they are typically small grain cover crops such as rye, oats, and alfalfa. However, cover crops can add monetary and opportunity costs to producers (Saleh et al., 2007).

Combinations of mitigation strategies would be ideal, especially if an edge-of-field strategy were incorporated (Dinnes et al., 2002). Edge-of-field mitigation strategies in use currently include vegetated buffer strips and constructed wetlands. Vegetated buffer strips are placed alongside rivers and creeks to filter nutrients from agricultural surface runoff, but have been shown to be ineffective under high flow conditions (Karthikeyan et al., 2002) and do not reduce nutrient losses from subsurface drainage. Constructed wetlands can replace the wetlands that were originally in the Midwest and are effective at

trapping excess nutrients. However, they can be expensive to construct and take up large amounts of land (Kovacic et al., 2006).

Agricultural bioreactors are an alternative edge-of-field method for removing nitrate-nitrogen (N) from subsurface drainage. Denitrifying bioreactors are enclosed anaerobic chambers that contain a carbon source (i.e. woodchips) that fuels heterotrophic microbial denitrification (Blowes et al., 1994; Schipper et al., 2010; Fenton et al., 2014), converting nitrate-N into di-nitrogen gas through a multi-step process (Averill and Tiedje, 1982). Denitrifying bioreactors are a remediation strategy that has been utilized all over the globe, from Canada (Robertson and Merkley, 2008) to New Zealand (Schipper and Vojvodic-Vukovic, 2000). Bioreactors are cost effective (Christianson et al., 2012) and, unlike constructed wetlands, donôt take land out of crop production (Jaynes et al., 2008). In fact, bioreactors can have a nitrate removal rate forty times higher than constructed wetlands (Robertson and Merkley, 2008).

Most agricultural denitrifying bioreactors are attached to tile drainage lines (pipelines placed under fields to control the water table) but if bioreactors were placed in drainage ditches they could potentially remediate a greater volume of drainage water. However, in order to be operational in drainage ditches, the bioreactors would need to be effective at nitrate-N reduction under high-flow conditions. High-flow conditions happen to correspond with high phosphorus loads in surface waters (Macrae et al., 2007), and therefore a drainage ditch bioreactor should be designed to reduce both nitrate-N and

dissolved reactive phosphorus (DRP) concentrations. Bioreactors should also be able to mitigate nitrate-N and DRP in cold temperatures; agricultural drainage releases excess nitrogen and phosphorus into both surface and ground water (Alexander et al., 2008; Fageria and Baligar, 2005) especially during times of snowmelt (Jin and Sands, 2003; Macrae et al., 2007). Our objective was to find bioreactor treatments that could sustain denitrification and DRP removal under high flow conditions and cold temperatures.

References:

Alexander, R.B., R.A. Smith, G.E. Schwarz, E.W. Boyer, J.V. Nolan, and J.W. Brakebill. 2008. Differences in phosphorus and nitrogen delivery to the Gulf of Mexico from the Mississippi River Basin. *Environmental Science and Technology* 42: 822 ï 830.

Anderson, K.A., T.J. Kelly, R.M. Sushak, C.A. Hagley, D.A. Jensen, and G.M. Kreag. 1999. Summary report on public perceptions of the impacts, use, and future of Minnesota lakes: results of the 1998 Minnesota lakes survey. A joint publication by the University of Minnesota Sea Grant Program (SH 1) and Minnesota Department of Natural Resources, Office of Management and Budget Services. 1-31.

Averill, B.A. and J.M. Tiedje. 1982. The chemical mechanism of microbial denitrification. *Febs Letters* 138 (1): 8 ï 12.

Blowes, D.W., W.D. Robertson, C.J. Ptacek, and C. Merkley. 1994. Removal of agricultural nitrate from tile-drainage effluent water using in-line bioreactors. *Journal of Contaminant Hydrology* 15: 207 ï 221.

Christianson, L.E., A. Bhandari, M.J. Helmers, K. Kult, T. Sutphin, and R. Wolf. 2012b. Performance evaluation of four field-scale agricultural drainage denitrification bioreactors in Iowa.ò *ASABE* 55 (6): 2163-2174.

Dinnes, D.L., D.L. Karlen, D.B. Jaynes, T.C. Kaspar, J.L. Hatfield, T.S. Colvin, and C.A. Cambardella. 2002. Nitrogen management strategies to reduce nitrate leaching in tile-drained Midwestern soils. *Agronomy Journal* 94: 153 ï 171.

Fenton, O., M.G. Healy, F. Brennan, M.M.R. Jahangir, G.J. Lanigan, K.G. Richards, S.F. Thornton, and T.G. Ibrahim. 2014. Permeable reactive interceptors: blocking diffuse nutrient and greenhouse gases losses in key areas of the farming landscape. *Journal of Agricultural Science*. 1 ï 11.

Jaynes, D.B., T.C. Kaspar, T.B. Moorman, and T.B. Parkin. 2008. In situ bioreactors and deep drain-pipe installation to reduce nitrate losses in artificially drained fields. *Journal of Environmental Quality* 37 (2): 429-436.

Karthikeyan, K.G., M.A. Tshabalala, and D. Wang. 2002. Use of lignocellulose materials as sorption media for phosphorus removal. *ASAE Meeting Presentation.*

Kovacic, D.A., R.M. Twait, M.P. Wallace, and J.M. Bowling. 2006. Use of created wetlands to improve water quality in the Midwest ï Lake Bloomington case study. *Ecological Engineering* 28: 258 ï 270.

Macrae, M.L., M.C. English, S.L. Schiff, and M. Stone. 2007. Intra-annual variability in the contribution of tile drains to basin discharge and phosphorus export in a first-order agricultural catchment. *Agricultural Water Management* 92: 171 ï 182.

Meinch, T. 2015a. Water Works plans to sue three counties. http://www.desmoinesregister.com/story/news/2015/01/06/des-moines-water-works-nitrate-lawsuit/21365355/. Accessed 16 May 2016.

Meinch, T. 2015b. Water Works votes to sue 3 Iowa counties over nitrates. http://www.desmoinesregister.com/story/money/agriculture/2015/03/10/utility-consider-pursuing-water-quality-suit/24728999/. Accessed 16 May 2016.

MPCA. 2014. The Minnesota nutrient reduction strategy. Accessed 5 January 2016. https://www.pca.state.mn.us/sites/default/files/wq-s1-80.pdf

NOAA. 2015. Gulf of Mexico dead zone 'above average'. http://www.noaanews.noaa.gov/stories2015/080415-gulf-of-mexico-dead-zone-above-average.html. Assessed 20 March 2016.

Rabalais, N.N., R.E. Turner, and W.J. Wiseman. 2002. Gulf of Mexico hypoxia, a.k.a. ñThe Dead Zoneò. *Annual Review of Ecology and Systematics*. 33 (1): 235 ï 263.

Robertson, W.D. and L.C. Merkley. 2008. In-stream bioreactor for agricultural nitrate treatment. *Journal of Environmental Quality* 38 (1): 230 ï 237.

Saleh, A., E. Osei, D.B. Jaynes, B. Du, and J.G. Arnold. 2007. Economics and environmental impacts of LSNT and cover crops for nitrate-nitrogen reduction in Walnut Creek Watershed, Iowa, using FEM and enhanced SWAT models. *Transactions of the ASABE* 50 (4): 1251 ï 1259.

Schipper, L.A., W.D. Robertson, A.J. Gold, D.B. Jaynes, and S.C. Cameron. 2010. Denitrifying bioreactors ï an approach for reducing nitrate loads to receiving waters. *Ecological Engineering* 36 (11): 1532 ï 1543.

Schipper, L.A. and M. Vojvodic-Vukovic. 2000. Rates of nitrate removal from groundwater and denitrification in a constructed denitrification wall. *Ecological Engineering* 14: 269 ï 278.

Tourism Economics. 2013. The economic impact of travel in Minnesota: 2013 analysis. Oxford Economic Company.

Chapter 1. Using unique carbon source combinations to increase nitrate removal rates in denitrifying bioreactors

Summary

Nitrogen losses from croplands contribute to impairment of water bodies. This study was conducted to test carbon source combinations for removal of nitrate-N from drainage ditch water via denitrifying bioreactors. The nitrate-N removal performance of candidate treatments ï corn cobs (CC), corn cobs with modified coconut coir (CC+MC), corn cobs with modified coconut coir and modified macadamia shell biochar (CC+MC+MBC), wood chips (WC), wood chips with hardwood biochar (WC+BC), and wood chips with continuous sodium acetate addition (WC+A) ï were tested in a laboratory upflow column experiment. Effluent was sampled after hydraulic residence times (HRT) of 1.5, 8, 12, and 24h. Columns were kept at 15°C for 14 weeks, 5°C for 13 weeks, and 15°C again for 7 weeks. Nitrate-N load reduction was highest for WC+A in all temperature runs, with 80.2%, 80.0%, and 96.8% reduction during the warm, cold, and rewarms runs, respectively. The nitrate-N removal rate was highest at the 1.5h HRT for the WC+A treatment for all temperatures. Corn cob treatments (CC, CC+MC, and CC+MC+MBC) had the second highest load reductions for all three temperature experiments, and WC and WC+BC had the lowest load reduction. Acetate addition improved wood chip bioreactor performance and could enhance nitrate-N removal under the cold and high flow rate conditions of spring drainage.

1. Introduction

Elevated crop productivity in the Midwest has had the unintended environmental and economic consequences of excess nitrogen in waterways. Less than 50% of the nitrogen fertilizer applied to soils is harvested with the crop grain (Fageria and Baligar, 2005), resulting in leaching of nitrate-N into the Mississippi River Basin (Alexander et al., 2008). Excess nitrate-N contributes every year to hypoxic conditions in the Gulf of Mexico (CENR, 2000), and removing excess nitrate-N from drinking water sources can be expensive. For example, the Des Moines Water Works (Des Moines, IA) is suing three agricultural counties in northwest Iowa because of the expense of removing nitrate from rivers used for sources of drinking water (Eller, 2015). In Minnesota, the Minnesota Pollution Control Agency (MPCA) has set a 45% reduction goal for nitrate-N loads in the Mississippi by 2040 (MPCA, 2014).

Agricultural best management practices (BMPs) can be used as mitigation strategies (Miller, et al., 2012). Nutrient management and cover crops are in-field strategies that can reduce nitrate-N loss, but cover crops can add costs on the producer (Saleh et al., 2007) and inherent soil properties can create nitrate-N losses even when fertilizers are managed well (Dinnes et al., 2002; Kovacic et al., 2006) Vegetated buffer strips and constructed wetlands are edge-of-field strategies that can be used in conjunction with on-field strategies. Buffer strips do not treat subsurface flow such as tile drainage, however, and wetlands can be expensive to construct and occupy large amounts of land (Kovacic et al., 2006).

Agricultural denitrifying bioreactors have the potential to be a better edge-of field strategy: they do not take land out of crop production (Jaynes et al., 2008) and are cost effective compared to other mitigation strategies (Christianson et al., 2012b). Denitrifying bioreactors are enclosed anaerobic chambers that contain a carbon source (i.e. woodchips) that fuels heterotrophic microbial denitrification (Blowes et al., 1994; Fenton et al., 2014; Schipper et al., 2010), converting nitrate-N into di-nitrogen gas through a multi-step process (Averill et al., 1982). Denitrifying bioreactors treating agricultural water typically are connected to the end of tile drainage lines (Christianson et al., 2012a; Jaynes et al., 2008; Moorman et al., 2010; Woli et al., 2010), but could also be placed in agricultural drainage ditches to treat water at a larger scale (Robertson and Merkley, 2008).

In order to operate in the volume of water found in an agricultural drainage ditch, high nitrate-N removal rates (NRR) in bioreactors are needed. Effective carbon sources will be vital in promoting biofilm development and high rates of complete denitrification; properties desirable in materials for biofilm development include small particle size, large surface area, and availability of carbon (Andersson et al., 2008). However, the carbon material size needs to be balanced with hydraulic conductivity properties (Feyereisen and Christianson, 2015; Ghane et al., 2014) as well as economic and environmental costs. Existing bioreactors in the Midwest generally rely on wood chips for a carbon material (e.g., Christianson et al., 2012b; Moorman et al., 2010). Wood chips are relatively accessible, cost effective per treatment area, and can remove 33%- 65% of nitrate-N load

(Jaynes et al., 2008; van Driel et al., 2006; Woli et al., 2010; Christianson et al. 2012). However, wood chip bioreactors have not exhibited high NRR under low temperature conditions (Schmidt and Clark, 2013), which limits their ability to remove nitrate-N in colder months. In MN, about 75% of infiltration between March and June is converted to drainage and snow melt makes up approximately 28% of annual drainage (Jin and Sands, 2003), so it is vital that denitrification is supported in bioreactors during cold temperatures.

Corn cob bioreactors have been shown to support higher NRRs than those with wood chips (Cameron and Schipper, 2010; Feyereisen et al., 2016), and corn cobs are readily available in the Midwest. More novel carbon materials such as biochar could be utilized in bioreactors also (Bock et al, 2014). Biochar is defined as a biomass that has been pyrolyzed under low-oxygen, high-temperature conditions (Gaunt and Lehmann 2008). Biochar additions to soil have been found to decrease nitrate leaching (Beck et al., 2011) and increase microbial biomass (Lehmann et al., 2011), specifically in bacterial families that are involved with denitrification (Anderson et al. 2011). When mixed with wood chips in bioreactors, biochar made out of hardwood has been shown to improve overall NRR performance (Bock et al. 2014).

Soluble carbon sources such as sodium acetate and methanol can also improve bioreactor performance by ensuring carbon availability for denitrification (Cantafio et al., 1996), especially during colder temperatures when bioreactor materials such as wood chips and

corn cobs are ineffective at promoting denitrification (Feyereisen et al., 2016; Schmidt and Clark, 2013).

In order to further increase NRR in ditch bioreactors, including an anion exchange material with the bioreactor material could increase nitrate retention time, which in turn could extend contact time with denitrifying microorganisms and thus increase nitrate reduction. Manufactured anion exchange products can be expensive but carbon materials, such as coconut coir and biochar, can be altered to become anion exchangers (Baes et al. 1997; Jansen and van Bekkum 1994; Orlando et al., 2003).

We sought to combine various carbon materials to create a denitrifying bioreactor that would reduce nitrate-N during high flow events and cold temperatures. Our objective was to evaluate and identify materials with the potential to increase NRR with respect to woodchip bioreactors, using simulated flow events and temperatures that mimic drainage ditch conditions during spring and summer flows in the Midwest, USA. We hypothesized that 1) biochar and liquid sodium acetate additions to woodchips would increase NRR compared to woodchips alone, and 2) corn cob performance would be enhanced by amine modified coconut coir and biochar additions.

2. Methods

Eighteen columns of PVC pipe (15.2 cm i.d. X 49.5 cm long) were packed with six unique treatments (n=3) in an upflow laboratory column experiment. The six treatments

were: (i) wood chips (WC), (ii) wood chips and woodchip biochar (WC+WBC), (iii) woodchips and sodium acetate (WC+A), (iv) corn cobs (CC), (v) corn cobs and modified coconut coir (CC+MC), and (vi) corn cobs, modified coconut coir, and modified macadamia biochar (CC+MC+MBC). Wood chips were approximately 13 ï 25 mm and were composed of a mixture of hard and soft woods (Mulch Store (Empire, MN)). Corn cobs were typically 5 ï 15 cm long and were obtained from the University of Minnesota ï Morrisô inventory of corn cobs intended for the campus biomass-fueled boiler. The hardwood biochar (Cowboy Charcoal) was mostly 13 ï 25 mm in size, similar to the wood chips, while the macadamia biochar (Biochar Brokers) was mostly < 6 mm in size. Chemical and physical properties of the biochars are listed in Table A.1. Coconut coir was obtained from J. Alderink LLC (Oak Park, MN) and fibers were typically 5 ï 15 cm long. Complete particle size descriptions are given in Table A.2. Coconut coir and macadamia biochar media were modified using an amination procedure similar to one described in Jansen and van Bekkum (1994). A solution of 10% nitric acid was added to a beaker of material and heated at 90°C for 4 hours. Material was then rinsed 10 times with DI water, or until pH was circumneutral. Baking soda was used to reach neutrality for all batches of materials.

Columns were built out of Schedule 40 PVC pipe with corresponding caps for top and bottom of pipe. A PVC plate (1.3 cm thick) with holes drilled in for drainage was screwed onto bottom of pipe, a corresponding cap was glued over pipe end with plate, and then a stainless steel mesh wire circle was placed inside on top of PVC plate. Before

packing columns, materials were oven dried at 70°C for 48 hours and then soaked in tap water for 48-72 hours. Materials were allowed to drip dry for 10 minutes and then packed in batches using the following method: batches of 400g of corn cobs or woodchips were packed into columns, with an 1812 g weight being used to tamp down each batch layer. 1g of biological inoculum (Biofloc^{TM} Biologicals, Innovative Turf Solutions, Cincinnati, OH) was weighed and divided between all batch layers before packing to provide denitrifiers for the columns. See Appendix II for more information on the biological inoculum. Single material treatments had ~10 layers. Columns with treatments consisting of several carbon materials were packed in alternating layers. Sub-samples were used to measure moisture content in order to determine dry mass of materials in columns. Percent by weight of different carbon materials was calculated for mixed treatments to determine how much modified coir or biochar was mixed in with the wood chips or corn cobs (Table A.2). Once all layers were packed into columns, a stainless steel mesh was placed on top of the material and another PVC plate was tapped down on top, leveled, and screwed into place. Drainable porosity was found for each column by filling columns with water, topping them off in the morning to make sure media were saturated, and then draining columns for 24h. Columns were capped using silicon caulk and glue and allowed to set for 24 h. Sensors measuring oxidation-reduction potential (ORP) were screwed into the top caps and were connected to a multiplexer controlled by a data logger that took readings every hour.

The experiment was conducted for fourteen weeks at 15°C (i.e., ñwarm runò) and for thirteen weeks at 5°C (i.e., ñcold runò) in a controlled temperature chamber. A second 15°C experiment (i.e., ñrewarm runò) was conducted for 7 weeks to verify results were not influenced by loss of labile carbon from the warm to the cold run, since media were not changed between temperature experiments. Flow rates were adjusted to approximate the rise and decay of a typical drainage hydrograph using data from Morris, MN. The rise and decay of flow rates were fit to a week regimen and were associated with hydraulic residence times (HRT) of 1.5, 8, 12, and 24 h (see Figure 1.1) with durations for 6, 42, 48, and 72 h, respectively. Nitrate-N and dissolved reactive phosphorus (DRP) influent concentrations were 20 mg N L^{-1} as potassium nitrate and 0.3 mg P L^{-1} as potassium phosphate, respectively, in reverse osmosis water. Sodium acetate was pumped into the three WC+A columns at a final concentration of 40 mg C L^{-1} using a separate pump at 1% of the main pump flow rate. Influent and effluent water was sampled at each of the 4 flow rates and tested for nitrate-N, ammonia-N, DRP, total carbon (TC), total organic carbon (TOC), and pH. Air and water temperatures were taken hourly.

Dissolved oxygen (DO) was measured 4 times weekly during water sampling by the following method: The hand-held DO probe (HI 9142 (meter), HI 76407/4 (probe), Hanna Instruments, Woonsocket, RI) was inserted into a syringe body that had been connected via 6-mm diameter tubing to a valve on the top cap of the columns. The column effluent outlet valve was closed and the valve to the syringe body simultaneously opened to permit flow of water into the syringe body. The flow rate was changed to 10

mL min^{-1} to allow fresh water to flow continuously next to the probeôs membrane. There was minimal space between the wall of the syringe and the probe to reduce the potential for oxygen diffusion.

Water samples were collected in 250 mL polyethylene bottles every week at the end of each HRT run and preserved with sulfuric acid (Cleresci et al., 1998) for analysis using colorimetric flow injection analysis (Lachat QuikChem 8500, Hach Co., Loveland, CO) for nitrate-N and ammonia-N (methods 10-107-04-1-B and 10-107-06-2-A, respectively). Analyses of TC and TOC were done using EPA Methods 415.1 and 9060A (Tekmar-Dohrmann Phoenix 8000, Teledyne Tekmar, Mason, OH). Unacidified water samples were collected and measured for pH.

