Impact of Substrate on Nutrient Removal in In-Ditch Bioreactors

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ABSTRACT

Drainage ditches, or grassed waterways, collect nutrient-laden runoff from agricultural fields and transport it to nearby waterbodies. The high nitrogen and phosphorus content in this water leads to negative effects, such as eutrophication in the receiving waters. In-ditch bioreactors are a simple, inexpensive treatment technology that could potentially remove nitrogen and phosphorus from agricultural runoff. In-ditch bioreactors are intended to reduce flow rate and stimulate denitrification and sedimentation. Using experimental ditch segments and simulated runoff, this study evaluated nutrient removal in 1) vegetated ditches, 2) vegetated ditches with woodchip bioreactors and 3) vegetated ditches with combination woodchip and biochar bioreactors. Biochar was added in an effort to increase phosphorus removal. Inlet and outlet concentrations of nitrate, ammonium and phosphate were measured for each of the three treatments in triplicate. There were no statistically significant differences between treatments on load removed for any of the three nutrients of interest. Issues in measuring outlet flow rate made drawing definitive conclusions on nutrient load reductions difficult. Further experimentation using adjusted outlet flow measuring methods and bioreactor design would help establish whether in-ditch bioreactors are suitable for use as a nutrient removal technology in agricultural grassed waterways.

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GENERAL AUDIENCE ABSTRACT

Drainage ditches, or grassed waterways, are located at the edge of agricultural fields where runoff migrates naturally. These ditches help to direct runoff from the field to receiving waterbodies while reducing erosion. Agricultural runoff often contains high levels of nitrogen and phosphorus from fertilizer added to promote crop growth. When runoff with a high nutrient content reaches a waterbody, it reduces the quality of the water for the plants and animals that live in it and for human recreation or consumption. In-ditch bioreactors are a simple, inexpensive treatment technology that could potentially remove nitrogen and phosphorus from agricultural runoff. In-ditch bioreactors have the potential to remove nitrogen from the water by creating optimal conditions for the microorganisms that transform nitrogen in the water to nitrogen in the air. Phosphorus removal has the potential to be enhanced by in-ditch bioreactors that reduce flow and allow for phosphorus to settle out of the water. In addition, settling of phosphorus may be increased by adding a material, such as biochar, that phosphorus can attach to. Using experimental ditch segments and simulated runoff, this study looked at nutrient removal in 1) vegetated ditches, 2) vegetated ditches with woodchip bioreactors installed and 3) a vegetated ditch with combination woodchip and biochar bioreactors installed. Concentrations of two nitrogen compounds and one phosphorus compound were measured before and after passing through each ditch. There were no significant differences between any of the three ditch types on how much of each compound they could remove. These results are inconclusive due to inaccuracies in measuring flow rate at the outlet of the ditches. Further experimentation using improved flow measuring techniques and bioreactor designs would likely help establish whether in-ditch bioreactors are suitable for use as a nutrient removal technology.

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Chapter 1. Background

1.1 Introduction

Agricultural activities are linked to excess nutrient levels in rivers, streams, lakes and wetlands (USEPA, 2017). Adding nutrients such as nitrogen (N) and phosphorus (P) to agricultural lands improves the growing conditions for plants and increases crop yield. Unfortunately, much of the N and P applied as fertilizer can enter surface waters either through runoff or leaching through aquifers (Sutton et al., 2013). Both the natural environment and human health suffer consequences as a result of increased nutrient levels in surface water.

One opportunity to reduce N and P inputs is to treat agricultural runoff as it is transported towards surface water. Most farms use some type of drainage practice to reduce waterlogging and erosion on their fields. Over 22 million ha of farmland in the United States is drained by subsurface pipes, called tile drains, and almost 18 million ha of farmland in the United States is artificially drained by ditches (USDA NASS, 2019). Drainage ditches not only help to reduce erosion, but also provide a location for water quality improvements.

Vegetation within ditches plays an important role in facilitating the removal of N and P from runoff, thereby reducing the amount transported to surface waters. Nitrogen can be taken up by plants and used as part of their biomass or released into the atmosphere through the biological process of denitrification. Phosphorus is also taken up by plants, but relies on adsorption and sedimentation that retains P in the ditch system rather than transporting it further downstream. Ditches can be optimized to enhance these naturally occurring processes either through design or technology.

In tile drainage, denitrifying bioreactors (DNBRs) are a technology that decreases N in agricultural runoff by creating optimal conditions for denitrification to occur (USDA-NRCS, 2015). A similar approach may be able to be applied in surface ditches and could offer a more approachable and affordable option for farmers. In-ditch bioreactors could also act as a barrier within the ditch to reduce water velocity and increase sedimentation making them advantageous for both N and P removal. Nutrient removal could also be improved through the inclusion of a material with a high sorption capacity, such as biochar. Biochar has been shown to increase N and P removal in DNBR applications and could work similarly in an in-ditch bioreactor. This project aims to test the feasibility of in-ditch bioreactor ‘socks,’ with and without biochar, to reduce N and P levels in runoff using a small-scale outdoor lab.

1.2 Research Hypotheses

Simulated runoff events were conducted in artificial drainage ditches to test the effectiveness of in-ditch bioreactors as a nutrient removal technology. A total of nine separate diches were used, with each of the three treatments of interest replicated in triplicate. The treatments were: 1) control ditches with no bioreactors; 2) ditches with woodchip bioreactors; and 3) ditches with woodchip and biochar mixed bioreactors. All ditches were of the same dimensions and vegetated with the same rye grass mixture. A solution of laboratory chemicals and well water was pumped into each ditch at a constant flow rate to imitate nutrient laden agricultural runoff. Runoff samples were collected at the ditch outlet and water quality analysis was performed to determine nutrient content.

Hypothesis 1: The addition of in-ditch bioreactors results in greater N and P reductions than in the vegetated ditches alone.

Hypothesis 2: In-ditch bioreactors containing both woodchips and biochar results in greater P removal than an in-ditch bioreactor with only woodchips.

Chapter 2. Literature Review

2.1 Agricultural Nutrient Pollution

Modern agricultural practices are characterized by pushing the land to the limits of its potential productivity. This has led to large quantities of fertilizer being added to promote crop production and support dense pastures for livestock. Runoff from crops and pastures contains high levels of nitrogen (N) and phosphorus (P); nutrients that are essential to life, but harmful to the natural environment at elevated levels. The majority of N and P applied as fertilizer is often not utilized by crops and ends up in the surrounding environment (Sutton et al., 2013). The agriculture industry has been identified as a major pollutant source to freshwater systems in the United States with N and P being the most widespread pollutants (USEPA, 2017). About half of all N and P pollution in the United States has been linked to agriculture (Wurtsbaugh et al., 2019). The need for food production for a growing population must be balanced with reducing pollution inputs to freshwater systems.

Excess nutrients in a body of water, also called eutrophication, has been linked to harmful algal blooms, fish kills, taste and odor issues in drinking water, and greenhouse gas release (Wurtsbaugh et al., 2019). Reducing N and P contributions from agricultural sources is fundamental to solving problems of eutrophication. Historically, the main source of eutrophication in the United States was untreated or insufficiently treated municipal sewage in urban areas. The most notable examples of such eutrophication occurred in Lake Washington and Lake Erie in the last half of the 20th century (Edmondson et al., 1956; Wurtsbaugh et al., 2019). In response to these issues, municipal sewage was collected and treated in wastewater treatment facilities before being released back into the environment, resulting in partial recovery in these lakes (Wurtsbaugh et al., 2019). These changes were relatively easy to implement as the source and the party responsible for the nutrient inputs were known; however, implementing technology to combat the effects of eutrophication can be expensive. For example, sourcing water from a eutrophic lake such as Lake Erie requires a drinking water plant to spend about $3 million dollars per year to remove cyanotoxins generated by algal blooms (Wurtsbaugh et al., 2019). It is clear that excess nutrients in natural waterbodies cause harmful and lasting effects, and practices that decrease nutrient inputs should be identified and applied. However, approaches vary in difficulty and scale depending on the characteristics of the pollution source.

Municipal sewage is an example of a point source (PS) pollution that is easily identifiable and collected at a point, unlike non-point source (NPS) pollution which is highly spatially variable and difficult to track. Non-point sources are any pollution sources that do not fit under the legal definition of PS pollution from Section 502(14) of the Clean Water Act which defines a PS as "any discernible, confined and discrete conveyance" (CWA, 1972). Non-point source pollution from agricultural activities has persisted despite efforts to reduce nutrient pollution in freshwater systems. The very nature of NPS pollution makes it difficult to collect for treatment and to identify the responsible party. Non-point source pollution is temporally and spatially variable, and is influenced by precipitation, land cover and topography. Agricultural and urban runoff are examples of non-point sources and are significant contributors of N and P to freshwater systems (Carpenter et al., 1998). There are, however, some opportunities to implement treatment technologies along the path of this runoff to reduce nutrient loads that enter freshwater systems.

