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Improving the nutrient removal capabilities of woodchip bioreactors

Emily Ann Martin
Iowa State University

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Improving the nutrient removal capabilities of woodchip bioreactors

by

Emily Ann Martin

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Environmental Science

Program of Study Committee:
Michelle Soupir, Major Professor
Thomas Isenhart
Thomas Moorman

The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this thesis. The Graduate College will ensure this thesis is globally accessible and will not permit alterations after a degree is conferred.

Iowa State University

Ames, Iowa

2018

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ABSTRACT

Intensive farming, fertilizer application, and the mineralization of soil organic matter in the Upper Midwestern United States, coupled with an extensive subsurface tile drainage, has contributed to excessive nutrient loading to surface waters. Woodchip bioreactors are a cost effective and minimally invasive edge-of-field practice designed to remove nitrate (NO$_3^-$N) from subsurface drainage. The NO$_3^-$N removal efficiency of woodchip bioreactors can be impacted by several factors, including hydraulic residence time (HRT) and the type of carbon source used.

The first study (Chapter 3) examined the impact of three HRTs, 2 hours, 8 hours, and 16 hours, on denitrification in a set of nine highly controlled pilot-scale woodchip bioreactors in Central Iowa. The 2 h HRT had the highest NO$_3^-$N mass removal rate (MRR) at 9.0 g m$^{-3}$ day$^{-1}$, followed by the 8 h at 8.5 g m$^{-3}$ day$^{-1}$, and the 16 h at 7.4 g m$^{-3}$ day$^{-1}$, all of which were statistically different (p < 0.05). When accounting for bypass flow, the 2 h HRT still removed more NO$_3^-$N by mass than the other HRTs. NO$_3^-$N concentration reduction was significant for all HRTs from the inlet to the outlet (p < 0.05). The 16 h HRT removed the most NO$_3^-$N by concentration (7.5 mg L$^{-1}$) and had the highest percent mass removal rate (PMRR) at 53.8%. The 8 h HRT removed an average of 5.5 mg L$^{-1}$ NO$_3^-$N with a PMRR of 32.1%. The 2 h HRT removed an average of 1.3 mg L$^{-1}$ NO$_3^-$N with a PMRR of 9.0%. Significant explanatory variables for PMRR were HRT (p < 0.001) and influent NO$_3^-$N concentration (p < 0.001) (r = 0.80).

The second study (Chapter 4) examined the potential to expand the nutrients removed by bioreactors to include P by adding biochar as an amendment to the carbon substrate. Six biomasses [Red Oak (*Quercus rubra*), Ash (*Fraxinus spp.*), Mixed Pine (*Pinus spp.*), Loblolly Pine (*Pinus taeda*), Switchgrass (*Panicum virgatum*), and Corn Stover (*Zea mays*)] were
pyrolyzed into biochar at three temperatures (400°C, 600°C, and 800°C). Experiments were conducted at the batch scale to test for removal of NO$_3^-$N and phosphate (PO$_4^{3-}$) by woodchips mixed with biochar at a ratio by weight of 12:1 (8.3% application rate). Each batch contained 3 L of nutrient solution with 30 mg L$^{-1}$ NO$_3^-$N and 10 mg L$^{-1}$ PO$_4^{3-}$ and were sampled at 0, 4, 8, 12, and 24 hours. NO$_3^-$N removal was not correlated with particle size, but was positively correlated with pyrolysis temperature ($r = 0.58$, $p < 0.05$). None of the biochars removed significantly more NO$_3^-$N than the woodchip control. The 800°C biochar and herbaceous biochars removed significantly more PO$_4^{3-}$ than the control, but the woodchips leached PO$_4^{3-}$ into solution, meaning significance may not be the same after the initial leaching period ($p < 0.05$). Results indicate that biochar is not suited to be an amendment to enhance either NO$_3^-$N or PO$_4^{3-}$ removal in woodchip bioreactors.
CHAPTER 1. GENERAL INTRODUCTION

1.1 Introduction

In the Upper Mississippi River Basin, intensively fertilized row crop agricultural systems and mineralized soil organic matter with subsurface tile drainage are a major source of nitrate pollution of surface waters (Cordell et al., 2009). Nitrate (NO$_3^-$) in agricultural drainage is quickly exported to local surface waters (U.S. EPA, 2016), and eventually, the Mississippi River and the Gulf of Mexico (Goolsby et al., 2001). Excessive NO$_3^-$ and phosphate (PO$_4^{3-}$) contribute to harmful algal blooms (HAB), which causes hypoxic or anoxic zones and leads to an imbalance of aquatic life (Rabalais et al., 2002). Once NO$_3^-$ enters a human body, it can be converted to nitrite (NO$_2^-$), which can combine with amines to form carcinogenic nitrosamines if consumed at concentrations significantly above the drinking water standard set by the United States Environmental Protection Agency at 10 mg L$^{-1}$ (U.S. EPA NPDWR, 2017; McCasland et al., 2012; DeSimone, 2009).

The extensive subsurface tile drainage network in the Upper Midwest typically combines flow from multiple farms to drainage district networks, making regulation and adoption of farm-level conservation practices challenging. In Iowa, over 40 million hectares of agricultural land are tile drained, and farms average 140 hectares (Christianson et al., 2018). To reduce NO$_3^-$ and PO$_4^{3-}$ export, widespread changes are needed in the agricultural landscape. The Iowa Nutrient Reduction Strategy (INRS) estimates that a 41% reduction in NO$_3^-$ loading from non-point sources is needed to meet the 45% total load reduction goal set by the U.S. EPA (INRS, 2017).

Edge-of-field practices will play a critical role in meeting the nutrient reduction goals set by the INRS. In-field practices are expected to remove anywhere from -3 to 41% of NO$_3^-$ by concentration, while edge-of-field practices are expected to remove 33 to 91% of NO$_3^-$ (INRS,
Currently, woodchip bioreactors are not included in the INRS phosphorus (P) reduction goals, but edge-of-field practices are expected to remove 57 to 85% of P loads (INRS, 2017). Woodchip bioreactors are a cost effective and minimally invasive strategy to remove NO$_3^-$N from subsurface drainage through denitrification (Christianson et al., 2010). Denitrification occurs when the system is anaerobic, there is a readily available carbon source, and microbes can utilize the oxygen in NO$_3^-$N (Schipper and Vojvodic-Vukovic, 2001). The ideal final product of denitrification is N$_2$ (gas). Incomplete denitrification can result in the generation of nitrous oxide (N$_2$O), a volatile greenhouse gas that contributes to positive radiative forcing (Moorman et al., 2010; IPCC, 2014).

Hydraulic residence time is an important factor impacting nitrate removal and the overall effectiveness of bioreactors. According to Christianson et al. (2013), the treatment of the majority of total annual drainage volume is not necessary, but instead bioreactors should be designed to treat flow rate at a 6-8 hour HRT. In another study, Christianson et al. (2012a) reported the performance of a single field-scale bioreactor and found that flow rates were not optimized to maximize nitrate removal. The bioreactor experienced ineffective volume utilization due to the HRT being too high and the flow too low. While they suggested minimum design HRT should be increased, they cautioned that low flow conditions could lead to the creation of harmful by-products.

While bioreactors are designed for NO$_3^-$N removal, recent work highlights growing concerns over the transport of P into subsurface tile drainage across the Midwest (Christianson et al., 2016; Clement and Steinman, 2017; Smith et al., 2015; Kinley et al., 2006; King et al., 2014). It was reported by Smith et al. (2015) that 48% of total P losses from a farm-scale research site occurred through the subsurface tile drainage discharge. King et al. (2014) found similar
numbers at 40% of the total P exported from the field leaving through tile discharge. Finally, Clement and Steinman (2016) reported 60% of the total P lost from a farm field left through tile drainage. A substantial amount of nutrient loading is not being accounted for by only treating P associated with surface runoff. Much of the research performed on bioreactors has focused on woodchips and their ability to serve as a carbon source for microbial transformation of NO$_3$N, leaving room to study the impacts of different carbon sources on multi-contaminant removal.

Various carbon substrates have been researched to be used in place of or in tandem with woodchips to improve and expand the nutrient removal capacity of bioreactors. Biochar has been suggested for application in agricultural settings as a nutrient remover because of its ability to retain more water than soil, its large specific surface area, higher cation exchange capacity (CEC), and proven ability to reduce N and P leaching (Laird et al., 2010a; Laird et al., 2010b; Yao et al., 2012; Bock et al., 2015). Biochar is biomass that has undergone pyrolysis to become a highly structured and stable substance with varying chemical properties. The ability of biochar to remove different contaminants depends on several factors, including biomass source, temperature of pyrolysis, type of pyrolysis, post-pyrolysis treatment, age, particle size, and application (Fidel et al., 2017; Jindo et al., 2014).

1.2 Goals and Objectives

The goal of the woodchip bioreactor study (Chapter 3) was to understand the impact of HRT on NO$_3$N removal and differences in processes between in-bioreactor sampling locations.

Objectives of the study were:

i. To determine the effect of HRT on NO$_3$N removal

ii. To compare nitrate removal between in-reactor treatment zones

iii. To compare HRT by the amount of NO$_3$N removed when considering bypass flow
The goal of the biochar study (Chapter 4) was to understand how differences in production of biochar impacts its capability to extend woodchip bioreactors nutrient removal abilities. The objective of the study was:

i. To assess biochar biomass source and pyrolysis temperature to enhance denitrification and P removal in woodchip bioreactors.

1.3 Hypothesis

The hypotheses of the woodchip bioreactor study were:

i. HRT has a significant impact on NO₃⁻N concentration and percent removal in woodchip bioreactors

ii. As HRT increases, NO₃⁻N removal will increase

iii. HRT does not impact mass load removal rates of NO₃⁻N

The hypothesis of the biochar study was:

iv. Pyrolysis temperature and biomass source will have significant impacts on N and P removal by biochar mixed with woodchips

1.4 Thesis Organization

This thesis was organized into five chapters. The first chapter provides a general introduction. The second chapter is a literature review that outlines information relevant to water quality issues and woodchip bioreactors. Chapter three is a manuscript that focuses on the role of HRT in woodchip bioreactors in a highly controlled system. Chapter four is a manuscript that discusses biochar as an amendment to woodchip bioreactors and how its properties are impacted by biomass source and pyrolysis temperature. Chapter 5 consists of the general conclusions to the thesis as well as the implications of the research and future studies.
1.5 References


CHAPTER 2. LITERATURE REVIEW

2.1 The Impact of Nutrients on the Environment, Economy, and Human Health

The loading of nitrogen and phosphorus into water bodies from agricultural regions has been known to cause environmental issues. Hypoxic or anoxic conditions in surface water bodies, namely the northern Gulf of Mexico, are often cited as the biggest concern as a result of current agricultural practices. Hypoxic conditions are defined as having dissolved oxygen (DO) levels $< 2-3 \text{ mg L}^{-1}$ in a water body, whereas anoxic conditions are $0 \text{ mg L}^{-1}$, both partially caused by an excess supply of limiting nutrients entering the ecosystem (U.S. EPA Hypoxia Task Force, 2017). Also called eutrophication, the excess nutrients promote the growth of algae, which die and decompose on the bottom of the Gulf, consuming oxygen and creating low oxygen levels. The fecal pellets of zooplankton that eat algae can also sink to the bottom and decompose (ESA, 2012). The water flowing in from the Mississippi River is less dense and warmer than the deeper saline water of the Gulf, causing stratification that prevents the surface water that is rich with oxygen from mixing with the oligotrophic water toward the bottom of the Gulf. The bottom waters then become oxygen limited, strengthening the hypoxic conditions (U.S. EPA Hypoxia Task Force, 2017).

Because of the hypoxic to anoxic conditions created by nutrient loading, fish kills are common (ESA, 2012). Without fish stocks, fishing boats and tourism are negatively impacted. While adult fish can swim elsewhere, young fish cannot move as easily because they typically need to stay in certain habitats for protection. Nurseries for young fish and shellfish are affected, eliminating populations because they cannot reach adulthood. Other animals such as clams do not have the ability to move to cleaner water. When aquatic life is impacted, any predators, such as birds and mammals, that relied on the area for food are also negatively impacted (ESA, 2012).
In 2017, the “Dead Zone” in the Gulf measured 22,720 km$^2$, reaching above the five-year average with the cause cited as above average discharge and high nitrate concentrations from the Mississippi River (Louisiana Universities Marine Consortium, 2017; NOAA, 2017).

Nitrogen and phosphorus loading can have local impacts as well. The United States Environmental Protection Agency (U.S. EPA) set the nitrate-nitrogen drinking water standard at 10 mg L$^{-1}$ in order to prevent human health issues (U.S. EPA NPDWR, 2017). Blue Baby Syndrome, or Methemoglobinemia, is often cited as the most common human health issue related to consuming nitrate in drinking water. However, in the United States, there are very rarely any cases reported due to better control of water supplies. Occasionally a case is reported from rural areas due to the use of private water wells in agricultural regions but is not related to surface water body pollution (Knobeloch et al., 2000; WHO, 2018). There has been some speculation that ingesting water above the standard nitrate concentration set by the EPA for long periods of time can cause cancer via the formation of nitrosamine, though there is yet to be concrete evidence of this connection (McCasland et al., 2012; DeSimone, 2009).

Local ecosystems can be impacted by nutrient loading in the same way that the Gulf of Mexico is. Algal blooms in Iowa are caused by excess nutrients and warm, slow moving water, usually occurring in the late summer to early fall (IDPH, 2018). These algae blooms are frequently caused by cyanobacteria, also called blue-green algae. Cyanobacteria can produce toxins dangerous to humans when swallowed or breathed in as water droplets. Symptoms can range from vomiting and diarrhea in typical cases to liver failure in the most severe cases (IDPH, 2018). The Iowa Department of Natural Resources (DNR) Beach Monitoring program tests 39 of Iowa’s State Park beaches for microcystin, one of the toxins produced by cyanobacteria. When microcystin exceeds 20 µg L$^{-1}$, a warning is posted advising people to stay out of the water, a
standard set by the World Health Organization (Iowa DNR, 2017). From 2006 to 2017, of the 39 beaches monitored, they were shut down a collective of 190 times due to high microcystin levels (Iowa DNR, 2017). In 2017, of the total 145 lakes and reservoirs listed as impaired by the Iowa DNR, over 90 were impaired by algal blooms (Iowa DNR, 2017).