Between each temperature run, there was a brief shut-down period. During this time, caps were removed and media samples (30-90g wet weight, ~20g dried weight) were taken for carbon analysis from each column and stored at 4°C and -80°C. Columns were then prepared for plumbing and capping, and temperature was changed as needed.

Nitrate removal rate (NRR) was calculated for each water sampling date as the difference between the nitrate-N mass into (N_{in}) and out of (N_{out}) the bioreactor. For each column, the N_{in} mass was determined by multiplying the volume of water that passed through the column by the concentration of nitrate-N at the inlet, and N_{out} was the volume of water that passed through the column multiplied by the nitrate-N concentration of that column.

$$NRR = \frac{N_{in} - N_{out}}{(t_n - t_{(n-1)})\, Vol_{medium}}$$

In the NRR equation, (t_n ï $t_{(n-1)}$) is the difference in sampling times and Vol_{medium} is the gross volume occupied by the medium (Schipper et al, 2010).

Nitrate-N percent load reduction was calculated for each column by first summing the N_{in} mass and summing the N_{out} mass during the entire temperature run, and then taking 1 ï (ΣN_{out}/ ΣN_{in}). Load reductions for each column were then averaged by treatment.

Total carbon (TC) and total organic carbon (TOC) production were both calculated for each column as the difference between the flow weighted grams of carbon in and carbon out of the bioreactor over the entire temperature experiment. Total inorganic carbon (TIC) was calculated for each column as the difference between TC production and TOC production for that column.

Percent carbon, percent nitrogen, and C:N were analyzed using original carbon materials and bioreactor material sampled after the rewarm run. Samples were oven dried at 60°C, ground, and analyzed via combustion (vario MAX CN, elementar Americas Inc, Mt. Laurel, NJ).

Once the experimental runs were complete, data were prepared for statistical analysis. The first two weeks of each temperature experiment were excluded as well as any weeks that had instrumental or experimental error (water pump failure, acetate pump failure, low water level, etc.). In all, this yielded eight weeks of data for both the warm and cold runs, and six weeks for the rewarm run. Nitrate-N concentration, nitrate-N load reduction by percentage, NRR, TC, TOC, and TIC were analyzed with a linear mixed-effects model in R using nlme package (Pinheiro et al, 2014). The linear mixed-effects model was run with and without a correlation argument, and fit criteria AIC and BIC showed that the correlation argument had the higher quality of statistical model in all analyses. Statistical treatment differences were determined by running Tukey multiple comparisons using lsmeans package (Lenth, 2015) at a significance level of P Ò0.05. See Appendix III for the R code.

3. Results

3.1 Experimental Conditions

Mean water temperatures were 14.6 ± 0.8 °C, 5.5 ± 0.5 °C, and 14.9 ± 0.4 °C for the warm, cold, and rewarm runs, respectively. Mean inlet nitrate-N concentration for all experimental runs was 19.74 ± 0.99 mg L^{-1}. Mean sodium acetate concentration was 46.83 ± 8.23 mg L^{-1}. Average HRT in the columns at the four flow rates (1.5, 8, 12, and 24h HRT) were 1.8, 8.8, 12.5, and 25.3h in the warm run, 2.0, 9.1, 12.9, and 24.1h in the

cold run, and 1.8, 8.9, 12.7, and 22.6h in the rewarm run (Table A.3). Phosphorus data are reported and discussed in Chapter 2.

3.2 Nitrate Concentration

Effluent nitrate-N concentrations for all treatments decreased from the inlet concentration at all HRT in the warm, cold, and rewarm temperature runs (Table 1.1). The warm run had effluent nitrate-N ranging 0.1 ï 7.9 mg N L^{-1} at 24h HRT and 14.0 ï 19.5 mg N L^{-1} at 1.5h HRT, the cold run ranging 0.2 ï 18.1 mg N L^{-1} at 24h HRT and 15.0 ï 19.3 mg N L^{-1} at 1.5h HRT, and the rewarm run ranging 0.1 ï 8.4 mg N L^{-1} at 24h HRT and 2.0 ï 19.1 mg N L^{-1} at 1.5h HRT. At 1.5h and 8h HRT, WC+A had the lowest effluent nitrate-N concentrations for all temperature runs. At 12h and 24h HRT the corn cob treatments (CC, CC+MC, CC+MC+MBC) were statistically as low as WC+A in the warm and rewarm run. In the cold run, WC+A was lower than the corn cob treatments at 12h and 24h HRT. WC and WC+BC had the highest effluent nitrate-N concentrations at all HRT in all temperature runs.

3.3 Nitrate Removal Rate

Most treatments had a NRR that was higher at 1.5h HRT and lower at 24h HRT in all temperature runs (Table 1.2). The only treatment that did not exhibit this trend was WC+BC, which had the inverse. The warm run had NRR ranging 6.0 ï 10.2 g N m^{-3} d^{-1} at 24h HRT and 2.5 ï 43.0 g N m^{-3} d^{-1} at 1.5h HRT, the cold run ranging 1.0 ï 11.8 g N m^{-3} d^{-1} at 24h HRT and -0.7 ï 29.9 g N m^{-3} d^{-1} at 1.5h HRT, and the rewarm run ranging

6.1 ï 10.6 g N m^{-3} d^{-1} at 24h HRT and 4.6 ï 120.6 g N m^{-3} d^{-1} at 1.5h HRT. The WC+A treatment had the highest NRR at 1.5h HRT in the warm and rewarm runs. At all other HRT, generally the WC+A, CC, CC+MC, and CC+MC+MBC treatments had higher NRR than the WC and WC+BC treatments. In the cold run the WC+A treatment had the highest NRR at all HRT, and the WC and WC+BC treatments had the lowest.

3.4 Q_{10} Values

Q_{10} values were -4.0 ï 7.3 for comparisons between the warm and cold run (W/C) treatments and -6.6 ï 9.1 for comparisons between the rewarm and cold run (R/C) treatments for all HRT (Table A.4). The WC+A treatment had a Q_{10} value of 1.0 and 0.9 in the W/C and R/C comparisons, respectively, for 8h, 12h, and 24h HRT. The CC, CC+MC, and CC+MC+MBC treatments had values of 1.4 ï 3.9 and the WC and WC+BC treatments were 4.8 ï 9.1 at 8h, 12h, and 24h HRT at both comparisons. At 1.5h HRT, WC+A had a value of 1.7 for the W/C comparison, but a value of 4.2 for the R/C comparison. In both comparisons, the negative Q_{10} values for 1.5h HRT associated with the WC+BC treatment are due to a negative NRR during the cold run at the 1.5h HRT (Table 1.2).

3.5 Nitrate Load Reduction

Cumulative nitrate-N load reductions for the treatments were 24.1 ï 80.2% in the warm run, 3.5 ï 80.0% in the cold run, and 26.9 ï 96.8% in the rewarm run (Figure 1.1). In the warm run, the WC+A, CC, CC+MC, and CC+MC+MBC treatments had the highest load

reduction, and WC and WC+BC had the lowest. In the cold run WC+A had the highest nitrate-N load reduction followed by the corn cob treatments, and WC and WC+BC treatments had the lowest nitrate-N load reductions. The rewarm run exhibited the same pattern as the cold run, except that the cumulative nitrate-N load reduction for the WC+BC treatment was less than that of the WC treatment.

3.6 Dissolved Oxygen

Mean dissolved oxygen (DO) concentrations at the inlet were 7.1, 8.9, and 8.3 mg O L^{-1} for the warm, cold, and rewarm runs, respectively (Table 1.3). All treatments during warm and rewarm runs had mean outlet DO concentrations of <1 mg O L^{-1}. During the cold run, WC+A mean outlet DO concentration was 0.3 mg O L^{-1} while the remaining treatments ranged from 2.0 to 2.6 mg O L^{-1}.

3.7 pH

Mean inlet pH was 7.3 during the warm run, 7.2 during the cold run, and 6.8 during the rewarm run (Table 1.3). Effluent pH in the WC+A treatment ranged between 7.0 and 9.4 in the warm and rewarm runs and between 8.2 and 9.6 in the cold run, with pH being higher during the 1.5h HRT than all other HRT (Figure A.2). All other treatments had effluent pH readings of ~7.0.

3.8 Oxidation Reduction Potential

The average oxidation reduction potential (ORP) ranges for all treatments across all HRT were -459 to +599 mV in the warm run, -191 to +670 mV in the cold run, and -279 to +448 mV in the rewarm run (Table 1.3). During the warm run the WC+BC was the only treatment with completely positive ORP values, and during the cold run the WC+A treatment was the only treatment with negative ORP values. Table A.5 contains ORP ranges for all six treatments during the warm, cold, and rewarm runs under each of the four HRT.

3.9 Carbon

The cumulative mean TC production loads were 2.8 ï 19.5 g in the warm run and 1.3 ï 13.2 g in the cold run for all treatments (Table 1.4) during eight weeks of data in both temperature runs. The WC+A treatment had the greatest TC load in the cold run, but during the warm run was statistically similar to the CC, CC+MC, and CC+MC+MBC treatments. In both runs, the WC and WC+BC cumulative TC production loads were significantly lower than all the other treatments. The cumulative mean TOC production loads were 1.1 ï 15.1 g in the warm run and 0.4 ï 8.6 g in the cold run (Table 1.4). The warm run TOC load exhibited a similar pattern to the warm run TC load, but during the cold run WC+A had a higher TOC production load than all other treatments. The cumulative mean TIC (total inorganic carbon) production loads were 1.4 ï 5.0 g in the warm run and 0.9 ï 4.7 g in the cold run, and showed a similar pattern to the TC load in both the warm and cold runs (Table 1.4). Rewarm carbon data are not presented due to equipment failure.

3.10 Media Analysis

Percent carbon, percent nitrogen, and C:N data of all media showed nitrogen enrichment compared to original samples except for hardwood biochar (Table 1.5). Corn cobs that were in bioreactor treatments had lower C:N values than the original corn cob material (157.6) and also had similar values to each other: 116.6 for CC, 115.0 for CC+MC, and 112.5 for CC+MC+MBC. Wood chips in bioreactor treatments also had lower C:N values than original wood chip material (299), but WC+A chips were much closer in value to original ones: wood chips from WC and WC+BC had C:N values of 169.5 and 163.2, respectively, while from WC+A they had a C:N value of 229.3. The hardwood biochar from the WC+BC treatment had a C:N value of the 146.8 and the original material had 108.

4. Discussion

4.1 Nitrate Removal Performance

The data support our hypothesis that additions of sodium acetate to woodchips increases NRR compared to wood chips alone; NRR for the WC+A treatment was higher than for the WC treatment in all HRT in the cold run, and for 1.5h, 8h, and 12h HRT in the warm and rewarm runs. The highest mean NRR was 120.6 ± 12.9 g N m^{-3} d^{-1} at 1.5h HRT during the rewarm run, and to our knowledge this is higher than any existing study. Fewer differences between treatments were seen at 24h HRT, with lower NRR being observed for WC+A, but this could be due to nitrate limiting conditions. Nitrate-N concentrations less than 1 mg N L^{-1} in effluent can limit NRR (Elgood et al., 2010),

which occurred in this experiment. One reason could be the initial concentration in this experiment was 20 mg N L^{-1} versus the 50 mg N L^{-1} that has been used in other laboratory column experiments (Greenan et al., 2009; Feyereisen et al., 2016).

The addition of biochar to a wood chip bioreactor did not increase NRR. During all HRT and temperature runs, there were no significant differences between WC and WC+BC NRR. This is in direct contradiction to what has been found by other researchers. Bock et al. (2014) found that a bioreactor with a mixture of hardwood biochar and wood chips exhibited nitrate-N reductions of 86% after 18h versus a wood chip-only bioreactor with a reduction of only 13%. When added to soil, biochar has been found to have nitrate-N reductions of 79-97% (Beck et al., 2011). Our column study only showed nitrate-N load reductions of 4-27% in the WC+BC treatment, and in fact it was the worst performing treatment in this regard. With a negative average NRR at 1.5h HRT in the warm run, the WC+BC may have even desorbed or leached nitrate-N. It is unclear why biochar has performed so differently in other laboratory experiments. Our hardwood biochar pieces were larger than that in Bock et al. (2014), but they found that particle size did not cause significant differences in NRR, so the size of the biochar should not have affected the WC+BC performance.

The WC treatment exhibited NRR values of 5.8 ï 7.3 g N m^{-3} d^{-1} in the warm run, 0.9 ï 2.2 g N m^{-3} d^{-1} in the cold run, and 7.1 ï 7.8 g N m^{-3} d^{-1} in the rewarm run. The ranges for all runs fall within reported removal rates for both field and laboratory-scale experiments

for wood chip medium at variable temperatures (Feyereisen et al., 2016; Christianson et al., 2012a; Schipper et al. 2010).

As for the corn cob treatments, our hypothesis that that amine-modified coconut coir would improve NRR of a corn cob bioreactor is partially supported. Under 1.5h HRT conditions in both warm and rewarm runs, the CC+MC treatment had a significantly higher NRR than the CC treatment. It could be that the anion exchange capacity in the amine-modified coconut coir sorbed nitrate-N at this high flow rate in comparison, or that the coconut coir itself generated more surface area for microbes to create biofilms and uptake nutrients. CC+MC did not have a higher NRR than CC in the cold run, however, so this treatment seems to be temperature dependent. Amine-modified macadamia biochar, on the other hand, did not improve corn cob NRR performance. There were no significant differences between NRR for CC+MC+MBC and CC treatments at any HRT during any temperature run. As for the differences between CC+MC+MC and CC+MC treatments, CC+MC had a higher NRR at 1.5h HRT in the warm run but were otherwise similar in performance.

The CC treatment NRR ranged from 9.3 to 24.5 g N m^{-3} d^{-1} in the warm run, 7.2 to 10.1 g N m^{-3} d^{-1} in the cold run, and 9.8 to 24.3 g N m^{-3} d^{-1} in the rewarm run for the 1.5 to 24h HRT. This range is similar to results from other corn cob bioreactor column experiments. Cameron and Schipper (2010) found mean NRR of 34.6 and 19.8 g N m^{-3} d^{-1} in 1 ï 10 and 10 ï 23 month experiments, respectively, at 14°C with a HRT of 44h. Feyereisen et

al. (2016) had mean NRR of 34.9 g N m^{-3} d^{-1} in a 15°C experiment and 7.4 g N m^{-3} d^{-1} in a 1.5°C experiment with a HRT of 12h. The CC treatment NRR in this experiment falls at the lower end of other observed corn cob NRR, but both of these other experiments used higher nitrate-N concentrations (159 mg L^{-1}, Cameron and Schipper, 2010; 50 mg L^{-1}, Feyereisen et al., 2016), and it is possible that the lower initial nitrate-N concentration in our study influenced NRRs (Elgood et al., 2010).

Nitrate-N load reduction data exhibits a similar pattern to NRR data; WC+A performed better than WC. In fact, in the cold and rewarm runs the WC+A outperformed all other treatments with load reduction means of 80.0% and 96.8%, respectively. The nitrate-N load reduction data also mirrors the NRR data in that the biochar additions to both corn cobs and wood chips did not improve bioreactor performance. In the rewarm run, WC+BC actually had a lower load reduction than WC; the WC+BC treatment had a 27% nitrate-N load reduction while the WC treatment had almost 40%.

Q_{10} values showed that the WC+A treatment was not temperature dependent, unlike the other treatments. Out of all the treatments, WC+A had the lowest Q_{10} value in the warm run/cold run comparison (W/C), which corresponds to its high NRR in these temperature runs. In the rewarm run/cold run comparison (R/C), WC+A has a relatively high Q_{10} value of 4.2, but this is likely due to the NRR in the rewarm run being 120 g N m^{-3} d^{-1} versus 29.9 g N m^{-3} d^{-1} in the cold run.

Q_{10} values for wood chip bioreactors have been reported between 1.0 and 5.7 (Christianson et al., 2012b; Schmidt and Clark, 2013; Warneke et al., 2011b; Feyereisen et al., 2016). Q_{10} values for our WC treatment, as well as the WC+BC treatment, are higher than this reported range for wood chips. This could be due to their relatively low NRR in the cold run versus in the warm and rewarm runs. For corn cob bioreactors, Q_{10} values between 0.8 to 3.4 have been reported (Cameron and Schipper, 2010; Feyereisen et al., 2016). Our corn cob treatments fall on the higher end of this range, which could again be due to low NRR in the cold run for these treatments.

4.2 Factors Influencing Denitrification

Treatments that had high NRR generally had ORP ranges that fell within the range for denitrification. The ORP range that promotes denitrification in wastewater is -50 to +50 mV (Gerardi, 2007). All treatments in the warm and rewarm runs were generally in this range except for WC and WC+BC, which corresponds with high NRR in corn cob and WC+A treatments versus low NRR in the WC and WC+BC treatments. In the cold run, the only treatment for which ORP readings were in this range for all flow rates was WC+A. In fact, it is the only treatment with negative ORP values in the cold run, which lines up with its comparatively high NRR during the cold run.

Dissolved oxygen values indicated that all treatments contained a suitable oxygen environment for denitrification. In both the warm and rewarm runs, DO values for all treatments were <1.0 mg O_2 L^{-1}, which indicates conditions appropriate for denitrification

(Elgood et al., 2010). During the cold run, DO values were higher for all treatments (2.0 ï 2.6 mg O_2 L^{-1}) except the WC+A treatment had values <1.0 mg O_2 L^{-1}. This corresponds with WC+A having NRR significantly higher than all other treatments in the cold run.

Treatments with higher NRR and nitrate load reduction had higher TC and TOC production loads. In the warm run, the corn cob treatments and WC+A had the highest TC and TOC values, while WC and WC+BC had significantly lower values. During the cold run, both TC and TOC were significantly higher in the WC+A treatment when compared to all other treatments. This could explain why this treatment had such high NRR and nitrate-N load reduction in the cold run; if the denitrifying bacteria were able to get access to carbon, they could potentially be able to continue denitrification in colder temperatures. The fact that the other treatments had such low TC and TOC values in the cold run could indicate that they were carbon limited compared to the WC+A treatment, and organic carbon is necessary for the conversion of nitrate-N to di-nitrogen gas (Elgood et al, 2010). Warneke et al. (2011a) and Feyereisen et al. (2016) both found that differences between treatments in nitrate-N removal could be linked to carbon bioavailability.

Both warm and cold runs had higher TIC in treatments that had higher NRR; corn cob treatments and WC+A treatment have the highest TIC in the warm run, and WC+A has the highest in the cold run. TIC can be an indirect measure of microbial respiration of

CO_2 during the denitrification process (Elgood et al., 2010). WC+A is the only treatment to have a significantly similar TIC value in both the warm and cold run, which could indicate that denitrification was occurring at similar efficiencies.

4.3 Potential for Adverse Effects

Acetate addition to the wood chip bioreactor improved nitrate-N removal to the point that caution needs to be taken to ensure that complete nitrate-N removal conditions would not persist in the bioreactor. Nitrate-N concentrations dropping below 0.5 to 1 mg L^{-1} nitrate-N can result in adverse side effects including emission of methane and methylation of mercury (Robertson and Merkley, 2008). If most of the nitrate-N is converted to di-nitrogen and the bioreactor remains anaerobic, then microorganisms will instead use sulfate and then carbon dioxide as electron acceptors, producing hydrogen sulfide and methane (Healy et al., 2012; Warneke et al. 2011a). Sulfate-reducing conditions can indicate potential for methylation of mercury in a bioreactor. Hydrogen sulfide and methane production occur at ORP values of -50 to -250mV and -175 to -400mV, respectively (Gerardi, 2007). We measured ORP values for WC+A that fell within this range. However, field studies of bioreactors have found relatively low levels of methyl mercury (Natarajan, 2015) and methane (Warneke et al., 2011a). Since we did not sample dissolved gases or methyl mercury concentrations in our laboratory column experiment we do not know if this would be an area of concern for the WC+A treatment, but when this treatment moves to a field-scale study it should be monitored for pollutants and greenhouse gas emissions.