2.2 Agricultural Drainage Ditches

Proper drainage of agricultural soils improves crop production, reduces soil erosion, and creates accessible flow pathways for runoff to be collected and managed for improved water quality. The term "drainage ditch" refers broadly to a channel-like depression in the landscape where water flows away from an area of concern and towards a waterbody (Needelman et al., 2007). Drainage ditches are usually located where surface water collects naturally, such as at the bottom of a slope, but they can also receive piped flow. Drainage ditches exist in a range of sizes and designs that serve a variety of different purposes depending on their setting. Each agricultural area has an individualized drainage system that is designed to accommodate the natural land cover, topography and resources available (Easton et al., 2016). Two important purposes of drainage ditches are draining wet areas to make them suitable for crop production and providing pathways for overland stormwater flow at the edge of agricultural fields to reduce erosion and the formation of gullies. Despite having different purposes, the biogeochemical and physical processes that occur in these ditches tend to overlap and many of the same scientific principles can be applied across designs and uses.

Agricultural fields are drained in two primary ways: subsurface tile drainage and surface drainage ditches. Tile drainage consists of perforated piping below the ground surface that collects water from the soil matrix (Easton et al., 2016). These pipes are placed at a uniform depth below ground and are graded such that the pipe is never under excessive pressure flow conditions (Hofstrand, 2010). Tile drainage outlets can drain either directly into natural waterbodies, enter a larger piped underground drainage matrix or enter into surface drainage ditches (Easton et al., 2016). Tile drains require piping materials and construction equipment to lay the pipes into the ground which results in high initial costs for farmers. These costs can be offset by increased production as a result of improved drainage, but it may take several years to recoup the investment (Hofstrand, 2010). Tile drainage is popular in the midwestern region of the United States (Midwest) and in coastal areas with low slopes and high water tables (Bock et al., 2018). For example, almost 90% of farmland in Iowa is drained by tile (USDA-NASS, 2019). For many smaller farms, it is more practical to use surface drainage ditches as the primary runoff pathway.

Surface drainage ditches (ditches) are a surface drainage system located where runoff migrates to naturally (i.e., edge of field, swales, or bottoms of hillslopes) and the runoff flow is channelized. Ditches range in design from straightened streams containing stream-bottom sediments to small depressional systems that are dug into the land surface (Needelman et al., 2007). While ditches do remove a portion of land from production, they are less expensive to install than piped tile drainage systems especially when located in areas where runoff naturally collects (Easton et al., 2016). In Virginia, the land drained by ditches is almost double that which is drained by tile (USDA-NASS, 2019).

Both tile drainage and ditches primarily serve as conduits for excess flow, but they also provide an opportunity for water quality improvements before the runoff enters a downstream waterbody. Historically, drainage ditches were only used to drain wetland areas and convert them into land for agricultural production (Cooper et al., 2002). Management practices were focused on increasing the hydraulic capacity of ditches to minimize flooding and soil moisture upstream. Woody vegetation at the ditch edge was removed to enable access to the ditch for mowing and silt removal (Evans et al., 2007). It was thought to be advantageous to limit vegetation within the ditch, through mowing and dredging, to reduce any flow resistance that might be present (Cooper et al., 2002; Evans et al., 2007). It has only been in the last two decades that researchers have begun to understand and appreciate the environmental benefits ditches can provide beyond water conveyance.

2.3 Nutrient Removal Mechanisms within Vegetated Ditches

While both N and P contribute to eutrophication, their chemical, physical and biological properties necessitate different approaches for their removal from water (Dollinger et al., 2015). Nitrogen exists in several different aqueous compounds, the most relevant of which are those containing nitrate (NO_3^-) and ammonium (NH_4^+). Nitrogen compounds are often quantified in terms of how much elemental N they contain and are referred to as nitrate-N (NO_3-N) or ammonium-N (NH_4-N) to specify the form in which N is currently present while recognizing the ease with which N undergoes biochemical changes. Transformations of N from one compound to another are done through biologically-mediated redox reactions, but no such processes exist for P (Brezonik & Arnold, 2011). Phosphorus removal relies on plant uptake and the physical processes of adsorption and sedimentation (Sharpley et al., 2007). Treatment technology designs are informed by the removal mechanisms of both N and P. These processes occur naturally and can be enhanced by controlling environmental conditions.

Ditches have characteristics of, and interact with, the natural environment unlike the plastic pipes of tile drained systems. Approaches to ditch management that aim to make them as pipe-like as possible through straightening, mowing and dredging prevent ditches from achieving their full treatment potential. Vegetated ditches have properties of both streams and wetlands, conveying water through the landscape and containing zones of low flow where microbial processes and sedimentation can occur (Needelman et al., 2007). Previous ditch management practices have focused on their function as conduits to rapidly move water away from agricultural fields and ignore the valuable ecosystem services that they can provide (Moore & Locke, 2020). Hydric soils and hydrophytes are often found in ditches, emphasizing their role as wetlands that link agricultural fields to receiving waters (Kröger et al., 2007). Wetlands are recognized as a unique ecosystem where nutrient cycling occurs, and constructed wetlands are a popular final step for treatment of nutrient laden waters. Optimizing ditches to function more like wetlands than conduits increases their pollutant processing potential and could reduce the nutrient load from ditches to receiving water bodies.

Vegetation plays an important role in facilitating nutrient removal within ditches. Limiting mowing and dredging to allow vegetation to become established within ditches is a simple step that can increase their nutrient removal capacity. Studies of vegetated and unvegetated ditches consistently show that vegetated ditches have higher nutrient removal than unvegetated ditches (Tyler et al., 2012; Moore et al., 2016; Kumwimba et al., 2020). Vegetated ditches also have greater bacterial abundance and diversity than unvegetated ditches (Cui et al., 2020). Vegetation removes nutrients directly through plant assimilation and indirectly by providing locations for microbial activity (Tyler et al., 2012). During assimilation, plants uptake N and P and incorporate these nutrients into their biomass (Tyler et al., 2012; Taylor et al., 2020) . In a study of vegetated drainage ditches in the Czech Republic, plant assimilation accounted for 26% of removed N load and 14% of removed P load (Vymazal & Březinová, 2018). Plant uptake is a significant contribution to a ditch's nutrient retention, but presents some limitations during dormant seasons.

While the plant is alive and growing, N and P are effectively stored within its biomass, but nutrient loss occurs when the plants begin to senesce. During late fall and winter as plants die, they release N and P from their biomass back into the water column, rendering the nutrient removal only temporary (Kumwimba et al., 2020). Harvesting of vegetation at the end of the growing season is recommended to maximize nutrient removal from plant assimilation (Chen et al., 2017; Kumwimba et al., 2020). However, many studies of plant senescence measure leaching rates in columns containing only water, which represents a worse scenario case. Columns of water are not representative of intact systems which contain sediments, root systems and microorganisms in addition to the senescing plant biomass. In a study of nutrient export from an intact ecosystem, leaching of P from plants during winter storm events was minimal and was attributed to the translocation of P to the plant root system (Taylor et al., 2020). While harvesting the vegetation prevents nutrient leaching back into the system, it disturbs the surrounding soil and biofilms causing a negative impact on nutrient cycling (Strock et al., 2007; Taylor et al., 2020). Nutrient cycling facilitated by microorganisms in soil and biofilms is especially important for N removal in ditches.

Several biogeochemical processes act on bioavailable forms of N in aquatic systems such as ditches. Bioavailable forms of nitrogen include NO_3^-, NH_4^+ and organic N. Both NO_3^- and NH_4^+ can be assimilated and become organic N through plant uptake, but NH_4^+ is preferred by plants (Strock et al., 2007; Brezonik & Arnold, 2011). Most importantly, the coupled process of nitrification and denitrification transforms bioavailable forms of N into N_2 gas which permanently removes N from the aquatic system. In nitrification, NH_4^+ is sequentially transformed to nitrite (NO_2^-) and nitrate (NO_3^-) by autotrophic bacteria under aerobic conditions

(Brezonik & Arnold, 2011). In denitrification, NO_3^- is converted to dinitrogen gas (N_2) by chemo-heterotrophic denitrifying bacteria under anoxic conditions (Brezonik & Arnold, 2011; Christianson et al., 2021). Anoxic conditions refer to a depleted oxygen state in which microbes cannot use the most preferred electron acceptor, oxygen, and are forced to use the next best electron acceptor, NO_3^-.

Denitrification relies on the presence of NO_3^- and dissolved organic carbon (DOC) under anoxic conditions (Hassanpour et al., 2017). DOC must be present in the solution because DOC is used as the terminal electron acceptor in denitrification (Hassanpour et al., 2017). Anoxic conditions are created through extended periods of inundation which slow the diffusion of oxygen into an aquatic system. As microbial respiration continues, oxygen is consumed at a greater rate than it is replenished and thus anoxic conditions are formed. A carbon substrate such as woodchips, rice straw, pine sawdust or biochar will release DOC into the solution as it decays (Liu et al., 2015; Hassanpour et al., 2017). An excess of DOC in the system makes N the limiting factor in denitrification and mitigates the potential of N leaching. Denitrification changes the nature of N entirely, and N that is converted to N_2 is removed from the water column as gas and does not easily reenter.

