Nutrient Removal Performance Of A Wood Chip Bioreactor Treatment System Receiving Silage Bunker Runoff

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NUTRIENT REMOVAL PERFORMANCE OF A WOOD CHIP BIOREACTOR TREATMENT SYSTEM RECEIVING SILAGE BUNKER RUNOFF

A Thesis Presented

by

Deborah Kraft

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The Faculty of the Graduate College

of

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In Partial Fulfilment of the Requirements
For the Degree of Master of Science
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ABSTRACT

Silage bunker runoff is a form of agricultural pollution that contributes to aquatic ecosystem degradation. Current handling and treatment methods for this process wastewater are often ineffective or expensive. A woodchip bioreactor is an emerging treatment technology designed to facilitate denitrification through the provision of an anaerobic, carbon rich environment. A wood chip bioreactor treatment system, consisting of three pre-treatment tanks, two wood chip bioreactors, and one infiltration basin, was constructed at the Miller Research Complex in South Burlington, Vermont in 2016. Runoff and leachate from an adjacent silage storage bunker is directed into the system. The pre-treatment tanks include two settling tanks and one aeration tank. The former allows for sedimentation of organic matter, while the latter is designed to allow for nitrogen transformations that will help maximize nitrogen removal in the bioreactors. During the summer and fall of 2017, sampling occurred at four points within the system in order to determine the efficacy of various treatment steps. Samples were analyzed for nitrate (NO$_3^-$-N), ammonium (NH$_4^+$-N), total nitrogen (TN), soluble reactive phosphorus (SRP), and total phosphorus (TP) in order to compare inflow and outflow pollutant concentrations and loads. Results indicate that this treatment system significantly reduced nutrient loads in the runoff. Over the entirety of the sampling period, the influent TN and TP mass load were both reduced by approximately 44%.
ACKNOWLEDGEMENTS

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CHAPTER 1: COMPREHENSIVE LITERATURE REVIEW

1.1 Introduction

The 2017 EPA National Water Quality Inventory: Report to Congress identifies agricultural pollution as a leading source of water quality impairment on lakes, wetlands, rivers and streams. Agricultural wastewater is an umbrella term, referring to runoff sources ranging from swine waste and cattle slurry to dairy parlor washings and irrigation tailwater (EPA, 2005). Surplus nitrogen and phosphorus from agricultural sources can wash into nearby surface water or leach into groundwater, directly impacting water quality (Elrashidi et al., 2013). Fertilizer runoff and liquid animal waste have been identified as major sources of excess nitrogen and phosphorus in predominantly agricultural watersheds (EPA ROE, 2008), but there are additional highly polluting agricultural wastewaters that deserve equal attention. Silage leachate, a natural byproduct of silage production, is one example of agricultural wastewater that can have immediate negative impacts on nearby surface water (Arnold et al., 2000).

Silage, which is prepared by the controlled anaerobic fermentation of a high moisture content crop (generally grass or corn), plays a large role in dairy farming and provides crucial fodder during the winter months (Gebrehanna et al., 2014, Arnold et al., 2000). The fermentation process associated with modern day silage is not without criticism as the complicated chemical changes within the crop lead to ecologically hazardous byproducts (Woolford, 1978) and many pollution events (Deans and Svoboda, 1992). Silage leachate, created from the expulsion of plants juices during fermentation, is an extremely powerful pollutant (Arnold et al., 2000). Silage leachate is often mixed with stormwater and snowmelt, either through infiltration of the stored silage or combined
runoff surfaces. This combination of liquids is known as silage runoff. While runoff is more dilute than leachate, the production of runoff creates an overall larger volume of polluted water that must be managed. This runoff contains elevated nutrient levels (phosphorus and nitrogen), is highly acidic, and has a high biological oxygen demand (Deans and Svoboda, 1992; Galanos et al., 1995).

Reactive nitrogen, defined as all nitrogen compounds except for inert nitrogen gas (N₂), includes compounds such as nitrate (NO₃⁻), ammonia (NH₃), gaseous nitrous oxide (N₂O) and organic forms of nitrogen (Erisman et al., 2011). Nitrogen is essential for all life forms on Earth (Reddy and Delaune, 2008); however, anthropogenic creation of reactive nitrogen has surpassed natural terrestrial production (Galloway et al., 2003). An overabundance of reactive nitrogen is artificially produced through a variety of methods, such as fossil fuel combustion, agricultural processes, septic treatment, and fertilizer production (Galloway et al., 2003; Erisman et al., 2011). This excess has led to an accumulation of reactive nitrogen in the environment, especially in surface waters.

Phosphorus, another essential element for all forms of life, is also being discharged in excess to surface waters as a common constituent of agricultural fertilizers, manure, and organic wastes in sewage and industrial effluent (USGS, 2018; Elser and Bennett, 2011). The environmental flow of phosphorus into the biosphere has quadrupled as a result of human activity since the middle of the twentieth century (Falkowski et al., 2000). Buildup of excess nitrogen and phosphorus in surface waters contributes to eutrophication, which leads to habitat degradation and a loss of biodiversity in coastal and terrestrial areas alike (Howarth et al., 2002, EPA ROE, 2008). Water that is rich in nitrogen and phosphorus compounds provides a good medium for the growth of
microorganisms. This growth rapidly depletes the levels of dissolved oxygen and leads to large fish kills events, as well as the death of other aquatic fauna (Woolford, 1978). These combined effects of eutrophication, loss of habitat and wildlife mortalities are detrimental to natural ecosystems (Osiadacz et al., 2010). Reactive nitrogen compounds can also pose health risks to humans (such as infant methemoglobinemia) if ground water or other drinking water sources are contaminated (Campbell, 1952).

While recognizing that phosphorus compounds are a contributing factor to pollution potential of silage runoff, this review will focus mainly on the effects of nitrogen compounds and the potential for their removal from silage runoff. The potential for phosphorus removal will be discussed further in Chapter 2.

Varying disposal methods have been used in the past to manage silage leachate and runoff, such as spreading on land and feeding to animals, but there are drawbacks associated with these techniques, due to the nature of the runoff and the timing at which it is produced (Woolford, 1984). Additionally, although numerous technologies for removing nitrogen from wastewaters exist (e.g. batch sequencing, ammonia volatilization, ion exchange, methanol dosing and reverse osmosis), these methods are often cost prohibitive, require maintenance, and are difficult to implement for treatment of small volume point source discharges, such as silage runoff (Koch and Seigrist, 1997; Kapoor and Viraraghavan, 1997). Furthermore, even when these treatments are applied, often some nitrogen remains in the final effluent (Cameron and Schipper, 2010). Some affordable, passive technologies that have been used to treat agricultural wastewaters include vegetative treatment areas (VTAs) (Larson and Safferman, 2012) and constructed wetlands (Smith et al., 2006). While these methods may be more appealing to farmers
than traditional options, limitations still exist for the treatment of silage runoff, such as insufficient capacity for highly acidic runoff and an inability to allow for necessary nitrogen transformations (Faulkner et al., 2011; Larson and Safferman, 2012; Holly and Larson, 2016)

One emerging technology that is being used to treat many forms of agricultural and domestic runoff is known as a denitrifying bioreactor. These bioreactors are commonly found in the form of a denitrification bed or wall (Bock et al., 2015). This review will focus on the beds, which are generally a large, lined cavity filled with a carbon-rich reactive media, that provide an ideal environment for microbes to perform heterotrophic denitrification. Denitrifying bioreactors have the potential to address some of the aforementioned limitations of VTAs and constructed wetlands in that they are not damaged by highly acidic runoff and that they allow for denitrification (although denitrification may be limited by low pH) (Bock et al., 2018). Denitrifying bioreactors are starting to be favored for their practicality, which is demonstrated in the low installation cost, small footprint, and minimal required maintenance (Blowes et al., 1994, Cameron and Schipper, 2010). Wood chips are an affordable and commonly available resource that are often used as a reactive media in denitrification beds (Schipper et al., 2010b). This review will focus on the specific characteristics and performance of wood chip bioreactors with respect to denitrification of silage runoff.
1.2 Silage and Silage Runoff

1.2.1 Background

A mural dating from 1500 to 1000 BC indicates that ancient Egyptians were familiar with the process of ensiling grain (Doelle et al., 2009), and silos found in the ruins of Carthage demonstrate that forage was ensiled there in about 1200 BC (Squires, 2011; Schukking, 1976). The use of early silage is still debated. Some historians argue that historical silos may have been used simply for storage (without fermentation occurring), or as a way to conceal grain from marauding tribes (Woolford, 1984). Other historians claim that the importance of anaerobic conditions has been known since ancient times (Doelle et al., 2009). Regardless of end product and whether it was intended to feed humans or animals, mankind has recognized the need to store and preserve grain in seasons of plenty for approximately 3,000 years. (Squires, 2011).

1.2.2 Ensilage Process

Crops destined for silage will be harvested and stored in a variety of sealed, airtight containers or arrangements, such as vertical silos, trench silos, stack silage and silage bunkers (Davis, 2016). Anaerobic storage conditions allow fermentation to occur. During fermentation, numerous types of bacteria convert water-soluble carbohydrates (mainly sugars) into a mixture of acids, alcohol, and carbon dioxide (Schukking, 1976). The creation of these products will lower the pH of the ensiled material, inhibiting the growth of spoilage organisms and ensuring that no further deterioration will occur; this preserves the crop as silage (Weinberg and Muck, 1996; Schukking, 1976). The chemical changes that occur in stored silage (due to respiration, fermentation, potential spoilage and aerobic
deterioration) result in the loss of nutrients (Woolford, 1984). Discharge of leachate from a silage storage structure is another source of nutrient loss (Woolford, 1984).

1.2.3 Leachate Production

The quantity of leachate that is produced during ensilage primarily depends on the dry matter content of the crop and also the degree of compaction that is experienced during storage (Stephens et al., 1997). Factors that have minor impacts on the amount leachate production include mechanical pre-treatment, preservative additions, air exposure, crop characteristics, and type of fermentation (Woolford, 1984).

In order for silage to ferment properly, there is an optimal moisture content (MC) to strive for before storage. At a proper MC, crops will be able to ferment while creating minimal amounts of natural leachate. While optimal MC during ensiling is recommended to prevent excess leachate, it is difficult to achieve due to weather conditions, available labor, and attempts to optimize crop yield and quality (Gebrehanna et al., 2014). Additionally, while optimal MC ensiling may prevent initial leachate, it will not protect from later storm events and snow melt that are able to infiltrate an open storage area, or a covered storage area with an improper seal. Previous research suggests that most attempts to manage silage leachate focus on limiting initial production. However, because this approach is unpredictable and silage leachate cannot always be avoided, it is important that researchers also focus on proper containment, treatment and disposal of this runoff.
1.2.4 Environmental Impacts and Biochemical Characteristics

The environmental impacts of silage leachate are far reaching. Spillane and O'Shea (1973) found silage leachate to be almost 200 times stronger than raw domestic sewage in terms of BOD (90,000 mg O$_2$/l compared to 500 mg O$_2$/l). The same study also found silage leachate to have the highest BOD by far when comparing common agricultural pollutants of watercourses, such as pig and cow slurry (35,000 mg O$_2$/l and 5,000 mg O$_2$/l, respectively). Since silage leachate is rich in plant materials that are highly nutritious to microorganisms, microbial activity is stimulated when the runoff reaches surface waters (Deans and Svoboda, 1992). This increased activity can result in rapid oxygen depletion. This deoxygenation, combined with an inevitable decrease of pH, can kill fish and other aquatic life (Arnold et al., 2000). The eutrophication that occurs when silage leachate reaches surface waters can also lead to large algae blooms. When these algae blooms die and are consumed by bacteria, oxygen is further depleted (NOAA, 2008). These effects damage aquatic ecosystems and can also be economically detrimental to coastal and lakeside towns as tourism is negatively impacted (cite).