Despite the wide-scale pollution, the nitrogen load reaching the Gulf of Mexico has somewhat stabilized due to conservation practices increasing in utilization in the Mississippi River Basin (Louisiana Universities Marine Consortium, 2017; NOAA, 2017). Ribaudo et al. (2008) estimate that nonpoint sources of nutrients are contributing over 90% of the pollution in 33% of nitrogen-impaired water bodies in the United States. The task then falls on states like Iowa to do a better job at mitigating its nutrient loading into surface water bodies. Recent efforts and events have raised Iowa’s awareness of nutrient pollution from agriculture. In early 2018, a bill was passed approving $282 million to go toward water quality initiatives in Iowa, including those set forth by the Iowa Nutrient Reduction Strategy (INRS) (Pfannenstiel, 2018). Although dismissed in 2017, a lawsuit filed by Des Moines Water Works brought attention to non-point source pollution of nitrate from three Northwest Iowa counties. The utility was seeking damages for the cost of operation to remove nitrate in 2015, reaching a total of $1.2 million (Elmer, 2017). Conversation in Iowa has turned toward developing and implementing practices that can prevent nutrient loading into surface water bodies both locally and downstream.

2.2 Transport of Nutrients in Tile Drainage

Beginning in the 1800s, the plow was used to convert tallgrass prairie into farmable land in the Midwest. By the late 1800s, the invention of drainage tile opened the door for farmers to till native wetlands, also known as prairie potholes, which once existed in Iowa. In 1908, an amendment to the Iowa Constitution Bill of Rights called for the creation of drainage districts, which allowed farmers to work together to construct tile drainage (Constitution of the State of
Iowa, 2010). In 150 years, over 90% of Iowa’s prairie potholes were drained and converted to cropland (NRCS, 2005). Financial assistance provided by federal and local government in the 1920s and 1930s furthered the drainage process not only in Iowa, but in Missouri, Minnesota, Illinois, Indiana, and Ohio as well (University of Illinois Extension, 2018). Iowa currently has 22% of its land (3,643,660 ha) tile drained, largely focused to the Des Moines Lobe glaciated region that has low saturated conductivity that restricts downward drainage (Kalita et al., 2007).

The benefits of draining farm land are numerous, including more consistent crop yields, less soil compaction, reduction of financial risk on famers, and earlier planting, to name a few (Iowa State University Extension, 2010). Despite turning the Midwest into one of the most profitable agricultural regions in the world, tile drainage has created concerns, from removing native habitat in the Midwest to lowering water quality both locally and downstream. Nitrogen has the ability to be mobile with water as the anion nitrate (NO\text{\text{3}}^-) because it does not readily sorb to negatively charged soil (Masarik et al., 2014). Nitrate can travel with water that percolates through soil profile and into the tile drainage where it enters surface water bodies. The use of commercial nitrogen fertilizer to increase productivity has caused increased nitrate levels being leached from agricultural land (Masarik et al., 2014). Soil nitrogen is also a major contributor to the nitrate pool with lack of plant uptake of nitrogen in the spring months cited as a major factor in nitrogen loss through tile drainage (Dinnes et al., 2002). Of nitrogen reaching the Gulf of Mexico, 52% is reported to originate from corn and soybean (USGS, 2014). Iowa, Illinois, and Indiana contribute 11.3, 16.8, and 10.1% of nitrate exported to the Gulf of Mexico, respectively (USGS, 2014).

It is highly unlikely that Midwestern states will give up farming any time soon, so conservation practices are being developed and utilized to lessen the impact of agricultural
drainage on water quality. The INRS was developed in response to the 2008 Action Plan set forth by the Mississippi River/Gulf of Mexico Watershed Nutrient Task Force asking the 12 states that border the Mississippi river to develop nutrient reduction plans. In the plan, several strategies are outlined and organized into three main groups: Nitrogen Management, Land Use, and Edge-of-Field (INRS, 2017). The focus of Nitrogen Management is controlling nitrogen as it is being applied to crops and before it leaves the field. This includes things like timing of application, source of fertilizer, application rates, inhibiting nitrification, and using cover crops. Next is Land Use, which puts an emphasis on farming practices such as growing perennial crops, using the Conservation Reserve Program (CRP), and practicing extended rotations that include corn, soybean, alfalfa, and wheat. Finally, the Edge-of-Field group aims to target nitrogen loss after it leaves the farm field but before it enters other water systems. Wetlands are one type of conservation practice that is working to restore Iowa’s native prairie potholes through the CRP, Conservation Reserve Enhancement Program (CREP), and Farmable Wetlands Program (FWP). Wetlands are expected to be able to remove up to 52% of influent nitrate concentrations by the INRS. Saturated buffers remove an average of 50% of influent nitrate. Finally, woodchip bioreactors are expected to remove up to 43% of nitrate concentration to meet the INRS goals (INRS, 2017).

2.3 Woodchip Bioreactors

Woodchip bioreactors are an edge-of-field conservation practice that intercepts subsurface tile drainage with the goal of removing nitrate from water before it enters surface water bodies. Bioreactors are trenches buried under the soil and filled with a carbon source, typically woodchips, and naturally occurring microbes from the woodchips and soil. Woodchips are typically used because of their high C:N ratio and low cost (Healy et al., 2012). The goal of bioreactors is to transform nitrate into N₂ (gas), an inert greenhouse gas (GHG).
Bioreactors transform nitrate into $N_2$ through oxidation-reduction (redox) reactions, specifically through a process called denitrification. The primary driver of denitrification in bioreactors is generally considered to be heterotrophic microorganisms under anaerobic conditions. Other processes have been found in bioreactors, but they were not the main process for nitrogen removal. These two processes were dissimilatory nitrate reduction to ammonium or ammonia (DNRA) and nitrogen immobilization to organic matter, both of which only accounted for less than 4% of the total nitrate removal (Greenan et al., 2006). Oxygen is the most favorable electron acceptor, but when oxygen is not readily available (anaerobic conditions), denitrifying microbes will use nitrate as the next best alternative electron acceptor, with carbon from the woodchips serving as the electron donor (Schipper et al., 2010). Denitrification takes place following the process shown in Equation 2.1. In order to successfully remove nitrate in a bioreactor to a less harmful environmental product, denitrification must happen completely.

Potential problems with bioreactors using include the production of $N_2O$ and $CH_4$ as gases, production of hydrogen sulfide, and production of methylmercury, which could bioaccumulate in aquatic life (Moorman et al., 2010).

Equation 2.1.

$$2 \text{NO}_3^- + 10 \text{e}^- + 12 \text{H}^+ \rightarrow \text{N}_2 + 6 \text{H}_2\text{O}$$

The first published study on bioreactors was Blowes et al. (1994), where they demonstrated nitrate removal in agricultural drainage with a bioreactor filled with woodchips, tree bark, and leaf compost. They were able to reduce the NO$_3^-$N concentration from 3-6 mg NO$_3^-$N L$^{-1}$ down to $< 0.2$ mg NO$_3^-$N L$^{-1}$. Since that paper was published, many other promising studies have been published on bioreactors with varied results and applications. A wide range of removal rates in woodchip bioreactors have been reported for the Upper Midwest. Removal rates depend on many
different factors, including but not limited to the age of the carbon substrate, weather conditions, temperature, influent nitrate concentrations, microbial community, maintenance of the system, size of land drained, and hydraulic residence time. Woodchip bioreactors in Iowa, Illinois, and Minnesota average between 15 to 60% nitrate load removed each year (Christianson and Helmers, 2011). In another case in Northeastern Iowa, a bioreactor ranged from no removal at all to almost 100% removal in the course of one year (Christianson et al., 2011). Addy et al. (2016) found that as temperature increases in the bioreactor, so does nitrate removal. In yet another study, Hoover et al. (2015) calculated the effects of hydraulic residence time (HRT) and found that nitrate removal increased from 8 to 55% as HRT increased incrementally from 1.7 hours to 21.2 hours. Due to the variability in results from studies all around the Midwest, Christianson et al. (2012b) suggest that more field-scale studies are needed to enhance understanding of NO$_3^-$N removal in woodchip bioreactors.

2.4 The Dynamics of Denitrification

Microbial denitrification in woodchip bioreactors can be manipulated by design and by the environment in a number of ways. For complete denitrification to occur in bioreactors, the four following conditions must be met: a microbial community of denitrifying bacteria, anaerobic conditions, NO$_3^-$N present to act as the electron acceptor, and a sufficient supply of carbon to act as the electron donor. There are a number of environmental conditions that can impact the efficiency of denitrification, including temperature of subsurface tile drainage water, dissolved oxygen (DO) concentrations in the water, and influent NO$_3^-$N concentrations. Some factors can be designed to alter NO$_3^-$N removal rates, including HRT, carbon type, and the rate of carbon degradation depending on the carbon source.
2.4.1 Temperature

The temperature of water and the woodchip bioreactor can have a significant impact on the rate of denitrification. Hoover et al. (2015) found in a lab-scale study that NO$_3^-$N removal percentages were influenced by increasing temperature from 10 to 20°C in an exponential response. Another lab-scale experiment found that the percent NO$_3^-$N removal increased from 29% reduction at 10°C to 96% reduction at 21.5°C (Soupir et al., 2018). Cameron and Schipper (2011) used passive solar heating on a denitrification bed to increase the temperature of a bioreactor by 3.4°C, which did not significantly increase the N removal rate. In the Upper Midwest, water temperatures tend to be lowest in the spring when flow rates and N loads are highest; to combat this, it is suggested that HRT be increased in the spring to increase nitrate removal (Christianson et al., 2012b). Because microbes are the main driver of denitrification in woodchip bioreactors, it is important to recognize the impact of temperature on their populations. Increasing the temperature of a bioreactor from 16.1°C to 27.1°C increased the concentration of genes required for denitrification by four times (Warneke et al., 2011).

2.4.2 Dissolved Oxygen

Dissolved oxygen concentrations in a woodchip bioreactor can have a significant impact on its denitrification efficiency. Denitrification begins to occur when DO levels are below 2 mg L$^{-1}$, but the process can be impeded with DO levels as low as 0.2 mg L$^{-1}$ (Hoover, 2012; Metcalf and Eddy, 2003). When oxygen is present, microbes prefer to use it as a terminal electron acceptor because it releases a higher amount of free energy than NO$_3^-$N when reduced within a cell (Schipper et al., 2010). Denitrification can still take place at a lower efficiency inside the anaerobic microsites of woodchips when DO is above 2 mg L$^{-1}$, but the pattern tends to be that as DO concentrations increase, denitrification efficiency decreases (Hoover, 2012). In a comprehensive review of woodchip bioreactors, Christianson et al. (2012b) reported that most
bioreactors are able to reduce DO concentrations to 0.5 mg L\(^{-1}\) within 25% of the length of bioreactor starting from the inlet. There is also potential for denitrifying microbial biofilms to have reduced growth when DO concentrations are high for long periods of time (Gomez et al., 2001).

### 2.4.3 Influent NO\(_3\)^-N Concentrations

Because there is high variation in site conditions where woodchip bioreactors are installed, the impact of influent NO\(_3\)^-N concentration on denitrification rates must be understood. Current studies differ in their conclusions on the influence of influent NO\(_3\)^-N concentrations. In one study, Hoover et al. (2015) tested the impact of influent NO\(_3\)^-N concentrations ranging from 10 to 50 mg L\(^{-1}\). They found that when environmental conditions are controlled for, NO\(_3\)^-N load reduction increases with higher influent concentrations, but the rate of increase slows the higher the NO\(_3\)^-N concentration. They suggested that N-saturation occurs between 30 to 50 mg L\(^{-1}\), which is indicative of Michaelis-Menten kinetics (Hoover et al., 2015). Ghane et al. (2014) state that the Michaelis-Menten kinetic model is the best for estimating NO\(_3\)^-N removal rates in denitrification beds that treat agricultural subsurface drainage water. Robertson et al. (2010) found that zero-order reaction kinetics were best to model denitrification reactions in woodchip bioreactors because NO\(_3\)^-N was not the rate-limiting substrate. Christianson et al. (2012a) found that besides flow rate, NO\(_3\)^-N removal rates were most impacted by influent NO\(_3\)^-N concentrations in four field-scale woodchip bioreactors in Iowa. They suggested that first-order reaction kinetics would best explain NO\(_3\)^-N removal rates because the reaction rates were dependent on the availability of NO\(_3\)^-N.

### 2.4.4 Microbes

Denitrifying microbes come from the groups Archaea and Bacteria. The most abundant type of denitrifier is heterotrophic bacteria, which are able to use a variety of carbon compounds,
including sugars, amino acids, and organic acids as sources of electrons (Hiscock et al., 1991). *Pseudomonas* have been most commonly isolated and are most representative of active denitrifiers as they exist in the environment naturally. *Alcaligenes* and *Flavobacterium* are other important groups for denitrification. *Thiobacillus denitrificans* is a representative microbe for autotrophic denitrification (Hiscock et al., 1991). An important part in identifying these microbes is understanding which functional gene they possess to denitrify. Some microbes only contain a few of the genes necessary while some possess all of the genes necessary for denitrification and can carry it out alone. The process to get from NO$_3$-N to N$_2$ can sometimes take a symphony of microbes working together in various types of relationships in the right environmental conditions. Because there is such a high diversity of microbes that denitrify in a vast number of ecosystems, the conditions in which they can survive and thrive varies greatly. Archaeal denitrifiers, for example, have been found to be hyperthermophilic and salinophilic (Jones, 2010). Denitrifying organisms are polyphyletic, which makes it difficult to connect their activity to microbial community composition based on species alone (Jones, 2010).

### 2.4.5 Hydraulic Residence Time

Perhaps one of the most confounding variables influencing N removal rates in bioreactors is HRT. Having the correct HRT is vital to maximizing NO$_3$-N removal rates, reducing the amount of bypass flow, and minimizing negative byproducts of anaerobic conditions. Hoover et al. (2015) found in a laboratory study that increasing HRT from 1.7 hours to 21.2 hours incrementally resulted in consistent NO$_3$-N mass removal, not increasing. A study by Christianson et al. (2011a) on pilot-scale bioreactors in Central Iowa also found that NO$_3$-N mass removal rates were consistent as HRT ranged from 1.3 to 11.3 hours despite the percent NO$_3$-N reduction increasing with increasing HRT. Typically, as HRT increases, so does NO$_3$-N by both concentration and by percent removal because the flow is slower and denitrifying microbes have
more time to remove NO$_3$N from the water. Having a shorter HRT with faster flow delivers greater loads of NO$_3$N into the bioreactor assuming nitrate concentrations are constant. Delivering larger loads of NO$_3$N in the influent flow gives lower HRT bioreactors more opportunity to remove NO$_3$N by mass, but it also lowers their overall percent removal of NO$_3$N. Percent removal can be a deceiving value, however, because it is dependent on the specific site conditions of the bioreactor, including differences in influent NO$_3$N concentrations. Mass removal of NO$_3$N, reported as g m$^{-3}$ day$^{-1}$, could be a better indicator of bioreactor performance because it is not as dependent on the variables of the bioreactor.