Like wetlands, ditches have spatially and temporally separate areas of anoxic and aerobic environments, which is ideal for aqueous forms of N to be microbially transformed into N_2 gas (Kröger et al., 2007). In aerobic zones, such as shallow water or around plant roots, nitrifying microbes transform NH_4 to NO_3^- (Strock et al., 2007; Chen et al., 2017). In anoxic zones, this NO_3^- is then transformed into N_2 gas and released from the system. Denitrification in ditches can be enhanced through technology such as water-control structures, which reduce flow and increase hydraulic residence time (HRT), creating an anoxic environment in the pooled water and allowing time for microbial processes to occur (Needelman et al., 2007; Kröger et al., 2011). Phosphorous retention in ditches is also improved through management and technology that aims to reduce flow, although it does not go through biogeochemical transformations as N does.

Phosphorus can be transported either in its dissolved or particulate form, but in ditches P is most effectively removed in its particulate form. Unlike N which is transported through ditches in dissolved inorganic aqueous forms, P is primarily transported via particulate adsorption (Iseyemi et al., 2018). Particulate P is formed through two separate processes: adsorption of P onto sediment particles and precipitation of P from a dissolved form to a solid form (Dunne et al., 2007; Penn et al., 2007). Sorption capacity is dictated by the availability of binding sites within soil organic matter. Minerals, such as iron and aluminum oxides, bind to P under appropriate redox conditions, but release this bound P under reducing conditions (Needelman et al., 2007; Kröger et al., 2011). Phosphorus adsorption potential can be increased by adding negatively charged complexing agents such as crushed marble, calcium carbonate or biochar which all have a higher sorption potential than sediment alone (Dunne et al., 2007; Houston, 2018). The sorption capacity of soils is finite and soils can become fully saturated and unable to retain additional P (Sharpley et al., 2007). Soils act as a P sink when sediment is accumulating and able to adsorb P, but when capacity is reached, these soils can become a source of P if eroded (Needelman et al., 2007). Once P is in a particulate form, P can be removed from the water column and prevented from being transported further downstream through sedimentation. Sedimentation refers to any particle settling out of the water column and depositing at the sediment-water interface. Sedimentation rates increase when flow is reduced and lead to increased P retention in the ditch.

Increasing the rate of sedimentation by reducing flow velocity is an important process for P mitigation (Penn et al., 2007; Kröger et al., 2008; Kröger et al., 2011). Flow velocity can be reduced in a variety of ways, including adding low-grade weirs or inset floodplains within the flow path, by reducing ditch slope or by maintaining vegetation within the ditch (Sharpley et al., 2007; Kröger et al., 2011; Hodaj et al., 2017). Kröger et al. (2011) found that the addition of a low-grade weir within a drainage ditch reduced NO_3^- and dissolved inorganic P (DIP) concentrations when compared to a ditch without flow control. Decreased flow velocity was hypothesized as the reason for increased P retention. The use of a two-stage ditch, a ditch with the addition of an inset floodplain, was also found to significantly increase P removal during both the growing and dormant seasons (Hodaj et al., 2017). In the Hodaj et al. (2017) study, increased settling of sediment-bound P was attributed to the addition of vegetated benches which decreased flow velocity. However, immobilizing P through sedimentation is not as permanent a removal as is denitrification for N. Understanding the mechanisms for N and P removal inform the design of technologies aimed at decreasing nutrient concentrations in agricultural runoff.

Ditches have several properties that make them a potential location for nutrient removal from agricultural runoff. These properties can be enhanced through informed management and technologies that aim to increase these nutrient removal properties. Plant assimilation retains both N and P within the ditch system, but is most impactful during the growing season. Denitrification effectively and permanently removes N from water in ditches, but the microorganisms that carry out this process require specific conditions that are not always present. P removal is largely dependent on a low flow rate which increases sedimentation and the settling of particulates on which P is adsorbed.

2.4 Denitrifying Bioreactors as a Best Management Practice

Agricultural best management practices (BMPs) are officially recognized practices or technologies aimed at reducing water pollution associated with agricultural activities. Suggested practices target nutrient reductions at the source, in the field and along runoff pathways. Denitrifying bioreactors (DNBRs) are a popular BMP implemented within tile drained systems in which a carbon source, typically woodchips, is used to reduce the concentration of NO_3-N in subsurface drainage flow through denitrification (USDA-NRCS, 2015). Original studies on the use of DNBRs were published in the late 1990s and since then their implementation has grown rapidly, especially in the Midwest (Hassanpour et al., 2017). In tile drained systems, DNBRs are constructed as beds of material which runoff flow is piped into and are designed such that conveyance is not restricted. Bioreactor beds in the Midwest remove 20-40% of the annual NO_3-N from runoff with some individual beds having NO_3-N concentration reductions of 98% (Christianson et al., 2021). Bioreactor efficiency varies significantly between individual systems and, while there are design standards, each system should be designed with the specific site conditions in mind. Efficiency of a DNBR is primarily controlled by NO_3-N and DOC availability, temperature and HRT (Hassanpour et al., 2017). These four factors guide DNBR design and are based on the scientific principles of denitrification. As DNBRs became more widely used in the Midwest and were recognized as a proven NO_3-N removal technology, a USDA-NRCS Conservation Practice was developed to guide the design of future bioreactors (USDA-NRCS, 2015).

DNBR design and construction is outlined in the USDA-NRCS Conservation Practice 605: Denitrifying Bioreactor (USDA-NRCS, 2015). This conservation practice was based upon a few field studies of DNBRs already in use and allowed federal incentive payments to go towards the construction of DNBRs (Christianson et al., 2021). A DNBR is constructed as a media chamber dug below ground and lined with plastic, to avoid leaching, with a carbon substrate filling the entire chamber volume (USDA-NRCS, 2015). Woodchips are the most commonly used carbon substrate as they are easily accessible in most locations around the United States, but other carbon sources such as straw or sawdust may also be used (Liu et al., 2015; Christianson et al., 2021). The volume of a DNBR is chosen such that it maintains a HRT of 3 h at peak flow capacity for the given site and given porosity of the carbonaceous material (USDA-NRCS, 2015). DNBRs range in size from a few cubic meters to several hundred cubic meters, with a median volume around 20 m^3, and HRTs that are generally longer than the recommended time of three hours (Christianson et al., 2021). A potential disadvantage of DNBRs is their size and the requirement of heavy equipment to contrast them. DNBRs are usually designed with a method to bypass flow during high flow events such that the water that is able to pass through the bioreactor can do so at an adequate retention time (Bock et al., 2018; Christianson et al., 2021). The effectiveness of DNBRs in the Midwest has led to research on the potential use of similar technology in different geographic locations and use cases.

Best Management Practices such as cover crops, no-till agriculture, riparian buffers, and drainage control structures are examples of treatment technology that can be used to reduce nutrient concentration along runoff pathways. BMPs are one of the primary restoration approaches used in the Chesapeake Bay watershed despite their implementation by independent land owners being largely voluntary (Fox et al., 2021). Developing BMPs that are inexpensive and require little maintenance and effort to implement could result in more widespread use of these methods. BMPs that can be installed within an already constructed drainage ditch are able to access the benefits of the ditch itself. The location of a BMP can be just as important as the technology itself. BMPs should be placed such that they intercept as much of the runoff as possible while not interfering with the intended use of the agricultural field. Designing BMPs for already existing drainage ditches, such as those found in Virginia, takes advantage of the channelized flow in these ditches and their implementation could be potentially widespread.

2.5 In-Ditch Bioreactors as Potential BMP

One possible ditch BMP is an in-ditch bioreactor, also called a bioreactor "sock" or an organic channel barrier, which uses the principles of a DNBR on a smaller scale. Just as DNBRs create favorable conditions for denitrification within tile-drained systems, in-ditch bioreactors provide these same benefits within a ditch drainage system. In-ditch bioreactors consist of an organic carbon substrate, most often woodchips, contained within a plastic netting that is sized such that the width of the bioreactor spans the width of the ditch bottom. An anoxic environment is created within the in-ditch bioreactor as it becomes saturated with water and the organic carbon material undergoes microbial decomposition (Schipper et al., 2010). Several studies of in-ditch bioreactors confirmed their ability to reduce dissolved oxygen (DO) concentrations from inflow to outflow, especially during low flow conditions (Pfannerstill et al., 2016; USDA-NRCS, 2020). Studies of in-ditch bioreactors have been done in ditches receiving agricultural runoff and roadside ditches receiving road runoff (Liu et al., 2015; Pfannerstill et al., 2016; Pluer, 2018; USDA-NRCS, 2020; Maxwell et al., 2022). Findings from these studies are summarized in Table 1. Most studies of in-ditch bioreactors found that they successfully removed NO_3-N from the runoff and all suggested further studies to improve the design.