Deans and Svoboda (1992) attributed hundreds of pollution events in Scotland, England and Wales to silage leachate in the years 1987-1989, and Beck (1989) reported that 38% of farm pollution incidents in Yorkshire and North Humberside (England) were connected with silage. Lennox et al. (1998) found that 22.9% of silage leachate pollution sources in Northern Ireland had a moderate stream impact, and 10% had a severe stream impact.

Silage leachate production should be avoided because of the aforementioned pollution and ecosystem harm. In addition, the leachate can be detrimental to farm
operations. The high acidity of silage leachate has the potential to damage concrete and steel, materials that are often used in silage storage containers (Arnold et al., 2000). It is also undesirable to farmers because the presence of leachate indicates a loss of dry matter and reduces the nutritional value of silage (Barry & Colleran, 1982; Reynolds and Williams, 1995).

Table 1, adapted from Gebrehanna et al., 2014 with additional sources added, shows an organized summary of biochemical characteristics of silage leachate found in current literature. This table quantifies the extremely high levels of total nitrogen and BOD, in addition to the substantially low pH levels often found in silage leachate.
Table 1: Biochemical characteristics of various silage leachate samples.

<table>
<thead>
<tr>
<th>References</th>
<th>Silage type</th>
<th>Notes</th>
<th>BOD (mg/L)</th>
<th>TP (mg/L)</th>
<th>TN (mg/L)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spillane and O’Shea (1973)</td>
<td>unspecified</td>
<td></td>
<td>90,000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beck (1989)</td>
<td>unspecified</td>
<td></td>
<td>30,000-80,000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deans and Svoboda (1992)</td>
<td>grass</td>
<td>samples from a silo collection pit-stored for up to 1 month at 4°C</td>
<td>33,800</td>
<td>2920</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>grass</td>
<td></td>
<td>37,900</td>
<td>2750</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>grass</td>
<td></td>
<td>46,300</td>
<td>3340</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td>Galanos et al. (1995)</td>
<td>grass</td>
<td>sample 1: collected a few days after 2nd cut of grass was covered</td>
<td>68,500</td>
<td>800</td>
<td>4010</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>grass</td>
<td>sample 2: collected 40 days after sample 1</td>
<td>72,500</td>
<td>850</td>
<td>4120</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>grass</td>
<td>sample 3: collected a few days after 2nd cut of grass was covered</td>
<td>54,600</td>
<td>740</td>
<td>3620</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td>grass</td>
<td>sample 4: collected 35 dates after sample 3</td>
<td>61,800</td>
<td>840</td>
<td>3870</td>
<td>4.4</td>
</tr>
<tr>
<td>Stephens et al. (1997)</td>
<td>grass</td>
<td>silage squeezed to produce leachate</td>
<td>44,000</td>
<td></td>
<td></td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td>grass</td>
<td></td>
<td>64,000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arnold et al. (2000)</td>
<td>grass</td>
<td>leachate stored for one year</td>
<td></td>
<td></td>
<td></td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td>grass</td>
<td>leachate from 10 days after production</td>
<td></td>
<td></td>
<td></td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td>grass</td>
<td>leachate from heavy rain 3 weeks after production</td>
<td></td>
<td></td>
<td></td>
<td>4.2</td>
</tr>
<tr>
<td>Tattrie (2006)</td>
<td>unspecified</td>
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<td>170,000</td>
<td>769</td>
<td>4905</td>
<td>4.5</td>
</tr>
<tr>
<td>Holly and Larson (2016)</td>
<td>unspecified</td>
<td></td>
<td>17,000</td>
<td>388</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
1.2.5 Treatment and Disposal Methods

One common leachate disposal method that is often used (in addition to attempts to limit production) is land application (Woolford, 1984). However, because leachate has such high nutrient concentrations and low pH, it must be diluted or treated before land application to avoid risk of vegetative burning and additional leaching (Gebrehanna et al., 2014). Furthermore, the high BOD of untreated leachate may deplete soil oxygen, which could negatively affect plant growth (Galanos et al., 1995; Burford, 1976). Another attempted disposal method involves feeding the leachate to livestock (Gebrehanna et al., 2014, Woolford, 1984). Unfortunately, similar to land application, this method has its drawbacks. Silage leachate is most abundantly produced at a time of year when grass is plentiful, so very few farmers will make use of it for feed (Galanos et al., 1995). Also, a cost-benefit analysis by Weddell et al. (1988) showed a 24% deficit due to the costs associated with leachate preservation and storage for the intent of feeding it to livestock.

VTAs (Larson and Safferman, 2012) and constructed wetlands (Gottschall et al., 2007; Smith et al., 2006; Wood et al., 2008) are affordable natural systems that have shown success in treating dairy farm wastewaters. However, these systems are less promising for silage leachate treatment, and VTA studies have achieved mixed results in measuring reduction of nutrient load from such runoff. Larson and Safferman (2012) compared three VTAs located in Michigan, and found that the area receiving silage leachate had the poorest treatment performance and also obtained vegetative burning on the surface. Faulkner et al. (2011) found appreciable mass reductions in soluble reactive phosphorus and ammonium in three VTAs located in New York, but also observed a
200% increase in nitrate mass. This increase is attributed to the inability of nitrogen in the runoff to undergo denitrification. Holly and Larson (2016) compared two different vegetated filter strips in Wisconsin, where one was outfitted with pretreatments tanks designed to facilitate nitrification and denitrification. The study found no evidence of additional nitrogen removal from the pretreatment (excess nitrate was created but had no opportunity to undergo denitrification), and both systems showed no reduction in nitrates. VTAs are approved in some jurisdictions for treating dilute silage leachate, but Wright and Vanderstappen (1994) and Wright et al. (2004) concluded that concentrated leachate must be diverted to a storage tank.

Tattrie (2006) studied a constructed wetland that was treating a combination of agricultural and domestic wastewaters, including silage leachate. After the first phase of treatment, silage leachate was diverted from the constructed wetland because its low pH and high pollution levels inhibited treatment and decreased removal efficiencies. The constructed wetland recovered after the diversion, and Tattrie concluded that silage leachate could not be treated in the system. However, tests were not done with naturally diluted silage leachate, which may have been more viable (Gebrehanna et al., 2014). Overall, VTAs and constructed wetlands may be ideal systems for treating dilute silage leachate (silage runoff), but they are not equipped to handle the concentrated leachate that is produced in low-flow or dry weather conditions.
1.3 Wood Chip Bioreactors

1.3.1 Basic Design

As seen in Figure 1, denitrifying bioreactors or beds are carbon filled structures that are designed to enhance the natural process of denitrification for the passive removal of nitrate from polluted runoff (Christianson and Schipper, 2016). In a wood chip bioreactor, this carbon source is made up of readily available types of wood. Wood chip bioreactors are designed and installed to intercept wastewater before it drains into nearby surface waters or infiltrates down to groundwater.

![Diagram of a denitrification bed](image)

**Figure 1:** Schematic adapted from Schipper et al. (2010b) of a denitrification bed designed to treat concentrated discharges of leachate or drainage water.

1.3.2 Denitrification

Wood chip bioreactors are designed to facilitate denitrification. Denitrification is the microbial oxidation of organic matter, in order to obtain energy, where nitrate acts as the terminal electron acceptor and is converted into inert nitrogen gas (Osiadacz et al., 2010). During denitrification, nitrate is reduced along the following pathway: nitrate
(NO$_3^-$) -> nitrite (NO$_2^-$) -> nitric oxide (NO) -> nitrous oxide (N$_2$O) -> dinitrogen gas (N$_2$) (Averill and Tiedje, 1981). This is a heterotrophic process performed by facultative bacteria that are also capable of oxidizing organic matter using oxygen as the terminal electron acceptor (Seitzinger et al., 2006). The environment must be energetically favorable (meaning that little or no oxygen is available) for nitrates to act as the electron acceptor (Reddy and DeLaune, 2008). Due to these condition requirements, an organic carbon source plays two key roles in promoting denitrification; 1) maintaining an anoxic environment, and 2) acting as an electron donor (Schipper et al., 2010b). In the case of a wood chip bioreactor, the wood chips will serve as the carbon source and electron donor. Denitrifying bacteria are ubiquitous, and denitrification can occur in soils with the proper conditions (Seitzinger et al., 2006). However, Moorman et al. (2010) found that denitrification potential in wood chips was 31 - 400 times higher than that in soils.

Greenan et al. (2006) analyzed nitrate removal in denitrification beds of varying reactive media (including wood chips) and found that in all cases, denitrification was the dominant removal process. Immobilization into organic matter and dissimilatory nitrate reduction to ammonia were present, but accounted for <4% of nitrate removal in all treatments (Greenan et al., 2006).

1.3.3 Environmental and Design factors

Current literature indicates that the performance quality of a woodchip bioreactor, evaluated by its nitrate removal rates, is variable depending on a range of site specific factors. Primarily, these factors can be categorized as design (media characteristics and bioreactor parameters) and environmental (temperature and influent nitrate...
concentration). For treatment to occur within a wood chip bioreactor, the beds must be placed in the flow path of the runoff or the runoff must be directed to the bioreactor. While effective at removing nitrate, denitrification will not treat nitrogen in any other form. Many types of agricultural runoff, including silage leachate, contain additional forms of nitrogen compounds such as ammonium and organic nitrogen. Because of this, design considerations should be made to allow for pretreatment settling tanks and aeration tanks, such as in Holly and Larson's (2016) study, when necessary. These tanks will have the potential to convert total nitrogen into ammonium via mineralization, and ammonium compounds into nitrate via nitrification, allowing for maximum removal of total nitrogen later via denitrification (Reddy and DeLaune, 2006). A bioreactor laboratory study by Feyereisen et al. (2016), found a different type of pretreatment (placing a compartment of corn cobs before the wood chip bed) to also be effective in increasing N removal rates, in addition to reducing carbon losses.

If oxygen is introduced and the system becomes aerobic, it is likely that microbes will cease denitrification and focus on oxygen as their terminal electron acceptor instead, due to a lower reduction potential (Reddy and DeLaune, 2006).

Overall, microbial denitrification should occur while nutrient-rich runoff is in the bioreactor as long as:

1) The bioreactor remains anaerobic
2) There is sufficient nitrate available in the runoff
3) Temperatures are high enough to support microbial activity, and
4) Retention time is adequate
Design Factors - Media Characteristics

The current research shows that wood-based media has been the most widely used material in field trials. Wood based media is often chosen because of its affordability and abundance, but it is also desirable because of its high permeability and long durability (Robertson et al., 2009, Robertson, 2010). While other, more labile carbon sources, such as cracked corn, corn stalks, and straw, may result in higher nitrate removal rates, they may require more frequent replenishments due to rapid carbon depletion (Schipper et al., 2010b; Cameron and Schipper, 2010). However, since silage runoff is rich in organic carbon (Gebrehanna et al., 2014), carbon depletion in the media may be a non-issue. Future work is needed to determine if additional carbon is necessary to ensure that denitrification in a bioreactor does not become carbon-limited over time.