Another potential concern about the WC+A treatment would be the introduction of soluble carbon into the aquatic environment of nearby streams. If TOC production load is examined at 1.5h and 24h HRT during the warm and rewarm runs for the WC+A treatment, the load is much higher during 24h than during 1.5h HRT (Figure A.3). To prevent harm to ecosystems in waterways, sodium acetate could be pumped in at a lower concentration during low flow events to prevent dumping excess carbon into waterways.

It is possible that NRR values were over-estimated in this experiment, especially for the 1.5h HRT. Due to fact that most bioreactor laboratory experiments have used a steady flow rate or HRT, the NRR equation we used has not been applied to many experiments with variable HRTs. In order to determine how outlet nitrate-N concentrations varied during the 1.5h HRT, water samples were taken every hour during the six hours of one 1.5h HRT cycle. Outlet concentration increased non-linearly during this time (Figure A.4); Nitrate-N concentrations for most treatments increased rapidly after the flow rate was increased and then reached a plateau. The WC+A treatment exhibited a much lower nitrate-N concentration change than the other treatments during the six hours. This could effect how the NRR equation works on the different treatments, and could be overestimating NRR in the treatments that exhibit non-linear data. It is possible that a new equation could be developed to account for non-linear outlet concentrations over an HRT regimen.

5. Conclusions

The addition of biochar to wood chips did not improve the NRR of wood chip bioreactors, and the addition of amine-modified coconut coir and biochar did not improve the overall NRR of corn cob bioreactors. A higher proportion of modified or biochar material perhaps would be needed to increase NRR in a wood chip or corn cob bioreactor. However, the addition of sodium acetate to wood chips greatly increased the NRR of a wood chip bioreactor, and the WC+A treatment had the highest NRR of any treatment at 5°C and the highest nitrate-N load reduction in all temperature runs. Sodium acetate could be used to improve denitrification in bioreactors under the cold conditions of spring drainage when bioreactors can be carbon-limited. Further research on the use of soluble carbon should be conducted in field-scale bioreactors.

References:

Alexander, R.B., R.A. Smith, G.E. Schwarz, E.W. Boyer, J.V. Nolan, and J.W. Brakebill. 2008. Differences in phosphorus and nitrogen delivery to the Gulf of Mexico from the Mississippi River Basin. *Environmental Science and Technology*. 42 (3): 822-830.

Anderson, C.R., L.M. Condron, T.J. Clough, M. Fiers, A. Stewart, R.A. Hill, and R.R. Sherlock. 2011. Biochar induced soil microbial community change: implications for biogeochemical cycling of carbon, nitrogen and phosphorus. *Pedobiologia* 54 (5-6): 309ï 20.

Andersson, S., M. Nilsson, G. Dalhammar, and G.K. Rajarao. 2008. Assessment of carrier materials for biofilm formation and denitrification. *Vatten* 64: 201-207.

Averill, B.A., and J.M. Tiedje. 1982. The chemical mechanism of microbial denitrification. *Febs Letters* 138 (1): 8-12.

Baes, A.U., T. Okuda, W. Nishijima, E. Shoto, and M. Okada. 1997. Adsorption and ion exchange of some groundwater anion contaminants in an amine modified coconut coir. *Water Science and Technology* 35 (7): 89ï 95.

Beck, D.A., G.R. Johnson, and G.A. Spolek. 2011. Amending greenroof soil with biochar to affect runoff water quantity and quality. *Environmental Pollution* 159: 2111-2118.

Blowes, D.W., W.D. Robertson, C.J. Ptacek, and C. Merkely. 1994. Removal of agricultural nitrate from tile-drainage effluent water using in-line bioreactors. *Journal of Contaminant Hydrology*. 15: 207-221.

Bock, E., N. Smith, M. Rogers, B. Coleman, M. Reiter, B. Benham, and Z.M. Easton. 2014. Enhanced nitrate and phosphate removal in a denitrifying bioreactor with biochar. *Journal of Environment Quality*. 44 (2): 605-613.

Cameron, S.G., and L.A. Schipper. 2010. Nitrate removal and hydraulic performance of organic carbon for use in denitrification beds. *Ecological Engineering* 36 (11): 1588-1595.

Cantafio, A.W., K.D. Hagen, G.E. Lewis, T.L. Bledsoe, K.M Nunan, and J.M. Macy. 1996. Pilot-scale selenium bioremediation of San Joaquin drainage water with *Thauera selenatis*. *Appl. Environ. Microbiol.* 62 (9): 3298-3303.

CENR. 2000. Integrated assessment of hypoxia in the Northern Gulf of Mexico. National Science and Technology Council Committee on Environmental and Natural Resources: Washington, DC.

Christianson, L., A. Bhandari, and M.J. Helmers. 2011. Pilot-scale evaluation of denitrification drainage bioreactors: reactor geometry and performance. *Journal of Environmental Engineering*. 137: 213-220.

Christianson, L.E., A. Bhandari, and M.J. Helmers. 2012a. A practice-oriented review of woodchip bioreactors for subsurface agricultural drainage. *ASABE* 28 (6): 861ï 74.

Christianson, L.E., A. Bhandari, M.J. Helmers, K. Kult, T. Sutphin, and R. Wolf. 2012b. Performance evaluation of four field-scale agricultural drainage denitrification bioreactors in Iowa.ò *ASABE* 55 (6): 2163-2174.

Dinnes, D.L., D.L. Karlen, D.B. Jaynes, T.C. Kaspar, J.L. Hatfield, T.S. Colvin, and C.A. Cambardella. 2002. Nitrogen management strategies to reduce nitrate leaching in tile-drained Midwestern soils. *Agronomy Journal* 94: 153 ï 171.

Elgood, Z., W.D. Robertson, S.L. Schiff, and R. Elgood. 2010. Nitrate removal and greenhouse gas production in a stream-bed denitrifying bioreactor. *Ecological Engineering*. 36 (11): 1575-1580.

Eller, D. 2015. Des Moines water quality suit slated for trial in 2016. *The Des Moines Register*. Accessed 29 November 2015. http://www.desmoinesregister.com/story/money/agriculture/2015/07/15/des-moines-water-works-lawsuit-buena-vista-calhoun-sac-counties-water-quality/30191169/

Fageria, N.K., and V.C. Baligar. 2005. Enhancing nitrogen use efficiency in crop plants. *Advances in Agronomy*. 88: 97-185.

Fenton, O., M.G. Healy, F. Brennan, M.M.R. Jahangir, G.J. Lanigan, K.G. Richards, S.F. Thornton, and T.G. Ibrahim. 2014. Permeable reactive interceptors: blocking diffuse nutrient and greenhouse gases losses in key areas of the farming landscape. *The Journal of Agricultural Science* 152: 71-81.

Feyereisen, G.W., and L.E. Christianson. 2015. Hydraulic flow characteristics of agricultural residues for denitrifying bioreactor media. *ASABE*. 31 (3): 89-96.

Feyereisen, G., T. Moorman, L. Christianson, R. Venterea, J. Coulter, and U. Tschirner. 2016. Performance of agricultural residue media in laboratory denitrifying biroeactors at low temperatures. *Journal of Environmental Quality.* 45 (3): 779 - 787.

Gaunt, J.L., and J. Lehmann. 2008. Energy balance and emissions associated with biochar sequestration and pyrolysis bioenergy production. *Environmental Science and Technology* 42 (11): 4152-4158.

Gerhardi, Michael H. 2007. ORP management in wastewater as an indicator of process efficiency. *NEIWPCC*. www.neiwppc.org/iwr/reductionpotential.asp. Accessed 29 July 2015.

Ghane, E., N.R. Fausey, and L.C. Brown. 2014. Non-Darcy flow of water through woodchip media. *Journal of Hydrology*. 519: 3400-3409.

Greenan, C.M., T.B. Moorman, T.B. Parkin, T.C. Kaspar, and D.B. Jaynes. 2009. Denitrification in wood chip bioreactors at different water flows. *Journal of Environmental Quality*. 38 (4): 1664-1671.

Healy, M.G., T.G. Ibrahim, G.J. Lanigan, A.J. Serrenho, and O. Fenton. 2012. Nitrate removal rate, efficiency and pollution swapping potential of different organic carbon media in laboratory denitrification bioreactors. *Ecological Engineering*. 40: 198 - 209.

Jansen, R.J.J., and H. van Bekkum. 1994. Amination and ammoxidation of activated carbons. *Carbon* 32 (8): 1507-1516.

Jaynes, D.B., T.C. Kaspar, T.B. Moorman, and T.B. Parkin. 2008. In situ bioreactors and deep drain-pipe installation to reduce nitrate losses in artificially drained fields. *Journal of Environmental Quality* 37 (2): 429-436.

Jin, C.-X., and G.R. Sands. 2003. The long-term field-scale hydrology of subsurface drainage systems in a cold climate. *ASAE* 46 (4): 1011-1021.

Lehmann, J., M.C. Rillig, J. Thies, C.A. Masiello, W.C. Hockaday, and D. Crowley. 2011. Biochar effects on soil biota - a review. *Soil Biology and Biochemistry* 43 (9): 1812-1836.

Length, R.V. 2015. Lsmeans: least-squares means. R package version 2.19. http://cran.r-project.org/package=lsmeans.

Miller, T.P., J.R. Peterson, C.F. Lenhart, and Y. Nomura. 2012. The Agricultural BMP Handbook for Minnesota. Minnesota Department of Agriculture.

Moorman, T.B., T.B. Parkin, T.C. Kaspar, and D.B. Jaynes. 2010. Denitrification activity, wood loss, and N_2O emissions over 9 years from a wood chip bioreactor. *Ecological Engineering* 36 (11): 1567-1574.

MPCA. 2014. The Minnesota nutrient reduction strategy. Accessed 5 January 2016. https://www.pca.state.mn.us/sites/default/files/wq-s1-80.pdf

Natarajan, M.A. 2015. Mercury methylation in denitrifying bioreactors: an investigation in pollution swapping. Thesis, 89 p.

Orlando, U.S., T. Okuda, A.U. Baes, W. Nishijima, and M. Okada. 2003. Chemical properties of anion-exchangers prepared from waste natural materials. *Reactive and Functional Polymers*. 55: 311-318.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R Core Team. 2014. Linear and nonlinear mixed effects models. R package version 3.1-117. http://cran.r-project.org/package=nlme.

Robertson, W.D., and L.C. Merkley. 2008. In-stream bioreactor for agricultural nitrate treatment. *Journal of Environmental Quality* 38 (1): 230-237.

Saleh, A., E. Osei, D.B. Jaynes, B. Du, and J.G. Arnold. 2007. Economics and environmental impacts of LSNT and cover crops for nitrate-nitrogen reduction in Walnut Creek Watershed, Iowa, using FEM and enhanced SWAT models. *Transactions of the ASABE* 50 (4): 1251 ï 1259.

Schipper, L.A., W.D. Robertson, A.J. Gold, D.B. Jaynes, and S.C. Cameron. 2010. Denitrifying bioreactors - an approach for reducing nitrate loads to receiving waters. *Ecological Engineering* 36 (11): 1532-1543.

Schipper, L., and M. Vojvodic-Vukovic. 2000. Nitrate removal from groundwater and denitrification rates in a porous treatment wall amended with sawdust. *Ecological Engineering* 14: 269-278.

Schmidt, C.A., and M.W. Clark. 2013. Deciphering and modeling the physicochemical drivers of denitrification rates in bioreactors. *Ecological Engineering* 60: 276-288.

Van Driel, P.W., W.D. Robertson, and L.C. Merkley. 2006. Upflow reactors for riparian zone denitrification. *Journal of Environmental Quality* 35 (2): 412-420.

Warneke, S. L.A. Schipper, M.G. Matiasek, K.M. Scow, S. Cameron, D.A. Bruesewitz, and I.R. McDonald. 2011a. Nitrate removal, communities of denitrifiers and adverse effects in different carbon substrates for use in denitrifiation beds. *Water Research*. 45 (17): 5463-5475.

Warneke, S., L.A. Schipper, D.A. Bruesewitz, I. McDonald, and S. Cameron. 2011b. Rates, controls and potential adverse effects of nitrate removal in a denitrification bed. *Ecological Engineering* 37 (3): 511-522.

Woli, K.P., M.B. David, R.A. Cooke, G.F. McIsaac, and C.A. Mitchell. 2010. Nitrogen balance in and export from agricultural fields associated with controlled drainage systems and denitrifying bioreactors. *Ecological Engineering* 36 (11): 1558-1566.

Tables:

Table 1.1: Mean (standard deviation) of nitrate-N outlet concentration at each hydraulic residence time (HRT) during the warm, cold, and rewarm runs for the six treatments.

Run	Temp (°C)	Treatment	1.5h HRT	8h HRT	12h HRT	24h HRT
			----------Nitrate-N outlet concentration (mg N L^{-1})----------			
Warm	14.6	CC	16.3 (1.4) b†	4.8 (2.9) c	2.8 (2.2) bc	1.5 (1.8) c
		CC+MC	15.2 (1.8) bc	3.8 (1.7) c	2.3 (1.1) bc	1.1 (0.8) c
		CC+MC+MBC	16.5 (1.8) b	7.0 (4.1) b	4.2 (3.6) b	1.4 (1.5) c
		WC	18.9 (1.0) a	15.6 (1.9) a	13.5 (2.2) a	5.1 (3.2) b
		WC+BC	19.5 (0.9) a	16.5 (1.1) a	15.2 (1.0) a	7.9 (1.8) a
		WC+A	14.0 (2.3) c	1.5 (2.8) d	0.9 (1.7) c	0.1 (0.1) c
Cold	5.5	CC	17.8 (0.8) a	14.5 (1.2) b	12.9 (1.6) b	7.7 (3.1) b
		CC+MC	17.7 (1.0) a	14.9 (1.4) b	13.5 (1.5) b	8.2 (2.2) b
		CC+MC+MBC	18.2 (1.0) a	15.3 (1.2) b	13.7 (1.2) b	9.1 (3.4) b
		WC	18.9 (0.8) a	18.5 (1.0) a	18.3 (0.8) a	17.1 (1.3) a
		WC+BC	19.3 (1.0) a	18.5 (0.8) a	18.9 (1.0) a	18.1 (2.2) a
		WC+A	15.0 (1.5) b	0.3 (0.3) c	0.3 (0.4) c	0.2 (0.1) c
Rewarm	14.9	CC	15.8 (1.9) b	3.3 (2.0) c	1.3 (0.9) c	0.8 (0.5) c
		CC+MC	15.6 (1.0) b	3.2 (1.7) c	1.3 (1.0) c	0.4 (0.4) c
		CC+MC+MBC	15.5 (1.0) b	3.3 (1.7) c	0.7 (0.4) c	0.2 (0.1) c
		WC	18.6 (0.7) a	13.9 (1.3) b	11.1 (1.8) b	5.7 (1.4) b
		WC+BC	19.1 (0.4) a	15.7 (0.8) a	13.5 (1.0) a	8.4 (1.2) a
		WC+A	2.0 (1.3) c	0.2 (0.2) d	0.2 (0.1) c	0.1 (0.1) c

† Within a column for a run, means followed by the same lowercase letter are not significantly different (P Ò 0.05).

Table 1.2: Mean (standard deviation) of nitrate removal rate (NRR) at each hydraulic residence time (HRT) during the warm, cold, and rewarm runs for the six treatments.

Run	Temp (°C)	Treatment	1.5h HRT	8h HRT	12h HRT	24h HRT
			----------NRR (g N m^{-3} d^{-1})----------			
Warm	14.6	CC	24.5 (8.3) c†	21.4 (3.6) ab	17.4 (2.3) a	9.3 (1.3) a
		CC+MC	32.1 (11.2) b	22.6 (2.4) ab	17.8 (2.0) a	9.4 (1.0) a
		CC+MC+MBC	23.2 (10.8) c	17.6 (4.5) b	15.4 (3.1) a	9.1 (1.0) a
		WC	6.5 (5.6) d	5.8 (2.5) c	6.4 (2.1) b	7.3 (1.3) a
		WC+BC	2.5 (5.9) d	4.6 (1.3) c	4.9 (1.0) b	6.0 (1.0) a
		WC+A	43.0 (19.7) a	26.7 (5.4) a	19.6 (3.3) a	10.2 (1.1) a
Cold	5.5	CC	10.1 (5.3) b	7.3 (1.4) b	7.4 (1.9) b	7.2 (1.7) b
		CC+MC	10.3 (5.7) b	6.6 (1.4) b	6.8 (1.6) b	6.9 (1.3) b
		CC+MC+MBC	7.0 (6.8) b	6.0 (1.2) b	6.4 (1.4) b	6.2 (1.7) b
		WC	2.2 (5.6) c	0.9 (1.2) c	1.5 (1.6) c	1.6 (0.6) c
		WC+BC	-0.7 (7.5) c	1.0 (0.8) c	0.8 (1.7) c	1.0 (1.0) c
		WC+A	29.9 (8.8) a	29.8 (2.0) a	21.3 (1.6) a	11.8 (1.3) a
Rewarm	14.9	CC	24.3 (8.3) c	22.1 (2.3) b	17.3 (1.1) a	9.8 (1.0) ab
		CC+MC	29.0 (5.1) b	23.3 (1.8) ab	18.1 (1.2) a	10.6 (0.9) a
		CC+MC+MBC	28.7 (5.7) bc	22.3 (1.9) ab	18.0 (1.4) a	10.3 (0.8) ab
		WC	7.1 (3.8) d	7.8 (1.6) c	7.8 (1.5) b	7.1 (0.9) ab
		WC+BC	4.6 (2.7) d	5.7 (0.7) c	6.0 (0.7) b	6.1 (0.8) b
		WC+A	120.6 (12.9) a	26.5 (2.7) a	18.4 (1.6) a	10.3 (0.8) ab

† Within a column for a run, means followed by the same lowercase letter are not significantly different (P Ò 0.05).

Table 1.3: Mean (standard deviation) of dissolved oxygen (DO) and pH as well as the oxidation-reduction potential (ORP) range during the warm, cold, and rewarm runs for the six treatments.

Run	Temp (°C)	Treatment	DO ($mg\ L^{-1}$)	pH	ORP (mV)
Warm	14.6	Inlet	7.1 (0.6)	7.3 (0.1)	NA
		CC	0.6 (0.9)	6.6 (0.4)	-163 to +425
		CC+MC	0.4 (0.5)	6.7 (0.4)	-320 to +380
		CC+MC+MBC	0.6 (0.8)	6.8 (0.3)	-241 to +354
		WC	0.9 (0.9)	7.2 (0.1)	-84 to +599
		WC+BC	0.9 (1.0)	7.1 (0.1)	+102 to +554
		WC+A	0.3 (0.3)	8.3 (0.7)	-459 to +112
Cold	5.5	Inlet	8.9 (0.6)	7.2 (0.2)	NA
		CC	2.5 (2.2)	6.9 (0.1)	+173 to +620
		CC+MC	2.3 (2.0)	7.0 (0.1)	+13 to +642
		CC+MC+MBC	2.6 (2.2)	6.9 (0.1)	+226 to +670
		WC	2.0 (2.6)	6.8 (0.1)	+73 to +661
		WC+BC	2.1 (2.4)	6.8 (0.1)	+218 to +670
		WC+A	0.3 (0.7)	9.1 (0.4)	-191 to +267
Rewarm	14.9	Inlet	8.3 (0.5)	6.8 (0.1)	NA
		CC	0.5 (0.8)	6.7 (0.2)	-163 to +401
		CC+MC	0.5 (0.8)	6.7 (0.2)	-225 to +335
		CC+MC+MBC	0.5 (0.7)	6.7 (0.2)	-168 to +325
		WC	0.5 (0.6)	6.8 (0.1)	-196 to +383
		WC+BC	0.7 (1.0)	6.8 (0.1)	-191 to +448
		WC+A	0.1 (0.1)	8.0 (0.7)	-279 to +368

Table 1.4: Mean (standard deviation) of flow weighted total carbon (TC), total organic carbon (TOC), and total inorganic carbon (TIC) production load over eight weeks of each temperature experiment. TIC production load is an estimate found by subtracting TOC load from TC load. Carbon data from the rewarm run are not presented due to equipment failure.