A report by the USDA-NRCS (2020) found that in-ditch bioreactors in a rural roadside ditch successfully reduced NO_3-N concentrations in agricultural runoff by 30% on average. The main ditch of interest was located in northern Pennsylvania and received runoff from a 40-acre mixed use farmland that was amended with manure twice a year. Two bioreactor ‘socks’ were constructed in series within the ditch using polyester debris netting and a mixture of hardwood woodchips. Water samples were collected from above, between and below the two bioreactor socks during the summers of 2018 and 2019 and analyzed for NO_3-N, total P, and metals. A rating curve was developed to measure flow at the bioreactor inlet and outlet by measuring depth and flow data was used to estimate the HRT within the ditch. During the study, HRT ranged from 40 min to over 1.5 h. Nitrate removal efficiency was dependent on ditch flow and decreased significantly during high flows that overtopped the bioreactors. During low flows, a decrease in DO was observed as water passed through the bioreactors which supported the hypothesis that in-ditch bioreactors are capable of creating conditions that promote denitrification. Based on the results of this study, in-ditch bioreactors were recommended as a possible NRCS conservation practice or as an amendment to the current NRCS Denitrifying Bioreactor (605) standard (USDA-NRCS, 2015).

Two additional studies of field woodchip bioreactors reported their success at reducing NO_3-N concentrations and recommended them as a possible BMP (Pfannerstill et al., 2016; Pluer, 2018). Pluer (2018) installed a woodchip bioreactor “sock” in a roadside ditch receiving agricultural runoff from an 80-acre field in New York State and reported significant decreases in

NO_3-N across the bioreactor during both baseflow and stormflow. Both NH_4-N and total P concentrations were also measured in this study, but were only found in very small amounts. During low flows, some leaching of P from the woodchips was observed, but not enough to be of concern. The woodchip bioreactor in Pfannerstill et al. (2018) was constructed with wire mesh rather than plastic netting. Pfannerstill et al. (2018) reported a 28% reduction in NO_3 concentrations over all seasons, with the highest decrease in spring and autumn when a combination of low flows and high temperatures provided the best conditions for denitrification. Woodchips are a well-studied and accessible carbon substrate to use for in-ditch bioreactors, but they are not the only option.

The performance of rice straw, pine sawdust and activated carbon with sand were examined by Liu et al. (2015) for use in in-ditch bioreactors. To study these three different substrates, a simulated drainage ditch system with artificial wastewater influent was used. A representative artificial wastewater mixture was created using NO_3^-, NH_4^+ and phosphate (PO_4) containing salts. A similar approach to create a representative runoff influent was used in studies of vegetation performance (Tyler et al., 2012; Moore et al., 2016; Chen et al., 2017; Moore and Locke, 2020) . Importantly, this study outlined procedures for a simulated ditch experiment using an unnatural influent solution developed using laboratory chemicals. Each bioreactor was built to be the same width as the ditches, have a depth of 0.1 m and cover 25% of the ditch bottom. The nutrient laden mixture was pumped at a constant flow rate into the ditches to achieve a HRT of 12 h. Results from this study found that rice straw had the greatest NH_4 and NO_3 removal rates of any of the carbon substrates, with 73% and over 90% removal rates respectively. In areas like China where rice straw is highly accessible it makes an excellent alternative to woodchips in in-ditch bioreactors.

Findings from several studies indicate that in-ditch bioreactors are a worthwhile tool for nutrient removal that require no disturbance of the ditch bottom and are created from inexpensive materials. However, a recent study of in-ditch bioreactors in Illinois found them to have little effect on nutrient concentration (Maxwell et al., 2022). Nitrate removal was negligible, with an average reduction in NO_3-N concentration of -1%. This lack of removal was attributed to low hydraulic connectivity between the bioreactor and the water column both because there was no mechanism forcing water through the bioreactor and also because sediment accumulated on top of the bioreactor. Still, pore water samples from within the bioreactor exhibited signs that the bioreactor was working as intended on the water that it did come into contact with as these samples had lower DO and NO_3-N concentrations than the surface water. The Maxwell et al. (2022) study identifies several limitations of hydraulic connectivity within the in-ditch bioreactor technology which may make it less attractive as a BMP. It is important to keep in mind that part of the reason that in-ditch bioreactors are an attractive option for a BMP is because of their ease of construction and low cost. Modifications that may make them more efficient may also make them more expensive or more intensive to construct.

2.6 Increasing Nutrient Removal with Biochar

Biochar refers to a range of materials that have been formed through the pyrolysis of biomass. Pyrolysis is a thermochemical process which exposes biomass to high temperatures (300-700°C) in the absence of oxygen. Biochar comes from many different sources including corn stalks, rice straw, pine and mixed hardwoods and is useful in a variety of applications such as soil amendment and pollution remediation (Panahi et al., 2020). The addition of biochar to agricultural soils has been linked to increased crop productivity. Biochar not only contains high levels of nutrients, but also increases nutrient retention and cation exchange capacity within the soils (Panahi et al., 2020). Biochar is also used in pollution remediation due to its porous structure and high adsorption capacity. Biochar has the ability to remove both inorganic and organic pollutants in soils and in water.

A study of biochar in laboratory-scale woodchip DNBRs found that adding biochar significantly increased both NO_3^- and P removal (Bock et al., 2015). Phosphorus removal was attributed to both the adsorptive capacity of the biochar and the magnesium and calcium in the biochar which creates insoluble complexes with P. The increase in denitrification leading to increased NO_3^- removal was thought to be due to additional locations for microbial communities provided by the biochar. In another study of biochar as a potential nutrient removal mechanism, biochar was found to leach P at low P concentrations (<2.9 mg/L P) but successfully removed P at higher concentrations (Houston, 2018). Success of biochar in a range of applications relating to nutrient retention and removal warrant its incorporation into technologies that aim to reduce N and P concentrations. Just as biochar can be incorporated into DNBRs, it could also be incorporated into in-ditch bioreactors.

Table 2.1. Summary of nutrient removal percentages from studies of in-ditch bioreactors.

Study	Bioreactor Substrate	NO_3 Reduction	P Reduction
USDA-NRCS (2015)	Woodchip	30%	NR
Pfannerstill et al. (2016)	Woodchip	28%	0%
Pluer (2018)	Woodchip	25%	NR
Liu et al. (2015)[a]	Rice straw	95%	Low
Liu et al. (2015)[a]	Pine sawdust	35%	NR
Liu et al. (2015)[a]	Activated carbon with sand	10%	NR
Maxwell et al. (2022)	Woodchips	-1%	NR

[a]Values of concentration changes for all substrates in the Liu et al. (2015) study were estimated visually from Figure 2 within the paper. NR=Not reported.

Chapter 3. Methods

3.1 Site Description

Nine experimental drainage ditches were constructed at the Virginia Tech Prices Fork Research Farm (PFRF) within a pasture simultaneously being used as sheep pasture (Fig. 1). PFRF is located in Blacksburg, Virginia where the climate is temperate throughout the year with average summer and winter temperatures of 20°C and 0°C, respectively, and an annual rainfall of approximately 100 cm (NOAA Climate Explorer). The dominant soil type in this area is Groseclose silt loam and vegetation is a mix of grasses (Mendez et al., 1999; Williams, 2014). Previous experiments at this study site have focused on plot runoff using rainfall simulators (Habersack, 2002; Soupir et al., 2004; Teany, 2004; Mishra et al., 2006; Williams, 2014). One previous experiment at this site evaluated vegetated filter strips to treat runoff and found them to be effective at trapping pollutants (Mendez et al., 1999).

Three treatments were applied in these nine ditches: control ditches without a bioreactor, ditches with a woodchip only bioreactor, and ditches with a mixed woodchip and biochar bioreactor. An extra ditch was constructed and used for preliminary testing of methods to avoid compromising any of the study ditches. Each experimental run was conducted in an artificial drainage ditch that had not previously had nutrient laden water passed through it. All of the simulated runoff events took place over the course of three days during the October 2021.

3.2 Ditch Construction

The experimental ditches were designed to mimic a short section of an agricultural drainage ditch or grassed waterway. All ditches were constructed to the same dimensions and allowed approximately three months to establish grass along the bottom. Ditch length and width were scaled down by 3/8 from the 2 m wide x 16 m long ditches used in Liu et al. (2015). Dimensions of the ditch were primarily constrained by the 0.75 m width of the dirt scoop used to dig them. A tractor pulling a King Cutter (Winfield, AL) Dirt Scoop was used to dig the ditches (Fig. 2). Scaling the length of the ditch based upon a width of 0.75 m resulted in a ditch length of 6 m. Ditch depth was somewhat difficult to control when using the dirt scoop to dig the ditches, but a target depth of 15 cm was used for all ditches. Ditches were dug parallel to one another along the land contour such that they all had slopes of approximately 10%. Ditch design drawings are included in Figure 3. The distance between each ditch was 1.2 m to allow enough space to use the mower to mow between the ditches.

While in practice most drainage ditches or grassed waterways have sloped sides, flow input was controlled and bank erosion was not a concern in this experiment, therefore the cross section of the ditches was rectangular. The bottom of the ditch was leveled using hand tools and an I-beam level to remove any holes or dips to reduce ponding during flow events. All ditches were seeded with a perennial rye grass covered in a layer of straw (Fig. 4). Rye grass was chosen because it grows quickly and straw was used to protect the grass seeds and hold moisture to help the seeds germinate. Ditches were watered daily for the three months preceding the time of the study. During this time, the pasture was being grazed by sheep. Electric fencing was put around the experimental area to discourage sheep from entering.