The size of media particulates does not appear to have an impact on nitrate removal rates; van Driel et al. (2006) measured nitrate removal rates for fine and coarse-grained media and found similar results for each (5.5 and 5.9 g N/m$^3$·d, respectively). Cameron and Schipper (2010) found there to be no significant difference in nitrate removal rate for five different grain sizes (ranging from sawdust to 61 mm wood chips) of the same softwood media. The same study also found no significant difference in removal rates between softwood and hardwood media. Addy et al. (2016), came to the same conclusion after comparing the findings of 26 published studies through a meta-analysis. The study found average removal rates of 2.6 and 3.7 g N/m$^3$·d for softwood and hardwood, respectively, which was not reported as a significant difference. However, the study acknowledged that the 95% confidence interval for these values encompassed a large range of rates, indicating that there may be undetected differences between wood
types (Addy et al. 2016). In general, current literature suggests that it is not necessary to seek out a specific wood type, allowing for flexibility when sourcing media for wood chip bioreactors and an ability to source locally. However, more research is necessary to investigate conflicting results. For example, Yamashita and Yamamoto-Ikemoto (2014) compared two different bioreactors, one packed with aspen (hardwood) and one packed with cedar (softwood), and found that the aspen bed had higher denitrification rates. However, it should be noted that these beds were a combination of wood and iron (as opposed to only wood in the Cameron and Schipper (2010) study and the studies looked at in the Addy et al. (2016) meta-analysis) in an attempt to remove phosphate as well as nitrate. This difference in media composition could play a role in the varying denitrification rates.

Design Factors - Bioreactor Parameters

Adequate parameters of the bioreactor (adjusted for flow rate and influent volume) are important to ensure proper saturation within the bed, which will increase the lifespan of the media. Moorman et al. (2010) found that wood chips in the upper layers of a bioreactor degraded more quickly than the ones below. At 90-100 cm depth, an average of 50% wood loss was observed, as opposed to a 13% wood loss at 155-170 cm. This was attributed to the fact that wood chips in the upper layers were subject to aerobic conditions more often as the water table dropped to the level of the drainage pipe, accelerating wood decay.

Ideally, the total volume of wood chips will equal the saturated volume of the bioreactor so that all wood chip investment is being used for treatment. In practice,
though, a top layer of unsaturated wood chips is often installed to provide supplementary carbon as the initial chips are degraded (Christianson and Schipper, 2016).

*Environmental Factors - Temperature*

Christianson et al. (2012a) and Addy et al. (2016), found that temperature and influent nitrate concentration were highly important factors for N removal rate and percent N load reduction. Both temperature and influent concentration correlate positively with N removal, with factors varying from site to site with respect to climate and silage runoff characteristics. Biological activity is well known to have a direct relationship with temperature, and the meta-analysis done by Addy et al. (2016) agreed with this trend. Cameron and Schipper (2010), found that regardless of media type or grain size, bioreactors at higher temperatures had correspondingly higher removal rates.

*Environmental Factors - Influent N Concentration*

Addy et al. (2016) found that beds with a high influent N concentrations had greater removal rates than beds with intermediate or low concentrations. Specifically, this review comparing 26 studies found average rates of 9.3, 4.9 and 2.4 g N/m³·d for beds with high (>30 mg/L), intermediate (10-30 mg/L) and low (<10 mg/L) influent N concentrations, respectively. All of these rates were found to be significantly different from each other.

1.3.4 Hydraulics

Hydraulic retention time (interchangeable with residence time) or HRT has a direct impact on the nitrate removal rates of a bioreactor. HRT is a function of bioreactor size, media porosity and flow rate.
Christianson et al. (2012b) states that low retention times in bioreactors are not always sufficient to reduce dissolved oxygen in the influent to levels that allow denitrification to occur, and that higher retention times correlate with higher NO$_3^-$ removal. Addy et al. (2016), Chun et al. (2009) and Greenan et al. (2009), confirmed these statements. The meta-analysis from Addy et al. (2016) found average N removals of 6.7, 4.4 and 0.7 g N/m$^3$ for HRTs of >20 hours, 6-20 hours, and <6 hours, respectively. Chun et al. (2009) reported nitrate concentration reductions of 10-40% at retention times less than ~5 hours, and 100% removal with retention times of 15.6 and 19.2 hours. On a longer timescale, Greenan et al. (2009) reported removal efficiencies of 30% for a 2.1-day retention time and 100% for a 9.8-day retention time.

While a low retention time will not allow full denitrification to occur, an extended retention time is also not ideal because there is a greater risk for generating high levels of DOC and harmful byproducts such as hydrogen sulfide (Schipper et al., 2010b, Christianson et al., 2012b). As carbon structures break down, it is possible for saturated hydraulic conductivity to decrease. Decreases in hydraulic conductivity can affect hydraulic performance by causing clogging and buildup at the entrance of a bioreactor or overall treatment system. As hydraulic conductivity decreases, retention time will increase.

### 1.3.5 Performance

*Removal Rates*

A range of nitrate removal rates from wood chip bioreactors have been reported in the literature. Table 2 lists findings from a variety of different studies.
Table 2: Reported nitrate removal rates and efficiencies from various studies. HRT = hydraulic retention time, HLR = hydraulic loading rate.

<table>
<thead>
<tr>
<th>References</th>
<th>Wastewater Source</th>
<th>Experimental Variables</th>
<th>Mean Removal (g/m³d) (%)</th>
<th>Range (g/m³d) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pluer et al. (2016)</td>
<td>Vegetable farm tile drainage</td>
<td>Laboratory study</td>
<td>6.6</td>
<td>3.23 up to 20</td>
</tr>
<tr>
<td>Bell et al. (2015)</td>
<td>N-spiked pond water</td>
<td>8-hr HRT 2-hr HRT Average of all runs with HRTS of 2, 4, 6 and 8 hrs</td>
<td>5 98</td>
<td>30 20</td>
</tr>
<tr>
<td>Christianson et al. (2012a)</td>
<td>Corn and soybean tile drainage</td>
<td>Results averaged from 4 bioreactors of varying location, size, and media</td>
<td>45</td>
<td>0.38-7.76 12-76</td>
</tr>
<tr>
<td>Cameron and Schipper (2010)</td>
<td>Nitrate-dosed water</td>
<td>Operational period: 1-10 months, 14°C 10-23 months, 14°C 1-10 months, 23.5°C 10-23 months, 23.5°C</td>
<td>2.3-9.7</td>
<td>2-4.6</td>
</tr>
<tr>
<td>Schipper et al. (2010a)</td>
<td>Dairy farm runoff Greenhouse effluent Domestic runoff</td>
<td></td>
<td>1.4</td>
<td>0-4</td>
</tr>
<tr>
<td>Greenan et al. (2009)</td>
<td>Nitrate-dosed water</td>
<td>Influent flow rate: 2.9 cm/d 6.6 cm/d 8.7 cm/d 13.6 cm/d</td>
<td>2.94 100</td>
<td>4.15 64.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4.15 51.</td>
<td>4.51 30.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4.01 30.</td>
<td>1.3 11.</td>
</tr>
<tr>
<td>Healy et al. (2015)</td>
<td>Nitrate solution</td>
<td>HLR 10 cm/d HLR 5 cm/d HLR 3 cm/d</td>
<td>57.7-77.2</td>
<td>82.6-99.4 99.6-99.7</td>
</tr>
<tr>
<td>Pfannerstill et al. (2016)</td>
<td>Flood plain drainage ditch with farm drainage tile</td>
<td>Year round</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>David et al. (2016)</td>
<td>Corn and soybean tile drainage</td>
<td>Year 1 Years 2 and 3</td>
<td>23-44</td>
<td>1.2-11</td>
</tr>
<tr>
<td>Woli et al. (2010)</td>
<td>Corn and soybean tile drainage</td>
<td></td>
<td>6.4 33</td>
<td>12-99.5</td>
</tr>
<tr>
<td>Van Driel et al. (2006)</td>
<td>Corn and soybean field drainage</td>
<td>Upflow reactors in: Riparian zone groundwater spring Perennial stream</td>
<td>0.7</td>
<td>2.5 1.5-3.5</td>
</tr>
</tbody>
</table>
Christianson et al. (2014), the study that reported the largest range in percentage removal in the table above, found that temperature, influent nitrate concentration, and retention time were the most important factors affecting percent bioreactor nitrate load reduction and overall nitrate removal rate.

**Lifespan**

Since bioreactors are a fairly new technology, a conclusive answer does not yet exist about their overall lifespan. Robertson and Cherry (1995) indicated the potential for consistent nitrate removal for decades from wood-based reactors with minimal maintenance. However, this figure is based on many assumptions about media quality and reactions in the bioreactor, such as sulfate and dissolved oxygen reduction not depleting the media, and the carbon being sufficiently labile to contribute to continuous denitrification. The meta-analysis by Addy et al. (2016) included data from 27 different bed units, but only two of the beds were more than 36 months old. Robertson et al. (2008) appears to have studied the longest running bioreactor, which is still performing well at 15 years of age. Schipper et al. (2010b) concluded that it is unclear how long these types of systems will be effective because no studied wood chip bioreactors have been observed to fail.

**Maintenance and Management Considerations**

Wood chip bioreactors are relatively low maintenance treatment systems. Farmers and system managers will predominantly need to ensure that clogging does not occur at the inflow, and may occasionally be required to trim encroaching vegetation. Addy et al. (2016) claims that bioreactors are commonly viewed as needing decadal management.
This reinforces their low maintenance reputation, but also recognizes the need to monitor aging bioreactors to determine how operation changes over extended time periods.

Timely wood chip replenishment is one maintenance action that should be considered. Moorman et al. (2010) estimated that wood chips situated below the water table in a bioreactor have an approximate half-life of 36.6 years. However, the same study also estimated that wood chips located at the saturated/unsaturated interface of a bioreactor have an approximate half-life of 4.6 years. These estimations were based on the percentage of C content that remained in the wood chips after being in operation for multiple years. Wood chip replenishment will ameliorate drops in nitrate removal rates and efficiencies that occur as a result of wood chip degradation.

Overall, previous literature suggests that once a bioreactor is installed, it will be able to perform effectively with minimal assistance (Schipper et al., 2010b).

1.3.6 Bioreactor Concerns and Considerations

Denitrification byproducts

One of the main concerns associated with wood chip bioreactors is that if denitrification is not carried out to completion, producing inert N₂ gas, harmful nitrogenous byproducts will be released. These byproducts, such as nitrous oxide (N₂O) and nitric oxide (NO), have the potential for environmental harm through ozone depletion, greenhouse effects, and nitrite poisoning (Seitzinger et al., 2006). However, a number of laboratory and field studies of wood chip bioreactors indicate that N₂ production is occurring as intended, and that nitrogen oxide production is minimal. In a laboratory column study, Greenan et al. (2009) found that N₂O accounted for a very small
fraction of the denitrified nitrate (ranging from 0.003-0.033%), indicating that N₂ was the primary end product of denitrification. Moorman et al. (2010), during a 9-year study of a bioreactor in Iowa, found that wood chips did not significantly increase overall indirect N₂O emissions when compared to an untreated control plot. Elgood et al. (2010), in a study of a wood chip bioreactor in Ontario, found that N₂O production occurred primarily in the winter and spring months when NO₃⁻ was not fully removed. However, the amount of N₂O produced was only 0.6% of the consumed nitrate amount. During the summer, when NO₃⁻ removal was complete, the reactor acted as a sink for N₂O, confirming the importance of complete denitrification. (Elgood et al., 2010).