Addy et al. (2016) performed a comprehensive review on published bioreactor data and found a significant difference in NO$_3$N mass load removal between bioreactors with an HRT <6 hours and those ranging from 6 to 20 hours and >20 hours. They went on to suggest that the increasing use of control structure would allow for further examination of NO$_3$N removal with different HRT strategies (Addy et al., 2016). Another review concluded that having low HRT may reduce the ability of the bioreactor to remove influent DO to levels that allow for denitrification (Christianson et al., 2012b). Having an HRT that is high leads to increased NO$_3$N removal rates but could lead to oxidation reduction potentials (ORP) that cause undesirable processes such as mercury methylation and sulfate reduction (Christianson et al., 2012b). There have been a wide range of NO$_3$N removal rates reported for bioreactors in Central Iowa, which were compiled by Christianson et al. (2012b). Of the bioreactors they listed, 20 were located in the Upper Midwest, and of those, five were either pilot-scale or lab-scale and 15 were field-scale studies. Only nine of the bioreactors listed from the Upper Midwest had HRT reported. The data from those nine bioreactors is summarized in Table 2.1. The general trend is that as HRT increases, the amount of NO$_3$N removed increases. The HRT in Table 2.1 range from 26 min. to
9.8 days, and the percent NO₃-N reduction ranges from 10% to 100%. Despite all of the included studies happening in the Upper Midwest and across only three states, the variation in the impact of HRT is large. The impact of HRT depends on the site conditions and the specific bioreactor design. In order to better understand the role of HRT on denitrification in woodchip bioreactors, a highly controlled bioreactor system needs to be set up to account for variables over a range of HRT.

Table 2.1. A comprehensive list of bioreactor studies from the Upper Midwest that reported hydraulic residence time. Data and table information used from (Christianson et al., 2012b).

<table>
<thead>
<tr>
<th>Source</th>
<th>Site</th>
<th>Scale</th>
<th>Volume m⁻³</th>
<th>HRT</th>
<th>Percent Reduction</th>
<th>Nitrate-N Removal Rate</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moorman et al., 2010</td>
<td>Central IA</td>
<td>Field</td>
<td>38.9</td>
<td>24 h required to reduce influent to ≤10 mg L⁻¹</td>
<td>NA</td>
<td>23.6 mg N kg woodchip⁻¹ day⁻¹</td>
<td>Retention time conclusion based on field data</td>
</tr>
<tr>
<td>Chun et al., 2010</td>
<td>Decatur, IL (west)</td>
<td>Field</td>
<td>55.8</td>
<td>4.4 h</td>
<td>47% load</td>
<td>NA</td>
<td>2.0 ha treatment area</td>
</tr>
<tr>
<td>Wolf et al., 2010</td>
<td>East-Central IL (DeLand, IL)</td>
<td>Field</td>
<td>76.9</td>
<td>26 min. to 2.8 h</td>
<td>23%–50% load</td>
<td>6.4 g N m⁻³ day⁻¹</td>
<td>14 ha treatment area</td>
</tr>
<tr>
<td>Ranavoson et al., 2010</td>
<td>Claremont, MN</td>
<td>Field</td>
<td>NA</td>
<td>32 h for 50% conc. Reduction</td>
<td>18%–47% load</td>
<td>NA</td>
<td>10.5 ha treatment area</td>
</tr>
<tr>
<td>Christianson et al., 2011</td>
<td>Central IA</td>
<td>Pilot</td>
<td>0.71</td>
<td>4 to 8 h</td>
<td>30%–70%</td>
<td>3.8–5.6 g N m⁻³ day⁻¹</td>
<td>Mixed hardwood chips; different design geometries</td>
</tr>
<tr>
<td>Chun et al., 2009</td>
<td>IL</td>
<td>Lab column</td>
<td>0.30</td>
<td>2.6–12.0 h and &gt;15 h</td>
<td>10%–40% and 100% concentration (respective to retentions)</td>
<td>NA</td>
<td>Three parameter estimation, first order reaction</td>
</tr>
<tr>
<td>Greenan et al., 2009</td>
<td>Central IA</td>
<td>Lab column</td>
<td>0.01</td>
<td>9.8, 3.7, 2.8, and 2.1 days</td>
<td>100, 64, 52, and 30% load (respective to retentions)</td>
<td>11–15 mg N kg woodchip⁻¹ day⁻¹</td>
<td>NA</td>
</tr>
<tr>
<td>Cooke et al., 2001</td>
<td>IL</td>
<td>Lab column</td>
<td>0.001</td>
<td>8 h</td>
<td>Nearly 100% concentration</td>
<td>NA</td>
<td>Woodchips at 25°C</td>
</tr>
<tr>
<td>Doheny, 2002</td>
<td>IL</td>
<td>Lab column</td>
<td>0.001</td>
<td>10 h</td>
<td>60% concentration</td>
<td>NA</td>
<td>Woodchips at 10°C</td>
</tr>
</tbody>
</table>
2.4.6 Carbon Source

Besides NO$_3^-$N, carbon is the other most important factor in determining if denitrification happens or not. Typically, woodchip bioreactors are not limited by carbon source. Cameron and Schipper (2010) evaluated several different carbon sources, including five different grain sizes of *Pinus radiata*, eucalyptus woodchips, maize cobs, green waste, and wheat straw. Despite initial leaching of NH$_4^-$N and biological oxygen demand (BOD), maize was found to be the substrate that removed the most NO$_3^-$N in a 23 month period. They also found that substrates with more labile carbon (maize cobs, green waste, and wheat straw) removed significantly more nitrate than wood media. Over the same 23 month period, hardwood and softwood media showed no significant difference in NO$_3^-$N removal and that hardwood may have initially contributed to an unfavorably low pH for the microbes. They concluded that maize cobs could be a replacement for woodchips in bioreactors as long as the initial NH$_4^-$N and BOD leaching is handled properly. The issue with using maize cobs is that they degrade quickly, which could limit their application in the field. Addy et al. (2016) suggested that a long-term field study would be needed to assess the true lability and decomposition rates of corn maize before it can be successfully integrated into bioreactors.

In another study, Addy et al. (2016) examined the effects of hardwood and softwood substrates on NO$_3^-$N removal. From researching multiple published papers, they found that there was no significant difference in nitrate removal between the two types of woodchips; however, they did find that there could be potential impacts on the overall stability of the bioreactor and availability of carbon. It was expected that softwood would be more recalcitrant per mass, but overall the availability of carbon did not vary between the two substrates. Woodchip density was found to have a possible effect on decomposition rates. Because of its lower density, softwoods (such as *Pinus*) can allow oxygen into dead wood, which increases the rate of decay and loss of
carbon supply. Addy et al. (2016) went on to discuss the impacts of the age of the biomass, in which they stressed that the first year of results from a bioreactor can be misleading. New bioreactors can show higher nitrate removal rates but tend to reach more average rates in the second year and beyond.

The type of carbon media used can impact how water moves through the bioreactor, affecting HRT and nitrate removal. It is assumed that woodchip bioreactors generally have a drainable porosity of 0.70 for the purposes of calculating HRT (Hoover et al., 2017). Cameron and Schipper (2010) state that more labile carbon sources have not been well studied, especially the long-term permeability when compared to woodchips. They also state that woodchip bioreactors usually do not suffer loss in permeability over time (Van Driel et al., 2006). In their study of different types of biomass, they found that as grain size increased, hydraulic conductivity also increased. They stated that this was most likely due to gas bubbles becoming trapped by the larger particle sizes as they tended to lay on their flat surfaces whereas the smaller sizes were “lath-like.” They also concluded that there was no difference between softwood and hardwood in their decline of hydraulic conductivity.

In another study, Cameron and Schipper (2012) found that carbon substrate and temperature had more impact on nitrate removal than the media’s hydraulic efficiency. Smaller wood grain sizes were more hydraulically efficient than larger sizes, but the difference was small. They also found that temperature tends to increase media porosity due to loss of water from the cellulose which causes the wood particle to contract. Over all the media they tested, they found slight differences in hydraulic properties, but none were significantly different enough to impact nitrate removal rate.
2.4.7 Age of Carbon Source

The age of the carbon source used in woodchip bioreactors also has an impact on denitrification. Most studies find that the first year of operation of a bioreactor usually has the highest amount of carbon loss and sometimes the highest NO$_3$-N removal rates. In a laboratory column-scale study, 7-year-old woodchips were able to remove 9.1 mg N L$^{-1}$, remaining within 75% of the rate of two-year-old woodchips and within 40-59% of the rate for fresh woodchips (Robertson, 2010). In a field-scale woodchip bioreactor in Illinois, David et al. (2016) saw a decrease from 23-44 g N m$^{-3}$ d$^{-1}$ NO$_3$-N in the first year to 1.2-11 g N m$^{-3}$ d$^{-1}$ in the second and third year. They state that the greater N removal rates in the first year were likely due to the C in the woodchips being highly degradable (David et al., 2016). Moorman et al. (2010) studied a woodchip bioreactor over nine years and found that at 90-100 cm depth, the loss of wood to decomposition reached 75 percent, giving a half-life of 4.6 years for the wood. At 155-170 cm depth, 20% of the wood was lost with a half-life of 36.6 years. At this lower depth, oxygen was depleted and methane was depleted, indicating that sustained anaerobic conditions below the tile drainage line at 120 cm caused the difference in degradation rates (Moorman et al., 2010). In a field-scale woodchip bioreactor study, Hassanpour et al. (2017) added biochar as an amendment to improve NO$_3$-N removal. They found that biochar outperformed the woodchip-only bioreactor only for the first year at NO$_3$-N removal, and that a lot of the biochar was either lost at the beginning of the study or rapidly aged due to the presence of organic matter from the soil (Hassanpour et al., 2017).

2.4.8 Biochar

There is potential to expand the nutrient removal capacity of bioreactors beyond NO$_3$-N by using different substrates with various treatments. Biochar has been suggested for application in agricultural settings because of its ability to retain more water than soil, its large specific
surface area, higher cation exchange capacity (CEC), and proven ability to reduce N and P leaching (Laird et al., 2010a; Laird et al., 2010b; Yao et al., 2012; Bock et al., 2015) Biochar is material that has undergone high-temperature burning in a low to no oxygen environment. Bock et al. (2015) examined the effects of biochar on nitrate and phosphate removal, in which they concluded that biochar performed better than woodchip controls in both an 18 and 72 hour period. Biochar is characterized by its high specific surface area and high micropore volume, which allows it to be an effective sorbent (Laird et al., 2010a).

Hassanpour et al. (2017) studied the addition of biochar to woodchip bioreactors in New York State over a three-year period. The biochar they used was produced from pine woodchips between 1-2 cm in size using slow pyrolysis between 550°C and 600°C. They found that biochar significantly increased denitrification during the first year, but declined in quality during subsequent years due to aging. A study performed by Yao et al. (2012) tested 13 different biochars and found that none had a significant impact on either nitrate or phosphate removal. The aging of biochar can have highly variable effects depending on the biochar type. The performance of fresh biochars is not necessarily predictive of how the biochar will perform long-term (Aller et al., 2017). Aller et al. (2017) highlighted that biomass source and pyrolysis conditions need to be studied more closely to better understand the potential impacts of biochar.

2.5 Greenhouse Gases and Other Products of Woodchip Bioreactors

While the main goal of woodchip bioreactors is denitrification, there are many other processes that take place that could have negative environmental impacts. Fenton et al. (2014) deemed this “pollution swapping,” emphasizing the need to better understand if the amount of nitrate removal is worth the negative effects. These effects include the release of greenhouse gases such as methane and nitrous oxide at the surface and dissolved gases in discharge water. It also includes the creation of hydrogen sulfide and methylmercury in low flow systems that have
become too anaerobic for long periods of time and lack NO$_3$N as a terminal electron acceptor (Addy et al., 2016). Fenton et al. (2014) suggest that control of hydraulic residence times will be one of the best ways to control pollution swapping, paired with permeable reactive interceptors that help target more contaminants than just NO$_3$N.

In a nine-year study of a woodchip bioreactor in Central Iowa, Moorman et al. (2010) concluded that N$_2$O release from the bioreactor was not significantly different than the control (soil) release when using IPCC estimates for greenhouse gases. In another study, Healy et al. (2012) found that different media can have different impacts on greenhouse gas release, with carbon dioxide and methane fluxes being the largest and nitrous oxide having little to no flux due to denitrification. This is expected, because at a redox potential low enough for methane production to occur, NO$_3$N would be absent which causes N$_2$O to also be absent. They concluded that methane may be the more important greenhouse gas being released because nitrous oxide amounts were too low to have a significant impact, and that HRT in bioreactors should be adjusted accordingly to prevent methane production. In the several studies they examined, they found a range of 0.003-4.3% for the amount of influent nitrate that left as nitrous oxide from bioreactors.