Run	Temp (°C)	Treatment	TC	TOC	TIC
			----------production load (g)----------		
Warm	14.6	CC	19.5 (4.0) a†	15.1 (3.9) a	4.4 (0.1) ab
		CC+MC	17.0 (3.2) a	13.0 (3.1) a	4.0 (0.4) ab
		CC+MC+MBC	13.3 (5.8) a	9.5 (5.0) a	3.8 (0.8) b
		WC	2.9 (0.4) b	1.1 (0.2) b	1.7 (0.2) c
		WC+BC	2.8 (1.0) b	1.4 (0.9) b	1.4 (0.2) c
		WC+A	17.2 (0.1) a	12.2 (0.2) a	5.0 (0.2) a
Cold	5.5	CC	3.5 (0.8) b	1.3 (0.4) b	2.2 (0.4) b
		CC+MC	3.3 (0.3) b	1.2 (0.1) b	2.0 (0.2) b
		CC+MC+MBC	3.0 (0.4) b	1.1 (0.2) b	1.9 (0.3) b
		WC	1.4 (0.1) c	0.4 (0.1) b	1.0 (0.1) c
		WC+BC	1.3 (0.1) c	0.4 (0.1) b	0.9 (0.0) c
		WC+A	13.2 (0.4) a	8.6 (0.9) a	4.7 (0.6) a

† Within a column for a temperature run, means followed by the same lowercase letter are not significantly different (*P* Ò0.05).

Table 1.5: Mean (standard deviation) of percent carbon, percent nitrogen, and C:N of original carbon materials and carbon materials after the rewarm run (n=2). Modified biochar data were excluded because there was insufficient sample mass at conclusion of experimental run.

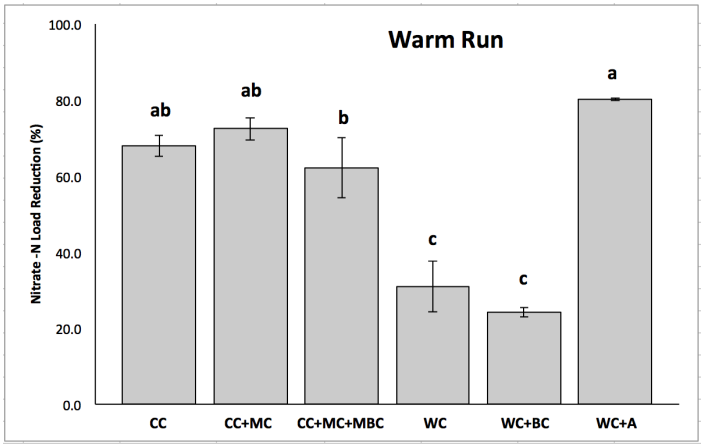
Media	Treatment	Carbon (%)	Nitrogen (%)	C:N
Corn cobs	Original	44.7†	0.3†	157.6†
	CC	43.7 (1.4)	0.4 (0.1)	116.6 (25.1)
	CC+MC	45.0 (0.0)	0.4 (0.0)	115.0 (7.2)
	CC+MC+MBC	43.5 (1.9)	0.4 (0.1)	112.5 (19.8)
Modified coconut coir	Original	45.5	0.8	54.8
	CC+MC	45.2	1.1	40.0
	CC+MC+MBC	42.9	1.1	40.8
Wood chips	Original	46.9	0.2	299.0
	WC	46.3 (0.2)	0.3 (0.1)	169.5 (52.6)
	WC+BC	45.7 (0.4)	0.3 (0.1)	163.2 (36.6)
	WC+A	47.3 (0.8)	0.2 (0.0)	229.3 (26.8)
Hardwood Biochar	Original	29.3	0.3	108.0
	WC+BC	32.5 (10.3)	0.2 (0.0)	146.8 (66.9)

† Values with no standard deviation are from only one set of samples

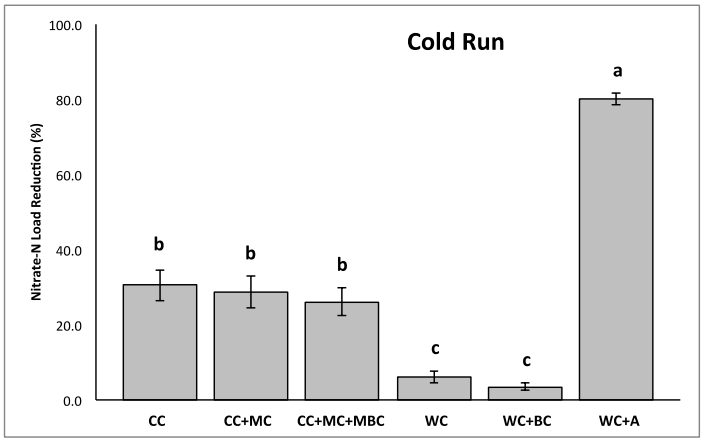
Figures:

Figure 1.1: Mean cumulative nitrate-N load reduction as a percentage for treatments in the a) warm run, b) cold run, and c) rewarm run. Error bars signify standard deviation. Within a temperature run, columns with the same lowercase letter indicate no significant difference between treatments (*P* Ò0.05).

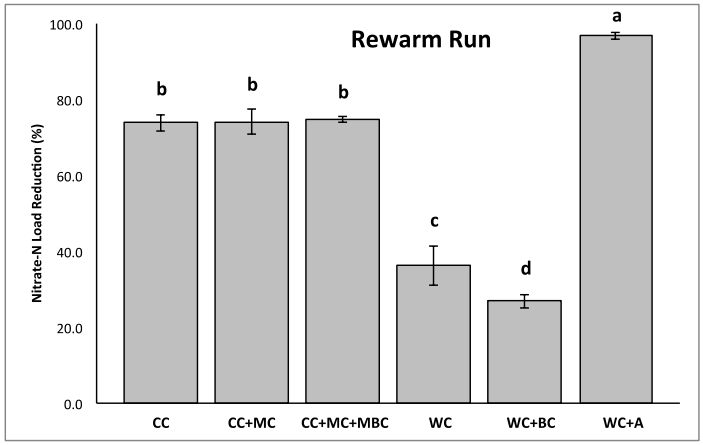
a)



b)



c)



Chapter 2. Phosphorus uptake in denitrifying bioreactors in a laboratory column experiment

Summary

Phosphorus is a limited resource in aquatic ecosystems and excess can lead to eutrophication. To mitigate excess phosphorus coming from agricultural cropland drainage, especially during cold temperatures and high flow conditions, drainage ditch bioreactors can be utilized. Bioreactors are typically employed for mitigating nitrate-N via denitrifying microorganisms, but dissolved reactive phosphorus (DRP) concentrations can potentially be reduced simultaneously through adsorption, precipitation, or microbial uptake. The DRP removal performance of candidate treatments ï corn cobs (CC), corn cobs with modified coconut coir (CC+MC), corn cobs with modified coconut coir and modified macadamia shell biochar (CC+MC+MBC), wood chips (WC), wood chips with hardwood biochar (WC+BC), and wood chips with continuous sodium acetate injection (WC+A) ï were tested in an upflow laboratory column experiment at two temperatures and various hydraulic residence times (HRT). Cumulative DRP load reduction was highest for the corn cob treatments - CC, CC+MC, and CC+MC+MBC - at 15°C, with 74.3%, 81.3%, and 67.4% load reduction, respectively. The WC+A treatment had the highest DRP load reduction at 5°C with 45.1% and a DRP concentration reduction of 36.5% during the highest flow rate at 5°C. WC+A would be the best treatment for removing DRP from effluent water in cold conditions and high flow conditions. Microscopy and EDS point analysis indicate that the mechanism for phosphorus removal was likely microbial uptake, and further study should be done to determine if DRP is susceptible to biological release from WC+A bioreactors.

1. Introduction

Phosphorus is a necessary nutrient for many organisms as it is used in the production of important cell components such as DNA, phospholipids, and ATP (Sylvia et al., 2005). Phosphorus can be a limited resource for freshwater aquatic organisms (Baker et al., 1998) and excess phosphorus in aquatic systems can stimulate excess algal growth, thus negatively affecting both local and downstream ecosystems through eutrophication (Correll, 1998). Sources of phosphorus can include animal manure, stormwater, and soil erosion (USEPA, 2015); 25% of phosphorus delivered to the Mississippi River Basin comes from corn and soybean acres (Alexander et al., 2008). It is vital to maintain water quality in water bodies for ecological as well as economic reasons; for example, 70% of Minnesotans use the stateôs lakes for recreation (Anderson et al., 1999) and recreational tourism accounts for approximately 15% of overall state tourism spending (Tourism Economics, 2013).

In order to reduce phosphorus loss from agricultural land, conservation strategies should be explored. Strategies can include in-field changes in nutrient management such as adjustments to fertilizer type and timing (MPCA, 2014). However, even when no fertilizer is used in a field, there can still be nutrient loss due to the properties inherent to the soil and drainage (Dinnes et al., 2002; Kovacic et al., 2006). Mitigation strategies will need to include edge-of-field structures such as wetlands and bioreactors to intercept nutrient loss from fields. Wetlands are effective at reducing phosphorus concentrations, but can be expensive, take up a lot of land area, and become less effective at removing

phosphorus over time (Kovacic et al., 2006; Ann et al., 2000). Agricultural denitrifying bioreactors have the potential to be a better edge-of field strategy; they do not take land out of crop production (Jaynes et al., 2008) and are cost effective compared to other mitigation strategies (Christianson et al., 2012b). Currently, most bioreactor studies focus on removal of nitrate-N via microbial denitrification (Christianson et al., 2012a; Feyereisen et al., 2016; Jaynes et al., 2008; Moorman et al., 2010; Robertson and Merkley, 2008; Woli et al., 2010), but there is some evidence that phosphorus can also be removed via bioreactors (Bock et al., 2014; Goodwin et al., 2015; Zhang, 2015).

There are several possible mechanisms by which phosphorus, specifically dissolved reactive phosphorus (DRP), could be removed from water in a bioreactor: precipitation, adsorption, and microbial uptake. Precipitation involves the DRP anion combining with cations to form a stable phosphate mineral and adsorption is the anion bonding with surface hydroxyl groups to form stable complexes (Baker et al., 1998). Since bioreactor bed media is usually a carbon-based material with little to no charge such as wood chips and corn cobs, a material with a high anion exchange capacity could be added to the bioreactor. Manufactured anion-exchanger products are often expensive but can be produced in the laboratory from carbon-based materials such as biochar and coconut coir (Baes et al., 1997; Jansen and van Bekkum, 1994; Orlando et al., 2003). Some bioreactor studies have achieved phosphate-P removal with calcium-rich material such as gypsum and crushed concrete (Bryant et al., 2012; Egemose et al., 2012) or iron-rich material such as steel slag (Penn et al., 2012). However, initial testing showed these materials

substantially increase alkalinity in treatment water (Figure A.7). This could create detrimental conditions for the denitrifying microbes or the downstream aquatic ecosystem, depending on if the material was placed before or after the carbon-based material intended for supporting denitrification. If phosphorus removal and denitrification are to occur simultaneously in a bioreactor, then conditions should not be detrimental to the denitrifying microorganisms. Furthermore, there is evidence that denitrifiers can be phosphorus-accumulating organisms (PAOs) (Kuba et al., 1993; Kerrn-Jespersen et al., 1994; Soejima et al., 2006). PAOs are a select number of organisms capable of storing phosphate as polyphosphate in their microbial bodies (Kerrn-Jespersen et al., 1994). Soluble carbon such as sodium acetate can be added to increase carbon availability and therefore enhance phosphorus removal efficiencies via denitrifying PAOs (Soejima et al., 2006).

Denitrifying bioreactors treating agricultural water typically are connected to the end of tile drainage lines (Christianson et al., 2012a; Jaynes et al., 2008; Moorman et al., 2010; Woli et al., 2010), but could also be placed in agricultural drainage ditches to treat water at a larger scale (Robertson and Merkley, 2008); tile drainages often empties into drainage ditches along agricultural fields, and ditches could be a location to treat more water with one structure. Macrae et al. (2007) found that phosphorus loading from tile drainage is greater during high flow events and snowmelt/thawing events.

We sought to combine various carbon-based materials to create a bioreactor that could remove phosphate-P during high flow events and cold temperatures. Our objective was to evaluate and identify carbon materials with the ability to mitigate phosphate-P in a drainage ditch environment. We hypothesized that 1) biochar and liquid sodium acetate additions to wood chips would increase phosphate-P removal when compared to wood chips alone, and 2) corn cob bioreactor phosphate-P removal would be enhanced by amine modified coconut coir and biochar additions.

2. Methods

Eighteen columns of PVC pipe (15.2 cm i.d. X 49.5 cm long) were packed with six unique treatments (n=3) in an upflow laboratory column experiment. The six treatments were: (i) wood chips (WC), (ii) wood chips and woodchip biochar (WC+WBC), (iii) woodchips and sodium acetate (WC+A), (iv) corn cobs (CC), (v) corn cobs and modified coconut coir (CC+MC), and (vi) corn cobs, modified coconut coir, and modified macadamia biochar (CC+MC+MBC). Wood chips were approximately 13 ï 25 mm and were composed of a mix of hard and soft woods (Mulch Store (Empire, MN)). Corn cobs were typically 5 ï 15 cm long and were obtained from the University of Minnesota ï Morrisô inventory of corn cobs intended for the campus biomass-fueled boiler. The hardwood biochar (Cowboy Charcoal) was mostly 13 ï 25 mm in size, similar to the wood chips, while the macadamia biochar (Biochar Brokers) was mostly < 6 mm in size. Chemical and physical properties of the biochars are listed in Table A.1. Coconut coir was obtained from J. Alderink LLC (Oak Park, MN) and fibers were typically 5 ï 15 cm

long. Complete particle size descriptions are given in Table A.2. Coconut coir and macadamia biochar media were modified using an amination procedure similar to one described in Jansen and van Bekkum (1994). A solution of 10% nitric acid was added to a beaker of material and heated at 90°C for 4 hours. Material was then rinsed 10 times with DI water, or until pH was circumneutral. Baking soda was used to reach neutrality for all batches of materials.

Columns were built out of Schedule 40 PVC pipe with corresponding caps for top and bottom of pipe. A PVC plate (1.3 cm width) with holes drilled in for drainage was screwed onto bottom of pipe, a corresponding cap was glued over pipe end with plate, and then a stainless steel mesh wire circle was placed on top of PVC plate. Before packing columns, materials were oven dried at 70°C for 48 hours and then soaked in tap water for 48-72 hours. Materials were allowed to drip dry for 10 minutes and then packed in batches using the following method: batches of 400g of corn cobs or woodchips were packed into columns, with an 1812 g weight being used to tamp down each batch layer. 1g of biological inoculum (Biofloc^{TM} Biologicals, Innovative Turf Solutions, Cincinnati, OH) was weighed and divided between all batch layers before packing to provide denitrifiers for the columns. See Appendix II for more information on biological inoculum. Single material treatments had ~10 layers. Columns with treatments consisting of several carbon materials were packed in alternating layers. Percent by weight of different carbon materials was calculated for mixed treatments to determine how much modified coir or biochar was mixed in with the wood chips or corn cobs (Table A.2).

Once all layers were packed into columns, a stainless steel mesh was placed on top of material and another PVC plate was tapped down on top, leveled, and screwed into place. Columns were capped using silicon caulk and glue and allowed to set for 24 h. Sub-samples were used to measure moisture content in order to determine dry mass of materials in columns. Drainable porosity was found for each column by filling columns with water, topping them off in the morning to make sure media were saturated, and then draining columns for 24h. Sensors measuring oxidation-reduction potential (ORP) were screwed into top cap and were connected to a multiplexer controlled by a data logger that took readings every hour.

The experiment was conducted for fourteen weeks at 15°C (i.e., ñwarm runò) and for thirteen weeks at 5°C (i.e., ñcold runò) in a controlled temperature chamber. A second 15°C experiment (i.e., ñrewarm runò) was conducted for 7 weeks to verify results were not influenced by loss of labile carbon from the warm to the cold run, since media were not changed between temperature experiments. Flow rates were adjusted to approximate the rise and decay of a typical drainage hydrograph in Morris, MN. The rise and decay of flow rates were fit to a weekly flow regime and were associated with hydraulic residence times (HRT) of 1.5, 8, 12, and 24 h (see Figure A.1) with durations for 6, 42, 48, and 72 h, respectively. Phosphate-P and nitrate-N influent concentrations were 0.3 mg P L^{-1} as potassium phosphate and 20 mg N L^{-1} as potassium nitrate, respectively, in reverse osmosis water. Sodium acetate was pumped into the three WC+A columns at a final concentration of 40 mg C L^{-1} using a separate pump at 1% of the main pump speed.

Influent and effluent water was sampled at each of the 4 flow rates and tested for dissolved reactive phosphorus (DRP), nitrate-N, ammonia-N, total carbon (TC), total organic carbon (TOC), and pH. Air and water temperatures were taken hourly.

Dissolved oxygen (DO) was measured 4 times weekly during water sampling by the following method. The hand-held DO probe (HI 9142 (meter), HI 76407/4 (probe), Hanna Instruments, Woonsocket, RI) was inserted into a syringe body that had been connected via 6-mm diameter tubing to a valve on the top cap of the columns. The column effluent outlet valve was closed and the valve to the syringe body simultaneously opened to permit flow of water into the syringe body. Flow rate was changed to 10 mL min^{-1} to allow fresh water to flow continuously next to the probeôs membrane. There was minimal space between the wall of the syringe and the probe to reduce the potential for oxygen diffusion.

Water samples were collected in 250mL polyethylene bottles every week at the end of each HRT and preserved with sulfuric acid (Cleresci et al., 1998) for analysis using colorimetric flow injection analysis (Lachat QuikChem 8500, Hach Co., Loveland, CO) for nitrate-N, ammonia-N, and DRP. Method number for DRP analysis was 10-115-01-1-A. Analyses of TC and TOC were done using EPA Methods 415.1 and 9060A (Tekmar-Dohrmann Phoenix 8000, Teledyne Tekmar, Mason, OH). Unacidified water samples were collected and measured for pH.

Between each temperature run, there was a brief shut-down period. During this time, caps were removed and media samples (30-90g wet weight, ~20g dried weight) were taken for carbon and microbial analysis from each column and stored at 4°C and -80°C. Columns were then prepared for plumbing and capping, and temperature was changed as needed.

The DRP inlet and outlet concentration data were used to find DRP concentration reduction and cumulative DRP load reduction. DRP concentration reduction as a percentage was found for each column by dividing the inlet concentration from the outlet concentration. Cumulative DRP load reduction as a percentage was found for each column by using the DRP mass into (P_{in}) and out of (P_{out}) the bioreactor. For each column, the P_{in} mass was determined by calculating the volume of water that passed through the column by the concentration of DRP at the inlet, and P_{out} was the volume of water that passed through the column multiplied by the DRP concentration of that column. Cumulative DRP load reduction was calculated by first summing the P_{in} mass and summing the P_{out} mass during the entire temperature run, and then taking 1 ï (ΣP_{out}/ ΣP_{in}). Load reductions for each column were then averaged by treatment.

Total carbon (TC) and total organic carbon (TOC) production were both calculated for each column as the difference between the flow weighted grams of carbon in and carbon out of the bioreactor over the entire temperature experiment. Total inorganic carbon (TIC) was calculated for each column as the difference between TC production and TOC production for that column.

Once the experimental runs were complete, data were prepared for statistical analysis. The first two weeks of each temperature experiment were excluded from analysis, as well as any weeks that had instrumental or experimental error (water pump failure, acetate pump failure, low water level, etc.). In all, this gave eight weeks of data for the warm and cold runs, and six weeks for the rewarm run. DRP concentration, DRP concentration reduction, DRP cumulative load reduction, TC, TOC, and TIC were analyzed with a linear mixed-effects model in R using nlme package (Pinheiro et al, 2014). The linear mixed-effects model was run with and without a correlation argument, and fit criteria AIC and BIC showed that the correlation argument had the higher quality of statistical model in all analyses. Differences in HRT within treatment for DRP concentration and DRP concentration reduction data, and treatment differences for all selected data were determined by running Tukey multiple comparisons using lsmeans package (Lenth, 2015) at a significance level of P Ò0.05. See Appendix III for R code.