Carbon Dioxide and Methane

Additional greenhouse gases, such as carbon dioxide and methane, can also be released from denitrification beds as a result of decaying organic matter. In the case of carbon dioxide, it is important to note that any carbon dioxide released does not result in a net increase in emissions because the carbon media in the bioreactor would have degraded anyway, regardless of its use (Schipper et al., 2010b). Regarding methane, Elgood et al., (2010) monitored methane production in a wood chip bioreactor and found seasonally opposite trends to N₂O production: methane production was stimulated in the summer by the complete removal of NO₃⁻. This concept is known as pollution swapping, which occurs when the capture of one pollutant results in the release of another. Pollution swapping has been studied in the context of denitrifying bioreactors, where the removal of nitrates from wastewater potentially contributes to environmental degradation via a gaseous release of carbon (Fenton et al., 2014). Preferred ecological engineering designs should seek to avoid or minimize pollution swapping.
1.4 Additional Research Needs

Silage leachate is a serious and problematic pollutant that is harming aquatic ecosystems (Woolford, 1978; Deans and Svoboda, 1992). With limited treatment or disposal options that are economical and environmentally safe, newer technologies are being explored as potential treatment options. Current literature indicates that the emerging technology of wood chip bioreactors has proven to be effective in removing nitrates from various kinds of agricultural, domestic and industrial runoff, making it a promising option for addressing silage leachate and runoff (Addy et al., 2016). Consisting of cheap, readily available materials, wood chip bioreactors are generally economically feasible (Blowes et al., 1994; Cameron and Schipper, 2010).

Wood chip bioreactors have been installed in the field in a select few locations. The predominant areas are the Midwest (Christianson et al., 2012; Moorman et al., 2010), as well as parts of Canada (Robertson et al., 2000; van Driel et al., 2006) and New Zealand (Schipper et al., 2010a). In order to assess the ability of wood chip bioreactors to perform in a variety of climates, additional field installations in contrasting climates are warranted.

Wood chip bioreactors have been used to treat many different types of runoff, but the agricultural application has been limited to tile drainage and other nonpoint source runoff. Based on the current research, extending this treatment to silage leachate has a high potential for success and should be studied. However, silage leachate contains nitrogen in multiple forms, and denitrification is only an applicable treatment for the nitrate compound. In the majority of denitrifying bioreactor treatment designs, the runoff
water is fed directly into the bed, without pretreatment. Research is therefore needed on pretreatment options, such as the gravel and settling tanks used by Holly and Larson (2016) that can convert other forms of available nitrogen into nitrate through aerobic nitrification.


CHAPTER 2: NUTRIENT REMOVAL PERFORMANCE OF A WOOD CHIP BIOREACTOR TREATMENT SYSTEM RECEIVING SILAGE BUNKER RUNOFF

2.1 Introduction

Globally, the overabundance of nitrogen and phosphorus accumulating in surface waters contributes to eutrophication, disrupts nutrient cycling in aquatic systems, and leads to habitat degradation and loss of biodiversity (Howarth et al., 2011, EPA ROE, 2008). These changes are detrimental to natural ecosystems and negatively impact the tourism and recreation industries (Osiadacz et al., 2010). Industrial, agricultural and urban land all yield polluted runoff that moves nutrients to surface water. In 2018, agriculture accounts for 18% of the land cover in the Lake Champlain basin, but it contributes 38% of annual phosphorus loading (Lake Champlain Basin Program, 2018). Farming operations are known to contribute high amounts of nutrients into surface water, primarily through fertilizer use and liquid animal waste (EPA ROE, 2008), but the less-studied runoff from silage storage areas also degrade water quality. Silage leachate is a natural byproduct of silage fermentation, which is a process used to preserve feed for cattle over the winter months when fresh fodder is not available (Gebrehanna et al., 2014, Arnold et al., 2000, Woolford, 1978). Silage leachate is an extremely polluting liquid (Arnold et al., 2000) with known characteristics of high biological oxygen demand (BOD), low pH, and high concentrations of nitrogen and phosphorus (Deans and Svoboda, 1992; Galanos et al., 1995). Silage runoff is created when stormwater mixes with silage leachate. While more dilute than the leachate, this runoff is still considered an agricultural wastewater that must be captured or treated.
Varying disposal methods have been used in the past to manage silage runoff, such as land application and feeding to animals, but there are drawbacks associated with these techniques due to the nature of the runoff and the timing at which it is produced (Woolford, 1984). Although numerous technologies for removing nitrogen from wastewaters exist (e.g. batch sequencing (Hajsardar et al., 2016), ammonia volatilization (Cameron and Schipper, 2010), ion exchange (Kapoor and Viraraghavan, 1997), methanol dosing (Koch and Seigrist, 1997) and reverse osmosis (Kapoor and Viraraghavan, 1997)), these methods are often cost prohibitive, require maintenance, and are difficult to implement for treatment of small volume point source discharges (Cameron and Schipper, 2010). Furthermore, even when these treatments are applied, some excess nitrogen often remains in the final effluent (Cameron and Schipper, 2010).

Some affordable, passive technologies that have been used to treat agricultural wastewaters include vegetative treatment areas (VTAs) (Larson and Safferman, 2012; Koelsch et al., 2006) and constructed wetlands (Smith et al., 2006). While these methods may be less intrusive and more appealing to farmers than traditional treatments, limitations still exist for their use with silage runoff. Studies done by Larson and Safferman (2012) and Faulkner et al. (2011) both concluded that studied VTAs had poor treatment performance when receiving silage runoff. Inadequate nutrient removal was observed, partially attributable to insufficient maintenance, and some VTAs were damaged by the acidity of the runoff. Even so, VTAs are approved in some jurisdictions for treating dilute silage runoff, but Wright and Vanderstappen (1994) and Wright et al. (2004) concluded that more concentrated runoff should be diverted to a storage tank. Tattrie (2006) studied a constructed wetland receiving a combination of wastewaters and
found that the low pH and high nutrient and BOD levels of silage runoff inhibited the performance of the system, concluding that silage runoff could not be treated in this way.

Denitrifying bioreactors are an emerging technology being used to treat many forms of agricultural and domestic runoff. Denitrifying bioreactors are structures filled with carbon-rich reactive media, designed to enhance the natural process of denitrification by providing an ideal environment for heterotrophic microbial activity (Christianson and Schipper, 2016). Denitrifying bioreactors are becoming favored as a treatment option due to their practicality, which is demonstrated by the relatively low installation cost, small footprint, and minimal required maintenance (Blowes et al., 1994; Cameron and Schipper, 2010). Wood based media is often chosen because of its affordability, abundance, high permeability and durability (Robertson et al., 2009; Robertson, 2010). Wood chip bioreactors have been proven to be effective in removing nitrates from various kinds of agricultural, domestic and industrial runoff in Ontario (Robertson and Merkeley, 2009; Driel et al., 2006, Robertson et al., 2000), Iowa (Greenan et al., 2009), and northern New Zealand (Schipper and Vojvodic-Vukovic, 2001). In Ontario, van Driel et al. (2006) directed drainage from a corn field and a golf course into two small wood chip bioreactors. Over a four-year time period, nitrate was reduced in the bioreactors by 32% and 53%, respectively. A Schipper and Vojvodic-Vukovic (2001) study from New Zealand found that a denitrifying bioreactor continuously removed more than 95% of the incoming nitrate in groundwater (from a farm spray-irrigated with dairy factory effluent), and also concluded that the design could support nitrate removal via denitrification for at least five years. Robertson et al. (2008) re-examined a denitrifying bioreactor fifteen years after its initial construction to treat a
septic plume, and found the nitrate removal rate to be about 4 g N m$^{-3}$ day$^{-1}$, only 50% lower than the rate measured in the first year of operation. Schipper et al. (2010) noted that there were currently no examples of denitrifying bioreactors that have failed due to depletion of carbon.

Denitrifying bioreactors have not previously been evaluated for silage runoff treatment in the Northeastern US, but their past performance makes them a promising treatment option. This study will assess the nutrient removal performance of a wood chip bioreactor treatment system receiving silage runoff from a horizontal, uncovered silage bunker. This treatment system is unique due to the presence of supplementary components designed to facilitate additional nitrogen transformations, which have the objective of maximizing subsequent denitrification in the bioreactor.

### 2.2 Objectives

The objectives of this study were to:

1) Collect additional data on silage bunker runoff characteristics.

2) Evaluate the performance of the aforementioned system by observing changes in nitrogen and phosphorus concentrations and loads, as well as changes in acidity

3) Provide recommendations for future designs based on the previous evaluation.
2.3 Methods:

2.3.1 Research Site

The wood chip denitrifying bioreactor treatment system, which began operation in May of 2017, is located at the University of Vermont Paul R. Miller Research Complex (UVM MRC), a teaching and research farm located in South Burlington, Vermont, that contains dairy and equestrian facilities. South Burlington (44° 27' 33.411" N, 73° 11' 21.9696") has an average annual rainfall of 93.4 cm and receives precipitation 151 days out of the year. The area’s average annual temperatures are 25.93 °C in the summer and -0.93 °C in the winter (U.S. Climate Data 1981-2010). The treatment system is located adjacent to the farm’s silage bunkers (Figure 2A) and drains a watershed of 2,767 m².
The surface of this drainage area is predominantly paved with asphalt, with seasonally varying amounts of plastic-covered silage filling the remaining area. The treatment system (Figure 2B) consists of a solids-screening forebay, three pre-treatment tanks in series, two wood chip bioreactors in parallel, and an infiltration basin. A process flow diagram of the system can be seen in Figure 2.
Auto samplers were placed at the locations A, B, C and D.

Runoff flows directly into the pre-processing unit and runoff following the remaining runoff path flows directly into the infiltration basin (one per bioreactor) which drain into the infiltration basin (8). Runoff following the high-flow path bypasses the pre-treatment (6) and enters into the bioreactors (7). The runoff is then split and directed consis of a settling unit (3) in an aeration unit with a blower (4), and another settling unit (5). The outlet is taken through either a low-flow path or extreme event path. Runoff following the low-flow path enters the excess of pre-treatment tanks which in turn flows through the seepage trough (2) through the infiltration basin (8). Figure 3: Cross-sectional process flow diagram of the treatment system showing the movement of runoff through these potential pathways.
During a storm event or a release of silage leachate, runoff flows from the silage bunkers and over an asphalt lot that is sloped towards the treatment area. A berm in the lot prevents additional farm runoff from outside of the bunker drainage area from entering the treatment system. The silage runoff is first directed through the stainless-steel screen assembly, composed of three screens in series that have decreasing mesh size openings (#1, Figure 3). This assembly serves to filter out silage particles that are transported with the runoff, as well as other debris that may be washed off of the bunker area.

Runoff then enters the flow diversion structure (#2, Figure 3). The flow diversion structure is equipped with three different outlets designed to accommodate flows of various intensities (Figure 3). The low-flow outlet, which has a diameter of 10.16 cm, is designed to handle non-storm-induced leachate and storm events up to an intensity of 45.72 mm/hr. This outlet directs runoff through the entirety of the treatment system (tanks, wood chip bioreactors, and infiltration basin). During larger storms, excess runoff is directed through the high-flow outlet. The high-flow outlet is designed to accommodate storms up to an intensity of 71.12 mm/hr. This outlet splits the runoff between two pipes, each 20.32 cm in diameter, which send the flow directly to the wood chip bioreactors, bypassing the pre-treatment tanks. This outlet is placed at a higher elevation than the outlet for the low flow in the flow diversion structure, to ensure that runoff will only bypass the pre-treatment tanks during high flow. During extreme storm events (i.e., greater than 71.12 mm/hr) that exceed the capacity of both the low- and high-flow outlets, there is an emergency spillway in the flow diversion structure that will allow runoff to flow directly into the infiltration basin, bypassing the tanks and bioreactors.
Runoff entering the low-flow outlet, which is the intended primary treatment path, will flow through a sequence of three pre-treatment tanks. These tanks are designed to remove any additional solids, lower BOD, and alter the nutrient composition of the runoff before it is directed into the wood chip bioreactors. The first of these tanks is a 7.57 m³ settling tank, which serves to dilute the low flow leachate and allow sedimentation to occur. This sedimentation assists in lowering BOD by removing organic solids, and also further helps to prevent clogging in the system. The second tank is a 3.79 m² aeration tank, outfitted with a 1.49 kW regenerative blower that is set to operate for 12 consecutive hours each day (approximately 1 am to 1 pm). The blower is intended to lower BOD in the runoff by increasing the amount of available oxygen. Additionally, the aeration tank is meant to promote mineralization of organic nitrogen and nitrification of ammonium by providing a highly aerobic environment. These processes allow more nitrogen removal to occur via denitrification in the wood chip bioreactors. The third tank is included to allow for sedimentation of any remaining suspended debris.