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CHAPTER 3. IMPACT OF CONTROLLED HYDRAULIC RESIDENCE TIME ON NITRATE REMOVAL IN PILOT-SCALE WOODCHIP BIOREACTORS

Modified from a manuscript to be published in a scientific journal

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3.1 Abstract

Nitrate (NO$_3$-N) export from row crop agricultural systems with subsurface tile drainage continues to be a major water quality concern for the Midwest and the Gulf of Mexico. Woodchip bioreactors are an effective edge-of-field practice designed to remove NO$_3$-N from tile drainage. The NO$_3$-N removal rate of woodchip bioreactors can be impacted by several factors, including hydraulic residence time (HRT). This study examined the impact of three HRTs, 2 h, 8 h, and 16 h, on NO$_3$-N removal in a set of nine controlled pilot-scale woodchip bioreactors in Central Iowa. The 2 h HRT had the highest NO$_3$-N mass removal rate (MRR) at 9.0 g m$^{-3}$ day$^{-1}$, followed by the 8 h HRT at 8.5 g m$^{-3}$ day$^{-1}$, and the 16 h HRT at 7.4 g m$^{-3}$ day$^{-1}$, all of which were statistically different (p < 0.05). When accounting for bypass flow, the 2 h HRT still removed more NO$_3$-N by mass than the other HRTs. NO$_3$-N concentration reduction from the inlet to the outlet was significant for all HRTs (p < 0.05). The 16 h HRT removed the most NO$_3$-N by concentration (7.5 mg L$^{-1}$) and had the highest percent mass removal rate (PMRR) at 53.8%. The 8 h HRT removed an average of 5.5 mg L$^{-1}$ NO$_3$-N with a PMRR of 32.1%. The 2 h HRT removed an average of 1.3 mg L$^{-1}$ NO$_3$-N with a PMRR of 9.0%. Significant explanatory variables for PMRR were HRT (p < 0.001) and influent NO$_3$-N concentration (p < 0.001) (r = 0.80).

3.2 Introduction

In the Upper Mississippi River Basin, intensively fertilized row crop agricultural systems and mineralized soil organic matter are a major source of nitrate (NO$_3$-N) to surface waters
because of subsurface tile drainage (Cordell et al., 2009). Nitrate in agricultural drainage is quickly exported to local surface waters (U.S. EPA, 2016), and eventually, the Mississippi River and the Gulf of Mexico (Goolsby et al., 2001). Excessive NO₃⁻N contributes to harmful algal blooms (HAB), which causes hypoxic zones and leads to reduced aquatic integrity (Rabalais et al., 2002). When NO₃⁻N is ingested by humans, it can be converted to nitrite (NO₂⁻N), which can combine with amines to form carcinogenic nitrosamines. If consumed at concentrations significantly above the drinking water standard set by the United States Environmental Protection Agency at 10 mg L⁻¹, this can lead to several types of cancer (U.S. EPA NPDWR, 2017; McCasland et al., 2012; DeSimone, 2009).

Extensive subsurface tile drainage in the Upper Midwest typically combines flow from multiple ownerships into drainage district networks, making regulation and adoption of farm-level conservation practices challenging. In Iowa, over 40 million hectares of agricultural land are tile drained, with farms averaging 140 hectares (Christianson, R. et al., 2018). To reduce NO₃⁻N export, multiple approaches are needed in the agricultural landscape. When accounting for point-source contributions, the Iowa Nutrient Reduction Strategy (INRS) estimates that a 41% reduction in NO₃⁻N loading from non-point sources is needed to meet the 45% total load reduction goal set by the U.S. EPA (INRS, 2017).

Edge-of-field practices will play a critical role in meeting NO₃⁻N reduction goals. In-field practices are estimated to remove from -3 to 41% of NO₃⁻N by concentration, while edge-of-field practices have potential to remove 33 to 91% of NO₃⁻N. Woodchip bioreactors are a cost effective and minimally invasive strategy to remove NO₃⁻N from subsurface drainage through microbial denitrification (Christianson et al., 2010) with an estimated NO₃⁻N percent mass removal rate (PMRR) of 43% (INRS, 2017). Denitrification occurs under anaerobic conditions
with a readily available carbon source (Schipper and Vojvodic-Vukovic, 2001). The ideal final product of denitrification is N\textsubscript{2} (gas). Incomplete denitrification can result in the generation of nitrous oxide (N\textsubscript{2}O), a volatile greenhouse gas (GHG) that contributes to positive radiative forcing (Moorman et al., 2010; IPCC, 2014).

As an edge-of-field practice, bioreactors have little to no impact on crop yield or soil quality, and they also require minimal land taken out of production (Christianson and Helmers, 2011). Bioreactors require minimal management, with the inlet and outlet control structures needing to be adjusted approximately twice per year (Christianson and Helmers, 2011). In Iowa, average bioreactor installation cost currently ranges from $10,000 to $20,000, with most installations being partially supported by cost sharing (Christianson and Helmers, 2011; McKinney, 2018). With an average removal rate of 43%, bioreactors have an estimated cost per kg of nutrient removed of $0.40 kg N\textsuperscript{-1} (Christianson et al., 2018). Woodchip bioreactors have a wide variety of reported NO\textsubscript{3}\textsuperscript{-N} removal rates, but typically range from 13 to 100% depending on conditions and location (Christianson et al., 2018; Greenan et al., 2009; Christianson et al., 2012b; Hassanpour et al., 2017). To maximize NO\textsubscript{3}\textsuperscript{-N} removal, bioreactors must be engineered to optimize denitrification through consideration of landscape placement, shape, biomass source, carbon source, drainage treated, peak flow conditions, and hydraulic residence time (HRT).

Hydraulic residence time is an important factor impacting NO\textsubscript{3}\textsuperscript{-N} removal within bioreactors. Ideal design for HRT is challenging based on studies in uncontrolled field settings because of high variability among sites. Christianson et al. (2012a) recommended that additional field-scale studies are needed to validate and enhance understanding of NO\textsubscript{3}\textsuperscript{-N} removal in woodchip bioreactors. Previous research on bioreactors has not included in-reactor sampling, which could help identify specific processes besides denitrification and where they occur within
the bioreactor. This study focused on the role of HRT on overall NO$_3$-N removal in controlled flow, triplicate pilot-scale woodchip bioreactors. Objectives were to (1) determine the effect of HRT on NO$_3$-N removal, (2) compare NO$_3$-N removal between in-reactor treatment zones, (3) compare each HRT by NO$_3$-N removal when considering bypass flow. The pilot-scale bioreactors provided a unique opportunity to study these processes in a controlled system to improve woodchip bioreactor design to maximize NO$_3$-N removal.

3.3 Materials and Methods

3.3.1 System Overview

The study was conducted in nine pilot-scale bioreactors located at the Iowa State University Agronomy and Agricultural Engineering Farm, located west of Ames, Iowa (42.019861, -93.776872). The system was described in detail by Hoover et al. (2017) and a schematic is shown in Figure 3.1. The bioreactors were installed in September 2014 and the water source was a 30.5 cm diameter tile drainage line. Water was pumped to three 11,356 L aboveground storage tanks, which held the water before entering the bioreactors. Water flowed by gravity to the reactors and was controlled by 5.1 cm brass gate valves. The nine bioreactors had individual internal dimensions of 5.79 m x 1.0 m x 1.07 m with a concrete frame, which created a closed system that allowed for estimation of water balance. The bioreactors were filled with local hardwood woodchips from Golden Valley Hardscapes (Story City, Iowa), which are described in detail by Christianson et al. (2010).
The bioreactors had four main structures: an inlet port, two sampling wells, and an outlet structure (Figure 3.1). Each bioreactor had two 1.8 m PVC sampling wells attached to the bottom of the bioreactor and positioned along the centerline lengthwise. The wells were slotted at 1.3 cm increments to a depth of 1.1 m that allowed the flow through of water for collecting samples. The two wells were considered sampling points “well A” and “well B” for each bioreactor, with well A located closest to the inlet port and well B located closest to the outlet structure (Figure 3.1). Well A was 1.42 m from the inlet, wells A and B were 2.95 m away from each other, and well B was 1.42 m from the outlet. Hydraulic residence times were randomly assigned in a complete block design to include triplicates of a 2 hour, 8 hour, and a 16 hour HRT. Target flow rates were calculated using media porosity and saturated bioreactor volume. Media porosity was determined by a potassium bromide tracer study (Hoover et al., 2017).

Figure 3.1. The layout of the set of nine pilot-scale woodchip bioreactors used in the study located in Central Iowa. They were constructed in 2014 and ran for this study from 2016-2017. There are three blocks with three different hydraulic residence times (HRT) set at 2 h, 8 h, and 16 h for a total of three repetitions per HRT.
3.3.2 System Sampling and Flow Control

To measure bioreactor HRT, inflow valves at the inlet ports were adjusted weekly using a stopwatch and bucket. The initial flow was recorded using a bucket and stopwatch at the inlet valves, and then flow was adjusted to the target flows for 2, 8, and 16 h HRTs shown in Table 3.1. Once the correct HRT was achieved, the bucket and stopwatch time was repeated twice to ensure accuracy. HRT was set to one of three times: 2 hours, 8 hours, and 16 hours, with three replicates of each across the nine bioreactors (Figure 3.1). HRT was calculated using the saturated flow volume and an estimated media porosity of 0.70 (Hoover, 2017). Saturated flow volume was calculated using internal bioreactor dimensions and the average water depth in the sampling wells for each bioreactor. Data was collected in 2016 from August to the end of October, but the system ran from June to the beginning of November. Boone County, IA experienced dry weather during June 2016, with precipitation 10.06 cm (3.96 in.) below annual averages (NOAA Climate Data, 2017). Precipitation was also below normal during 2017. Between June and July, rainfall totaled 15.37 cm (6.05 in.) below average, with drought conditions continuing through October (NOAA Climate Data, 2017). Valid data was collected from May through July for 2017.

During the winter months of 2016 and 2017, bioreactors were left filled with water to prevent woodchip degradation. When temperatures reached above consistent freezing temperatures in Iowa, the bioreactors were turned on for the season. Bioreactors flowed for one week before sample collection to allow for flushing of water held during the winter.

3.3.3 Sample Collection

Water and gas (dissolved and surface flux) samples were collected weekly during the testing season. A companion study analyzed the impact of HRT on GHG emissions (Davis et al.,
Water samples were collected at the inlet, well A, well B, and the outlet. At the inlet, water samples were collected by opening the valves and letting water flow out into the sample bottle. At well A, well B, and the outlet, a peristaltic pump with tubing long enough to reach into the bioreactor was used to extract the water sample. Before collecting the water sample, wells were evacuated by pumping out 8.2 L of water, a volume equivalent to the well volume. All sample bottles were rinsed with the sample water before the final grab for analysis. A total of 125 mL was sampled from each location. Samples were stored on ice in the field before being taken to the laboratory. NO$_3$-N and total ammonia nitrogen (TAN) samples were acidified with sulfuric acid (H$_2$SO$_4$).

3.3.4 Sample Analysis

Water samples were transported to the lab to be analyzed for NO$_3$-N and TAN. All samples were stored in a cooler at 4°C. Samples were analyzed using a Seal Analytical (Mequon, WI) AQ2 discrete autoanalyzer. NO$_3$-N samples were measured as NO$_3$-N+NO$_2$-N using AQ2 method EPA-114-A, Rev. 7 (equivalent to U.S. EPA method 353.2, ver. 2 (1993)) where the NO$_3$-N in the samples were reduced by copperized cadmium to NO$_2$. After reduction, NO$_2$ was measured spectrophotometrically at 520 nm with a detection limit of 0.03 mg N L$^{-1}$. If NO$_3$-N concentrations were below 0.25 mg N L$^{-1}$, the AQ2 method EPA-127-A, Rev. 7 (range 0.012-2.0 mg N L$^{-1}$) was used. TAN was measured as NH$_3$-N using AQ2 method EPA-103-A, Rev. 10 (equivalent to U.S. EPA method 350.1, Rev. 2.0) by allowing NH$_3$ to combine with hypochlorite (OCH) ions to form mono chloramine (NH$_2$Cl), which reacts with phenate, resulting in 5-aminophenate that oxidizes after being exposed to sodium nitroprusside. The reaction creates indophenol, a blue compound that is read in the spectrometer at 660 nm.
### 3.3.5 Data Analysis

Weekly instantaneous flow values were converted to daily flows (L day⁻¹). Flows and NO₃⁻N concentrations between collection days were linearly interpolated using R Studio statistical software. Flow rates were recorded two times per week during the sampling periods, so an interpolation was necessary to understand the daily flow rates going through each bioreactor. Knowing the initial flow rate before the flow was reset each week and the flow rate the bioreactor was set to allowed us to interpolate what the flow rates were between those points while accounting for drift from the target flow. Interpolation of flow and NO₃⁻N have been done in previous bioreactor studies (Herbstritt, 2014) and is a common practice with hydrologic flow data (Kratzer et al., 2006). Interpolated flows were used to calculate daily mass removal rate (MRR) of NO₃⁻N using Equation 3.1. Percent mass removal rate (PMRR) of NO₃⁻N was calculated using Equation 3.2. Removal (MRR or concentration) was analyzed with a linear mixed model using R Studio with interactions between the treatment (HRT) and sampling location within and across the bioreactors as a time series (with repeated measures). Time, treatment, sampling location, and block all had fixed effects. Reactor and sampling location by reactor had random effects. An ANOVA was performed using the mixed model comparing sampling location and treatment (HRT), which gave a Tukey pairwise comparison between all treatment zones. Flow was compared among the three treatments in an ANOVA test with a Tukey Test as the post-hoc analysis in R Studio.

**Equation 3.1.**

\[
MRR = V_t ([NO_3^- - N]_{influent} - [NO_3^- - N]_{effluent})
\]

**Equation 3.2.**

\[
PMRR = (\frac{V_t ([NO_3^- - N]_{influent} - [NO_3^- - N]_{effluent})}{[NO_3^- - N]_{influent}}) * 100\%
\]
In-reactor temperature, dissolved oxygen (DO), influent NO$_3$N, and HRT were used as explanatory variables in a multiple linear regression model to test the dependency of each on PMRR and MRR using R Studio. All data was run together in the model to allow explanatory parameters only to be used if they were statistically significant ($p < 0.05$). A Durbin-Watson test was run at 5% significance to detect for correlated errors between observations that were temporally near each other. Multicollinearity diagnostics were also run to test for relationships between the explanatory variables.

An assessment was conducted to estimate NO$_3$N removal when bypass flow is included to represent typical field conditions. Maximum flow to all reactors was set to the 2 h HRT maximum flow, but the maximum flow delivered to each reactor was the average flow for each HRT (Table 3.1), with excess flow to the 8 and 16 h HRTs being classified as bypass. NO$_3$N influent to each reactor was calculated using the percent of flow treated and the average influent NO$_3$N concentration for the 2016-2017 testing period.