To investigate fate of phosphorus, precipitation, adsorption, or microbial uptake, several analyses were carried out. To investigate biological uptake of phosphorus, bioreactor samples (n=2) were prepared for DAPI (4ô,6-diamidino-2-phenylindole) staining through an elutriation method similar to one used in Whitman et al. (2003): Caliber Tween 20 gelatin (1 mL) was added to 1L of phosphate-buffered saline (PBS) solution. This mixture was added to bioreactor samples in centrifuge tubes and shaken at 400 RPM for 20 min on a tabletop shaker (New Brunswick Scientific G-2, Edison, NJ). Tubes were

then left to sit for 20 min, and supernatant was transferred to another centrifuge tube and centrifuged at 7000 (5860 x g) for 15 min. Supernatant was then disposed and pellet saved.

Pellets were re-suspended and pipetted onto a labeled well place. Ethanol was placed on top of wells, and slide was allowed to dry. Fluorescent stain DAPI (4ô,6-diamidino-2-phenylindole) was added to each well, and placed in a hybridization chamber made of a Falcon tube and Kimwipe wetted with distilled water. The hybridization chamber was placed under a box to keep it dark and left there for 30 min. Slide was then dunked in a tube of DI water 10 times using a tweezers. Slide was brusquely shaken to remove excess water per method instructions, and then allowed to dry. To prepare slide for microscopy, a cover slip was adhered to the well plate using Vectashield antifade mounting medium (Vector Laboratories, Burlingame, CA). Procedure was carried out in a room with limited light exposure. At least one well was prepared using a standard known to contain polyphosphate. Slide was then viewed under a microscope for evidence of polyphosphate, and photographs were taken.

To investigate precipitation and adsorption of phosphorus, scanning electron microscope (SEM) imaging was done for the two treatments that exhibited the highest phosphate removal from effluent water during all temperature runs: CC+MC and WC+A. Samples taken after the cold run were coated with 100-micron carbon to increase conductivity of material. Once images were taken, elemental composition was determined using Energy

Dispersive X-ray Spectroscopy (EDS) point analysis method. As EDS is a semiquantitative analysis tool, relative elemental presence can be deduced from differences in peak heights.

3. Results

3.1 Experimental Conditions

Mean water temperatures were 14.6 ± 0.8°C, 5.5 ± 0.5°C, and 14.9 ± 0.4°C for the warm, cold, and rewarm runs, respectively. Mean inlet phosphate-P concentration for all experimental runs was 0.23 ± 0.05 mg L^{-1}. Mean sodium acetate concentration was 46.83 ± 8.23 mg L^{-1}. Average HRT in the columns at the four flow rates (1.5, 8, 12, and 24h HRT) was 1.8, 8.8, 12.5, and 25.3h in the warm run, 2.0, 9.1, 12.9, and 24.1h in the cold run, and 1.8, 8.9, 12.7, and 22.6h in the rewarm run (Table A.3). Nitrogen data are discussed in Chapter 1.

3.2 DRP Concentration

Effluent DRP concentrations in the warm and rewarm runs for all treatments decreased from the inlet concentration at all HRT (Table 2.1). The warm run had mean effluent DRP concentrations of 0.05 ï 0.14 mg L^{-1} at 24h HRT and 0.04 ï 0.21 mg L^{-1} at 1.5h HRT, the cold run had mean DRP concentrations of 0.10 ï 0.17 mg L^{-1} at 24h HRT and 0.16 ï 0.24 mg L^{-1} at 1.5h HRT, and the rewarm run had mean DRP concentrations of 0.07 ï 0.17 mg L^{-1} at 24h HRT and 0.07 ï 0.19 mg L^{-1} at 1.5h HRT. In both the warm and rewarm run all corn cob treatments (CC, CC+MC, and CC+MC+MBC) generally

had the lowest DRP effluent concentrations. During the 1.5h HRT in the warm run, CC+MC had a lower DRP concentration than the CC+MC+MBC treatment, but otherwise all corn cob treatments were similar. WC and WC+BC had the highest effluent DRP concentrations in the warm and rewarm runs, except during 12 and 24h HRT in the rewarm run when WC was similar to all corn cob treatments. The DRP concentrations for WC+A were significantly lower than the WC and WC+BC treatments at 1.5h and 8h HRT during the warm and cold runs, and at 1.5h HRT during the rewarm run.

For the cold run, effluent DRP concentrations decreased from the inlet for all HRT except 1.5h, where all treatments but WC+A had concentrations as high as the inlet DRP concentration. The WC+A effluent DRP concentration was lower at 1.5h and 8h HRT; all other treatments were higher and statistically similar to each other. During 12h HRT all the treatments were statistically similar. For the 24h HRT, the CC was statistically lower than the WC, WC+BC, and WC+A treatments.

The effluent DRP concentration within a treatment was generally more variable in the cold run than in the warm and rewarm runs between HRT. The CC+MC+MBC and WC+BC treatments had higher DRP concentrations at 1.5h HRT than any other HRT in the warm run, but CC and WC+A were similar in all HRT. In the cold run all treatments had the highest DRP concentrations at the 1.5h HRT except WC+A, which had a statistically similar values at 1.5 and 24h HRT. The CC+MC treatment was the only one

in the rewarm run to have statistically similar DRP concentrations at all HRT, but WC+A was the only treatment to have the lowest DRP concentration at 1.5h than any other HRT.

3.3 DRP concentration reduction

The DRP concentration reduction as a percentage for all treatments in the warm run was 35.9 ï 76.3% at 24h HRT and 0.1 ï 78.9% at 1.5h HRT, in the cold run 24.5 ï 55.8% at 24h HRT and 2.0 ï 36.5% at 1.5h HRT, and in the rewarm run 29.1 ï 71.0% at 24h HRT and 17.9 ï 67.8% at 1.5h HRT (Table 2.2). In the warm run, all corn cob treatments had the highest DRP concentration reduction for all HRT, except that at 1.5h HRT CC+MC was higher than CC+MC+MBC. All wood chip treatments (WC, WC+BC, and WC+A) were statistically similar to each other at 12h and 24h HRT, but at 1.5h and 8h HRT WC+A concentration reduction values were higher than WC+BC and WC. In the cold run, WC+A had the highest DRP concentration reduction under 1.5h and 8h conditions and all other treatments were lower and statistically similar. CC and CC+MC treatments had the highest reduction under 24h conditions and all wood chip treatments were lower and similar. In the rewarm run, the treatments with the highest DRP concentration reduction during the 1.5h HRT were CC, CC+MC and WC+A. At all other HRT, WC+A was lower than CC and CC+MC treatments and all corn cob treatments had higher concentration reduction for 8h, 12h, and 24h HRT. WC and WC+BC had the lowest DRP concentration reduction at 1.5h but were similar to WC+A for all other HRT.

The DRP concentration reduction within a treatment followed the same general trend as the effluent DRP concentration for differences between HRT; the cold run had more variable values between HRT than the rewarm and warm runs. The CC, CC+MC, and WC+A all had statistically similar percent reduction for all HRT. The WC and WC+BC treatment both had their highest DRP concentration reduction at 24h, and WC+BC had a reduction at 1.5h that was lower than the reduction at 12h and 24h HRT. During the cold run, all treatments had their lowest DRP concentration reduction at 1.5h HRT, except the WC+A treatment, which had values at 1.5h and 24h similar to each other. Treatments in the rewarm run also generally had their lowest DRP concentration reduction value at 1.5h HRT. WC+A was the only treatment that had a significantly higher reduction value at 1.5h HRT than any other HRT.

3.4 Cumulative DRP Load Reduction

Cumulative DRP load reduction for all treatments was 17.8 ï 81.3 % in the warm run, 24.4 ï 45.1 % in the cold run, and 34.0 ï 72.5 % in the rewarm run (Figure 2.1). CC, CC+MC, and CC+MC+MBC had the highest cumulative load reduction in the warm run, with WC+A being the second highest and WC and WC+BC being the lowest. In the cold run, WC+A had the highest load reduction and all other treatments were statistically similar. The rewarm run was similar to the warm run, except that CC and CC+MC+MBC were statistically similar to WC, WC+BC, and WC+A; CC and CC+MC+MBC were elevated compared to other treatments but not statistically different because of high standard deviation seen in CC+MC+MBC and WC+A treatments.

3.5 pH

Mean inlet pH was 7.3 during the warm run, 7.2 during the cold run, and 6.8 during the rewarm run (Table 2.3). Effluent pH in the WC+A treatment ranged from 7.0 and 9.4 in the warm and rewarm runs and between 8.2 and 9.6 in the cold run, with pH being higher during the 1.5h HRT than any other HRT (Figure A.2). All other treatments had effluent pH readings of ~7.0 which did not differ from the inlet.

3.6 Dissolved Oxygen

Mean DO at the inlet was 7.1, 8.9, and 8.3 mg O L^{-1} for the warm, cold, and rewarm runs, respectively (Table 2.3). All treatments during warm and rewarm runs had mean DO concentrations of <1 mg O L^{-1}, but during the cold run WC+A the DO concentration was 0.3 mg O L^{-1} while the remaining treatments ranged from 2.0 to 2.6 mg O L^{-1}.

3.7 Polyphosphate

Evidence of polyphosphate being stored inside microorganisms was observed in all treatment and control samples. Examples of microscopy from CC+MC and WC+A samples after the cold run are given in Figure 2.2. No quantification was done to determine number of cells that had evidence of polyphosphate versus number of cells that did not.

3.8 SEM/EDS Analysis

Multiple SEM images were taken of CC+MC and WC+A treatment samples (Figure 2.3). EDS point analysis did not detect any significant amounts of phosphorus in samples from the CC+MC or WC+A treatments (Figure 2.4).

4. Discussion

4.1 Dissolved reactive phosphorus data

The data support our hypothesis that DRP concentration reduction for wood chips could be improved by the addition of sodium acetate. During the warm and rewarm runs the WC+A treatment removed over twice as much DRP as the WC treatment at the 1.5h HRT, and during the cold run the WC+A had higher reductions than WC at 1.5, 8, and 12h HRT. Cumulative DRP load reduction for WC+A was greater than WC in both the warm and cold runs, but WC+A and WC values were statistically similar in the rewarm run. This could be due to the fact that the WC treatment had greater DRP load reduction in the rewarm run, or that WC+A had a high standard deviation for DRP load reduction. However, the biochar addition to the wood chips did not improve DRP concentration reduction performance when compared to the wood chip only bioreactor. No differences were revealed between WC and WC+BC treatments in cumulative DRP load reduction either.

The addition of amine modified coconut coir and biochar did not improve the DRP concentration reduction in corn cob bioreactors. For the corn cob treatments, CC+MC concentration reduction was statistically similar to that of CC at all HRT in all temperature treatments. The same is true for CC+MC+MBC except in the warm run when the DRP concentration reduction at 1.5h HRT was statistically lower than the CC+MC treatment. At 1.5h HRT in the rewarm run, the CC+MC+MBC and CC treatments were similar. This could be due to the concentration reduction data from CC+MC+MBC having a high standard deviation (Table 2.2).

The cumulative DRP load reduction data showed a similar trend; the CC+MC and CC+MC+MBC treatments were statistically similar to the CC treatment values. It could be that there was not enough modified material added to the corn cobs to increase anion exchange capacity appreciably, and this is why there was no observed increase relative to the other materials. Baes et al. (1997) found that anion contaminants could be mitigated using amine modified coconut coir, but they used coconut coir dust, which would not work well in a high-flow, high-volume drainage ditch scenario.

The biochar media used in our experiment performed differently than other biochar materials used in other bioreactor experiments. Bock et al. (2014) found 65% phosphorus removal from hardwood biochar at 18h HRT, and Zhang (2015) reported an average of 80% phosphorus reduction at 24h HRT. Beck et al. (2011) found that soil amended with biochar had a 42% reduction of total phosphorus. The highest mean DRP concentration

reduction seen in the WC+BC treatment was 43% at 24h HRT in the rewarm run, and cumulative DRP load reduction means were 17.8%, 25.4% and 34.0% in the warm, cold, and rewarm runs, respectively.

The corn cob treatments and WC+A had the highest DRP concentration reduction and cumulative load reduction values among treatments. In the warm run CC, CC+MC, and CC+MC+MBC all had higher load reductions than any wood chip treatment. In the rewarm run, CC+MC was the only corn cob treatment that had a higher load reduction than the wood chip treatments. WC+A had the highest load reduction of all treatments in the cold run. Both corn cob treatments and WC+A would potentially work well at removing DRP in a drainage ditch denitrifying bioreactor, but corn cob treatments have higher DRP load reduction than WC+A in the warm and rewarm runs so could be the better option. However, greater phosphorus loading of water bodies occurs under high flow and cold temperatures (Macrae et al., 2007) and WC+A had the highest DRP load reduction during the cold run and was the only treatment to have a higher DRP concentration reduction at 1.5h HRT than any other HRT. Our laboratory experiment wasn't able to perfectly mimic field conditions; we did not increase DRP concentrations with lower HRT. However, these results suggest that WC+A would be the ideal candidate for testing in a drainage ditch setting.

4.2 Mechanism for phosphate-P removal

The DAPI and EDS results indicate that a major mechanism of phosphate-P removal in these laboratory columns could be microbial uptake via denitrifying PAOs. There is evidence from the DAPI staining of polyphosphate storage in microbial bodies in all treatments, and the EDS point analysis found no phosphorus on the materials. Microbial uptake as the mechanism is supported by the nitrogen results presented in Chapter 1: WC+A had both the highest cumulative nitrate-N and DRP load reduction in the cold run, and corn cob treatments had comparable load reduction percentages for both nitrate-N and DRP. Also, the fact that the DRP load reduction values for the WC and WC+BC treatments were lower for corn cob treatments in the warm run and CC+MC in the rewarm run could point to the presence of denitrifying PAOs. Wood chip bioreactors have a smaller abundance of denitrifying microorganisms and lower rates of nitrate removal when compared to corn cob bioreactors at 15.5°C and 1.5°C (Feyereisen et al., 2016). Other systems with PAOs are usually exposed to alternating anaerobic and aerobic conditions (Kuba et al., 1993; Kerrn-Jespersen et al., 1994; Soejima et al., 2006), while our bioreactors operated continuously in an anaerobic state during the temperature runs. Further microbial studies should be done to look at community structure and confirm presence of PAOs. Also, if the mechanism for phosphorus removal is in fact microbial uptake, it is unknown what will happen to this stored polyphosphate if the bioreactors should freeze or dry out. Further study should be done with wetting and drying cycles to determine if there is a flush in phosphorus from the bioreactors.

4.3 Potential for Adverse Effects

It is possible that our sodium acetate additions to the wood chip bioreactor were greater than necessary for complete denitrification and phosphorus uptake by microorganisms. WC+A had the highest total carbon (TC) and total organic carbon (TOC) load production in the cold run and one of the highest in the warm run (Table A.6), and nitrate removal rates were very high for WC+A (Table 1.2) so denitrification was clearly being carried out. Too much carbon in the environment has the potential to negatively affect aquatic ecosystems (Correll, 1998). Further laboratory experiments should be done before this treatment is recommended for field remediation.

5. Conclusions

The data supported our hypothesis that sodium acetate additions could improve phosphorus removal in a wood chip denitrifying bioreactor. However, the addition of biochar or amine modified material at the levels used in this experiment did not improve DRP load reduction in corn cob or wood chip bioreactors. WC+A would be the best treatment option for a ditch bioreactor; the corn cob treatments had higher DRP load reduction in 15°C conditions, but WC+A had the highest DRP reduction in the 5°C conditions and high flow rates, which is when the phosphorus load of surface waters is of most concern. Microscopy and EDS point analysis indicate that the mechanism for phosphorus removal was likely microbial uptake, but further microbial work is needed to confirm this. Further research should also be done to determine if DRP is susceptible to biological release from these bioreactors.

References:

Alexander, R.B., R.A. Smith, G.E. Schwarz, E.W. Boyer, J.V. Nolan, and J.W. Brakebill. 2008. Differences in phosphorus and nitrogen delivery to the Gulf of Mexico from the Mississippi River Basin. *Environmental Science and Technology*. 42 (3): 822-830.

Anderson, K.A., T.J. Kelly, R.M. Sushak, C.A. Hagley, D.A. Jensen, and G.M. Kreag. 1999. Summary report on public perceptions of the impacts, use, and future of Minnesota lakes: results of the 1998 Minnesota lakes survey. A joint publication by the University of Minnesota Sea Grant Program (SH 1) and Minnesota Department of Natural Resources, Office of Management and Budget Services. 1-31.

Ann, Y., K.R. Reddy, and J.J. Delfino. 2000. Influence of chemical amendments on phosphorus immobilization in soils from a constructed wetland. *Ecological Engineering*. 14 (1-2): 157-167.

Baes, A.U., T. Okuda, W. Nishijima, E. Shoto, and M. Okada. 1997. Adsorption and ion exchange of some groundwater anion contaminants in an amine modified coconut coir. *Water Science and Technology*. 35 (7): 89-95.

Baker, M.J., D.W. Blowes, and C.J. Ptacek. 1998. Laboratory development of permeable reactive mixtures for the removal of phosphorus from onsite wastewater disposal systems. *Environmental Science and Technology.* 32 (15): 2308ï 2316.

Beck, D.A., G.R. Johnson, and G.A. Spolek. 2011. Amending greenroof soil with biochar to affect runoff water quantity and quality. *Environmental Pollution*. 159: 2111-2118.

Bock, E., N. Smith, M. Rogers, B. Coleman, M. Reiter, B. Benham, and Z.M. Easton. 2014. Enhanced nitrate and phosphate removal in a denitrifying bioreactor with biochar.ò *Journal of Environment Quality*. 44 (2): 605-613.

Bryant, R.B., A.R. Buda, P.J.A. Kleinman, C.D. Church, L.S. Saproito, G.J. Folmar, S. Bose, and A.L. Allen. 2012. Using flue gas desulfurization gypsum to remove dissolved phosphorus from agricultural drainage waters. *Journal of Environmental Quality*. 41: 664-671.

Christianson, L.E., A. Bhandari, and M.J. Helmers. 2012a. A practice-oriented review of woodchip bioreactors for subsurface agricultural drainage. *ASABE* 28 (6): 861ï 74.

Christianson, L., A. Bhandari, M. Helmers, K. Kult, T. Sutphin, and R. Wolf. 2012b. Performance evaluation of four field-scale agricultural drainage denitrification bioreactors in Iowa. *ASABE* 55 (6): 2163-2174.

Correll, D.L. 1998. The Role of Phosphorus in the Eutrophication of Receiving Waters: A Review. *Journal of Environmental Quality.* 27: 261ï 266.

Dinnes, D.L., D.L. Karlen, D.B. Jaynes, T.C. Kaspar, J.L. Hatfield, T.S. Colvin, and C.A. Cambardella. 2002. Nitrogen management strategies to reduce nitrate leaching in tile-drained Midwestern soils. *Agronomy Journal* 94: 153 ï 171.

Egemose, S., M.J. Sønderup, M.V. Beinthin, K. Reitzel, C.C. Hoffmann, and M.R. Flindt. 2012. Crushed concrete as a phosphate binding material: a potential new management tool. *Journal of Environmental Quality*. 41: 647-653.

Feyereisen, G., T. Moorman, L. Christianson, R. Venterea, J. Coulter, and U. Tschirner. 2016. Performance of agricultural residue media in laboratory denitrifying biroeactors at low temperatures. *Journal of Environmental Quality.* 45 (3): 779 - 787.

Goodwin, G.E., R. Bhattarai, and R. Cooke. 2015. Synergism in nitrate and orthophosphate removal in subsurface bioreactors. *Ecological Engineering*. 84: 559-568.