3.3 Bioreactor Construction

Bioreactor dimensions and design were informed by previous studies of in-ditch bioreactors (Liu et al., 2015; Pluer, 2018; USDA-NRCS, 2020). The dimensions of the bioreactors were scaled down based on the width, with the width of the bioreactor being equal to the width of the ditch (0.75 m) and the length (1.5 m) chosen such that the bioreactor would cover 25% of the ditch bottom area (Liu et al., 2015). Depth was approximately 10 cm, as was used in Liu et al. (2105) and Pluer (2018). Based on these dimensions, the total volume of carbonaceous material in each bioreactor is 0.1125 m^3 or about 112.5 L.

Woodchips were a mix of hard and soft woods and were sourced from the Virginia Tech Facilities storage pile. The woodchips were dried prior to being put into bioreactor and any noticeable non-woodchip material was removed. Mixed hardwood biochar leftover from a previous experiment was mixed with woodchips at a ratio of 10% by volume. The biochar was sourced from Biochar Now (Berthoud, CO) and was created using a slow (8-12 h) pyrolysis process at 550-600°C (Houston, 2018). In the mixed woodchip and biochar bioreactors, woodchips and biochar were mixed in a large trashcan to evenly coat the woodchips with the biochar before placing them in the mesh.

Design of the bioreactor was informed by the “sock” design where woodchips were contained within a flexible mesh fabric (Pluer, 2018; USDA-NRCS, 2020). The material used in this study was 100% high-density polyethylene (HDPE) garden insect netting from AgFabric

(Corona, CA) which was strong enough to hold the woodchips during moving, flexible enough to easily construct the bioreactors and had a mesh opening that allowed water to pass through but contained the woodchips and larger pieces of biochar. Bioreactors were sealed on the edges using plastic zip ties. When completed and ready for use, each bioreactor was transported to the study site and placed in the ditch 1 m from the outlet (Fig. 5). The bioreactor was adjusted manually such that it was as flush as possible to the ditch bottom, spanned the entire width of the ditch and was the same depth throughout.

3.4 Nutrient Amended Solution

Laboratory chemicals were used to mimic the aqueous forms of common nutrients in agricultural runoff (Tyler et al., 2012; Liu et al., 2015; Moore et al., 2016; Chen et al., 2017; Moore & Locke, 2020). The nutrients of interest in this study were nitrate (NO_3^-), ammonium (NH_4^+) and orthophosphate (PO_4^{-3}). Sodium nitrate (Formula Weight (FW) 84.99 g), ammonium sulfate (FW 132.14 g) and dipotassium phosphate (FW 174.18 g) were mixed with well water from the site to create a solution containing the appropriate concentration of nutrients. The well water was tested for background concentrations of each of these nutrients and results are listed in Table 1. Target concentrations of 20 mg/L of nitrate (4.5 mg/L NO3-N), 5 mg/L of ammonium (3.9 mg/L NH4-N) and 5 mg/L of phosphate (1.6 mg/L P) were used. These concentrations could only be targeted due to the inability to accurately measure the total volume of well water within the influent tank. Influent concentrations were sampled before pumping the mixture into the ditches.

To ease in the creation of the nutrient-amended solution, a 4 L concentrated solution was mixed off site. Total mass of each chemical was calculated based upon the target concentrations and an assumed tank volume of 1230 L. This concentrated solution was poured into the tank of well water at the study site prior to each simulated runoff event. Complete mixing of the solution was accomplished by pumping the mixture through a water pump for 5 min with the end of the hose going back into the tank after which the background sample of influent nutrient concentration was taken.

3.5 Simulated Runoff Flow

Simulated runoff flow was directed into ditches using a tank and pump system. Two plastic 1230 L tanks were strapped onto a trailer so they could be filled with well water located away from the site and brought back to the study location (Fig. 6). Only one tank was used for each simulated runoff event and was emptied of nutrient amended solution before being filled with well water for following floods. An ECHO Water Pump WP-1000 (ECHO Incorporated, Lake Zurich, IL) was attached to the in-use tank for solution mixing and flow production. Hoses ran from the pump to a flow spreading device placed at the top of each experimental ditch (Fig. 4). The flow spreader was a PVC pipe closed on both ends with holes along the surface so that flow coming out of a 2 cm hose was more evenly distributed across the 0.75 m wide ditch. This was done to more accurately represent flow patterns within ditches in the field and to maximize flow interaction with the bioreactor.

The nutrient amended solution was pumped into each ditch at a constant flow rate for enough time to create runoff at the outlet for 32.5 min. Flow rate was determined based upon guidance for compost socks in the North Carolina Erosion and Sediment Control Planning and Design Manual (NC-DEQ, 2013) and scaled down based on the dimensions of the bioreactors used in this study (Table 2). Based upon a bioreactor that is 100 cm thick (diameter) and 1.5 m long, the flow through rate should be 35.6 L/min. This flow rate was outside the range that the water pump could perform and so the highest flow rate that the pump could comfortably maintain for over 30 min, 26.5 L/min, was chosen. This was determined during pre-study tests in the aforementioned test ditch.

3.6 Measuring Flow Rate

Input flowrate was controlled with the ECHO (ECHO Incorporated, Lake Zurich, IL) Water Pump WP-1000 and measured using a RainPoint (Walnut, CA) Water Flow Meter. The flow meter was attached 1 m before the flow spreader along the set of hoses running from the pump to the flow spreader. The length of hose near the flow spreader was always straight throughout the experiment. Readings from the flow meter were in recorded in gallons per minute and were recorded every 5 min at the same time water-quality samples were taken.

Outlet flow rate was measured using a 90° V-notch thin plate weir (Fig. 7). The same weir was used in each ditch and was moved between simulated runoff events. The weir was hammered into the sides and bottom of the ditch to prevent leaking. No vegetation was removed and grass was present all through the ditch and down to the weir. A point gauge was used to measure depth of flow upstream of the weir at the same 5-min intervals used for inlet flow and sampling measurements. During each runoff event, a nappe formed on the downstream side of the weir and ponding occurred behind the weir such that where the point gauge was measuring was a flat surface. The ponding area was small enough to have no effect on runoff flow interaction with the bioreactor. Point gauge measurements were used to calculate discharge using equations for 90° V-notch thin plate weirs as described in section 3.8.2.

3.7 Sample Collection and Analysis

Sampling methodology was based on the National Research Project for Simulated Rainfall - Surface Runoff Studies protocols (SERA-17, 2008). The first sample was taken after 2.5 min of continuous runoff and at every 5-min interval over the course of 32.5 min of continuous runoff. Continuous runoff was defined as when the nappe formed at the weir, thus as soon as the nappe formed the runoff timer began. Samples were collected from the nappe that formed at the outlet weir in acid washed 250 mL HDPE bottles. The experimental plan was to record the time to fill a 250 mL bottle to calculate flow rate: however, the high flow rates made it impossible to accurately record this value so this approach was not used to develop the runoff hydrograph. Each simulated runoff event and associated ditch produced a total of 8 water-quality samples: a sample of the inlet tank water, and samples taken at 2.5, 7.5, 12.5, 17.5, 22.5, 27.5 and 32.5 min of continuous runoff. Samples were stored on ice on-site and transported at the end of each sampling day to the Virginia Tech Biological Systems Engineering Watershed Monitoring Laboratory to be stored in the 4°C cooler.

3.7.1 Sample Analysis for Water Quality Parameters

Samples were removed from the cooler and allowed to reach room temperature for at least 2 h before any further preparation or analysis was conducted. Measurements of pH and conductivity were taken for each sample using an Oakton (Environmental Express, Charleston, SC) 510 Series benchtop meter. Samples were prepared for further analysis by passing 200 mL through a 0.7 µm glass microfiber filter and collecting the filtrate in a new, unused container. All filters were dried in a 105°C oven prior to passing samples through them. Once filtrate was collected, the container was labeled and placed in the freezer. Sample preparation was combined with a test for Total Suspended Solids (TSS) on the raw samples (USEPA, 1971). Residue on the filters was dried overnight in the oven and dry mass measured. TSS was calculated using Equation 1. All samples remained in the freezer until the day of laboratory analysis.

$$\mathrm{TSS\ (mg/L)} = \frac{(\mathrm{A} - \mathrm{B}) * 1000}{\mathrm{C}} \tag{1}$$

where
A = mass of filter + residue, mg
B = mass of filter, mg
C = volume of sample filtered, mL

3.7.2 Sample Analysis for Nutrient Concentration

All analyses were performed in the Virginia Tech Biological Systems Engineering Water Quality Lab using the SEAL AutoAnalyzer3 (SEAL Analytical, Mequon, WI). Samples were taken out of the freezer and thawed the morning of analysis. All samples were manually diluted by a factor of five with Milli-Q water. Samples were analyzed for concentrations of nitrate and nitrite (assumed to be all nitrate), ammonia (assumed to be analogous to ammonium) and orthophosphate. Methods for testing all the nutrients use colorimetric analysis after combining the sample with a specific reagent. Nitrate is first reduced to nitrite using a copper-cadmium reduction column and then all the nitrite reacts with sulfanilamide to form a diazo compound (SEAL Analytical, 2010). This diazo compound then reacts with N-1-naphthylethylenediamine dihydrochloride to form a reddish-purple azo dye and the absorbance is measured. Ammonia reacts with salicylate ions to form a blue-green complex from which absorbance is measured (SEAL Analytical, 2012). Orthophosphate reacts with molybdate and antimony ions and is then reduced with ascorbic acid to form a blue phospho-molybdate complex and absorbance is measured (SEAL Analytical, 2008).