3.4 Results and Discussion

3.4.1 Flow

In this unique experimental design, HRT was the controlled treatment within triplicate pilot-scale bioreactors. Target and achieved flow for the three HRTs are shown in Table 3.1. All flows were significantly different from each other ($p < 0.05$). Observed and target flow for all three HRTs were within < 1.0 L min$^{-1}$ through the duration of 2016-2017. The 2 h HRT had the greatest standard deviation of 6,973 L day$^{-1}$, followed by the 16 h HRT (± 1,963 L day$^{-1}$) (Figure 3.2), and the 8 h HRT, with the lowest flow standard deviation at ± 1,024 L day$^{-1}$. The fast flow rate of the 2 h HRT and the slower flow of the 16 h HRT were difficult to maintain in the pilot-scale system.
Table 3.1. The summary data for the 2 h, 8 h, and 16 h hydraulic residence times from a set of pilot-scale woodchip bioreactors in Central Iowa, including flow and NO₃-N removal rates. Standard deviation values are in parentheses. Letters indicate which values were significantly different from one another. PMRR is percent mass removal rate. MRR is mass removal rate.

<table>
<thead>
<tr>
<th>Hours</th>
<th>Flow</th>
<th>Nitrate-N Removal</th>
<th>Nitrate-N Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Target Flow</td>
<td>Observed Flow</td>
<td>PMRR</td>
</tr>
<tr>
<td></td>
<td>L min⁻¹</td>
<td>L day⁻¹</td>
<td>L min⁻¹</td>
</tr>
<tr>
<td>2</td>
<td>31.4</td>
<td>45,187</td>
<td>31.7 a</td>
</tr>
<tr>
<td>8</td>
<td>7.9</td>
<td>11,298</td>
<td>8.2 b</td>
</tr>
<tr>
<td>16</td>
<td>3.9</td>
<td>5,649</td>
<td>4.4 c</td>
</tr>
</tbody>
</table>

Figure 3.2. The standard deviation in flow during the 2016-2017 testing season, grouped by HRT at 2 h, 8 h, or 16 h. The 2 h HRT had the greatest deviation at ±6,973 L day⁻¹, followed by the 16 h HRT at ±1,963 L day⁻¹. The 8 h HRT had the lowest deviation in flow at ±1,024 L day⁻¹. All mean flows were significantly different from each other at p < 0.05.
3.4.2 Nitrate Removal by Concentration

All bioreactors at all HRTs exhibited a significant (p < 0.05) reduction in NO$_3^{-}$N concentration from the inlet to the outlet (Figure 3.3). In the 2 h HRT, average NO$_3^{-}$N removal was 1.3 (±0.58) mg L$^{-1}$. The average percent of NO$_3^{-}$N removed for the 2 h HRT was 9.0% (±3.69). The 8 h HRT removed an average of 5.5 (±1.32) mg L$^{-1}$ NO$_3^{-}$N by concentration from the inlet to the outlet. Average percent NO$_3^{-}$N removal for the 8 h HRT was 32.1% (±5.18). The 16 h HRT removed an average of 7.5 (±4.11) mg L$^{-1}$ NO$_3^{-}$N by concentration. The average percent of NO$_3^{-}$N removed for the 16 h treatment was 53.8% (±17.55). As HRT increased, NO$_3^{-}$N PMRR and removal by concentration also increased. These results are in agreement with previously published studies that also found a positive relationship between increasing HRT and NO$_3^{-}$N percent removal (Nordstrom and Hebert, 2017; Hoover et al., 2015; Christianson et al., 2012a).

![Figure 3.3. The NO$_3^{-}$N removal rates by concentration over the length of the bioreactor, grouped by HRT. The significance is indicated by the p-values next to the corresponding line on the graph in the matching color indicated by the legend. All NO$_3^{-}$N concentrations for all HRTs were significantly lower (p < 0.05) from the inlet to the outlet.](image-url)
When evaluating NO$_3^-$N concentration removal within bioreactors, well A, well B, and the outlet were significantly different across all treatments (p < 0.05). The only exception was the inlet concentration, which was expected because the same water source was provided to all bioreactors. The majority of NO$_3^-$N removal in the 2 h HRT occurred from well A to well B with 66.2% of the total reduction. NO$_3^-$N concentration did not drop significantly between the inlet and well A (p = 0.75) or well B to the outlet (p = 0.79) (Figure 3.3) in this HRT. In the 8 h HRT, 69.9% of the total NO$_3^-$N removal occurred between wells A and B. The only point that did not have statistically significant NO$_3^-$N concentration removal was between well B and the outlet, where an increase in concentration (p = 0.78) was observed. In the 16 h HRT, 53.8% of the total NO$_3^-$N removal occurred between wells A and B. Similar to the 8 h HRT reactors, the only area of the bioreactor that did not significantly differ in NO$_3^-$N concentration was well B to the outlet (p = 0.09).

For all HRTs, the majority of NO$_3^-$N removal occurred between wells A and B, which can be explained in several ways. First, this is the longest bioreactor length at 2.95 m versus the 1.42 m between all other sampling points. However, when normalizing for the extra length, the majority of NO$_3^-$N removal still occurred over the central portion of the bioreactors. Dissolved oxygen concentrations were still elevated in the first section of the bioreactors, potentially leading to less efficient denitrification. The trend in lowered DO from inlet to well A is shown in Figure 3.4. Warneke et al. (2011a) also found that the carbon substrate closest to the inlet mainly serves as the removal area for DO. Between well B to the outlet, NO$_3^-$N removal could be impeded by lower concentrations of NO$_3^-$N, switching from zero-order kinetics to first-order kinetics. Both have been observed in other bioreactors with a transition expected as NO$_3^-$N concentration decreases (Schipper et al., 2010).
An increase in NO$_3^-$ N concentration was observed between well B and the outlet in the 8 h HRT (Figure 3.3). This trend was most common in a single bioreactor (#4). This pattern in NO$_3^-$ N increase from well B to the outlet was observed for differing time periods in almost every bioreactor. This observation coincided with sampling points where there was also either TAN production, or TAN entered the bioreactor with the influent flow. Figure 3.5 shows that at the same point from well B to the outlet there was a decrease in TAN. The increase in NO$_3^-$ N during these periods could be explained by nitrification. Nitrification is a process carried out by chemoautotrophic bacteria in which ammonia or ammonium is oxidized to NO$_3^-$ N (Chun et al., 2009). Nitrifying bacteria are obligate aerobes, but they are microaerophiles, which means they
thrive under low oxygen conditions (Ward, 2008). Although maximum nitrification typically occurs at 3 mg L\(^{-1}\) DO and 30°C, it only ceases once DO levels are < 0.5 mg L\(^{-1}\) and temperature is < 5°C (Sharma and Ahlert, 1977). The DO levels in the bioreactors were never less than 0.5 mg DO L\(^{-1}\) at any point during the study (Figure 3.4), and temperature never dropped below 11.5°C, indicating that nitrification of TAN is a possible explanation for the observed increase of NO\(_3^-\)N between well B and the outlet.

![Figure 3.5](image.png)

*Figure 3.5. The total ammonia nitrogen (TAN) concentrations over the length of the bioreactors for all HRTs. Over the course of the study period, each of the three HRTs had a significant net production of TAN (p < 0.05). TAN concentrations were not statistically different between any of the HRTs (p > 0.05).*

In a review of denitrifying bioreactors, Christianson et al. (2012b) stated that the bioreactor denitrifying community can vary with time of year, flow direction, and depth of water within the bioreactor. The microbial community within the pilot-scale bioreactors could have adjusted throughout the year, possibly explaining the inconsistent timing of NO\(_3^-\)N increases
between B and the outlet. Moorman et al. (2010) found that their bioreactor woodchip decomposition rates were controlled by sustained anaerobic conditions below the tile drainage line. Our woodchip bioreactors could have experienced similar variation with different areas within the bioreactors exhibiting different rates of processes or differing microbial communities. The decrease in TAN concentration cannot fully explain the increase in NO$_3^-$N from well B to the outlet in the 8 h HRT. There could also have been a preferential flow path within the bioreactors may have routed water around well B in the 8 hour HRT and falsely shown an increase in NO$_3^-$N at the outlet.

### 3.4.3 Nitrate Mass Removal Comparison

Average mass NO$_3^-$N removal rates were 9.0 ($\pm$4.01) g m$^{-3}$ day$^{-1}$ (2 h HRT), 8.5 ($\pm$2.42) g m$^{-3}$ day$^{-1}$ (8 h HRT), and 7.4 ($\pm$2.52) g m$^{-3}$ day$^{-1}$ (16 h HRT) (Table 3.1). When comparing all points (inlet, A, B, and outlet) across HRTs, NO$_3^-$N mass load removal rates were significantly different ($p < 0.05$). Inlet mass NO$_3^-$N loads were significantly different between HRTs due to difference in flows.

In the 2 h HRT, a statistically significant NO$_3^-$N mass removal ($p < 0.05$) was observed between the inlet and outlet (Figure 3.6). Nitrate mass removal was not significant between the inlet to well A ($p = 0.82$) and well B to the outlet ($p = 0.47$). However, NO$_3^-$N removal rate was significant between sampling points A and B ($p < 0.05$). In the 8 h HRT, when comparing the inlet to A ($p = 0.20$), A to B ($p = 0.12$), and B to the outlet ($p = 0.19$), no statistically significant differences in NO$_3^-$N mass load removal rate were observed. When comparing longer portions of the bioreactor, (inlet to outlet, A to outlet, and inlet to B) all had significant differences in NO$_3^-$N MRR ($p < 0.05$). For the 16 h HRT, the only points that were statistically different from each other were the inlet to the outlet and the inlet to point B ($p < 0.05$).
Our observed pattern in NO$_3$N mass load removal rate versus HRT is in contrast to previous studies. Hoover et al. (2015) found in a laboratory study that incrementally increasing HRT from 1.7 h to 21.2 h resulted in consistent NO$_3$N mass removal, not increasing.

Christianson et al. (2011a) studying pilot-scale bioreactors in Central Iowa also observed that NO$_3$N removal rates were consistent as HRT ranged from 1.3 to 11.3 h, despite the percent NO$_3^-$ N reduction increasing with increasing HRT. Because this was a controlled study and flows did not vary as they do in the field, influent NO$_3$N mass loads were more consistent over time at each HRT. The mean influent load for the 2 h HRT was 103.3 g m$^{-3}$ day$^{-1}$ ($\pm$25.98), the 8 h HRT was 26.4 g m$^{-3}$ day$^{-1}$ ($\pm$5.34), and the 16 h HRT was 14.8 g m$^{-3}$ day$^{-1}$ ($\pm$1.87). Despite its low

Figure 3.6. The NO$_3$N mass load removal over the length of the bioreactors, grouped by HRT. Significance level is indicated as $p$-values next to lines with corresponding color as indicated by the legend. All NO$_3$N mass load removal rates for all HRTs were significantly lower ($p < 0.05$) from the inlet to the outlet.
NO$_3$N PMRR, the 2 h HRT removed the greatest NO$_3$N by mass (p < 0.05) because it treated a significantly greater volume of water in a 24-hour period when compared to the reactors set at a higher HRT.

3.4.4 Bypass Flow

The pilot-scale bioreactors in this study did not have bypass flow, but as is typical under normal field conditions, excess flow would bypass the bioreactors and be discharged as untreated tile drainage (Christianson et al., 2012a). To account for this difference, the mass load removal rates were calculated by assuming each bioreactor received 45,675 L of tile drainage in a 24-h period, the maximum amount of flow that could be treated by the 2 h HRT. The average flow treatable by each HRT in a 24-h period was used, which is shown in the maximum flow treatable column in Table 3.2. Average influent NO$_3$N concentration from the study was used to calculate the amount of NO$_3$N by mass that needed to be treated in the 24-h period, which was 103.1 g m$^{-3}$ day$^{-1}$. Average PMRR for each HRT was used to calculate how much NO$_3$N each HRT could remove of the total influent NO$_3$N mass load. Only the bioreactors set at 2 h HRT could treat 100% of the flow and removed a mass load of 9.3 g m$^{-3}$ day$^{-1}$ (Table 3.2). The 8 h HRT was able to treat 25.7% of the flow, removing 8.5 g m$^{-3}$ day$^{-1}$. Finally, the 16 h HRT was able to treat 13.8% of the influent, removing 7.7 g m$^{-3}$ day$^{-1}$. The mass load of NO$_3$N in the outflow for each treatment was 93.8 g m$^{-3}$ day$^{-1}$ (2 h), 94.6 g m$^{-3}$ day$^{-1}$ (8 h), and 95.5 g m$^{-3}$ day$^{-1}$ (16 h). With bypass flow, the 2 h HRT had a PMRR of 9.0%, the 8 h had 8.3%, and the 16 h had 7.4%.

In the field, HRT varies with season, precipitation, and management of flow within the bioreactors. Understanding the performance over the full range of expected HRTs under field conditions is therefore important. The 2 h HRT was able to remove the most NO$_3$N by mass when not accounting for bypass flow because it can treat the greatest volume of water in a
24-h period. Despite its low PMRR, it would still outperform the other two treatments in typical field conditions. The 8 h HRT had a PMRR of 32.1% and because of its lower retention time, it was able to treat almost twice the tile drainage at the 16 h HRT in a 24-h period (Table 3.2). Because a high PMRR is related to high HRT, bypass flow is increased, along with the mass of NO$_3^-$ N that leaves untreated. Understanding the dynamics of bypass flow and HRT is critical to informing woodchip bioreactor design for use at the field-scale. There are also other important factors to consider beyond NO$_3^-$ N removal rates. If removal rates were the only aspect to consider, a 2 h HRT would be the most ideal for field conditions without factoring in the difficulty in maintaining flow. Taking greenhouse gas (GHG) emissions into account, a companion study by Davis et al. (2018 – in review) found that the 2 h treatment had the largest dissolved N$_2$O production (501.5 mg N$_2$O m$^{-3}$ day$^{-1}$). On the other hand, the 16 h treatment had the highest CH$_4$ (1.69 g C m$^{-3}$ day$^{-1}$) production with the 8 h treatment a close second (1.50 g C m$^{-3}$ day$^{-1}$) (Davis et al., 2018 – in review). When comparing the production of N$_2$O and CH$_4$ across all three treatments, the 2 h HRT had the greatest (p < 0.05) global warming potential (Davis et al., 2018 – in review). When considering all factors, including GHG emissions, NO$_3^-$ N MRR and PMRR, and NO$_3^-$ N removal rates with bypass flow, the optimal HRT for the woodchip bioreactors used in this study was 8 h.