The bioreactors, referred to as west wood chip bioreactor (WWB) and east wood chip bioreactor (EWB), are filled with mixed hardwood bole chips (individual chips are approximately 5.08 cm x 5.08 cm x 0.64 cm), with a species composition of 60% Ash (Fraxinus), 20% Yellow Birch (Betula alleghaniensis), and 20% Silver Maple (Acer saccharinum). The dimensions of each bioreactor are 18.29 m by 12.19 m with a depth of 1.37 m. The bioreactors are lined with 45-mil ethylene propylene diene monomer (EPDM). Runoff following the low-flow path (middle arrow in Figure 3, second in legend) enters the bioreactors through perforated pipes placed along the upper perimeter under the wood chip surface. Runoff following the high-flow path (top arrow in Figure 3,
third in legend) enters the bioreactors from discrete pipe outlets that discharge onto the surface. After percolating down through the wood chips, runoff is collected by a 10.16-cm diameter underdrain pipe and directed into a 76-cm diameter outflow water level control structure (one per bioreactor; #7 in Figure 3). When the outflow water level control structure fills, runoff will overflow into a sump and then a 20.32 cm diameter upturned elbow pipe (#7a, Figure 3) that is outfitted with a 15.88-cm diameter sharp-crested weir. This pipe discharges into an earthen infiltration basin.

### 2.3.2 Sampling

*Autosampling and flow measurements*

Four autosamplers (Teledyne ISCO 6712, Lincoln, NE) were placed at different locations throughout the treatment system to collect runoff samples for water quality analysis. The first sampler collected untreated runoff as it entered the first pre-treatment tank, the second collected runoff in between the pre-treatment tank and the wood chip bioreactors, and the third and fourth collected runoff from the outflow of the WWB and the EWB, respectively.

The samplers were activated using a flow-based sampling protocol. Flow into the treatment system was calculated using a combination of a compound weir (Thel-mar, Brevard, NC) and a water level sensor that was linked to the first sampler (Teledyne ISCO 730 Bubbler Flow Module, Lincoln, NE). During storm events, the water level above the weir was measured and recorded every two minutes. A table of values provided by the weir manufacturer was used to convert the water level data recorded by the flow module into flow rates. Flow into tank one was assumed to be the same as flow out of
tank three. The first and second autosamplers were connected by a communications cable that synchronized sampling times. The autosamplers sampling the wood chip bioreactor outflows were also outfitted with water level sensors. Runoff exiting the wood chip bioreactors flowed through the 15.88-cm diameter openings of the upturned elbow pipes in the water level control structures (#7a in Figure 3). These pipe openings were treated as sharp crested rectangular weirs without end contractions. To calculate the flow rate exiting the bioreactors, the following equation was used:

\[ Q = 3.33LH^{3/2} \]

Where \( Q \) is flow (m\(^3\)/s), \( L \) is the weir length or pipe circumference (m), and \( H \) is the water level above the weir (m). The retention time in the bioreactors was calculated by dividing the pore volume of the bioreactors by the flow rate of the exiting runoff.

To calculate volume from flow, the following equation was used:

\[ V = \int Q(t) \, dt \]

Where \( V \) is volume (m\(^3\)), \( Q \) is flow (m\(^3\)/s), and \( t \) is time (s). In the event that the high-flow path was used during a storm, the volume of the high-flow was calculated by subtracting the tank runoff volume and the bioreactor direct precipitation volume from the volume of runoff exiting the bioreactors.

Autosamplers were set to take and store a 500-mL sample of runoff after a set amount of volume had been measured. This unique volume varied by storm event and
was determined by using the forecasted rainfall depth in the Curve Number equation (Tollner, 2002) for the bunker drainage area, and dividing by 24 (the maximum number of bottles that could be filled by the autosampler). This volume was programmed into the sampler in advance and allowed for samples to be taken throughout the entire duration of a storm event. Eighteen storm events were sampled during the summer and fall of 2017, between the months of June and November. A rain gauge (Onset RG3 Hobo Rain Gauge Data Logger, Bourne, MA) installed by the treatment site collected data during the entirety of the sample season.

2.3.3 Water Quality Analysis

Nutrient analysis

The bottles stored in each autosampler were collected within 24 hours of the end of a storm event. Samples were analyzed for concentration of nitrate/nitrite (NO$_3$-N), ammonium (NH$_4^+$-N), total nitrogen (TN), soluble reactive phosphorus (SRP), and total phosphorus (TP). Analysis was completed with flow injection analysis instruments (Lachat QuickChem8000 AE, Hach Inc., Loveland, CO) in the University of Vermont Agricultural and Environmental Testing Laboratory and the Vermont Agriculture and Environmental Laboratory (both located on the University of Vermont campus) using identical methods. Samples designated for total nutrient analysis were prepared by persulfate digestion, while soluble nutrient samples were processed through a 0.45-um pore nylon mesh filter. Due to instrument error, several storms were not analyzed for all five analytes. Out of the nineteen sampled storms, thirteen were fully analyzed and six were missing data for one or more target analytes.
Samples from thirteen out of nineteen storm events were composited before nutrient analysis. Samples were partially composited within the autosampler (the autosampler was programmed to store multiple samples in one bottle), and composited further in the lab after collection if needed (depending on how many bottles were filled). Compositing in the lab consisted of taking an equal volume from each filled sampled bottle and combining into one sample (this volume varied by storm and depended on how many bottles had been filled). Composite sampling was deemed to be an acceptable method because the autosamplers were programmed to follow a flow-based sampling protocol, which produces more useful information for mass load calculations than time-based sampling (Harmel et al., 2003). King and Harmel (2003) also concluded that composite sampling using a flow-based protocol provided no statistical advantage or disadvantage over flow-based discrete sampling, and that fewer samples could be analyzed while maintaining the same absolute error.

**Nutrient mass removal**

Nutrient mass loads for all sampled storms were calculated using runoff volume and nutrient concentrations from the following equation:

\[ M = \Sigma (VC) \]

Where \( M \) is mass load (g), \( V \) is volume (L) of runoff passing by a sampling point during a defined interval, and \( C \) is nutrient concentration (g/L) of the runoff during the same interval. The entire storm volume recorded by an autosampler was divided by the number
of samples taken to determine the final interval volume used in the equation. This final volume was multiplied by the analyte concentration of each sample to determine loads.

During low-flow storm events, the influent mass load to the pre-treatment tanks was identical to the whole system’s influent mass load because no runoff bypassed the tanks. During storms that experienced high-flow, the whole system’s influent mass load was a combination of 1) runoff that entered the pre-treatment tanks and 2) runoff that bypassed the tanks and directly entered the wood chip bioreactors. For every storm, the mass load entering the tanks was calculated based on the volume and concentrations measured by the first autosampler. For storms that experienced high-flow, the mass load influent of the whole system was calculated by adding the influent mass load of the tanks to the mass load of the bypass flow. The concentration of the bypass flow was the same as the tank influent, and the volume of the bypass flow was calculated by subtracting the volume received by the tanks from the volume exiting the bioreactors (recorded by autosamplers 3 and 4), with a small adjustment for direct precipitation on to the bioreactors.

From mass load values, the percentage mass load reduction \( R^\text{n} \) was calculated for the entire system and for individual treatment steps using the following equation:

\[
R^n = \frac{(M_i-M_o)}{M_i} * 100
\]

Where \( M_i \) is the nutrient mass load of the inflow (g) and \( M_o \) is the nutrient mass load of the outflow (g).
2.3.4 pH and Dissolved Oxygen Analysis

A portion of samples collected from the treatment area were also analyzed for acidity. A bench meter (Mettler Toledo Five Easy, Columbus, OH) was used to measure pH of individual samples after collection and before compositing. Samples collected from the beginning, middle, and end of a storm event were measured, and the results were averaged to one value per autosampler per storm.

On June 20\textsuperscript{th} -21\textsuperscript{st}, 2018, the runoff water in tank two was monitored directly at the site for 21 hours using a probe (YSI ProDSS, Yellow Springs, OH). From 5:26 pm to 2:26 pm, measurements of DO, temperature, and pH were taken every ten minutes.

2.3.5 Wood Chip Total Phosphorus Analysis

Three samples of wood chips were analyzed for total phosphorus content. Samples were ground, ashed, and digested with nitric acid before being analyzed with an inductively coupled plasma optical emission spectroscopy instrument (ICP-OES) (Optima 3000DV, Perkin Elmer Corp, Norwalk, CT). The three samples consisted of a control (wood chips collected at the time of bioreactor construction and stored in an air-tight container), a composite sample with wood chips from ~15 cm under the surface of each bioreactor (damp but not saturated), and a composite sample with wood chips from below the water level of each bioreactor at ~30 cm (saturated).

2.3.6 Hydraulic Retention Time

The hydraulic retention time (HRT) of the runoff in the bioreactors was calculated for each sampled storm. HRT was calculated by dividing the volume of runoff that can be
held in the bioreactors by the average flow rate of the exiting runoff. The volume of runoff in the bioreactors was calculated by multiplying the bioreactor dimensions by the porosity of the wood chips. The porosity of the wood chips was determined in the lab by filling a known-volume container with wood chips and measuring how much water the container was then able to hold.

2.3.7 Statistical Analysis

Changes in storm event nutrient concentrations were compared between the influent and effluent of the pre-treatment tanks, the influent and the WWB effluent, and the influent and the EWB effluent using a Wilcoxon Signed-Rank (WSR) nonparametric paired test. Changes in nutrient mass loads were compared between total influent (flow into the pre-treatment tanks and bypass flow) and the combined bioreactor effluent, as well as between the influent and effluent of the pre-treatment tanks also using a WSR nonparametric test. Tests were considered significant if \( p \) was less than 0.05, and marginally significant if \( p \) was between 0.05 and 0.1. Statistical models were run using Rstudio, Version 1.1.453.

2.4 Results

The treatment system area received 46.99 cm of precipitation during the sampling period, as measured by the rain gauge. According to the Curve Number equation, this precipitation led to approximately 1,283,000 liters of runoff entering the treatment system. The mean depth of the sampled storm events was 1.37 cm, with a minimum of 0.10 cm and a maximum of 4.04 cm. Figure 4 shows the distribution of depths of
sampled storm events. While the month of June received significantly more precipitation than the historical normal (>5.88 in vs. 3.7 in, respectively), every other month during the sample season received slightly less precipitation than the historical normal (U.S. Climate Data 1981 - 2010).

![Figure 4: Histogram showing the distribution of sampled storm event depths. The storms were split into five categories: 0-1 cm, 1-2 cm, 2-3 cm, 3-4 cm, and 4-5 cm.]

### 2.4.1 Nutrient Removal Performance

*Nitrogen and Phosphorus Concentration*

The mean concentration of all measured nitrogen and phosphorus species at each sample location is displayed in Table 3.
Table 3: Mean concentrations of nitrogen and phosphorus species at the sample locations in the treatment system, followed by standard deviation in parentheses. Sample locations correspond with Figure 3 locations A-D. WWB = west wood chip bioreactor. EWB = east wood chip bioreactor. * indicates that concentrations were significantly different from the influent concentration.