3.8 Calculations and Statistics

3.8.1 Normalized Nutrient Concentrations

This study aims to understand how the nutrient content of runoff water changes as it passes through a vegetated ditch and/or a bioreactor. The difference between the inlet and outlet nutrient content informs whether the ditch or bioreactor increased or decreased the nutrient content in the water. As discussed above, influent nutrient concentrations were targeted for certain values but could not be perfectly controlled due uncertainties in the exact volume of water put into the tank. When analyzing changes in nutrient content across the triplicates of each treatment we focused on comparing the outlet values in reference to the corresponding inlet values for each ditch. To accomplish this, nutrient concentrations in each of the nine ditches were normalized to the influent concentration of the ditch using Equation 2, based on the percent difference equation. Equation 2 leads to seven normalized concentration values that correspond to a specific point in time for each of the nine ditches. These values are between 0 and 1 and indicate how much the concentration has changed at each time from the input concentration. Positive values indicate an increase in concentration and negative values a decrease in concentration.

$$\text{Normalized Concentration} = \frac{C_{Out} - C_{in}}{C_{in}} \tag{2}$$

where
C_{out} = output nutrient concentration at one of t=2.5, 7.5…32.5 min, mg/L
C_{in} = input nutrient concentration, mg/L

After all outlet concentration values were normalized to inlet concentration values for each ditch, the mean values were calculated for each treatment for every sampling time (n=3).

3.8.2 Outlet Flow Rate

The 90° thin plate V-notch weir was intended to be used to get accurate estimates of the outlet flow rate for each ditch. A point gauge was attached to a bar across the top of the weir and measured the depth of water 58 mm upstream of the weir. Before each experimental run, the point gauge was lined up parallel to the V-notch and zeroed out at the point to easily measure the depth of water (in mm) above the V-notch. Unfortunately, the small size of the weir and low flow rates used in this study were well below the limits of the Cone equation commonly used for 90° V-notch weirs (USBR, 2001). The Cone equation (Equation 3) is intended for use with discharges between 85 to 7220 L/min and requires measured depths to be greater than 60 mm and measured at a distance of at least two times the depth upstream of the weir (USBR, 2001). Flow rates used in this experiment were all below 30 L/min, depths around 30 mm and measured at a distance slightly less than two times the depth upstream of the weir. Due to these conditions, the Cone equation was not appropriate to use in estimating flow rate.

$$Q = 2.49h^{2.48} \tag{3}$$

where
Q = discharge, ft^3/s
h = head on weir, ft

Since the Cone equation was not applicable for the weir used in this experiment, alternative weir equations were sought out. Eli (1986) tested two different calibration methods for V-notch weirs: measuring depth at the plane of the weir and measuring the width of flow using a caliper. Using results from measurements taken by several different people, Eli (1986) reported equations for both calibration methods over a range of discharges all with correlation coefficients greater than 0.99. Equation 4 comes from this study and is applicable for the depth of flow measured at the notch for discharges between 0 and 102 L/min. Point gauge measurements taken in mm were used to calculate discharge in m^3/s from Equation 4 and then converted to L/min.

$$Q = 1.0967h^{2.361} \tag{4}$$

where
Q = discharge, m^3/s
h = depth of flow at notch, m

3.8.3 Infiltration Rate Calculations

Mean infiltration rate was calculated using the area of the ditch, the length of the experimental flow event and calculated inflow and outflow. The volume infiltrated was assumed to be the difference between the volume in and volume out of the ditch. Volume was calculated from flow rate assuming that each measurement was representative of a 5-min period, similar to our calculation of load. The time of the experiment was approximately 0.5 h and this was used to calculate how quickly the water infiltrated. The area of the ditch (0.75 x 6 m) was used to get the volume infiltrated in terms of depth such that infiltration was reported in mm/h.

3.8.4 Nutrient Load Removed Calculations

Averaged load removed was calculated for each of the three nutrients of interest in two ways: 1) by calculating load in and out for each ditch and then averaging across the triplicates for each treatment, and 2) using average concentrations and flows calculated from the triplicates to calculate load. In calculating load for both methods, flow rates in and out at each sampling time were assumed to be representative of a 5-min block of time. For example, the flow rate calculated at 2.5 min was multiplied by 5 to get an approximate volume in in the first 5 min, then the flow rate calculated at 7.5 min was multiplied by 5 to get an approximate volume for the next five minutes and so on and so forth. Multiplying each 5-min volume interval by the corresponding nutrient concentration results in a nutrient load in units of mass (mg or g) for that 5-min period. We then used the cumulative sum of those 5-min intervals to estimate total load in and out.

In the first load calculation method, no triplicate averages were taken until the very last step. The inlet nutrient concentration (treated as constant over time) was multiplied by the volumes calculated from flow rates measured at each sampling time but remained relatively constant. Both the outlet concentration and flow rate were measured at each of the seven sampling times and the corresponding concentration measured and volume calculated at each time were multiplied to get total load at the outlet. For each ditch (n=9) and each nutrient (n=3) a total load removed was calculated by subtracting the cumulative load out from the cumulative load in. Finally, the load removed for each nutrient was averaged across the triplicates of each treatment (n=3).

In the second load calculation method, we first averaged 1) nutrient concentration in, 2) nutrient concentration out, 3) flow rate in and 4) flow rate out across the triplicates of each treatment. Because calculating load requires a concentration, the unitless normalized values calculated previously for concentration could not be used. Instead, nutrient concentration in was simply averaged across the three triplicates the concentrations out were averaged across the triplicates at each point in time. Flow rate in and out were also averaged across the three triplicates at each point in time. Again, flow rate at each time interval was multiplied by five to get a volume for the 5-min period bracketing the sampling time. For each nutrient, average concentration in was multiplied by volume in for each time interval and average concentration out was multiplied by volume out for each time interval. Cumulative load in and out was calculated for each nutrient (n=3) and each treatment (n=3), and load removed was calculated by taking the cumulative sum in minus the cumulative sum out for each nutrient and treatment combination.

3.8.4 Statistical Analysis

Using the load removed values from the first calculation method (per ditch), an ANOVA test was run to evaluate statistical differences between load removed across all three treatments. A p-value of 0.05 was used to test for significance. Shapiro and Bartlett tests were run on the data to test for normality and homoscedasticity before performing the ANOVA.

Linear regressions were run between load removed for each of the three nutrients and quasi steady-state flow, infiltration rate and inlet nutrient concentration. Quasi steady-state flow was calculated as the average between the three outlet flow measurements taken at 17.5, 22.5 and 27.5 min as this was determined visually from the plots to be where flow leveled out in most of the ditches.

Figure 3.1. Study site location in Blacksburg, Virginia at Prices Fork Research Farm.


Figure 3.2. Ditch construction at PFRF using dirt scoop.

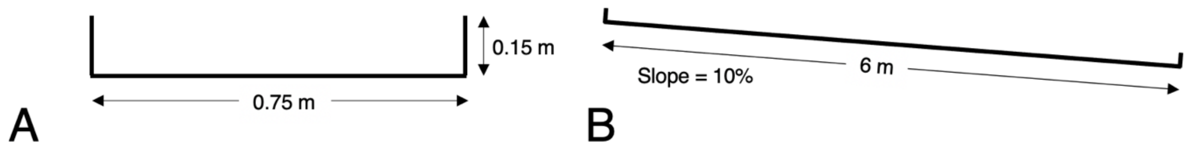

Figure 3.3. (A) Cross-section and (B) longitudinal view of ditch design.