Jansen, R.J.J., and H. van Bekkum. 1994. Amination and ammoxidation of activated carbons. *Carbon.* 32 (6): 1507-1516.

Jaynes, D.B., T.C. Kaspar, T.B. Moorman, and T.B. Parkin. 2008. In situ bioreactors and deep drain-pipe installation to reduce nitrate losses in artificially drained fields. *Journal of Environmental Quality* 37 (2): 429-436.

Kerrn-Jespersen, J.P., M. Henze, and R. Strube. 1994. Biological phosphorus release and uptake under alternating anaerobic and anoxic conditions in a fixed-film reactor. *Water Resources.* 28 (5): 1253-1255.

Kovacic, D.A., R.M. Twait, M.P. Wallace, and J.M. Bowling. 2006. Use of created wetlands to improve water quality in the Midwest - Lake Bloomington case study. *Ecological Engineering*. 28: 258-270.

Kuba, T., G. Smolders, M.C.M. van Loosdrecht, and J.J. Heijnen. 1993. Biological phosphorus removal from wastewater by anaerobic-anoxic sequencing batch reactor. *Water Science and Technology*. 27(5): 241-252.

Length, R. V. 2015. "lsmeans: Least-Squares Means." R package version 2.19. http://cran.r-project.org/package=lsmeans.

Macrae, M.L., M.C. English, S.L. Schiff, and M. Stone. 2007.Intra-annual variability in the contribution of tile drains to basin discharge and phosphorus export in a first-order agricultural catchment. *Agricultural Water Management.* 92 (3): 171-182.

Moorman, T.B., T.B. Parkin, T.C. Kaspar, and D.B. Jaynes. 2010. Denitrification activity, wood loss, and N_2O emissions over 9 years from a wood chip bioreactor. *Ecological Engineering* 36 (11): 1567-1574.

MPCA. 2014. The Minnesota nutrient reduction strategy. Minnesota Pollution Control Agency. Accessed 5 January 2016. https://www.pca.state.mn.us/sites/default/files/wq-s1-80.pdf

Orlando, U.S., T. Okuda, A.U. Baes, W. Nishijima, and M. Okada. 2003. Chemical properties of anion-exchangers prepared from waste natural materials. *Reactive and Functional Polymers*. 55: 311-318.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R Core Team. 2014. "Linear and Nonlinear Mixed Effects Models." R package version 3.1-117. http://cran.r-project.org/package=nlme

Penn, C.J., J.M. McGrath, E. Rounds, G. Fox, and D. Heeren. 2012. Trapping phosphorus in runoff with a phosphorus removal structure. *Journal of Environmental Quality* 41 (3): 672 - 279.

Robertson, W.D., and L.C. Merkley. 2008. In-stream bioreactor for agricultural nitrate treatment. *Journal of Environmental Quality* 38 (1): 230-237.

Soejima, K., K. Oki, A. Terada, S. Tsuneda, and A. Hirata. 2006. Effects of acetate and nitrite addition on fraction of denitrifying phosphate-accumulating organisms and nutrient removal efficiency in anaerobic/aerobic/anoxic process. *Bioprocess and Biosystems Engineering.* 29: 305-313.

Sylvia, D., P. Hartel, J. Fuhrmann, and D. Zuberer. 2005. Principles and applications of soil microbiology, 2nd ed. New Jersey: Pearson Prentice Hall. 640 p.

Tourism Economics. 2013. The economic impact of travel in Minnesota: 2013 analysis. Oxford Economics Company. Accessed 10 January 2016. http://www.exploreminnesota.com/industry-minnesota/research-reports/researchdetails/?nid=915.

USEPA. 2015. Nutrient Pollution: Sources and Solutions. United States Environmental Protection Agency. Accessed 26 December 2015. http://www.epa.gov/nutrientpollution/sources-and-solutions

Whitman, R.L., D.A. Shively, H. Pawlik, M.B. Nevers, and M.N. Byappanahalli. 2003. Occurrence of *Escherichia coli* and Enterococci in *Cladophora* (Chlorophyta) in nearshore water and beach sand of Lake Michigan. *Applied and Environmental Microbiology*. 69 (8): 4714-4719.

Woli, K.P., M.B. David, R.A. Cooke, G.F. McIsaac, and C.A. Mitchell. 2010. Nitrogen balance in and export from agricultural fields associated with controlled drainage systems and denitrifying bioreactors. *Ecological Engineering* 36 (11): 1558-1566.

Zhang, L. 2015. Exploring N and P reduction in bioreactors. Thesis, 50 p.

Tables:

Table 2.1: Mean (standard deviation) of dissolved reactive phosphorus (DRP) outlet concentrations at each hydraulic residence time (HRT) during the warm, cold, and rewarm runs for the six treatments. Target inlet DRP concentration was 0.3 mg P L^{-1}. Mean actual concentration was 0.23 ± 0.05 mg P L^{-1}.

Run	Temp (°C)	Treatment	1.5h HRT	8h HRT	12h HRT	24h HRT
			----------DRP outlet concentration (mg L^{-1})----------			
Warm	14.6	CC	0.06(0.05) bc†Aÿ	0.05 (0.04) cA	0.04 (0.03) bA	0.06 (0.04) bA
		CC+MC	0.04 (0.03) cAB	0.03 (0.02) cAB	0.03 (0.01) bB	0.05 (0.04) bA
		CC+MC+MBC	0.09 (0.06) bA	0.07 (0.06) cbB	0.04 (0.04) bB	0.05 (0.04) bB
		WC	0.17 (0.04) aA	0.16 (0.03) aA	0.15 (0.05) aA	0.11 (0.04) aB
		WC+BC	0.21 (0.05) aA	0.16 (0.05) aB	0.16 (0.05) aB	0.14 (0.05) aB
		WC+A	0.10 (0.03) bA	0.11 (0.05) bA	0.12 (0.05) aA	0.13 (0.05) aA
Cold	5.5	CC	0.22 (0.07) aA	0.19 (0.04) aB	0.14 (0.04) aC	0.10 (0.04) bD
		CC+MC	0.23 (0.06) aA	0.17 (0.04) aB	0.15 (0.04) aB	0.11(0.03) abC
		CC+MC+MBC	0.24 (0.06) aA	0.19 (0.05) aB	0.16(0.05)aBC	0.14(0.04) abC
		WC	0.23 (0.07) aA	0.18 (0.05) aB	0.15 (0.04) aB	0.16 (0.04) aB
		WC+BC	0.23 (0.07) aA	0.18 (0.05) aB	0.15 (0.05) aC	0.16(0.05)aBC
		WC+A	0.16 (0.06) bA	0.10 (0.05) bB	0.12 (0.05) aB	0.17 (0.05) aA
Rewarm	14.9	CC	0.11 (0.06) abA	0.06 (0.03) bcB	0.07(0.05)bAB	0.07(0.05)bAB
		CC+MC	0.09 (0.05) abA	0.05 (0.03) cA	0.05 (0.04) bA	0.08 (0.07) bA
		CC+MC+MBC	0.14 (0.10) abA	0.06 (0.05) bcB	0.05 (0.03) bB	0.10(0.10) abA
		WC	0.18 (0.06) aA	0.15 (0.04) aAB	0.13(0.03)abAB	0.12 (0.04)abB
		WC+BC	0.19 (0.06) aA	0.16 (0.04) aAB	0.18 (0.03) aA	0.14 (0.06)abB
		WC+A	0.07 (0.04) bB	0.13 (0.04) abA	0.14 (0.04)abA	0.17 (0.06) aA

† Within a column for a temperature run, means followed by the same lowercase letter are not significantly different (*P* Ò0.05).

ÿ Within a row for a treatment, means followed by the same uppercase letter are not significantly different (*P* Ò0.05).

Table 2.2: Mean (standard deviation) of dissolved reactive phosphorus (DRP) concentration reduction as a percentage at all hydraulic residence times (HRT) during the warm, cold, and rewarm runs for the six treatments.

Run	Temp (°C)	Treatment	1.5h HRT	8h HRT	12h HRT	24h HRT
			----------DRP concentration reduction (%)----------			
Warm	14.6	CC	72.4 (19.0) ab†Aÿ	75.6 (15.0) aA	80.3 (11.7)aA	72.5 (15.5)aA
		CC+MC	78.9 (12.9) aA	84.5 (9.0) aA	85.1(7.5)aA	76.3(17.0) aA
		CC+MC+MBC	57.9 (22.1) bcB	64.7(30.9)aAB	77.0(16.8)aA	75.5 (16.4) aA
		WC	15.8 (23.5) dB	15.9 (20.1) cB	20.7 (24.4)bB	51.6 (17.1) bA
		WC+BC	0.1 (27.4) dC	12.7(25.6)cBC	18.3 (25.4)bB	35.9(22.2)bA
		WC+A	48.6 (18.3) cA	40.2 (30.3) bA	36.4 (25.4)bA	41.1 (22.2) bA
Cold	5.5	CC	12.2 (21.9) bD	25.1 (14.3) bC	40.8(16.7)abB	55.8 (14.7) aA
		CC+MC	6.9 (18.2) bC	30.2 (13.3) bB	39.8(15.4)abAB	50.0 (10.9)aA
		CC+MC+MBC	2.0 (20.5) bC	24.7 (20.0) bB	33.1 (20.3)bAB	39.5 (15.1)abA
		WC	8.0 (19.5) bB	28.9 (20.0) bA	37.5(24.2)abA	31.0 (14.3) bA
		WC+BC	17.8 (23.3) bC	38.9 (20.0) bB	41.3(19.5)abA	29.8(25.6)bAB
		WC+A	36.5 (20.2) aB	60.2 (16.2) aA	53.2 (18.1)aA	24.5 (20.8) bB
Rewarm	14.9	CC	56.8 (25.3) aB	75.0 (15.4)abA	73.0(18.6)abAB	71.0(16.3)aAB
		CC+MC	61.3 (19.8) aB	78.8 (10.8) aA	78.9 (14.3)aA	66.0(26.5)aAB
		CC+MC+MBC	41.7 (37.4) abB	77.3 (20.1)abA	80.7 (12.7)aA	58.9 (34.4) aB
		WC	25.2 (24.1) bB	41.1 (18.2) cA	48.3(16.9)bcA	51.6 (14.1)abA
		WC+BC	17.9 (26.8) bB	38.4 (17.0) cA	31.5(13.6)cAB	43.1 (22.0)abA
		WC+A	67.8 (17.4) aA	48.8 (17.0)bcB	45.1(14.7)bcB	29.1 (24.0) bC

† Within a column for a temperature run, means followed by the same lowercase letter are not significantly different (*P* Ò0.05).

ÿ Within a row for a treatment, means followed by the same uppercase letter are not significantly different (*P* Ò0.05).

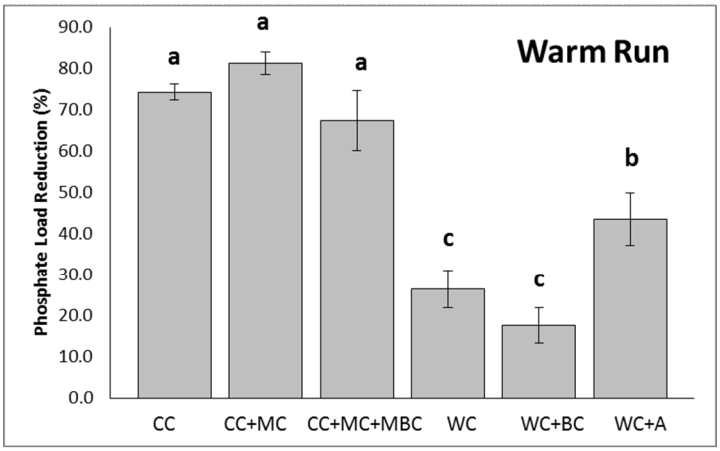
Table 2.3: Mean (standard deviation) of dissolved oxygen (DO) and pH during the warm, cold, and rewarm runs for the six treatments.

Run	Temp (°C)	Treatment	DO (mg L^{-1})	pH
Warm	14.6	Inlet	7.1 (0.6)	7.3 (0.1)
		CC	0.6 (0.9)	6.6 (0.4)
		CC+MC	0.4 (0.5)	6.7 (0.4)
		CC+MC+MBC	0.6 (0.8)	6.8 (0.3)
		WC	0.9 (0.9)	7.2 (0.1)
		WC+BC	0.9 (1.0)	7.1 (0.1)
		WC+A	0.3 (0.3)	8.3 (0.7)
Cold	5.5	Inlet	8.9 (0.6)	7.2 (0.2)
		CC	2.5 (2.2)	6.9 (0.1)
		CC+MC	2.3 (2.0)	7.0 (0.1)
		CC+MC+MBC	2.6 (2.2)	6.9 (0.1)
		WC	2.0 (2.6)	6.8 (0.1)
		WC+BC	2.1 (2.4)	6.8 (0.1)
		WC+A	0.3 (0.7)	9.1 (0.4)
Rewarm	14.9	Inlet	8.3 (0.5)	6.8 (0.1)
		CC	0.5 (0.8)	6.7 (0.2)
		CC+MC	0.5 (0.8)	6.7 (0.2)
		CC+MC+MBC	0.5 (0.7)	6.7 (0.2)
		WC	0.5 (0.6)	6.8 (0.1)
		WC+BC	0.7 (1.0)	6.8 (0.1)
		WC+A	0.1 (0.1)	8.0 (0.7)

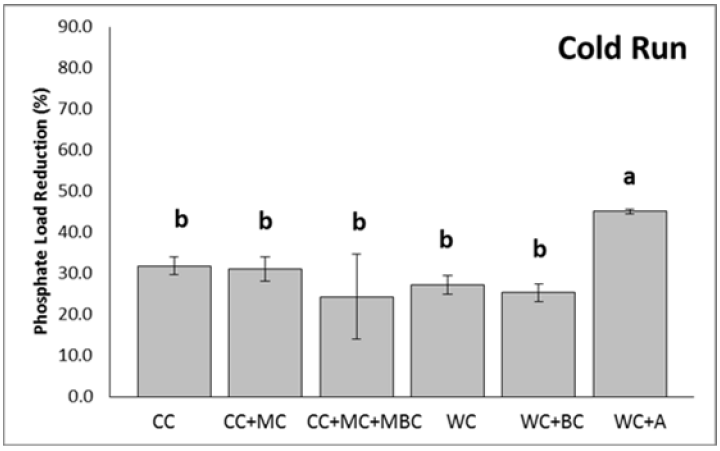
Figures:

Figure 2.1: Mean cumulative dissolved reactive phosphorus (DRP) load reduction as a percentage for treatments in the a) warm run, b) cold run, and c) rewarm run. Error bars signify standard deviation. Within a temperature run, columns with the same lowercase letter indicate no significant difference between treatments (*P* Ò0.05).

a)



b)



c)

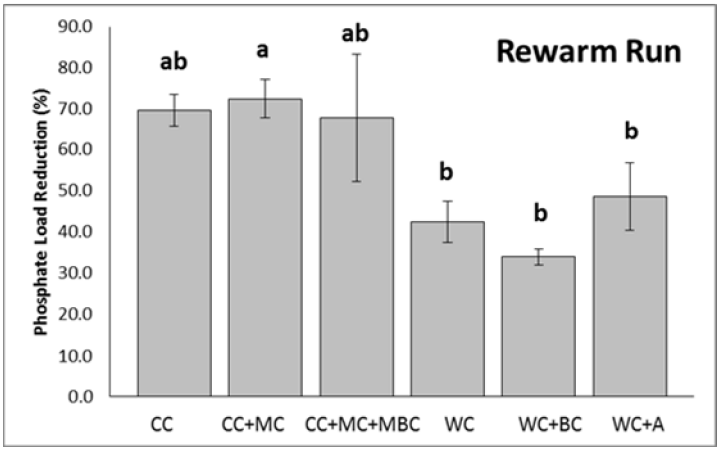
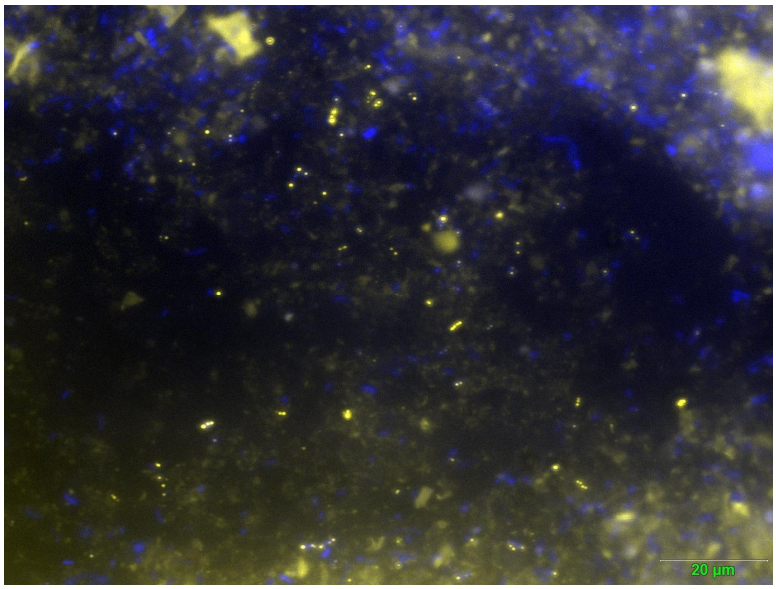


Figure 2.2: Microscope photographs of DAPI (4ǫ,6-diamidino-2-phenylindole) fluorescent-stained samples from a) CC+MC and b) WC+A treatments after the cold run. The microorganisms are stained light blue and the polyphosphate is stained yellow. If polyphosphate and microbial bodies line up, it is evidence for polyphosphate being stored inside cell bodies.

a)



b)

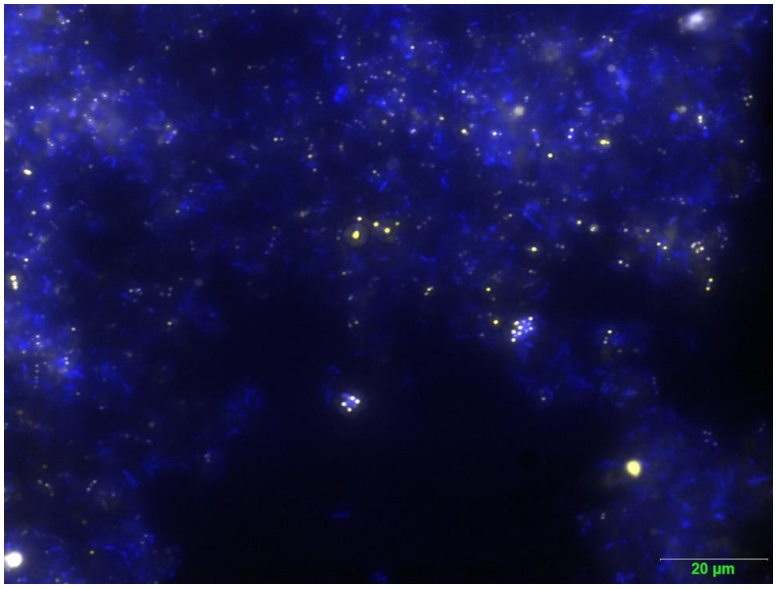
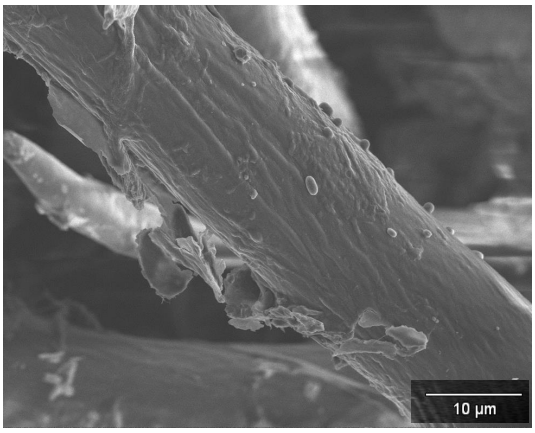
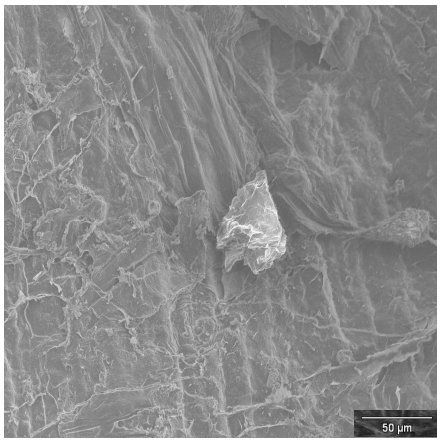