<table>
<thead>
<tr>
<th></th>
<th>(A) Influent</th>
<th>(B) Tank 3 effluent</th>
<th>(C) WWB effluent</th>
<th>(D) EWB effluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO\textsubscript{x}-N (mg/L)</td>
<td>0.15 (± 0.23; n=16)</td>
<td>0.05* (± 0.01, n=13)</td>
<td>0.07* (± 0.06, n=16)</td>
<td>0.08 (± 0.09, n=12)</td>
</tr>
<tr>
<td>NH\textsubscript{4}-N (mg/L)</td>
<td>28.38 (± 14.26, n=15)</td>
<td>96.42* (± 85.33, n=12)</td>
<td>54.26* (± 17.51, n=15)</td>
<td>80.3* (± 39.09, n=12)</td>
</tr>
<tr>
<td>TN (mg/L)</td>
<td>131.1 (± 90.89, n=14)</td>
<td>131.16 (± 78.13, n=12)</td>
<td>72.76* (± 23.13, n=15)</td>
<td>101.44 (± 45.06, n=11)</td>
</tr>
<tr>
<td>SRP (mg/L)</td>
<td>50.44 (± 36.32, n=17)</td>
<td>37.09 (± 27.15, n=13)</td>
<td>25.71* (± 8.36, n=17)</td>
<td>30.43* (± 12.64, n=13)</td>
</tr>
<tr>
<td>TP (mg/L)</td>
<td>62.02 (± 44.86, n=16)</td>
<td>52.86 (± 32.24, n=13)</td>
<td>30.23* (± 6.28, n=16)</td>
<td>36.53* (± 11.92, n=12)</td>
</tr>
</tbody>
</table>

When comparing changes in nutrient concentrations, all effluent samples were compared to the influent (as opposed to comparing the bioreactor effluent to the tank effluent) because a large portion of runoff followed the high flow path and entered the bioreactors directly, bypassing the tanks.

The pre-treatment tanks were found to have a marginally significant effect on reduction of influent NO\textsubscript{x}-N concentration \((p=0.096)\), the WWB was found to significantly reduce influent NO\textsubscript{x}-N concentration \((p=0.022)\), and the EWB was found to have no significant impact on influent NO\textsubscript{x}-N concentration \((p=0.272)\), all via a Wilcoxon Signed-Rank test.

The pre-treatment tanks, the WWB, and the EWB were all found to have significantly higher concentrations of NH\textsubscript{4}-N than the influent via a Wilcoxon Signed-Rank test \((p=0.004, p<0.001, \text{ and } p=0.008, \text{ respectively})\).
The pre-treatment tanks and the EWB were found to have no significant effect on the influent concentration of TN via a Wilcoxon Signed-Rank test ($p=0.765$ and $p=0.375$, respectively), while the WWB significantly reduced influent TN concentration ($p=0.009$).

For the mean inflow and outflow concentrations of individual storm events, five out of eleven storms (45%) showed a reduction in TN between influent and pre-treatment tank effluent. Twelve out of fourteen (85.7%) showed a reduction in TN between influent and WWB effluent, and seven out of ten (70%) showed a reduction in TN between influent and EWB effluent.

The pre-treatment tanks did not significantly affect the influent SRP or TP concentration ($p=0.191$ and $p=0.455$, respectively), but both the WWB and EWB were found to significantly decrease influent SRP and TP concentration via a Wilcoxon Signed-Rank test ($p<0.001$ and $p=0.003$, respectively for SRP, and $p=0.001$ and 0.007, respectively for TP).

Comparing mean inflow and outflow concentrations of individual storm events, six out of twelve storms (50%) showed a reduction in TP between influent and pre-treatment tank effluent. Fourteen out of sixteen (87.5%) showed a reduction in TP between influent and WWB effluent, and nine out of twelve (75%) showed a reduction in TP between influent and EWB effluent.

**Nitrogen and Phosphorus Mass Removal**

Figures 5 and 6 show the total mass load per storm of NO$_x$-N, NH$_4$-N, TN, SRP, and TP in the influent and effluent runoff of the entire treatment system. Tables 6 and 7 in the supplementary materials section show the exact mass load values for each analyte and storm, as well as mass load values for runoff entering and exiting only the pre-
treatment tanks. Due to runoff taking the high-flow path and adding mass load to the bioreactors and not the tanks, data are presented in these two separate tables so as to compare total system removal to the removal by the pre-treatment tanks alone. Figures 5 and 6 both show $p$-values from Wilcoxon Signed-Rank tests for significant differences between the influent and effluent mass load of each analyte. The treatment system as a whole significantly decreased the mass load of $\text{NO}_x$-N, TN, SRP and TP, while significantly increasing the mass load of $\text{NH}_4$-N. The series of pre-treatment tanks also significantly increased the mass load of $\text{NH}_4$-N, but had no significant impact on the mass load of any other species.
Figure 5: Total mass load in the influent and effluent of the entire system for all nitrogen species. p-values are from WSR tests comparing influent and effluent values for each species.
Figure 6: Total mass load in the influent and effluent of the entire system for all phosphorus species. $p$-values are from WSR tests comparing influent and effluent values for each species.

Table 4 shows the overall mass load removal performance of the entire treatment system during the sample season, as well as the overall mass load removal performance of solely the pre-treatment tanks during the sample season. These mass load removal values are based on the measured influent and effluent load of sampled storms. Since not
every storm was captured by the samplers, the true mass load in to the system was most likely higher than the collected data indicated, meaning that the overall removal was likely higher as well. The results are reported separately again to compare performance of the entire system (tanks and bioreactors combined) vs. the performance of the tanks. Due to storms experiencing high-flow and inducing bypass flow around the tanks, a direct comparison between solely bioreactors and tanks was unattainable. However, because the majority of the runoff bypassed the tanks during most storm events, the total system results are representative of the bioreactor performances. The results indicate an overall higher treatment potential in the bioreactors than the tanks.

Table 4: Seasonal total mass load removal (by kg and %) of all measured nutrient species in the entire treatment system and in the tanks.

<table>
<thead>
<tr>
<th>Nutrient Species</th>
<th>Total System Mass Load Removal (kg)</th>
<th>Total System Mass load Removal (%)</th>
<th>Tank Mass Load Removal (kg)</th>
<th>Tank Mass load removal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO\textsubscript{3}N</td>
<td>1.65</td>
<td>88.25</td>
<td>0.004</td>
<td>17.73</td>
</tr>
<tr>
<td>NH\textsubscript{4}N</td>
<td>-114.62</td>
<td>-93.74</td>
<td>-39.49</td>
<td>-257.54</td>
</tr>
<tr>
<td>TN</td>
<td>228.38</td>
<td>44.08</td>
<td>-14.73</td>
<td>-0.23</td>
</tr>
<tr>
<td>SRP</td>
<td>60.572</td>
<td>35.17</td>
<td>-0.18</td>
<td>-0.79</td>
</tr>
<tr>
<td>TP</td>
<td>103.99</td>
<td>44.36</td>
<td>-1.14</td>
<td>-0.04</td>
</tr>
</tbody>
</table>

2.4.2 Influent TN Concentration and Reduction

An apparent correlation was observed between influent TN concentration of a storm and the corresponding % reduction in TN, as seen in Figure 7. A Spearman correlation test produced a \( p \)-value < 2.2x10\textsuperscript{-16}, confirming the observed correlation.
2.4.3 pH

Sample pH was recorded for thirteen individual storm events. As seen in Table 5, pH values were all between 5 and 8. An increase in pH between the inflow and outflow was observed in every storm. Three storms showed a slight decrease in pH between the tank effluent and the mean of the two bioreactors’ effluents.

Table 5: Mean pH of runoff samples followed by standard deviation in parentheses.

<table>
<thead>
<tr>
<th>Storm Date</th>
<th>Influent pH</th>
<th>Tank Effluent pH</th>
<th>Mean pH of WWB and EWB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jun 29</td>
<td>5.18 (± 0.22)</td>
<td>6.19 (± 0.07)</td>
<td>6.72 (± 0.52)</td>
</tr>
<tr>
<td>Jul 13</td>
<td>6.22 (± 0.97)</td>
<td>7.19 (± 0.06)</td>
<td>7.95 (± 0.16)</td>
</tr>
<tr>
<td>Aug 22</td>
<td>6.41 (± 0.46)</td>
<td>7.41 (± 0.14)</td>
<td>7.37 (± 0.25)</td>
</tr>
<tr>
<td>Sept 7</td>
<td>5.86</td>
<td></td>
<td>6.88</td>
</tr>
<tr>
<td>Oct 4</td>
<td>6.62</td>
<td>7.51</td>
<td></td>
</tr>
<tr>
<td>Oct 8</td>
<td>6.43 (± 0.40)</td>
<td></td>
<td>7.62 (± 0.31)</td>
</tr>
<tr>
<td>Oct 9</td>
<td>5.32</td>
<td>7.45</td>
<td>6.93 (± 0.21)</td>
</tr>
<tr>
<td>Oct 24</td>
<td>6.28</td>
<td>7.14</td>
<td>7.00 (± 0.10)</td>
</tr>
<tr>
<td>Oct 26</td>
<td>5.76 (± 0.84)</td>
<td>6.73 (± 0.91)</td>
<td>7.21 (± 0.23)</td>
</tr>
<tr>
<td>Oct 29</td>
<td>5.91</td>
<td>6.15</td>
<td>6.86 (± 0.03)</td>
</tr>
<tr>
<td>Nov 3</td>
<td>6.37 (± 0.04)</td>
<td>6.66 (± 0.02)</td>
<td>7.08 (± 0.00)</td>
</tr>
<tr>
<td>Nov 6</td>
<td>6.24</td>
<td>6.80 (± 0.08)</td>
<td>7.20 (± 0.06)</td>
</tr>
<tr>
<td>Nov 18</td>
<td>6.45 (± 0.01)</td>
<td>7.13 (± 0.10)</td>
<td>7.22 (± 0.02)</td>
</tr>
</tbody>
</table>
2.4.4 Dissolved Oxygen in Aerobic Tank

Over the 21-hour time frame where measurements of DO, pH, and temperature in the aeration tank were recorded every 10 minutes, noticeable drops in both DO and pH were observed when the blower cycled off (Figure 8). While the blower was on, the tank water was at near saturation DO levels with a mean of 97.41% or 8.74 mg/L. Less than two hours after the blower turned off, dissolved oxygen content in the tank had dropped to 0.00 mg/L. Four hours after the blower turned off, pH dropped to the lowest recorded value of 7.6 (down from a mean of 8.34). When the blower cycled back on, DO readings returned to near saturation within an hour and pH readings returned to the former mean within an hour and twenty minutes. During this 12-hour time frame, temperature in the tank varied by less than 0.5°C (20.67 – 20.22°C).

Figure 8: Dissolved Oxygen and pH Changes in the Aerobic Tank due to the Blower Cycle
2.4.5 Total Phosphorus Content of Wood Chips

The results of the nitric acid digest and ICP-OES analysis of the TP content of three wood chip samples are presented below in Figure 9. The sample from above the water level was 37.6% higher than the control, and the sample from below the water level was 24.7% higher than the control.