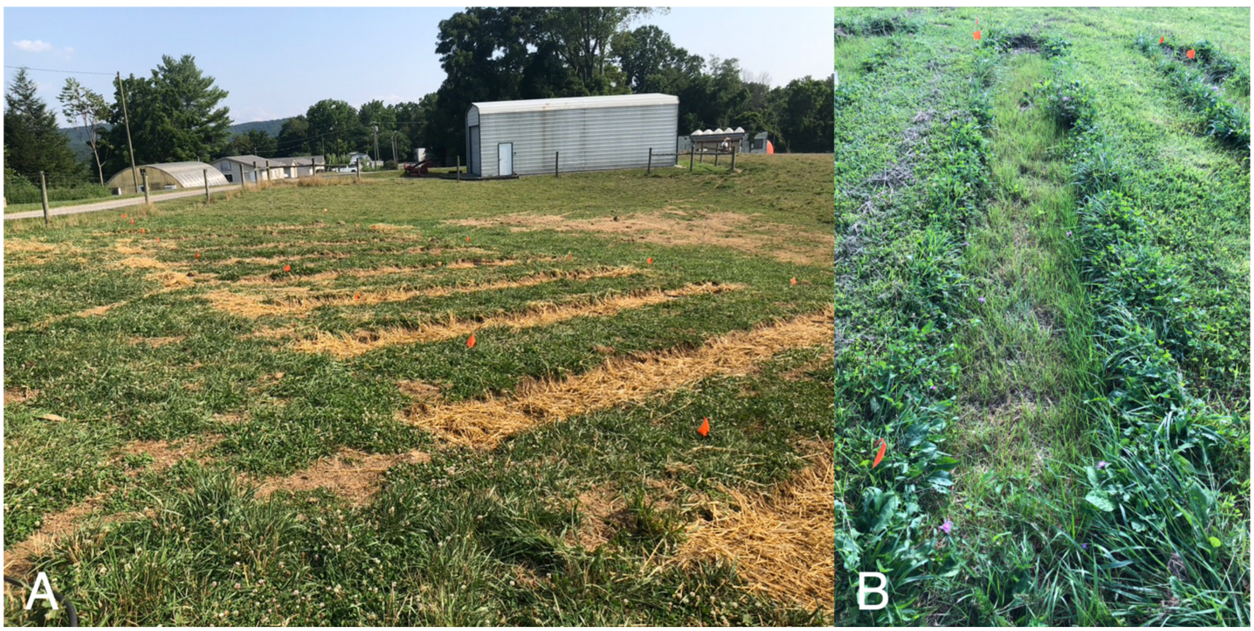

Figure 3.4. (A) Ditches seeded with rye grass and covered with a layer of straw in July 2021. (B) An example of an established ditch just before the simulated runoff events in October 2021.

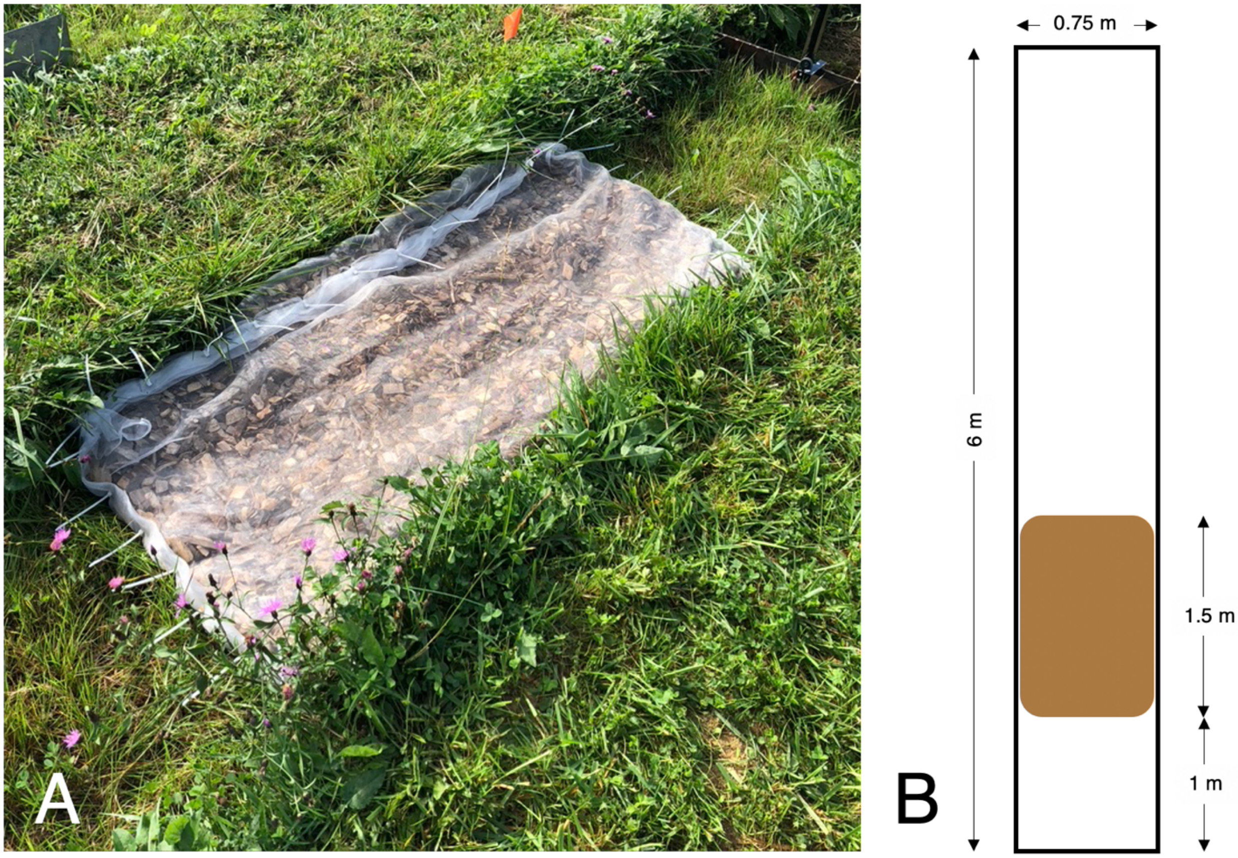

Figure 3.5. (A) Image of a bioreactor in a ditch and (B) a design drawing of bioreactor placement in the ditch.

Figure 3.6. (A) Simulated flow set up shows two tanks, containing the nutrient amended solution, on trailer and an Echo pump. (B) Flow spreader within ditch that was attached to hose from Echo Pump during simulated runoff events.

Figure 3.7. Outlet flow measuring device, including weir and point gauge, during simulated runoff event. Bioreactor is visible behind the point gauge.

Table 3.1. Background nutrient concentrations in well water.

Well Water Sample	Nitrate (mg/L N)	Ammonia (mg/L N)	Phosphate (mg/L P)
First Flush	1.23	<0.01	<0.01
After 5 min	1.68	<0.01	<0.01
After 10 min	1.72	0.02	<0.01

Table 3.2. Compost Sock Initial Flow Rates from NC-DEQ (2013).

Compost Sock Design Diameter	Maximum Slope Length (<2%)	Hydraulic Flow Through Rate
200 mm	183 m	94 L/min/m
300 mm	229 m	141 L/min/m
450 mm	305 m	188 L/min/m
600 mm	396 m	281 L/min/m
800 mm	500 m	374 L/min/m

Chapter 4. Results and Discussion

Each of the nine experimental ditches were randomly assigned to the three treatments: control (CT), woodchip-only bioreactor (WT) or woodchip and biochar bioreactor (BT). A sketch of the experimental ditch setup is provided in Figure 4.1.

4.1 Comparison of Nutrient Concentrations from Ditch Treatments

Samples taken from the inlet tank and at the outlet were analyzed for NO3-N, NH3-N and PO4-P. Inlet concentration was only sampled once and assumed to remain constant throughout each experimental runoff event. Table 4.1 provides the mean and standard deviation of actual inlet concentrations used in the experiment as compared to the target inlet concentrations. For all three nutrients, the mean inlet concentration in the experiment was greater than the target concentration. This is likely because the target concentrations were calculated assuming a tank volume of 1230 L (the total volume of the tank), but in practice the tank was not completely filled to avoid spilling of water during transport from the filling station to the experimental site. The concentration of NO3-N is also higher than the target concentration due to the background levels of NO3-N in the well water which were not accounted for when calculating the target concentration.

All treatments showed decreases in NH3-N concentrations, which appear to be correlated with increases in NO3-N concentration for CT and WT (Fig. 4.2-4.4). Nitrification is a microbially mediated aerobic process that transforms NH3-N to NO3-N and it is likely that this process was occurring in all the treatments. The greater removal of NH3-N from the system is also paired with an increase in NO3-N in the system when comparing CT and WT. This may be due to increases in DOC, which has been shown to increase both nitrification and denitrification (Bernhardt and Likens, 2002; Hassanpour et al., 2017). WT increased NH3-N removal, likely through nitrification, which resulted in an increase in NO3-N greater than what was observed in CT. It seems that nitrification was occurring in all ditches, as seen in the decreases in NH3-N concentration (Fig. 4.2). However, increases in NO3-N reflect a lack of denitrification occurring in the bioreactors due to the inability to adequately saturate the socks and develop anoxic conditions.

While denitrification was probably not occurring in any of the treatments, the addition of biochar in BT did reduce NO3-N concentrations when compared to CT and WT. The decreases in NH3-N concentration for BT are greater than in both CT and WT indicating that biochar may have increased microbial activity. Based on the trend seen between CT and WT (Fig. 4.2), we expected that the greater decrease in NH3-N in BT would be paired with a greater increase in NO3-N. However, the mean NO3-N concentration in BT appears to be decreasing slightly or staying the same as the input concentration (Fig. 4.2). While the addition of biochar in bioreactors has been shown to increase NO3-N removal, the removal was attributed to increased microbial activity prompting more denitrification (Bock et al., 2015). There is low confidence that anoxic conditions were developed in any of the bioreactors in this study. The NO3-N concentrations for BT are highly variable and raw nutrient data do not exhibit this same trend (Fig. 4.5). This observation may only be due to the opposite trends in Ditch 6 and 9 cancelling each other out when the mean is taken across the triplicates. Further experimentation is required to test whether biochar promotes denitrification in bioreactor socks when anoxic conditions can be confirmed.