**Table 3.2.** The bypass flow comparison for all treatments using the 2 h HRT as the base flow to be treated in a 24-hour time period. Values were used from Table 3.1 to calculate NO$_3^-$ N removal when bypass was accounted for.

<table>
<thead>
<tr>
<th>HRT Hours</th>
<th>Flow to treat L day$^{-1}$</th>
<th>Maximum flow treatable L day$^{-1}$</th>
<th>Flow left untreated L day$^{-1}$</th>
<th>Percent of flow untreated</th>
<th>Percent of flow treated</th>
<th>Amount of nitrate to be treated in flow g m$^{-3}$ day$^{-1}$</th>
<th>Maximum nitrate treatable g m$^{-3}$ day$^{-1}$</th>
<th>Nitrate removal efficiency %</th>
<th>Final nitrate removal g m$^{-3}$ day$^{-1}$</th>
<th>Total nitrate untreated g m$^{-3}$ day$^{-1}$</th>
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<tr>
<td>2</td>
<td>45675</td>
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<td>103.1</td>
<td>14.3</td>
<td>53.8</td>
<td>7.7</td>
<td>95.5</td>
</tr>
</tbody>
</table>
3.4.5 Parameters that Impact Nitrate Removal

In a multiple regression model analysis, PMRR was found to be most strongly positively correlated with HRT and influent NO₃⁻N concentration (p < 0.001) (Table 3.3). The same relationships for HRT and influent NO₃⁻N concentrations with PMRR were found in other bioreactors in Iowa (Christianson et al., 2012a). Temperature was also correlated with PMRR in this study but not as strongly as the other parameters (p < 0.001). This is in contrast to Christianson et al. (2012a), who observed that temperature was most strongly correlated with PMRR. The relationship between temperature and PMRR was negative due to large removal rates when influent NO₃⁻N concentrations were high and temperatures were low. The only environmental parameter not significantly correlated with PMRR was DO (p = 0.06).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Model Intercept</th>
<th>HRT hours</th>
<th>Temperature °C</th>
<th>DO mg DO L⁻¹</th>
<th>Influent Nitrate Concentration mg NO₃⁻N L⁻¹</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent Mass Removal Rate</td>
<td>-8.68</td>
<td>3.10***</td>
<td>-0.51**</td>
<td>[a]</td>
<td>1.98*** (±0.21)</td>
<td>0.80</td>
</tr>
<tr>
<td>Mass Removal Rate</td>
<td>7.31***</td>
<td>-0.12***</td>
<td>-0.26***</td>
<td>-0.38***</td>
<td>0.66*** (±0.06)</td>
<td>0.37</td>
</tr>
</tbody>
</table>
When running the same multiple regression model analysis for MRR, all parameters were equally significant ($p < 0.001$) (Table 3.3). All explanatory parameters except influent NO$_3^-$N concentration had a negative correlation with MRR. For HRT, this means that as HRT increased, the mass removal rate of NO$_3^-$N decreased. As DO increased, the MRR of NO$_3^-$N decreased in the woodchip bioreactors. Like PMRR, temperature had a negative correlation with MRR because of large removal rates when influent NO$_3^-$N were high and temperatures were low.

As found by Christianson et al. (2012a), this regression modeling approach is limited because of autocorrelated errors based on a time dependency. Their model failed the Durbin-Watson test ($p > 0.05$) because values that are closer in time dependency tend to be related. Their model passed the multicollinearity test, indicating that none of their variables were linear combinations of each other (Christianson et al., 2012a). Similarly, the regression model used in this study also failed the Durbin-Watson test and all variables passed the test for multicollinearity with variance inflation factors less than four.

Model results indicate that for every 1 mg NO$_3^-$N L$^{-1}$ increase in influent concentration, there was a 0.7 g N m$^{-3}$ day$^{-1}$ increase in removal, resulting in a 1:0.7 ratio. A relationship of 1:0.9 strongly indicates first-order reaction kinetics, which were seen in other field bioreactors (Christianson et al., 2012a; Chun et al., 2010). Data were limited because the model assumed all other parameters were held constant, which may not be true under field conditions which can have a number of variables that affect kinetics. The influent NO$_3^-$N concentrations during the study period ranged from 10.7 mg N L$^{-1}$ to 18.0 mg N L$^{-1}$. Other studies that were able to determine the impact of influent NO$_3^-$N concentrations on removal rates typically had larger ranges and higher concentrations (Ghane et al., 2015; Hoover et al., 2015). More variation in influent NO$_3^-$N concentration in the pilot bioreactors will help to better determine kinetics.
Temperature was a significant explanatory parameter for both PMRR and MRR, but both were negative correlations. In-reactor water temperature ranged from 11.5°C to 19.5°C over the 2016-2017 testing period. Other lab-scale and field-scale studies have reported increasing removal rates with increasing temperature (Christianson et al., 2012a; Hoover et al., 2015; David et al., 2016). David et al. (2016) indicated that during the first three years of bioreactor operation, NO$_3$-N removal performance can vary greatly in response to temperature, woodchip age, and woodchip quality. The pilot-scale bioreactors for this study were built in 2014, which puts the 2017 testing season near the three year mark for operation time. Longer-term data in these systems will help elucidate the effects of temperature on NO$_3$-N removal. A wider range of temperatures will also be necessary to perform a Q$_{10}$ analysis.

In the pilot-scale woodchip bioreactors, DO concentrations were not significantly different between any of the HRTs at any point (Figure 3.5). The average starting DO concentration was 7.1 (±1.48) mg L$^{-1}$ and the DO level never fell below 0.5 mg DO L$^{-1}$ from the inlet to the outlet for any duration of the study period. The majority of the DO was removed between the inlet and well A, which constitutes 24.5% of the total length of the bioreactor. Differences in DO from the inlet to the outlet were significant in all bioreactors (p < 0.05).

Dissolved oxygen levels in a woodchip bioreactor can be an indicator of its denitrification efficiency. Dissolved oxygen concentrations can inhibit denitrification rates at levels as low as 0.2 mg L$^{-1}$ (Metcalf and Eddy, 2003). When DO is present, facultative aerobic microbes will utilize oxygen over NO$_3$-N, making complete saturation of woodchip bioreactors necessary for complete denitrification to occur. Christianson et al. (2012b) reported that several field woodchip bioreactor sites were able to reduce DO concentrations to 0.5 mg DO L$^{-1}$ within 25% of the length from the inlet. The pilot-scale bioreactors used in this study did not achieve
similar DO concentration reductions. The NO$_3$-N removal rates in this study could have been impacted by higher DO concentrations within the bioreactors, causing the microbial community to utilize DO instead of NO$_3$-N over the entire length of the bioreactors (Christianson et al., 2011a). Warneke et al. (2011b) studied lab-scale bioreactors and observed a DO reduction from 6 mg L$^{-1}$ at the inlet to less than 2 mg L$^{-1}$ at the outlet with an average NO$_3$-N removal rate between 1.3 to 6.2 g N m$^{-3}$ day$^{-1}$. They concluded that the substrate closest to the inlet serves to make conditions within the bioreactor anaerobic (Warneke et al., 2011b). In another study on a field-scale denitrification bed, Warneke et al. (2011a) observed removal rates of 7.6 g N m$^{-3}$ day$^{-1}$ with DO levels above 0.5 mg L$^{-1}$, which they concluded did not limit NO$_3$-N removal.

### 3.4.6 Total Ammonia Nitrogen Production

Dissimilatory reduction of nitrate to ammonia or ammonium (DRNA occurs under anaerobic conditions when NO$_3$-N (the electron acceptor) is scarce, pH is above 7.0, carbon (the electron donor) is abundant, and microbes need to optimize the use of available oxidants to regenerate NAD+ (Mohan and Cole, 2007). DRNA is a two-step process, reducing nitrate to nitrite, and then reducing nitrite to ammonium. While the ideal final product of denitrification in bioreactors is N$_2$ (g), DRNA can alter the desired final product. TAN production and release can be directly toxic to aquatic ecosystems in high concentrations (Nordstrom and Herbert, 2017). TAN could have also been produced through the mineralization of N by the microbial decomposition of the woodchips or microbial biomass. Mineralization of N cannot occur at a C:N ratio that is higher than 16:1 (Enwezor, 1975). Woodchips have a high C:N ratio, sometimes as high as 513:1 (Nolan et al., 2011). This suggests that DRNA was the dominant TAN production process in the woodchip bioreactors.
Each of the three treatments had a statistically significant net production of TAN from the inlet to the outlet when averaged over the study period (p < 0.05). Concentrations were not significantly different between any of the HRTs. The 2 h HRT produced 0.05 (±0.90) mg N L\(^{-1}\) TAN, the 8 h HRT produced 0.1 (±0.09) mg N L\(^{-1}\) TAN, and the 16 h HRT produced 0.1 (±0.06) mg N L\(^{-1}\) TAN (Table 3.4). The U.S. EPA has set ammonia standards at 1.9 mg TAN per liter at a pH of 7 at 20°C over a 30-day average duration (U.S. EPA, 2017). The U.S. EPA also recommends that the highest four-day average in that period should not exceed 2.5 times the 1.9 mg TAN L\(^{-1}\) limit (USEPA, 2017). When taking these criterion into account, the woodchip bioreactors did not exceed the limits set by the U.S. EPA for any duration while they were running. Healy et al. (2012) found that ammonium (NH\(_4^+\)N) production increased in concentration along a lab-scale column, and that a shorter HRT had lower concentrations of NH\(_4^+\)N. Greenan et al. (2006) estimated that DRNA accounted for <4% of NO\(_3^−\)N removal in bioreactors. Herbstrittt (2014) had ammonium concentrations of 0.1 mg NH\(_4^+\)N L\(^{-1}\) in field-scale bioreactors, and they occasionally also saw increases from the inlet to the outlet.

Table 3.4. The total ammonia nitrogen (TAN) concentrations across the bioreactors. Standard deviations are in parentheses. Over the course of the study period, each of the three HRTs had a significant net production of TAN (p < 0.05). TAN concentrations were not statistically different between any of the HRTs (p > 0.05). Letters indicate which values were significantly different from one another.

<table>
<thead>
<tr>
<th>HRT Hours</th>
<th>Total Ammonia Nitrogen Concentration (mg L(^{-1}))</th>
<th>Inlet</th>
<th>A</th>
<th>B</th>
<th>Outlet</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td></td>
<td>0.01</td>
<td>0.02</td>
<td>0.08</td>
<td>0.05 (±0.016) a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(±0.060) ab</td>
<td>(±0.277) ab</td>
<td>(±0.085) b</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>0.01</td>
<td>0.04</td>
<td>0.12</td>
<td>0.10 (±0.016) a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(±0.114) ab</td>
<td>(±0.182) ab</td>
<td>(±0.085) b</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>0.01</td>
<td>0.04</td>
<td>0.13</td>
<td>0.10 (±0.016) a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(±0.044) ab</td>
<td>(±0.157) ab</td>
<td>(±0.061) b</td>
</tr>
</tbody>
</table>
3.5 Conclusions

This study was one of the first to collect samples at several points within bioreactors instead of only the inlet and outlet. Substantial variability in NO$_3$N removal was observed under this increased spatial sampling, identifying the need for additional research from more bioreactors to better understand the drivers of the observed variability within bioreactors. Understanding the impact of HRT from a controlled pilot-scale system is crucial to optimizing woodchip bioreactor design under varied field conditions. Our results indicated that if peak mass removal rate of NO$_3$N is the major design objective for woodchip bioreactors, a lower HRT could be best. When HRT was increased, the bioreactors removed more NO$_3$N by concentration and had higher efficiency, but the mass removal rate decreased. When considering the future of bioreactor data reporting, especially as it applies to policy planning, NO$_3$N mass removal rates will be more informational than NO$_3$N percent and concentration removal rates. NO$_3$N removal rates were not the only environmental impact that was taken into account in our study. When considering all of the factors discussed, the ideal HRT for the bioreactors used in this study was 8 hours to achieve maximum NO$_3$N removal while reducing the impact from GHG emissions.

3.6 References


CHAPTER 4. IMPACT OF PYROLYSIS TEMPERATURE AND BIOMASS SOURCE ON BIOCHAR AS A WOODCHIP BIOREACTOR AMENDMENT

Modified from a manuscript to be published a scientific journal as a short communication.

E.A. Martin, M.L. Soupir

4.1 Abstract

Woodchip bioreactors are a cost effective and minimally invasive edge-of-field practice designed to remove nitrate (NO$_3$N) from subsurface drainage. There is potential to expand the range of pollutants removed by woodchip bioreactors to include phosphorus (P) by adding biochar as an amendment. This study assessed biochar biomass source and pyrolysis temperature to identify optimal treatment for enhanced denitrification and P removal in woodchip bioreactors. Six biomasses [Red Oak (Quercus rubra), Ash (Fraxinus spp.), Mixed Pine (Pinus spp.), Loblolly Pine (Pinus taeda), Switchgrass (Panicum virgatum), and Corn Stover (Zea mays)] were pyrolyzed into biochar at three temperatures (400°C, 600°C, and 800°C). Experiments were conducted at the batch scale to test for removal of NO$_3$N and phosphate (PO$_4^{3-}$) by woodchips mixed with biochar at a ratio by weight of 12:1 (8.3% application rate). Each batch contained 3 L of nutrient solution with 30.0 mg L$^{-1}$ NO$_3$N and 10.0 mg L$^{-1}$ PO$_4^{3-}$ and were sampled at 0, 4, 8, 12, and 24 hours. NO$_3$N removal was not correlated with particle size, but was positively correlated with pyrolysis temperature ($r = 0.58$, $p < 0.05$). None of the biochars removed significantly more NO$_3$N than the woodchip control. The 800°C biochar and herbaceous biochars removed significantly more PO$_4^{3-}$ than the control, but the woodchips leached PO$_4^{3-}$ into solution, meaning significance may not be the same after the initial leaching period ($p < 0.05$). Results indicate that biochar is not suited to be an amendment to enhance either NO$_3$N or PO$_4^{3-}$ removal in woodchip bioreactors.
4.2 Introduction

In the Upper Midwestern United States, two major nutrients pollute surface waters: nitrogen and phosphorous. Both nutrients come from the application of fertilizers on row crop agricultural land and the mineralization of nitrogen in soil organic matter, resulting in nonpoint source water pollution (Cordell et al., 2009). Nitrogen (N) as nitrate (NO$_3^-$) and phosphorous (P) as phosphate (PO$_4^{3-}$) travel from agricultural land with water as surface and subsurface flows into streams, lakes, and eventually the Gulf of Mexico (U.S. EPA, 2016). The widespread use of subsurface tile drainage in the Midwest allows for more rapid transport of water from farm fields, which also expedites N and P movement to surface waterbodies (Smith et al., 2015). When excessive N and P reaches waterbodies, they can lead to eutrophication and eventually hypoxia (dissolved oxygen levels < 2 mg L$^{-1}$), which alters ecosystem structure (Rabalais et al., 2002).