![Figure 9: Comparison of TP Concentration in Three Different Wood Chip Samples](image)

2.4.6 Hydraulic Retention Times

Hydraulic retention time (HRT) in the bioreactors varied based on storm duration and intensity. Retention time per storm of each bioreactor was compared to an estimate of TN mass load percent removal per storm in the corresponding bioreactor. No correlation between retention time and TN reduction was observed (p=0.501 for WWB and p=1 for EWB in a Spearman correlation test). Time since the last storm event was also compared to percent TN mass load reduction in the combined bioreactor effluent, and again no correlation was observed (p=0.66 in a Spearman correlation test). The estimated mean
HRT in the WWB during the sample season was 189 hours (7.88 days), and the estimated mean HRT in the EWB during the sample season was 3405 hours (141.88 days). However, this retention time is an estimate based on the fixed bioreactor volume and individual storm flow rate. Subsequent storms will affect the actual retention time by directing new runoff into the bioreactors that forces out previous runoff.

The flow data recorded by the autosamplers showed that there were unequal volumes of runoff entering and exiting the two bioreactors, indicating an uneven split to the bioreactors from the high-flow path and from the pre-treatment tanks. The unevenness of the split also varied from storm to storm. Comparing flow data from twenty individual storm events indicated that on average, only 35% of the runoff from a given storm event entered the EWB, while the rest entered the WWB. However, this percentage ranged from less than 1% to over 90% throughout the season, and 25% of storms saw the majority of runoff flowing into the EWB instead of the WWB.

2.5 Discussion

2.5.1 Nitrogen Transformations and Removal

The NH₄-N and NOₓ-N concentration and load data indicated that mineralization to ammonium occurred in the tanks, but did not indicate that the mineralization was followed by nitrification to nitrate (Table 4). However, the TN data suggest that denitrification occurred in the bioreactors due to the reduction in concentration and load (Figure 5 and Table 4). Since there was not a significant change in NOₓ-N concentration observed in the tanks, it can be speculated that coupled nitrification and denitrification was occurring in the wood chip bioreactors. This phenomenon of coupled nitrogen
transformation has been observed by many researchers to contribute to N loss in media that experiences oxic and anoxic conditions (Xia et al., 2017; Marchant et al., 2016; Penton et al., 2013), but most notably in an alternate wetting and drying (AWD) irrigation system by Verhoeven et al. (2018). This AWD irrigation in an Italian rice paddy allowed an initially flooded paddy to exist in a cycle of draining and re-flooding, similar to the conditions experienced by the upper layers of the wood chip bioreactors during and after storm events. That study found that the oxic conditions created by the AWD irrigation facilitated nitrification that was tightly coupled to denitrification.

While runoff and precipitation temporarily saturated the upper layers of the bioreactor during storm events, the same layers would return to an unsaturated state when the storm event passed and runoff subsided. This is because the standing level of water in the bioreactors, determined by the height of the pipe in the outflow water level control structures, sits 6 cm below the perforated inlet pipe to the bioreactors. Between storms, this unsaturated layer became more aerobic than the lower layers, creating ideal conditions in the bioreactors for both nitrification and denitrification to occur (Penton et al., 2013). The existence of aerobic and anaerobic layers in the bioreactors would have also created an oxic/anoxic zone interface. This oxic/anoxic interface has been shown to support coupled nitrification and denitrification as well (Brune et al., 2000).

During the sample season, some plants took up residence in the upper layers of the bioreactor edges. Plant growth was predominantly seen around the outflows of the high-flow pipes that brought runoff directly from the flow diversion structure to the bioreactors. Wetland plants have the unique ability to translocate oxygen to their roots, which allows some oxygen to diffuse into the surrounding sediment (or in this case, wood
chips) (Titus, 1992). Oxygen diffusing from plant roots in constructed wetlands is believed to be utilized by nitrifying bacteria (Titus, 1992). Ammonia that is not taken up by plants has been observed to be nitrified in aerobic zones, followed by transportation by concentration gradient and then denitrification in anoxic zones (Good and Patrick, 1989). While the bioreactors are not equivalent to constructed wetlands and the aforementioned plants were not identified, it is likely that the plants were wetland plants or similar due to the partially saturated nature of the bioreactor. Coupled nitrification and denitrification has been observed in the rhizosphere of various saturated settings by Gersberg et al. (1983), Penton et al. (2013), Arth et al. (1998), and Nicolaisen et al. (2004). Nitrification in the rhizosphere of the bioreactors followed by denitrification in the saturated, anoxic zone of the bioreactors could be another potential pathway for nitrogen removal in the wood chip bioreactor treatment system.

If nitrification occurred in the bioreactors, creating nitrate that was then consumed through denitrification, this explains how TN was potentially reduced through denitrification even though no significant changes in NO$_3$-N concentration were observed. This theory would also explain the reduction in NH$_4$-N concentration observed between the tank effluent and the bioreactor effluent.

A comparison of percent reduction of TN mass load in the pre-treatment tanks versus the entire system shows that the bioreactors were the component responsible for the majority of TN reduction throughout the season (Table 4), ruling out the likelihood that coupled nitrification and denitrification was occurring to a significant extent in the tanks. It is possible that minimal coupled nitrification and denitrification occurred in the tanks where aerobic and anaerobic environments were both present (providing an
explanation for the slight reduction of NO$_3$-N observed in the tanks), but it did not occur to a great enough extent to reduce TN. Furthermore, the occurrence of annamox (anaerobic ammonium oxidation) was unlikely due to high levels of ammonium and the presence of organic carbon (Strous et al., 1999; Fernandez et al., 2012; Dapena-Mora et al., 2007; Tang et al., 2010; van de Graaf et al., 1996; Chamchoi et al., 2008). This suggests that coupled nitrification and denitrification occurring in the bioreactors was the dominant pathway for nitrogen removal.

As seen in figure 7, influent TN concentration has a strong positive correlation with N removal. This is consistent with previous findings in literature from Christianson et al. (2012a) and Addy et al. (2016). However, even though runoff with the same TN concentration was entering both bioreactors, the WWB exhibited a superior TN reduction performance in comparison to the EWB. This difference in performance is most likely due to the uneven split of runoff between the two bioreactors. While the volume of runoff directed to each bioreactor varied from storm to storm, the WWB received an overall larger volume of runoff over the course of the monitoring season, and some storms generated no flow through the EWB. This means that the cycle of saturated and unsaturated conditions necessary for coupled nitrogen transformations would have occurred more frequently in the WWB, increasing that bioreactor’s capacity for treatment.

2.5.2 Phosphorus Removal

When comparing concentrations and loads of both TP and SRP between the tank influent and effluent, significant reductions were not observed (Table 3 and 4). The data
also show that SRP (a soluble species) comprises ~75% of the TP load into the system, indicating that any settling of organic P in the tanks would not largely impact TP values. These results suggest that phosphorus was being removed predominantly in the bioreactors.

Wood chip TP data in Figure 8 indicated that the wood chips themselves may be responsible for at least a portion of phosphorus reduction that occurred in the treatment system. Extrapolating the data from the samples in Figure 8 to the entire bioreactor volume indicated that if all the wood chips were responsible for 20 g/kg of TP uptake (the lower end of the observed uptake), wood chip removal would account for more than the amount of TP removed from the runoff during the sample season. This extrapolation was calculated by multiplying the approximate weight of wood chips in the bioreactors by 20 g/kg, which yielded ~1,400 kg. Reducing this number by half to account for the fact that not all areas of the bioreactors came in contact runoff yields 700 kg. According to Table 4, ~104 kg of TP was removed from the system. Assuming that not all areas of the bioreactors were able to uptake the same amount of TP, the total amount of TP removed by the system could still be attributed to the wood chips.

It is unclear exactly how the phosphorus is being removed, as literature indicates that wood chip bioreactors need additional resources to remove P, such as biochar (Kortbein and Rajendran, 2016), mixed-media (Husk et al., 2018), or filters (Christianson et al., 2017; 2018; Hua et al., 2016). One theory is that mycelium growing in the wood chip bioreactors is absorbing the phosphorus, as the incorporation of fungal treatment has been shown to help remove phosphorus in other wastewaters (Singh, 2006). Since the majority of the TP in the system is SRP, much of the phosphorus is readily available for
plant and fungi uptake. Mycelium was not observed in the sample of wood chips taken from below the water level, but was seen in an abundant layer above the water level, from which the second wood chip sample was taken (Figure 6). Runoff would have passed through this mycelium layer during storm events as it percolated down through the wood chip bioreactors towards the underdrain pipe. The analysis of these samples indicated that the wood chips taken from within the mycelium layer had a higher concentration of TP, suggesting that P uptake was occurring. Similar mycoremediation has been observed by Thomas et al. (2003), who reported phosphorus reduction of up to 46% in dairy lagoon waste through mycoremediation, and Hultberg and Bodin (2017), who reported phosphate reductions between 28.3 and 44% in a fungi-based treatment of brewery wastewater. Zhou et al. (2012) studied the use of fungi-algae pellets as wastewater treatment, and found an 89.83% reduction in TP from centrate and an 84.7% reduction in TP from diluted swine manure wastewater.

The presence of mycorrhiza, a fungus that forms a symbiosis with the roots of plants (Smith, 2008), could also have assisted in the removal of phosphorus from the silage runoff. Mycorrhiza receive sugars from plants, and in turn they improve a plant’s capacity to absorb water and nutrients (Plenchette et al., 2005; Smith, 2008; Smith et al., 2003). This increased capacity for absorption has been attributed to the increase in surface area that comes with mycorrhiza hyphae (Abbott and Robson, 1977), as well as the hyphae’s ability to enter small pore spaces that root hairs cannot explore (Bjorkmann, 1949). Since mycorrhizae are widespread and naturally occurring (Bolan, 1991), the plants that were observed growing around the perimeter of the bioreactors could be likely
hosts for mycorrhizae. This relationship would help to improve the efficiency of any phosphorus uptake that was already occurring because of the plants.

2.5.3 Influence of High Flow, Mixing, and Uneven Split to Bioreactors

High-flow (top arrow in Figure 3, third in legend) occurred during every storm that was sampled after June 29\textsuperscript{th}, 2017 (prior to this date, the treatment system was still retaining runoff in order to fill the tanks and saturate the bioreactors as designed). The number of high-flow events was not intended at the outset of this research and was due to inaccurate leveling of the high-flow path pipe, where the pipe’s opening overlapped the upper level of the low flow path opening. This led to a mean of 85\% (\pm 13.1) of the inflow volume bypassing the pre-treatment tanks for each storm that experienced significant high-flow. Despite the significant volume of runoff that bypassed the pre-treatment tanks during the sampling season, the pH, nutrient concentration and load data indicate that the treatment system performed effectively and reduced the polluting potential of the silage runoff (Tables 3, 4, and 5).

Due to runoff from different storm events mixing in the tanks and bioreactors, analyzing the inflow and outflow of individual storm events is not indicative of the treatment system’s efficacy. A small number of sampled storms showed an increase in TN and TP mass load between the influent to the effluent (Tables 6 and 7 in Supplementary Materials). While nitrogen transformations are occurring, nitrogen and phosphorus mass must be conserved. This suggests that runoff from a previous storm, with a higher TN or TP concentration, is mixing with lower concentration runoff in the system, rendering an individual storm inflow and outflow comparison ineffective.
The increase between influent (Tank 1) and effluent (Tank 3) in TN, SRP, and TP load observed in the pre-treatment tanks from the summed season-long data (Table 4) could also be explained by this mixing. Since not all storms were sampled and analyzed, un-monitored runoff was entering the tanks throughout the sample season. It is likely that the nutrient mass load from this unmeasured runoff was mixed in and sampled in the tank outflow of a later storm, creating an artificially high load measurement that does not correlate to the inflow. The analysis of total inflow and outflow nutrient load for all storms sampled during the monitoring season was a more effective indicator of performance because it accounted for mixing of individual storms, as well as varying retention times between and during storms.