Figure 2.3: Scanning electron microscope (SEM) photographs of a) CC+MC and b) WC+A treatments from the cold run. Each photo had at least two analysis points, determined by textures and shapes seen in image, for energy dispersive x-ray spectroscopy (EDS) composition analysis.

a)

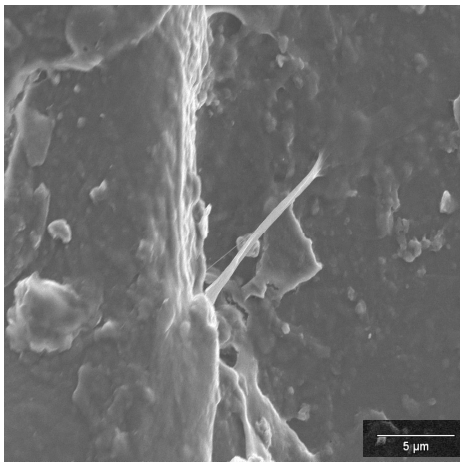


x5000 magnification

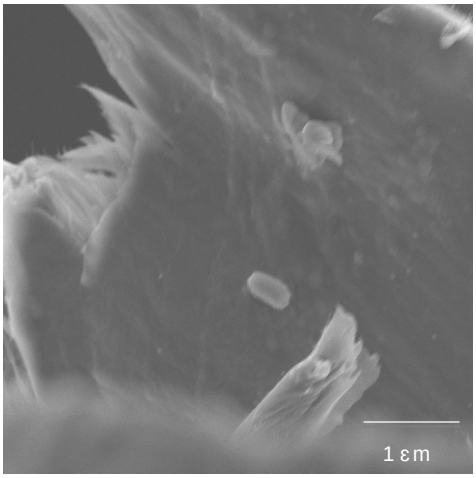


x500 magnification

b)



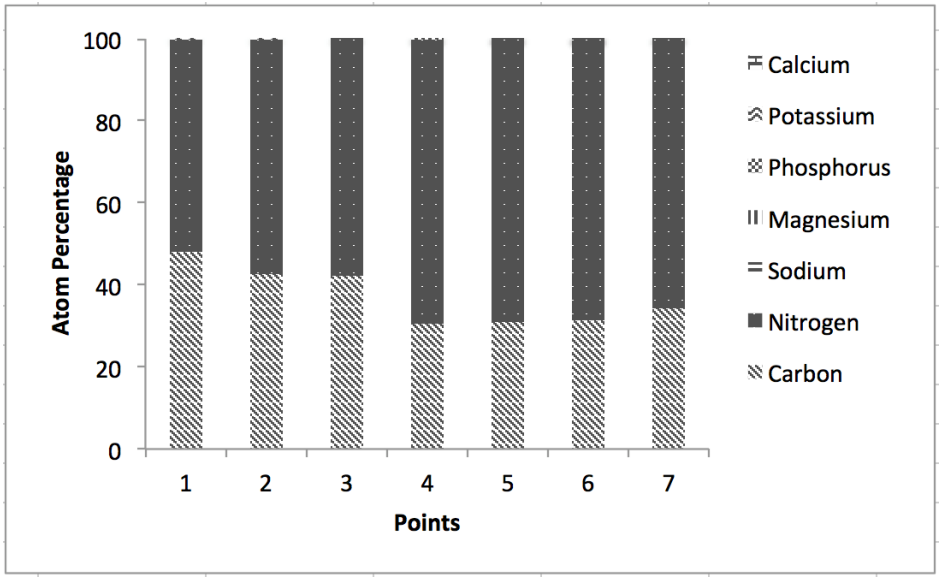
x5000 magnification



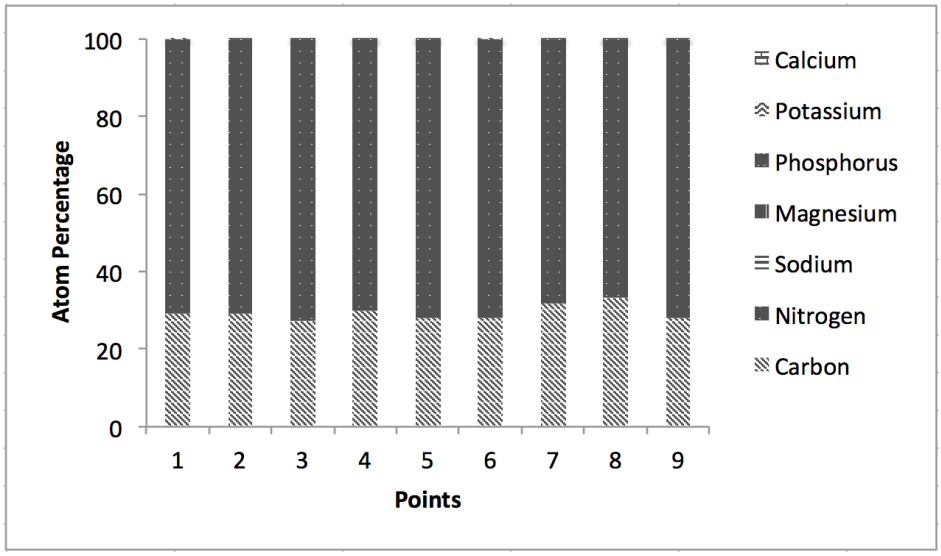
x8500 magnification

Figure 2.4: Elemental composition from Energy Dispersive X-ray Spectroscopy (EDS) data of one point on a) corn cob sample and b) wood chip sample. Corn cobs were from CC+MC treatment and wood chips from WC+A treatment after the cold run. Elemental composition presented is representative of other EDS sample points on the material.

a)



b)



Conclusions

Our objective was to find bioreactor treatments that could sustain denitrification and DRP removal under high flow conditions and cold temperatures. WC+A was the treatment that had both the highest nitrate removal rate (NRR) and cumulative dissolved reactive phosphorus (DRP) load reduction in 5°C conditions and in a 1.5h hydraulic residence time (HRT). The corn cob treatments also had high NRR and DRP load reduction in the 15°C, but did not in the 5°C conditions. It is possible that too little modified coconut coir and biochar was added to the corn cobs and therefor did not create optimal levels of anion exchange capacity in the bioreactor. Further exploratory tests should be carried out on the WC+A treatment to determine effects of excess carbon on aquatic ecosystem and evidence of any greenhouse gases or methylation of mercury. Also, the DRP removal mechanism in these bioreactors is potentially microbial uptake and therefore DRP could be susceptible to release in a field bioreactor.

Appendix I: Supplemental

Table A.1: Chemical and physical properties of hardwood and macadamia nut biochars used in column experiments.

Feedstock	Pyrolysis Temp (°C)	pH	% C	% N	% Ash	% O	% H	Surface Area (m^2 g^{-1})	Volatile %
Macadamia Nut Shell	500	6.55	88.3	0.25	2.4	7.02	2.42		
Hardwood	600-700†	6.2	84	0.6	2	2	2.3	0.4	15.2

† indicates an estimate, as the company did not disclose the pyrolysis temperature

Table A.2: Original solid carbon materials used in column bioreactor experiments, along with particle description, source, and percentage by weight in columns when mixed with other carbon materials. WC+BC is the wood chip and hardwood biochar treatment, CC+MC is the corn cob and modified coconut coir treatment, and CC+MC+MBC is the corn cob, modified coconut coir, and modified macadamia nut biochar treatment.

Media	Particle Description		Source	% by weight in column (if mixed)
Wood chips	Sieve Size (mm)	Mass (%)	The Mulch Store, Empire, MN	WC+ BC: 69%
	>38	0		
	25 ï 38	8		
	19 ï 25	21		
	13 ï 19	55		
	10 ï 13	11		
	6 ï 10	4		
	<6	1		
Corn cobs	Length (cm)	Numeric Frctn (%)	U of M, Morris, MN	CC+MC: 94% CC+MC+MBC: 86%
	0 ï 5	20		
	5 ï 10	46		
	10 ï 15	29		
	15 ï 20	5		
Hardwood Biochar	Sieve Size (mm)	Mass (%)	Cowboy Charcoal, Brentwood, TN	WC+BC: 31%
	>38	0		
	25 ï 38	18		
	19 ï 25	27		
	13 ï 19	45		
	10 ï 13	4		
	6 ï 10	2		
	<6	4		
Macadamia Biochar	Sieve Size (mm)	Mass (%)	Biochar Brokers (EternaGreen™), CO	CC+MC+MBC: 8%
	>38	0		
	25 ï 38	0		
	19 ï 25	0		
	13 ï 19	6		
	10 ï 13	19		
	6 ï 10	27		
	<6	48		
Coconut Coir	Length (cm)	Numeric Frctn (%)	J. alderink LLC, Oak Park, MN	CC+MC: 6% CC+MC+MBC: 6%
	0 ï 5	10		
	5 ï 10	42		
	10 ï 15	25		
	15 ï 20	19		
	20 ï 26	5		

Table A.3: Mean (st. dev.) treatment hydraulic residence times (HRT) for each predicted HRT during the warm, cold, and rewarm runs for the six treatments and an overall mean for each temperature run.

Run	Temp (°C)	Treatment	1.5h	8h	12h	24h
			----------HRT----------			
Warm	14.6	CC	1.8 (0.2)	8.8 (0.8)	12.5 (1.2)	25.3 (2.8)
		CC+MC	1.9 (0.2)	9.3 (0.8)	13.2 (1.2)	26.9 (2.7)
		CC+MC+MBC	1.8 (0.2)	9.1 (0.8)	13.0 (1.3)	26.1 (2.7)
		WC	1.8 (0.2)	8.8 (0.9)	12.5 (1.3)	25.3 (2.9)
		WC+BC	1.7 (0.2)	8.3 (0.8)	11.8 (1.2)	23.9 (2.6)
		WC+A	1.7 (0.2)	8.3 (0.7)	12.0 (1.1)	24.0 (2.5)
		Overall	**1.8 (0.1)**	**8.8 (0.4)**	**12.5 (0.6)**	**25.3 (1.2)**
Cold	5.5	CC	2.0 (0.3)	9.1 (1.0)	13.0 (1.1)	24.4 (3.0)
		CC+MC	2.0 (0.3)	8.8 (0.9)	12.5 (0.8)	23.3 (2.5)
		CC+MC+MBC	1.9 (0.3)	8.8 (1.0)	12.4 (1.1)	23.3 (2.8)
		WC	2.2 (0.3)	9.9 (0.9)	14.0 (0.8)	26.3 (2.6)
		WC+BC	2.0 (0.2)	9.0 (0.9)	12.9 (1.2)	24.0 (2.5)
		WC+A	1.9 (0.3)	8.8 (0.9)	12.7 (1.4)	23.2 (2.4)
		Overall	**2.0 (0.1)**	**9.1 (0.4)**	**12.9 (0.6)**	**24.1 (1.2)**
Rewarm	14.9	CC	1.8 (0.4)	8.7 (1.2)	12.3 (1.6)	22.1 (3.5)
		CC+MC	1.7 (0.2)	8.2 (0.9)	11.7 (1.1)	20.9 (2.0)
		CC+MC+MBC	1.8 (0.2)	8.8 (1.1)	12.6 (1.4)	22.4 (2.4)
		WC	2.1 (0.3)	9.9 (1.0)	14.0 (1.3)	24.8 (2.2)
		WC+BC	1.8 (0.2)	9.0 (0.8)	12.7 (1.0)	22.6 (1.5)
		WC+A	1.8 (0.2)	8.9 (0.8)	12.7 (1.0)	22.7 (1.7)
		Overall	**1.8 (0.1)**	**8.9 (0.6)**	**12.7 (0.8)**	**22.6 (1.3)**

Table A.4: Treatment Q_{10} reaction rates, or the rate of change due to increasing the temperature by 10°C, calculated for the difference between the warm and cold runs (W/C) and the rewarm and cold runs (R/C). Calculation is (NRR_{warm}/NRR_{cold})/(($temp_{warm}$ ï $temp_{cold}$)/10) and was done for each HRT.

Comparison	Treatment	1.5h	8h	12h	24h
		----------Q_{10} values----------			
W/C	CC	2.8	3.3	2.6	1.4
	CC+MC	3.6	3.8	2.9	1.5
	CC+MC+MBC	3.8	3.3	2.7	1.6
	WC	3.4	7.3	4.9	5.2
	WC+BC	-4.0	5.3	6.9	6.7
	WC+A	1.7	1.0	1.0	1.0
R/C	CC	2.5	3.2	2.4	1.4
	CC+MC	2.9	3.7	2.8	1.6
	CC+MC+MBC	4.3	3.9	2.9	1.7
	WC	3.4	9.1	5.5	4.8
	WC+BC	-6.6	6.1	7.8	6.4
	WC+A	4.2	0.9	0.9	0.9

Table A.5: Complete range of treatment oxidation-reduction potentials (ORP) for each HRT during the warm, cold, and rewarm runs for the six treatments.

Run	Temp (°C)	Treatment	1.5h	8h	12h	24h
			---------- ORP(mV) ----------			
Warm	14.6	CC	-155 to +425	-160 to +276	-163 to +178	-139 to +125
		CC+MC	-320 to +380	-178 to +199	-173 to +166	-158 to +157
		CC+MC+MBC	-241 to +325	-99 to +354	-176 to +245	-157 to +113
		WC	+296 to +599	-84 to +555	-36 to +445	+47 to +463
		WC+BC	+292 to +554	+228 to +441	+181 to +406	+102 to +334
		WC+A	-459 to -20	-261 to +112	-284 to +111	-233 to +9
Cold	5.5	CC	+186 to +574	+340 to +620	+343 to +603	+173 to +413
		CC+MC	+162 to +642	+214 to +465	+218 to +474	+13 to +358
		CC+MC+MBC	+226 to +570	+377 to +670	+379 to +595	+245 to +414
		WC	+73 to +588	+337 to +661	+324 to +498	+181 to +453
		WC+BC	+218 to +653	+420 to +622	+405 to +670	+299 to +493
		WC+A	-191 to +267	-128 to +234	-167 to +265	-168 to +64
Rewarm	14.9	CC	-153 to +401	-139 to +290	-140 to +125	-163 to +14
		CC+MC	-225 to +335	+70 to +230	+10 to +143	-93 to +29
		CC+MC+MBC	-149 to +325	+35 to +244	-168 to +133	-166 to +7
		WC	-196 to +383	+233 to +336	+199 to +309	+172 to +289
		WC+BC	-191 to +448	+237 to +389	+179 to +343	+144 to +316
		WC+A	-197 to +368	-261 to -159	-279 to -125	-241 to -146

Table A.6: Mean (standard deviation) of net flow weighted total carbon (TC), total organic carbon (TOC), and total inorganic carbon (TIC) production load over eight weeks of each temperature experiment. TIC production load is an estimate found by subtracting TOC load from TC load. Carbon data from the rewarm run is not presented due to equipment failure.

Run	Temp (°C)	Treatment	TC	TOC	TIC
			----------production load (g)----------		
Warm	14.6	CC	19.5 (4.0) a†	15.1 (3.9) a	4.4 (0.1) ab
		CC+MC	17.0 (3.2) a	13.0 (3.1) a	4.0 (0.4) ab
		CC+MC+MBC	13.3 (5.8) a	9.5 (5.0) a	3.8 (0.8) b
		WC	2.9 (0.4) b	1.1 (0.2) b	1.7 (0.2) c
		WC+BC	2.8 (1.0) b	1.4 (0.9) b	1.4 (0.2) c
		WC+A	17.2 (0.1) a	12.2 (0.2) a	5.0 (0.2) a
Cold	5.5	CC	3.5 (0.8) b	1.3 (0.4) b	2.2 (0.4) b
		CC+MC	3.3 (0.3) b	1.2 (0.1) b	2.0 (0.2) b
		CC+MC+MBC	3.0 (0.4) b	1.1 (0.2) b	1.9 (0.3) b
		WC	1.4 (0.1) c	0.4 (0.1) b	1.0 (0.1) c
		WC+BC	1.3 (0.1) c	0.4 (0.1) b	0.9 (0.0) c
		WC+A	13.2 (0.4) a	8.6 (0.9) a	4.7 (0.6) a

† Within a column for a temperature run, means followed by the same lowercase letter are not significantly different (*P* Ò0.05).

Figure A.1: Flow rate and hydraulic residence time (HRT) determined for the laboratory column bioreactor experiment. Flow rate is the solid line associated with the left y-axis and HRT is the dotted line associated with the right y-axis. Flow rates were determined using data from a drainage hydrograph in Morris, MN.

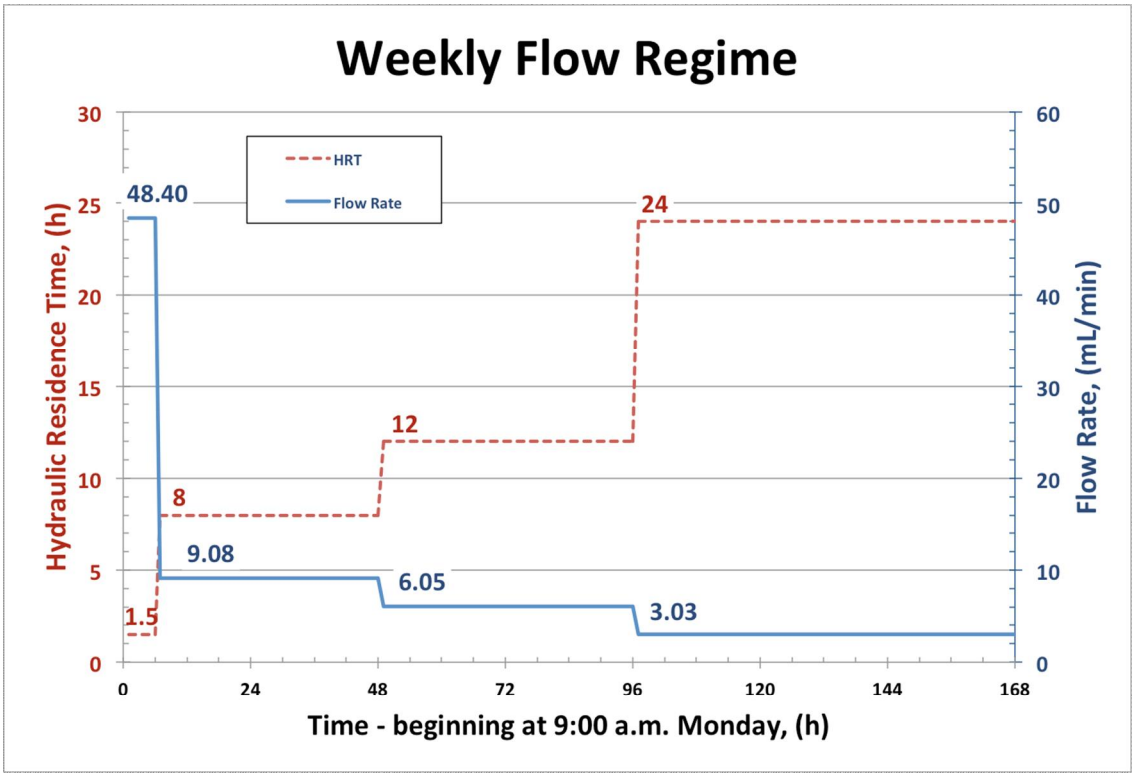


Figure A.2: Mean pH of various treatments and inlet during six weeks of the rewarm run. Graph starts with pH reading at 1.5h HRT, then 8h, 12h, and 24h HRT and then the series repeats. Highest pH values in WC+A treatment correspond with 1.5h HRT.