Many different conservation practices are currently being implemented across the Midwest that address water quality issues associated with drainage. Of these, woodchip bioreactors are a promising edge-of-field strategy that has an average removal of 43% of influent NO$_3^-$ N concentration (Christianson and Schipper, 2016; INRS, 2017). Woodchip bioreactors are installed to directly intercept subsurface tile drainage for removal of NO$_3^-$ N by creating a favorable environment for heterotrophic denitrifying microbes. The woodchips provide a carbon source that is consumed by microbes for respiration, and the low lability of the carbon in the woodchips contributes to its longevity as a carbon source (Christianson and Helmers, 2011). Anaerobic conditions and the presence of a carbon source allow the microbes to transform NO$_3^-$ N into N$_2$ (gas) that is released from the bioreactor.
While bioreactors are designed for NO$_3$N removal, recent work highlights growing concerns over the transport of P into subsurface tile drainage across the Midwest (Christianson et al., 2016; Clement and Steinman, 2017; Smith et al., 2015; Kinley et al., 2006; King et al., 2014). Smith et al. (2015) reported that 48% of total P losses from a farm-scale research site occurred through the subsurface tile drainage discharge. King et al. (2014) similarly reported 40% of the total P exported from the field leaving through tile discharge, and Clement and Steinman (2016) reported 60%. A substantial amount of total P loading is not being accounted for by focusing treatment efforts on P associated with surface runoff.

Much of the research performed on bioreactors has focused on woodchips and their ability to serve as a carbon source for microbial transformation of NO$_3$N. Various other carbon substrates have been researched to be used in place of or in tandem with woodchips to improve and expand the nutrient removal capacity of bioreactors. Biochar has been suggested for application in agricultural settings because of its ability to retain more water than soil, its large specific surface area, higher cation exchange capacity (CEC), and proven ability to reduce N and P leaching (Laird et al., 2010a; Laird et al., 2010b; Yao et al., 2012; Bock et al., 2015). Biochar is biomass that has undergone pyrolysis to become a highly structured and stable substance with varying chemical properties. The ability of biochar to remove contaminants depends on several factors, including biomass source, temperature of pyrolysis, type of pyrolysis, post-pyrolysis treatment, age, particle size, and application (Fidel et al., 2017; Jindo et al., 2014). Hardwood and herbaceous biomasses generally have a higher pH than softwood biomass, with herbaceous having the highest average pH (Fidel et al., 2017). Hardwood tends to have larger particle sizes, thus allowing easier flow-through of water (Zhang et al., 2004; Jung and Kim, 2014). Slow pyrolysis tends to yield more biochar from its original biomass while maintaining larger particle
sizes, making for a more cost-efficient product and there is a general trend that the higher the temperature, the lower the yield of biochar from pyrolysis (Jindo et al., 2014; Antal and Gronli, 2003; Novak et al., 2009).

Pyrolysis temperature can have a significant impact on the final product of biochar and its ability to remove nutrients. Lawrineko (2014) reported that varied pyrolysis temperature exhibits different oxidization of carbon (C). Lower temperature (300°C-500°C) pyrolysis creates biochar with more oxygen groups that are more likely to participate in ligand exchange with P and N (Zhao et al., 2017). Zhao et al. (2017) also showed that with increasing pyrolysis temperature, CEC, ratios of O:C and H:C, and O and H all decrease. Lower temperature pyrolysis produces biochar with volatile and easily labile compounds, potentially making C more accessible to microbes (Jindo et al., 2014; Christianson et al., 2011).

Higher pyrolysis temperatures (700°C-900°C) yield biochar that is functionalized with oxidation, making it more suited to anion exchange capacity (AEC) with NO₃⁻ and PO₄³⁻ due to the formation of oxonium functional groups during pyrolysis (Lawrinenko, 2014). Jung and Kim (2014) reported that as pyrolysis temperature increases, the pH of the biochar also increases due to the loss of acidic functional groups and high content of alkaline and alkali earth metals in biochar. The authors concluded that a maximum surface area of biochar was achieved at 800°C at 249 m² g⁻¹.

While results vary depending on the type of biochar tested, it is clear there is potential for biochar to be utilized as a multi-contaminant removal substrate. In a lab-scale study, Pluer et al. (2016) studied the impact of biochar mixed with woodchips on NO₃⁻ and nitrite (NO₂⁻) removal and found that the biochar-woodchips mixture removed more NO₃⁻ than woodchips alone. Bock et al. (2016) found that when influent NO₃⁻ concentration is above 5 to 10 mg L⁻¹,
bioreactors that include biochar have greater N removal than those without biochar. Zhang (2015) compared biochar to mixed hardwood chips and corn cobs and found that all three removed similar amounts of orthophosphate. In another study, it was found that the addition of biochar into a bioreactor significantly reduced P concentration by up to 75% when compared to the woodchip bioreactor alone (Lassiter and Easton, 2013). An important yet unexplored aspect of this research is better understanding of the wide-range of variables that can be applied to the creation of biochar. The objective of this study was to expand knowledge of biochar chemistry by exploring biomass source paired with pyrolysis temperature to create highly specialized biochars to lessen the impact of agricultural pollution on water bodies.

4.3 Materials and methods

Biochars originating from six biomasses were sourced from the BioCentury Research Farm in Boone, IA and one additional biomass from the City of Ames, IA Public Works. Of the six biomasses (Table 4.1), two were hardwood woodchips, two were softwood woodchips, and two were herbaceous materials. Hardwood woodchips were Red Oak (*Quercus rubra*) and Ash (*Fraxinus spp.*), both sourced from Central Iowa. Softwood woodchips were Mixed Pine (*Pinus spp.*) from Michigan and Loblolly Pine (Pine-Southern Yellow) (*Pinus taeda*) from the Southern United States. Herbaceous materials were Switchgrass (*Panicum virgatum*) and Corn Stover (*Zea mays*), both harvested locally in Central Iowa. For the control, Ash (*Fraxinus spp.*) woodchips were used without biochar addition. The Ash woodchips used in the control were obtained from the City of Ames, IA Public Works. The biochar was created using three temperatures of slow pyrolysis by ARTichar (Prairie City, IA). Each biomass was produced at a peak temperature of 400°C, 600°C, and 800°C to create a total of 18 biochars.
4.3.1 Particle Size

Particle size analysis was performed using gravimetric particle size distribution to assess the effects of pyrolysis temperature on particle size and nutrient removal capabilities. Four sieves were used (2 mm, 1 mm, 0.5 mm, and 0.25 mm) with a bottom pan to capture anything less than 0.25 mm. Each biochar had some loss due to the fine particle sizes, which was accounted for as dust in Table 4.1. Before testing, the total amount of biochar was weighed. The pans were covered and shaken for four minutes. After shaking, the biochar and sieve or pan was weighed and recorded. The sieve or pan weight was subtracted to get the final total for each particle size.

4.3.2 Nitrate and Phosphate Batch Test

For the batch test, 350 grams of Ash woodchips was mixed with 30 grams of biochar for a ratio of 12:1 (8.3%) and put into a 5 L container with 3 L of deionized (DI) water. There were three replicates for each biomass and pyrolysis temperature. This ratio was similar to the ratio used by Christianson et al. (2011) of 7-14% in a study also examining the effects of pyrolysis temperature on biochar as a bioreactor amendment. Three denitrifying microbes (Bradyrhizobium japonicum, Pseudomonas stutzeri, and Raoultella spp.) were added with 5 mL of broth solution (Henry et al., 2004; Hendriks et al., 2000; Tiedje 1994). This mix was soaked for 24 hours to allow microbial acclimation and to allow the biochar and woodchips to flush any nutrients to avoid an initial spike during the test. After 24 h, the biochar woodchip mix was drained and 2 L of nutrient solution was added. The nutrient solution was made to 30.0 mg L\(^{-1}\) NO\(_3\)\(^-\)N and 10.0 mg L\(^{-1}\) PO\(_4\)\(^{3-}\) using KNO\(_3\) and KH\(_2\)PO\(_4\) - PO\(_4\) with DI water, respectively. The NO\(_3\)\(^-\)N concentration was chosen to be representative of high-level concentrations found in tile drainage in Iowa. The PO\(_4\)\(^{3-}\) concentration was over three times higher than observed P in field tile drainage to ensure that removal rates were not limited by P (Bock et al., 2015). 25 mL water
samples were collected at 0, 4, 8, 12, and 24 hours. The samples were immediately filtered through a 0.45 µm glass microfiber filter and acidified using sulfuric acid (H₂SO₄). Because the samples were filtered through a 0.45 µm glass microfiber filter, the P was considered dissolved reactive phosphorus (DRP). Samples were stored at 4°C until they were tested using the Seal Analytical AQ2 automated spectrophotometer. NO₃⁻N was measured as NO₃⁻N+NO₂⁻N using AQ2 method EPA-103-A, Rev. 10. DRP was measured using AQ2 method EPA-145-A, Rev. 1.

4.3.3 Statistics

Statistical analysis was performed using R Statistical Analytical Software and all data were tested for normality. An ANOVA with Tukey post-hoc pairwise comparison was used to compare each biochar biomass and temperature against the control of woodchips for NO₃⁻N and DRP removal. Linear regression was used to compare pyrolysis temperature to the change in NO₃⁻N and DRP concentration relative to the woodchip-only control.

4.4 Results and discussion

There was no statistical different in NO₃⁻N removal between biochar amended-woodchip bioreactors and woodchip-only bioreactors at any pyrolysis temperature, shown in Figure 4.1 and Table 4.1 (p = 0.32 for 800°C; p = 0.62 for 600°C; p = 1.0 for 400°C). Increased NO₃⁻N removal was observed as pyrolysis temperature increased (r = 0.58, p < 0.05). Biochars pyrolyzed at 800°C removed of 11.8 (±1.45) mg L⁻¹ NO₃⁻N. The 600°C removed an average of 11.2 (± 3.59) mg L⁻¹ NO₃⁻N over 24 hours. Average N removal using 400°C biochar was 9.7 (±0.90) mg L⁻¹ NO₃⁻N. The control Ash woodchip bioreactors removed 9.6 (±1.33) mg L⁻¹ of NO₃⁻N.

Higher temperature pyrolysis yields biochar with higher AEC, and therefore more readily available to adsorb PO₄³⁻ and NO₃⁻N (Lawrinenko, 2014). Because there was significant P removal but not N removal, one of the major physical processes could have been selective ion
exchange (Yang and Jiang, 2014). Jung and Kim (2014) stated that maximum surface area is achieved in biochar produced at 800°C. However, in a linear regression with particle size and the concentration reduction of NO$_3^-$N, there was no significant correlation ($r = 0.11$, $p = 0.68$). This suggests that microbial denitrification was likely the primary NO$_3^-$N removal pathway, and that biochar did not provide a significantly better carbon source for microbial denitrification. Biochar may have also removed some NO$_3^-$N through physical adsorption, but physical adsorption did not significantly outperform microbial denitrification at NO$_3^-$N removal. Due to the small volume ratio of biochar to woodchips, the adsorption process was expected to not have significant improvement on overall N removal with the presence of microbial denitrification.

Table 4.1. The summary data for all biochar types including the NO$_3^-$N removal rate at 24 hours, the amount of PO$_4^{3-}$ leached, and the particle size distribution. Negative values indicate leaching, positive values indicate removal. The particle size distribution for all biochars is percentage of weight at that particle size unless otherwise indicated. Letters indicate significant differences at $p < 0.05$ within that column. Groups are outlined. Standard deviation is in parentheses.

<table>
<thead>
<tr>
<th>Nutrient Removal</th>
<th>Particle Size (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>NO$_3^-$N Removed (mg N L$^{-1}$)</td>
<td>0.0</td>
</tr>
<tr>
<td>PO$_4^{3-}$ Removed (mg P L$^{-1}$)</td>
<td>0.0</td>
</tr>
</tbody>
</table>

- **Control**
  - 9.6 (1.33) a
  - -1.8 (0.99) b

- **Red Oak**
  - 10.9 (1.11) a
  - -0.9 (0.98) b

- **Mixed Pine**
  - 10.9 (1.11) a
  - -0.9 (0.98) b

- **Loblolly Pine**
  - 10.9 (1.11) a
  - -2.0 (1.46) b

- **Corn Stover**
  - 13.8 (4.59) a
  - 0.3 (0.75) b

- **Switchgrass**
  - 10.7 (2.04) a
  - 0.2 (0.50) b

- **Hardwood**
  - 11.8 (1.45) a
  - 0.5 (0.54) c

- **Softwood**
  - 10.4 (1.01) a
  - -1.1 (1.72) b

- **Herbaceous**
  - 12.3 (3.97) a
  - 0.1 (0.66) d
The 400°C biochar removed 9.7 (±0.90) mg L\(^{-1}\) of NO\(_3\)N on average. As mentioned previously, the properties of low temperature pyrolysis include more oxygen groups that could take part in ligand exchange (Zhao et al., 2017). It was also indicated that the 400°C biochar would be best suited toward CEC (Zhao et al., 2017). Like the adsorption of NO\(_3\)N by higher temperature pyrolysis using AEC, ligand exchange and CEC were not as significant as microbial denitrification. Christianson et al. (2011) performed a study of biochar made from *Pinus radiata* (softwood biomass) at 380°C and 550°C and tested at ratios with woodchips between 7-14%. They also found that the biochar did not remove significantly more NO\(_3\)N than woodchips without biochar (p = 0.91), which they attributed to biochar not increasing microsites or carbon

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**Figure 4.1.** The NO\(_3\)N concentration removed over a 24 hour period in a small-scale batch test compared by temperature of pyrolysis of biochar. None of the biochars, when grouped by pyrolysis temperature, removed significantly more NO\(_3\)N than the woodchip-only control (p > 0.05).
availability. The biochar that removed the least NO$_3$N in their study was the 380°C biochar at 7% application rate, followed by the 550°C, and finally the 380°C at 14% application rate, which was the only biochar to remove more NO$_3$N than the control (Christianson et al., 2011). The application rate in this study was 8.3%, similar to the lower application rate used by Christianson et al. (2011). Bock et al. (2015) found that biochar application rates of 10% and 30% did not significantly differ (p > 0.05) N and P removal. The application rates of biochar produced at 800°C should be studied further to better understand the role of application rates in both N and P removal rates. In another study, Hyland et al. (2010) concluded that biochar feedstock and increasing pyrolysis temperatures both have significant impacts on NO$_3$N removal rates.