While there is substantial literature that supports a positive relationship between retention time and increased nitrogen removal (Addy et al., 2016; Chun et al., 2009; Greenan et al, 2009; and Christianson et al., 2012b), there were too many uncontrollable variables in this study that rendered a true calculation and comparison of HRT impossible. The mixing in the system that occurred between storms, as well as the uneven split in runoff entering the bioreactors, both impacted the ability to calculate an accurate retention time.

2.5.4 Dissolved Oxygen and pH

The DO measurements taken from within the aerobic tank (Figure 3, Tank 2) indicated that the blower was extremely effective at oxygenating the runoff, but that the added oxygen was quickly consumed when the blower cycled off, returning the tank water to its original anoxic conditions (Figure 7). It is possible that a lack of access to a
steady supply of oxygen is responsible for limiting nitrification in the tanks. Gao et al. (2012) conducted a study on soil nitrogen mineralization in a tidal salt marsh, and found that mineralization rates remained relatively constant in the oxic and anoxic conditions experienced during tidal flooding. In a follow up study by Gao et al. (2018), nitrification was found to have stopped almost completely under the anaerobic environment, confirming similar findings from Lodhi et al., (2009). The conditions in Tank 2 alternated between oxic and anoxic as the blower cycled on and off. If mineralization was able to continue in both conditions but nitrification was inhibited, this could explain the buildup of NH\textsubscript{4}-N without a subsequent conversion to NO\textsubscript{3}-N. A lack of nitrifying bacteria in the tank environment could also be the reason behind limited nitrification in pre-treatment tanks.

The readings from these measurements also indicated that pH drops slightly when the blower turns off, but does not fall to levels seen in samples from influent into the system. This suggests a link between pH and DO, but the pH is generally shown in these data to increase the most in the bioreactors, a theoretically anoxic environment (Table 5). Most likely, pH is increased in the tanks due to dilution and settling out of acidic silage particles (lactic and acetic acid are produced during the fermentation of ensilage (Weinberg and Muck, 1996; Schukking, 1976). pH probably increases in the bioreactors due to the denitrification process, which produces bicarbonates and hydroxides (Rivett et al., 2008; Rust et al., 2000).
2.6 Conclusion

Despite significant runoff following the high flow path unexpectedly, the treatment system was still able to reduce nutrient load for TN and TP and reduce acidity during the monitoring period. While the majority of nutrient reduction was shown to happen in the bioreactors, this was in part due to the fact that the bioreactors received ~85% of the runoff and therefore ~85% nutrient load for the majority of the sampled storms. While the bioreactors were designed to be, and acted as, the main aspect of the treatment system, the pre-treatment tanks were still beneficial for mineralization, allowing more nitrogen to be removed at the next step in the process, presumably through coupled nitrification and denitrification. If design adjustments are made allowing the majority of the silage runoff to flow through the entirety of the system nutrient load reductions could be expected to increase due to additional nitrogen transformations. However, while the pre-treatment tanks assisted in nitrogen transformations, the treatment system proved its ability to function adequately in their absence because the storms that experienced significant high-flow still displayed nutrient removal. If cost and/or space are of concern for future designs, the tanks could theoretically be removed with minimal effect on treatment performance.

Overall, even without full functionality, this wood chip bioreactor treatment system provided an effective method for improving the quality of silage bunker runoff on a northern Vermont farm.
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Appendix A

ADDITIONAL WATER QUALITY ANALYSES

During the monitoring period, biochemical oxygen demand (BOD) was also measured in runoff samples collected from the system. However, only four storms events were measured for BOD, limiting the confidence of the results. Further BOD sampling should be performed before any strong conclusions can be made about the treatment system’s impact on BOD.

Methods

BOD analysis of samples from four individual storm events was performed using standard methods (APHA, 2011). Composite samples were processed by first adding buffered dilution water to create a solution that was 1% as potent as the original sample. Then, initial dissolved oxygen (DO) content of this solution was measured using a DO meter (YSI Pro20, Yellow Springs, OH). After a five-day incubation (temperature and light controlled), final DO content was measured using the same meter. The following equation was used to calculate BOD:

\[ \text{BOD}_5 = \frac{\text{DO}_i - \text{DO}_f}{P} \]

Where \( \text{BOD}_5 \) is biochemical oxygen demand after a 5-day incubation period (mg/L), \( \text{DO}_i \) is the initial dissolved oxygen content (mg/L), \( \text{DO}_f \) is the final dissolved oxygen content (mg/L), and \( P \) is the decimal volumetric fraction of sample used in the dilution water (unitless). A 1% dilution was used for the measured samples to guarantee that not all
dissolved oxygen would be consumed during the incubation period. This value was chosen after a series of tests comparing different dilutions ranging from 1% to 20%.

**Results**

As seen in Figure 10, two out of the four analyzed storms exhibited an increase in BOD between the system influent and the tank effluent, but overall BOD decreased between the inflow and outflow of the treatment system in every storm. BOD values ranged from 49 – 939 mg/L.

![Figure 10: BOD in the treatment system as measured in composited storm samples](image)

**Discussion**

This analysis indicated that, in addition to reducing nutrient load and acidity, the treatment system also successfully lowered the BOD of the silage runoff for the small number of sampled storms. While BOD was expected to be reduced predominantly in the tanks, due to the sedimentation of suspended organic matter in tanks 1 and 3 (Figure 3), BOD reduction by the bioreactors was consistently more significant. This was most likely due to two main factors: 1) sedimentation in the tanks only removed a portion of suspended organic matter, leaving enough organic material in the runoff to stimulate
oxygen-consuming microbial breakdown activities, and 2) the wood chip bioreactors acted as biofilters that were able to remove additional organic matter from the runoff, further reducing microbial breakdown activities and therefore reducing BOD as less oxygen was consumed. Similarly, Lens et al. (1994) found wood chips to successfully reduce BOD in domestic wastewater after a percolation treatment.
Appendix B

TREATMENT SYSTEM COMPLICATIONS

Due to design flaws in the system, laboratory equipment malfunctioning, and confines of lower detection limits, some aspects of the performance of the treatment system were not assessed fully. A number of other factors must be considered before the full impact of this treatment system can be assessed and understood. This appendix describes complications, limitations, and future considerations pertaining to this research.

Impact of Unexpected Bypass and of the Pre-treatment Tanks

An initial direct comparison of TN and TP influent load into the tanks was found to be significantly smaller than the combined effluent load of the bioreactors for fourteen out of eighteen total sampled storms (77.78%). This increase in load (but not concentration) was due to the fact that a much larger volume of runoff was exiting the bioreactors than was entering the tanks. This realization led to the discovery that the high flow path in the flow diversion structure, designed to accommodate high flow storms by directing excess flow directly to the bioreactors, was incorrectly placed at an elevation that was too low. The low flow outlet reaches from the cement base of the flow diversion structure to 18.3 cm above the base, and the high flow outlet starts at 5.5 cm above the base and extends to 29.1 cm above the base. This means that there is significant overlap of the two openings, allowing more water than intended to bypass the tanks and flow directly into the bioreactors.
Recognizing the need to account for this volume, a secondary comparison of TN and TP load in the total influent to the two bioreactors (bypass volume plus volume of the tank effluent) versus the combined effluent from both bioreactors was performed. This comparison showed a significant reduction in TN and TP for almost every storm.

**Bioreactor Performance Comparison**

Due to the fact that runoff flow into the two bioreactors was not identical by volume, a direct comparison between the two bioreactors needs to consider several caveats. The runoff that entered the bioreactors, either from the tank effluent or the bypass flow, was not split evenly between the two. This means that each bioreactor received varying percentages of pre-treated and untreated runoff with each storm. For this reason, the HRT and % TN load reduction comparison done in Chapter 2 (Figures 3 and 4), was based on many assumptions. First, since the individual influent concentration and mass load for each bioreactor was unknown, only storm events where the bypass accounted for over 85% of total influent were used in the analysis. For these storms, it was assumed that the high-flow runoff comprised the majority of the inflow to the bioreactors, and therefore influenced the bioreactors’ outflow samples’ water quality. To approximate the influent load to each bioreactor, the TN concentration of the bypass flow (which was identical to the TN concentration measured in the inflow to the tanks) was multiplied by the volume of each bioreactor outflow minus the precipitation volume received. The TN concentration and volume from the pre-treatment tank effluent was not included in this comparison because the volume that was directed to each bioreactor
could not be accurately identified. These comparisons, which were based on many assumptions, did not confidently identify any links between HRT and nitrogen removal.

In general, the WWB received a greater volume of runoff, but this was also inconsistent and varied with storm intensity. The difference in flow to the bioreactors explains the wide gap in their mean retention times. When comparing nutrient reduction, the WWB consistently outperformed the EWB. This seems counterintuitive because a sufficiently long retention time is necessary for the completion of denitrification, and the EWB generally had a longer retention time. However, since the WWB received more flow over the duration of the sample season, and the EWB didn’t receive any flow in some storms, the aerobic and anaerobic conditions discussed in Chapter 2 that were necessary for coupled nitrogen transformations would have occurred more frequently in the WWB. It is possible that this factor played a larger role in nitrogen removal than retention time.

There also appeared to be a leak in the sump of the EWB’s water level control structure, as the water level control structure from the EWB was often observed to be well below the zero level in between storm events. This loss of runoff impacted the retention time and effluent load from the EWB, further limiting the accuracy of the information obtained during sample events.

**Future Considerations**

While this study provided valuable insight into the operations of the treatment system, there are still many unstudied factors to consider. While it was found that the bioreactor with the shorter retention time was more successful in reducing nutrient loads
and concentration, a retention time that is too short could lead to a release of nitrous oxide from the bioreactor. Nitrous oxide, a greenhouse gas, is a byproduct of denitrification that is only at risk of being released if denitrification is not carried out to completion (Seitzinger et al., 2006). This risk grows as retention time shrinks. In future studies, it would be beneficial to monitor outgassing from the bioreactors and look for the presence of nitrous oxide. It would also be informative to place an autosampler that pulls from this upper, oxic layer of the bioreactors, in order to further monitor nitrate concentrations/loads and confirm that denitrification is in fact the dominant pathway for nitrogen removal. One could also test for the presence of annamox bacteria to confirm that it is not present.

Since design flaws greatly impacted the outcomes of this study, future modifications should be made that remedy the issues at hand. If the pre-treatment tanks are to remain in use, a plug should be placed in the high flow path outlet of the flow diversion structure, to prevent bypass flow, and the pipes directing runoff to the bioreactors should be adjusted so that flow is evenly split between the two. If flow to the two bioreactors is identical, then retention time can be varied by adjusting the height of the outflow pipe in the water level control structures. This would allow for more accurate comparisons of varying HRTs between two otherwise replica bioreactors.

Additionally, the third pre-treatment tank should be investigated to assess whether or not it is effectively removing solids. If sedimentation does not appear to be happening in this tank, then it would be recommended that future similar systems forego this aspect of treatment, as its main function of solids removal is already addressed in the first
settling tank. The third pre-treatment tank also seems to create an unnecessary anoxic zone between the aerobic tank and the bioreactors.

Lastly, a blower that operates on a different cycle would probably be more beneficial. Instead of creating an environment saturated in oxygen for ~12 hours and then an environment devoid of oxygen for ~12 hours, a blower that cycles on and off every hour would create a more stable environment. Oxygen levels would still rise and fall with the cycle, but there would most likely always be at least minor amounts of oxygen available. This could potentially promote nitrification, while also helping to maintain pH more effectively than before.
References