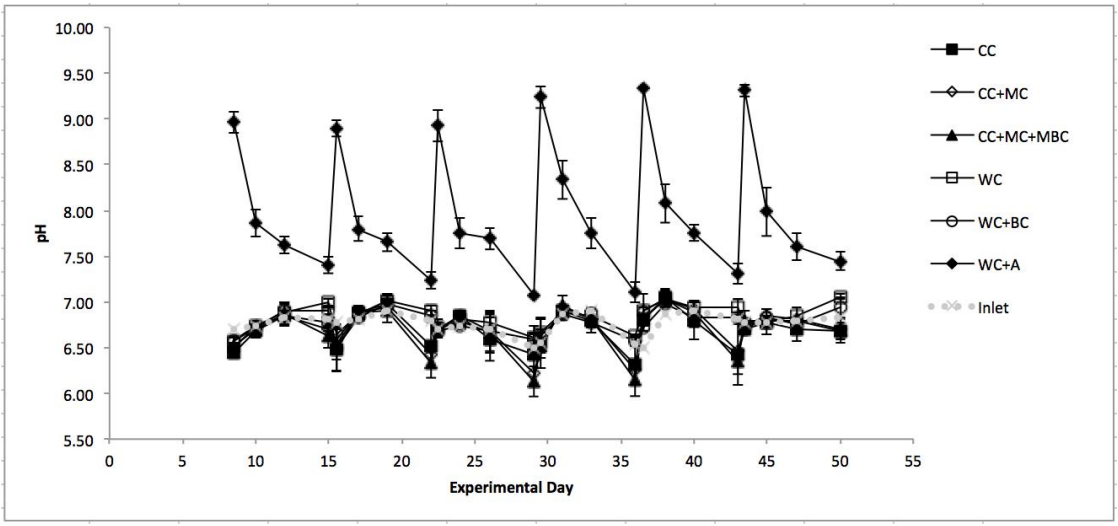


Figure A.3: Total organic carbon (TOC) outlet concentrations of WC+A bioreactor for the 1.5h and 24h HRT during the warm, cold, and rewarm runs. Error bars represent standard deviation (n=3).

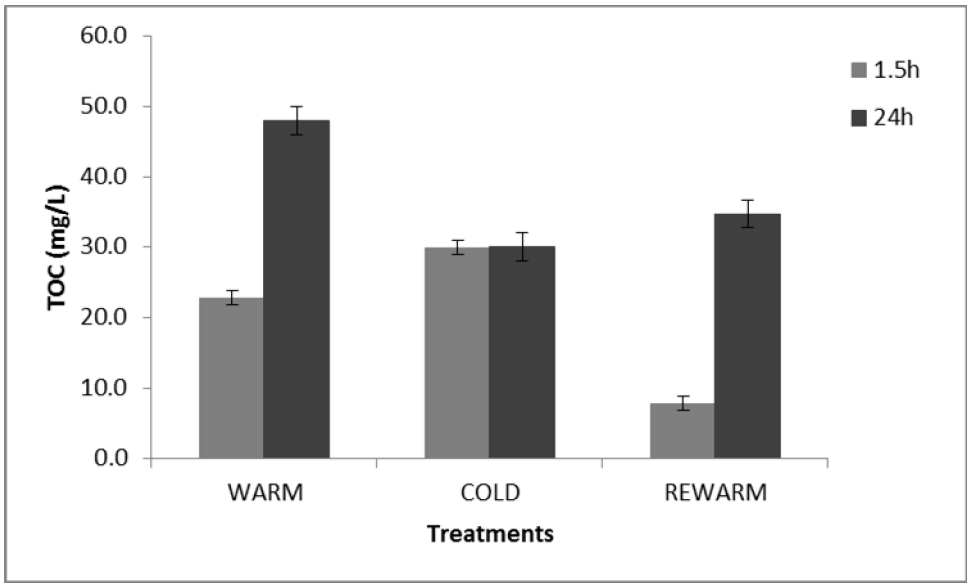
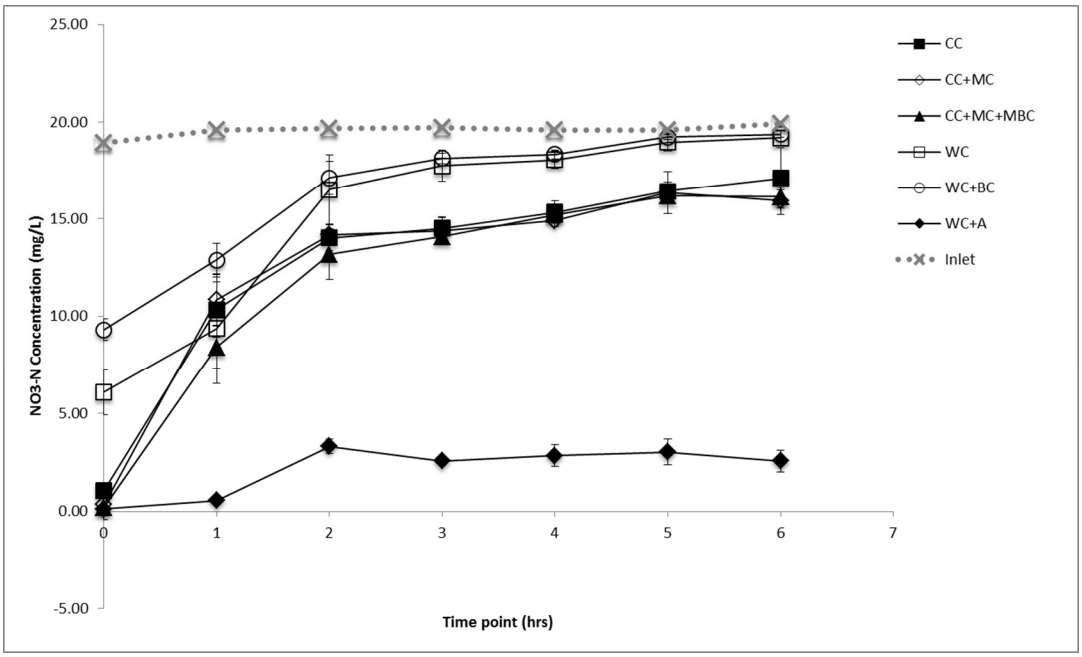


Figure A.4: Nitrate-N concentration of all bioreactor effluent and influent during one 1.5h HRT flow event. A sample was taken every six hours of the 1.5h HRT flow rate, with ñ0ò being when the pumps were changed from the flow rate for the 24h HRT to the flow rate for the 1.5h HRT, and ñ6ò being right before the pumps were set to the flow rate for the 8h HRT.



Appendix II: Inoculum

Product: Biofloc™ Biologicals

Contents:

- *Bacillus azotofixans* (2 strains)
- *Bacillus azotoformans* (3 strains)
- *Bacillus inegaterium* (2 strains)
- *Bacillus polymyza* (5 strains)
- *Bacillus licheniformis* (6 strains)
- *Bacillus pumulis* (2 strains)
- *Bacillus* spp. (3 strains)
- Other proprietary *Bacillus* (10 strains)
- *Pseudomonas* (5 strains)
- Pantothenic Acid
- Amotrol

Application rates: apply 60-100 pounds per 1,000,000 Liters (795,000 gallons).

Use in this experiment: 0.03 g/L

Appendix III: R code

```
###R code is from Felipe in Statistics Help Center and Dr. Jessica Gutknecht

#Import data to R Studio
#Set up R library

library(nlme)
library(ape)
library (car)
library(multcomp)
library(effects)
library(lsmeans)

#Make sure certain items are factors: HRT_pred (predicted HRT), Treatment, Cycle, and
ColNo (column number).

Marta_rewarm$HRT_pred <- factor(Marta_rewarm$HRT_pred)
Marta_rewarm$Treatment <- factor (Marta_rewarm$Treatment)
Marta_rewarm$Cycle <- factor(Marta_rewarm$Cycle)
Marta_rewarm$ColNo <- factor(Marta_rewarm$ColNo)

######Linear mixed-effects model: nitrate concentration (NO3_Conc) example

##Cycle is nested within columns as random factors, also with a correlation matrix for
the repeated measures covariation

NO3lmeW <-lme(fixed = NO3_Conc ~ Treatment*HRT_pred,random =
~1|ColNo/Cycle, correlation=corAR1(),na.action = "na.omit", data=Marta_rewarmrun,
method="ML")

##One concern is the correlation argument within the lme (linear mixed-effects model)
because of non-sequential weeks. The above model is using the correlation structure class
ñcorAR1ò, which is autoregressive process of order 1

##Can run model with and without correlation and compare the fit of both models

NO3lmeW2 <-lme(fixed = NO3_Conc ~ Treatment*HRT_pred,random =
~1|ColNo/Cycle, na.action = "na.omit", data=Marta_rewarmrun, method = "ML")

anova(NO3lmeW, NO3lmeW2)
```

```
Model          df      AIC      BIC    logLik   Test  L.Ratio p-value
NO3lmeAR        1 28 2054.695 2176.568 -999.3473
NO3lmeAR2       2 27 2074.247 2191.768 -1010.1233 1 vs 2 21.55198  <.0001
```

#Want to choose the model with the lowest AIC and/or BIC ï in this case the correlation argument does not matter

```
summary(NO3lmeW)
Anova(NO3lmeW)

##Tukey multiple comparisons using the lsmeans package

W1 <- lsmeans(NO3lmeW, ~ Treatment | HRT_pred)
summary(W1)
pairs(W1)

W2 <- lsmeans(NO3lmeW, ~ HRT_pred | Treatment)
summary(W2)
pairs(W2)
```

Appendix IV: Prototype

Prototype column experiments:

Materials were selected for potential phosphorus-sorbing properties: limestone, crushed concrete, steel slag, and coconut coir. Select materials were subjected to a 24h static test (n=3) with standard controls (n=2) in water with 0.46 mg L^{-1} P to determine ability to decrease dissolved reactive phosphorus (DRP) concentration (Table A.7). Materials were then subject to static test of variable time increments to determine DRP concentration reduction (Table A.8).

5 column arrangements were set up with steel slag (n=2), limestone (n=1), and crushed concrete (n=2) in 4-inch PVC columns. Wood chips, a material that has been shown in the literature to promote denitrification, was packed into 6-inch PVC columns. Material was inoculated using Biofloc^{TM} Biologicals (Appendix II). Caps were placed on all 6-inch columns to create an anaerobic environment, but the top of 4-inch PVC columns was left open to the air to produce an aerobic environment.

4-inch PVC columns were placed in brackets above the 6-inch columns on a metal rack. Tubing was placed between the 4-inch column and corresponding wood chip columns to have the inlet water to first come through the 4-inch or the 6-inch column, leading to water pumped through denitrification material, a.k.a wood chips, first (N to P) or through the potential phosphate-sorbing material first (P to N) (Figure A.5). Inlet water phosphate concentration was 0.30 mg L^{-1} P. Water was pumped at approximately 2.6 mL min^{-1} (24h hydraulic residence time from inlet to outlet).

In all treatments and structures, nitrate concentration was below 4 mg L^{-1}. Phosphate concentrations varied between treatments and structures (Figure A.6). All structures had a final outlet DRP concentration that was less than the inlet except for Structure 3 with wood chips and limestone (N to P structure). Structures 1, 3, and 4 had a similar pH to the inlet (~7.0) throughout the experiment, while structures 2 and 5 had a pH of ~9.0 towards the end of the experiment (Figure A.7).

Table A.7: Mean (standard deviation) of dissolved reactive phosphorus (DRP) concentration and pH of various materials after a 24h static test. Initial DRP concentration was 0.46 mg L^{-1} P.

Material	DRP concentration (mg L^{-1})	pH
Coconut Coir	2.75 (0)	6.38 (0.08)
Limestone	0.58 (0.20)	6.60 (0.17)
Crushed Concrete >6.3mm	0.21 (0.06)	12.07 (0.06)
Crushed Concrete 4-6.3mm	0.82 (0.84)	11.96 (0.03)
Standard	0.46 (0)	9.55 (0.25)

Table A.8: Dissolved reactive phosphorus (DRP) concentration reduction after a static test of variable time increments (min) from limestone, crushed concrete, and steel slag.

Material	Time (min)	DRP reduction (%)
Limestone	2	5.7
	4	22.9
	6	31.4
	8	17.1
	10	16.0
	12	40.0
Crushed Concrete >6.3mm	2	63.6
	5	84.0
	10	92.0
	15	77.3
Steel Slag < ¼ inch	2	52.4
	5	72.2
	10	83.3
	15	86.1
	30	80.6
Steel Slag ½ - 4 inch	2	6.6
	5	22.4
	10	27.7
	15	41.5

Figure A.5: Diagram of materials and structure arrangements used in the prototype laboratory column experiment. Arrows designate the direction the inlet water was pumped, and numbers designate the five different structures that included a potential denitrification column and phosphate-sorbing column.

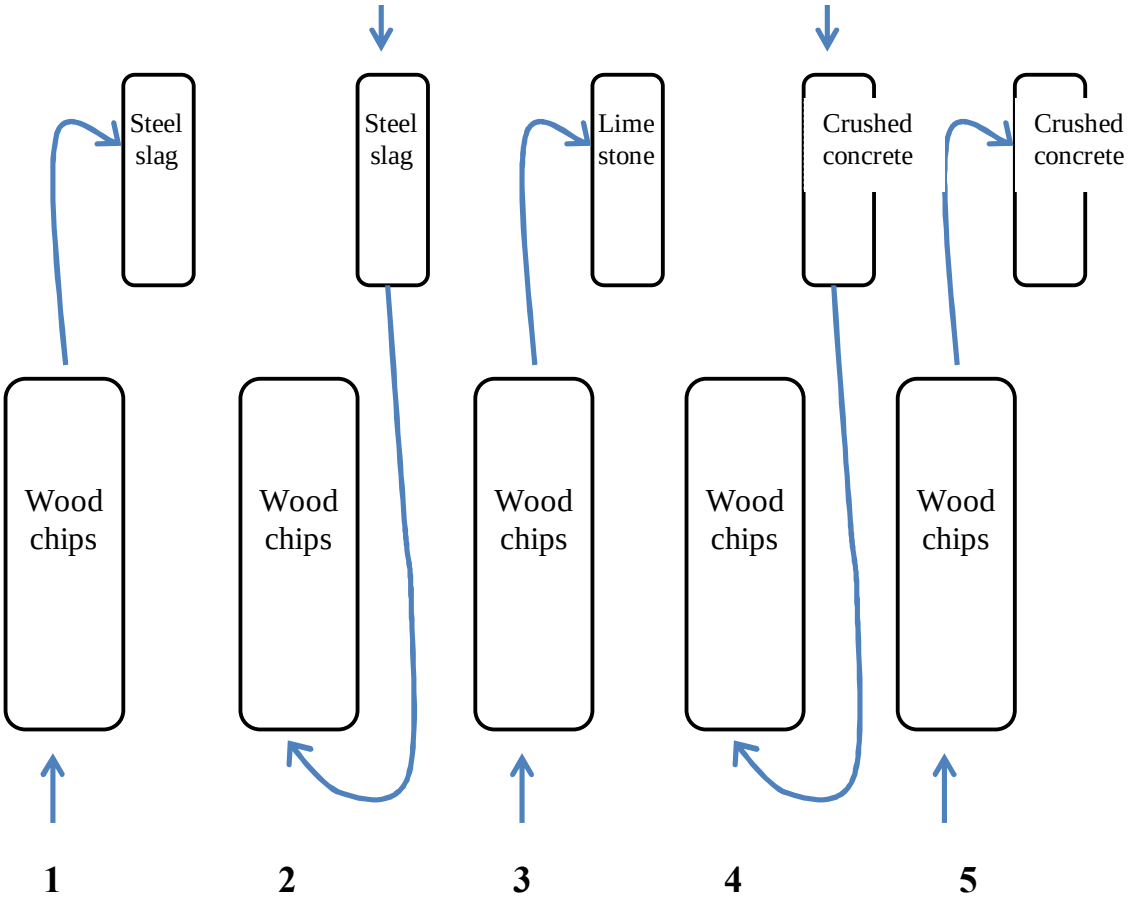
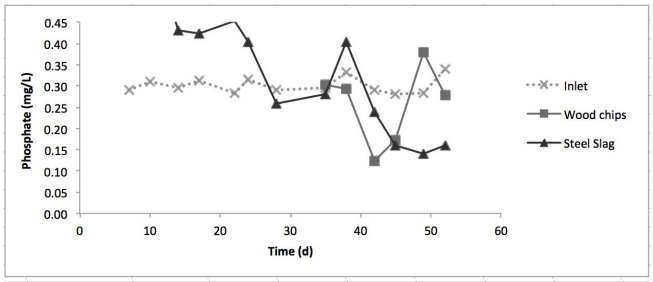
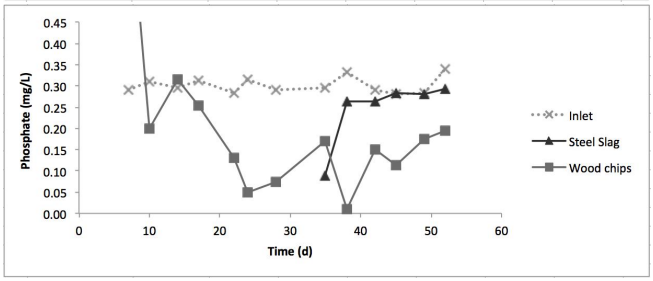


Figure A.6: Dissolved reactive phosphorus (DRP) concentration (mg L^{-1}) of inlet, midpoint, and outlet over time for each of the prototype structure arrangements. Numbers correspond to structures pictured in Figure A.5; 1 & 2 have steel slag, 3 has limestone, and 4 & 5 have crushed concrete. All structures had wood chips. 1, 3, and 5 structures had inlet flowing through wood chips first (N to P), and 2 and 4 structures had inlet flowing through steel slag and crushed concrete first, respectively (P to N).

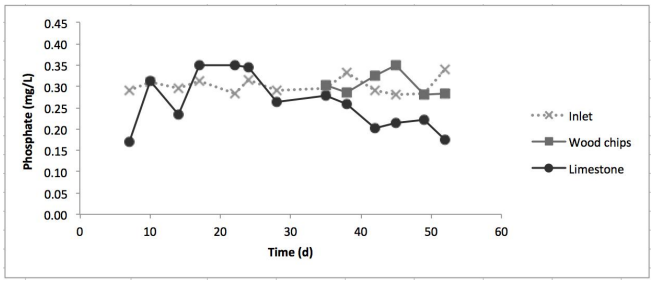
1)



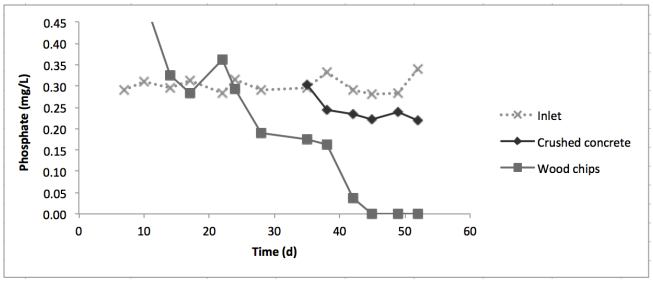
2)



3)



4)



5)

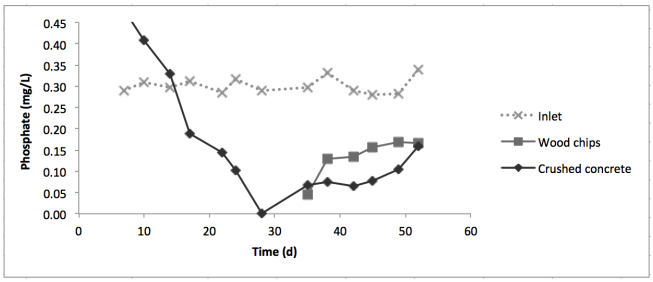


Figure A.7: The pH of the outlet of all prototype structures and the inlet over the entire prototype experiment. Structures 1, 3, and 5 had inlet flowing through wood chip material first (N to P), and structures 2 and 4 had inlet flowing through steel slag and crushed concrete first, respectively (P to N).