In a comparison between biomass type and the woodchip control, none of the biochars removed significantly more NO$_3$N than the control (Figure 4.2, Table 4.1). The biochars removed the following amounts of NO$_3$N by concentration (Figure 4.2): Corn Stover (13.8 ±4.59 mg L$^{-1}$) (p = 0.13), Loblolly Pine (10.9 ±1.11 mg L$^{-1}$) (p = 0.97), Switchgrass (10.7 ±2.04 mg L$^{-1}$) (p = .99), Red Oak (10.1 ±1.64 mg L$^{-1}$) (p = 1.0), Ash (10.0 ±0.78 mg L$^{-1}$) (p = 1.0), and Mixed Pine (9.9 ±0.49 mg L$^{-1}$) (p = 1.0).

Biochars were also tested for PO$_4^{3-}$ removal in the batch tests. PO$_4^{3-}$ concentration leached into the solution solely by biochar was calculated by subtracting PO$_4^{3-}$ concentration leached by the woodchip control from the total leached PO$_4^{3-}$ concentration by biochar-woodchip mixture. Only the 800°C biochar removed a significant amount of PO$_4^{3-}$ when compared to the woodchip-only control (p < 0.05) (Figure 4.3). The 800°C biochar also removed statistically more PO$_4^{3-}$ than both the 600°C (p < 0.05) and the 400°C biochars (p < 0.05). The 800°C biochar removed 0.5 (±0.54) mg P L$^{-1}$, the 600°C leached 1.3 (±1.69) mg L$^{-1}$, and the 400°C leached 1.9 (±0.93) mg P L$^{-1}$. As temperature of pyrolysis increased, the amount of P leached decreased (r = -0.64, p
< 0.05). Of the biomasses, hardwood leached 0.5 (±1.25) mg P L^{-1}, softwood leached 1.1 (±1.72) mg P L^{-1}, and herbaceous removed 0.1 (±0.66) mg P L^{-1}. The herbaceous biochars removed a statistically higher amount of PO_{4}^{3-} when compared to the control (p < 0.05). The significantly higher removal of PO_{4}^{3-} by the 800°C and herbaceous biochars was compared to a control that leached PO_{4}^{3-}. In a longer term study, biochar may show the same significance once the woodchips stop leaching PO_{4}^{3-}.

![Figure 4.2. The NO_{3}N removed over a 24 hour period in a small-scale batch compared by biomass source that was pyrolyzed into biochar. None of the biomasses had significantly greater NO_{3}N removal than the woodchip-only control, but Corn Stover had the largest removal.](image)

The U.S. EPA has a recommended limit range from 0.070 to 0.118 mg L^{-1} of total phosphorus (TP) for Iowa streams (U.S. EPA, 2016; INRS, 2017). In the Midwest, TP in tile drainage can vary greatly depending on the management of the land being drained and the time
of year. When selecting biochar as an amendment to remove P in woodchip bioreactors, it is important to consider influent P loads. One study observed a range from 0.03 to 0.27 mg TP L\(^{-1}\) in a Central Iowa watershed over a study period of two years (Kalkhoff et al., 2016). In another bioreactor study at Central Minnesota, Erickson et al. (2017) found that influent TP concentration in tile drainage ranged from 0.138 to 1.516 mg TP L\(^{-1}\). At these influent concentrations, the 800°C biochar removal rate of 0.5 mg P L\(^{-1}\) has the potential to meet the U.S. EPA TP standards over the majority of the year. The P removal rate of herbaceous biochar was significantly different than the control, but when compared to influent P loads seen in the field, it would not meet the U.S. EPA standard in most cases. The focus for biochar as a P amendment should be on those pyrolyzed at high temperatures.

Figure 4.3. The PO\(_4^{3-}\) removed or leached over a 24 hour period in a small-scale batch test compared by biomass source and pyrolysis temperature. Negative values indicate leaching, positive values indicate removal. Only the 800°C and herbaceous biochar groups removed significantly more PO\(_4^{3-}\) than the woodchip control (p < 0.05). 11 of the 18 biochars leached P into solution even after a 24-hour pre-soak period.
The leaching of P by 11 of the 18 of the biochars could be problematic for the application biochar in field water treatment systems. Different methods to perform the initial flushing need to be tested further to better understand the potential impacts of adding biochar to large-scale systems. According to Mukherjee and Zimmerman (2012), biochar can leach dissolved organic carbon (DOC), N, and P when first being used to filter water. Hassanpour et al. (2017) confirms that carbon and nutrients leaching from biochar was observed in full-scale bioreactor systems during initial startup and flushing period due to its small particle size in comparison to woodchips. In the batch test, the biochar and woodchips were soaked in DI water with denitrifying microbes for a 24-hour period. Before the soaking period, the woodchips were rinsed with DI water and after the soaking period, each bucket was completely drained and refilled with nutrient solution. This was done to simulate an initial flushing period. Even with the rinsing and 24-hour soaking period, 11 out of 18 biochars and the control woodchips leached PO$_4^{3-}$ into the solution throughout the testing period. The following biochars did not leach P: Corn Stover 600°C, Switchgrass 600°C, Red Oak 800°C, Ash 800°C, Corn Stover 800°C, Switchgrass 800°C, and Mixed Pine 800°C. It is also important to note that unlike microbial denitrification which transforms NO$_3^-$N into N$_2$ that can leave the bioreactor as a gas, PO$_4^{3-}$ would be adsorbed to the biochar and remain within the bioreactor. There are a limited number of sites within biochar than can adsorb and hold PO$_4^{3-}$, which means after a variable amount of time, biochar would stop removing PO$_4^{3-}$. If PO$_4^{3-}$ continues to enter the bioreactor, it could cause the PO$_4^{3-}$ held by the biochar to desorb and leach back into the water and leave the bioreactor. These potential negative impacts of biochar need to be studied on a longer time scale.

Particle size did not have a significant correlation with pyrolysis temperature ($r = 0.047$, $p = 0.85$). Although a particle size analysis was not performed on the biomass before it was
pyrolyzed as part of this study, we suspected that the biochar particle size was related to its original biomass particle size. Particle size also did not have a significant correlation with NO$_3$N removal ($r = 0.11$, $p = 0.68$) or PO$_4^{3-}$ removal ($r = 0.07$, $p = 0.80$). Bock et al. (2015) in a lab-scale column study also found that particle size did not have a significant effect ($p > 0.05$) on either NO$_3$N or PO$_4^{3-}$ removal. Christianson et al. (2011) proposed that lower temperature biochars would be the better microbial carbon source because they consist of more aliphatic functional groups. The authors also found that biochar did not perform significantly better at NO$_3$N removal than woodchips (Christianson et al., 2011). As previously discussed, physical reactions rather than microbial were responsible for both NO$_3$N and PO$_4^{3-}$ removal by biochar, with microbes preferring the labile carbon from the woodchips rather than the aliphatic functional groups found in biochar. When considering scaling up to field woodchip bioreactors, particle size will be a major factor. Because particle size was not significantly related to nutrient removal, it would be best to use larger particle sizes in the field. Having larger particle sizes will allow for better flow-through of water through the bioreactor while smaller particle sizes may clog the system or leach from the bioreactor altogether.

It is possible that on a larger scale, longer timescale, and at varying influent nutrient concentrations, biochar could perform differently than observed in the lab. In one study, Hassanpour et al. (2017) concluded that biochar improved NO$_3$N removal in a field-scale woodchip bioreactor for the first year in a three-year period, but due to aging of the biochar, performance decreased to that of a similar woodchip-only bioreactor. A pilot-scale study performed by Bock et al. (2016) found that biochar may outperform woodchip-only bioreactors when influent NO$_3$N levels are above 5 to 10 mg L$^{-1}$. Results of this study indicate there is not much future potential for the use of biochar in field- and pilot-scale bioreactors, but there are
many factors to consider. Biochar in this study did not outperform woodchips at NO$_3^-$N removal. Biochar pyrolyzed at 800°C and made from herbaceous materials significantly outperformed woodchips at PO$_4^{3-}$ removal, but only for the period where the woodchips were leaching PO$_4^{3-}$. The cost-benefit of creating biochar could negate its positive nutrient removal impacts. The application rates also need to be examined more closely, which could drive up costs if a bioreactor needs to be composed of mostly biochar to see significant nutrient removal impacts.

4.5 Conclusions

For biochar to be a viable amendment to woodchip bioreactors, the full range of properties created by biomass selection and pyrolysis temperature must be understood. The results from this study are just one piece of the puzzle. Other factors still need to be better understood, including impact of particle size on bioreactor hydrology, application rates of biochar, effect of biomass sources with a focus on herbaceous materials, and the changes in nutrient removal and leaching over time. Results from this study showed that biochars made from herbaceous materials and pyrolyzed at 800°C had the most potential to remove PO$_4^{3-}$ from agricultural drainage, but not at rates that warrant its immediate use in woodchip bioreactors. As pyrolysis temperature increased, so did NO$_3^-$N removal. As pyrolysis temperature increased, leaching of PO$_4^{3-}$ decreased and was even removed by herbaceous biochars and biochar pyrolyzed at 800°C. Initial flushing periods and potential downstream impacts will need to be considered when amending woodchip bioreactors with biochar in the field. Initial flushing could be reduced by using herbaceous biochars produced at high pyrolysis temperature, after a 24-hour soaking period. Results from this study should be used to inform biochar selection for future studies with a focus on high temperature pyrolysis biochars.
4.6 Acknowledgements

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4.7 References


CHAPTER 5. GENERAL CONCLUSIONS

5.1 Conclusions

Hydraulic residence time (HRT) had a significant impact on not only the percent mass removal rate (PMRR) of NO$_3^-$ N in woodchip bioreactors, but also on the NO$_3^-$ N mass load removal rate (MRR). As HRT increased PMRR increased and MRR decreased. The 2 h HRT removed 9.0 g m$^{-3}$ day$^{-1}$ with a PMRR of 9.0%. The 8 h HRT removed 8.5 g m$^{-3}$ day$^{-1}$ with a PMRR of 32.1%. The 16 h HRT removed 7.4 g m$^{-3}$ day$^{-1}$ with a PMRR of 53.8%. When accounting for bypass flow, the 2 h HRT still removed the most NO$_3^-$ N by mass. There was significant TAN production throughout the study period for all treatments, but TAN was not significantly different between the HRTs. The strongest explanatory variables for PMRR were HRT and the influent NO$_3^-$ N concentration. When considering numerous factors outlined by this study, an 8 h HRT performed best in this specific system at NO$_3^-$ N removal while minimizing negative environmental impact.

The biochar study showed that biochar made at 800°C during pyrolysis and from herbaceous materials removed significantly more PO$_4^{3-}$ than the woodchip-only control, but only for the period when the woodchip control was leaching PO$_4^{3-}$. 11 of the 18 biochars leached PO$_4^{3-}$ into the solution during a batch test. Leaching of nutrients by biochar during start-up of woodchip bioreactors needs to be addressed. None of the biochars removed significantly more NO$_3^-$ N than the woodchip control. Biochar should not be targeted to NO$_3^-$ N removal but instead should be used as a PO$_4^{3-}$ amendment after further studies confirm its PO$_4^{3-}$ removal capabilities. Biochar is not currently recommended for use in woodchip bioreactors based on the methods used in this study.
5.2 Future Work and Implications of Research

A major factor in improving the nutrient removal capabilities of woodchip bioreactors is understanding the role of HRT on NO\textsubscript{3}N removal. By having a controlled pilot-scale system with triplicate bioreactors at three HRTs, we were able to isolate HRT as an explanatory variable for NO\textsubscript{3}N removal. Previous studies found that changing HRT did not have a significant impact on MRR. One of the major conclusions of this study is that increasing HRT decreases the MRR, or in other words, HRT does have a significant impact on MRR. When considering the future of bioreactor data reporting, especially as it applies to policy planning, NO\textsubscript{3}N mass removal rates may have more impact than NO\textsubscript{3}N percent and concentration removal rates. The variation in NO\textsubscript{3}N concentrations within the bioreactors is also a major result as very few studies have had testing points within bioreactors. Variation and increases in NO\textsubscript{3}N within the bioreactors implies there are processes occurring that have not previously been reported. Future studies on woodchip bioreactors should focus on taking samples from within the bioreactor as done in this study to understand and identify the processes occurring that remove NO\textsubscript{3}N. It has been documented that woodchip bioreactors have different NO\textsubscript{3}N removal rates during the first 3 years of operation than is seen in the long-term. A long-term study needs to be performed on the pilot-scale bioreactors to better understand the role of explanatory variables such as temperature and dissolved oxygen (DO) in denitrification. Because woodchips are not as readily available in the Midwest, other carbon sources should be examined for their NO\textsubscript{3}N removal abilities and longevity for use in bioreactors.

Previous studies have found a wide variety of results and applications for biochar, but none have quantified the impact of biomass and pyrolysis temperature on such a large amount of biochars. We found that high temperature pyrolysis at 800°C has the most potential to be used to
remove $PO_4^{3-}$ in woodchip bioreactors. We also found that herbaceous biochar has some potential for use as a $PO_4^{3-}$ amendment. These results should be used by future studies to inform biochar selection. The leaching of $PO_4^{3-}$ needs to be addressed before taking future studies of biochar full-scale. A 24-hour pre-soak in deionized (DI) water helped reduce leaching, but it did not eliminate it. Particle size also needs to be better understood because the smaller the particle size of biochar, the more likely it is to impede bioreactor flow or leave the system altogether.

Other studies have found variable results from biochar as a nutrient remover because of changes in influent nutrient concentration, the age of the biochar, and differences in scaling from the lab to the field. All of these factors need to be researched using the biochars recommended by this study. One of the most important factors that needs to be studied is how long biochar can act as a $PO_4^{3-}$ remover before the adsorption sites are filled.