Phosphorus was highly variable throughout all triplicates and treatments making it somewhat difficult to find trends within the data (Fig. 4.2). Both WT and BT show decreasing P concentrations over time while P remains more constant in the CT. It does seem that in the WT ditches, the woodchips leached phosphorus initially and then began to retain more and more P as time went on. The addition of biochar in BT seems to combat the leaching tendencies of the woodchips and helps to retain P. This result is in line with previous findings that biochar helps to adsorb P and remove it from the water (Bock et al., 2015).

4.2 Comparison of Water Quality Parameters from Ditch Treatments

TSS was highest at the start of the experiment for BT (Fig. 4.6) which corresponded to visual differences between the sample at 2.5 min and 32.5 min (Fig 4.7). The same trend can be seen in WT to a lesser extent. TSS was relatively constant in CT likely because no additional particulate matter was added to the ditch and ditches were allowed to stabilize with rye grass for three months. The bioreactor socks were not pre-rinsed before the experiment likely causing the large spikes in TSS at the beginning of both WT and BT. In particular in BT where the biochar contained many small particles that were carried from the bioreactor to the outlet within the first few minutes of flow passing through the bioreactor. The TSS decreased back to influent values around the halfway point of the experiment (17.5 min). Future studies could explore whether and how much pre-rinsing the bioreactor sock decreases these leaching effects.

Both pH and conductivity remained almost constant over time for both CT and BT treatments. In WT, pH and conductivity remained constant throughout the flow event, but pH increased slightly and conductivity decreased slightly compared to the inlet solution (Fig. 4.6). In both WT and BT, woodchips were added, but the effect on pH and conductivity seen in WT is not present in BT. Because the changes in pH and conductivity are so slight (less than 5%), it is safe to assume that adding a WT or BT bioreactor did not have a significant effect on pH and conductivity.

4.3 Flow Rate Analysis

Inlet flow rate remained relatively constant across all ditches and treatments as expected. Outlet flow rate was calculated from point gauge measurements taken at the outlet weir. The calculation method and equation used are described previously in 3.8.2. Outlet flow rates varied widely between all the ditches, but generally showed a pattern of increasing initially and leveling out around 12.5 min (Fig. 4.8). Outlet flow rates for CT and BT both reach steady values of ~23 L/min in the last half of the experiment. Outlet flow rates for WT were lower with average values staying below 20 L/min. This was unlikely to be an indication that adding a bioreactor to a ditch decreases flow rate because the same effect was not seen in BT. Additionally, one of the WT triplicates (Ditch 2) had a significantly lower outlet flow rate which likely brought down the WT average outlet flow rate.

Infiltration rates varied widely throughout all ditches and treatments and showed no clear pattern associated with location or treatment type (Fig 4.9). There is no infiltration value for Ditch 7 because the volume in and out were approximately equal indicating no loss to infiltration. The soils in the study area are of Hydrologic Soil Group C, which have low infiltration rates of 1.3 – 3.8 mm/h (USDA-SCS, 1985). All of the calculated infiltration rates were well above this expected infiltration rate with most being approximately 50 times greater. This likely indicates that the calculated volume of water infiltrating into the soils was unreasonably large and the calculated outlet flows are underestimates.

4.4 Comparison of Load Removed from Ditch Treatments

The two different calculation methods used to calculate average load removed for each nutrient from each treatment lead to similar values and trends (Fig. 4.10-4.11). It is encouraging that both calculation methods resulted in similar mean values and increases confidence in claims made from these plots despite difficulty in some measurements (namely flow). Across all nutrients, WT resulted in the greatest load removed. Removal from BT was less than in WT, but greater than removal from CT. Removal from WT also showed the greatest variability and was likely impacted by the low outlet flowrate calculated for Ditch 2. When multiplying outlet flow rate by outlet concentration, the low flow would lead to a low load out and show as a large load removed. Error in flow rate measurements may exaggerate the load removal capacity of WT ditches. Flow calculation error also impacted NO3-N and PO4-P load in the CT ditches, specifically Ditch 7. In Ditch 7, the outlet flow rate was greater than the inlet flow rate for much of the experiment which lead to slightly negative load removed values. Mass removed for NO3-N and NH3-N was much greater than PO4-P across all treatments and differences between treatments were larger for these nutrients as well. All the ditches, regardless of treatment, had a greater impact on the removal of N species than they did on P.

To test whether the trends and differences described above were statistically significant, an analysis of variance (ANOVA) was performed for all three nutrients across the three treatments. Data used for this analysis came from load removal values calculated for each ditch, such that n=3 for each of the treatments for each nutrient (Fig. 4.10). Results were considered significant if the p-value was less than 0.05 (Table 4.2). Before performing the ANOVA, tests of normality (Shapiro test) and equal variance (Bartlett test) were performed to make sure that ANOVA was relevant. The null hypothesis for the Shapiro test is that the data are normally distributed thus p-values greater than 0.05 fail to reject this hypothesis. Similarly, the null hypothesis for the Bartlett test is that the variances are equal. Results of these tests suggest that ANOVA is an appropriate test to use to test for statistical difference between treatments on load removed. No statistical difference between treatments was found for any of the nutrients of interest (Table 4.2). While slight differences can be observed in the plots, they are not enough to show that a WT or BT bioreactor statistically increases nutrient removal when compared with an unamended ditch (CT).

The linear regressions run on nutrient load removed data show statistically significant correlations between load removed and quasi stead-steady flow, and load removed and infiltration rate (Table 4.3). This is to be expected as all of these variables are dependent on the calculated flow. The regression between load removed and quasi steady-state has a negative slope, indicating that as flow rate increases load removed decreases (Fig. 4.12-4.14). The slope between infiltration rate and load removed is slightly positive, indicating that increases in infiltration result in increases in load removed. Both of these results highlight the importance of low flow rates within ditches as a method of nutrient removal. There is no statistically significant relationship between inlet nutrient concentration and load removed of that nutrient. This is a positive result that shows that, even though the inlet concentrations were somewhat variable, this variability had no significant effect on load removed results.

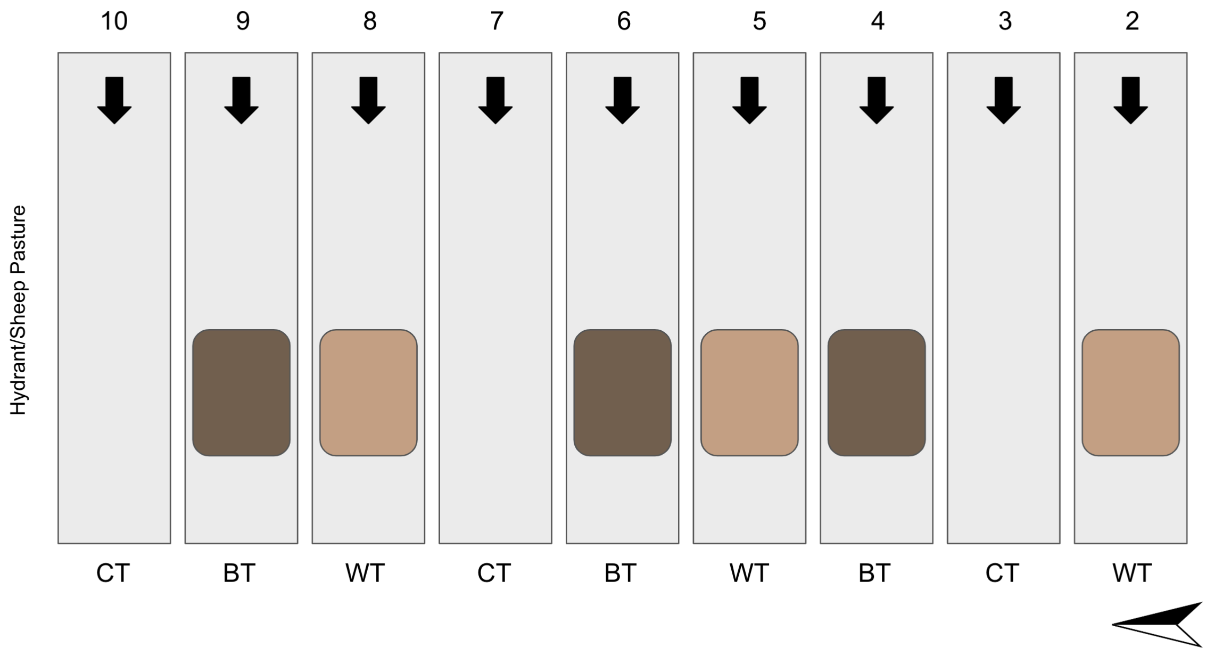

Figure 4.1. Diagram of experimental ditch set-up and treatment location. Brown rectangles represent location of bioreactor socks, black arrows show flow direction.

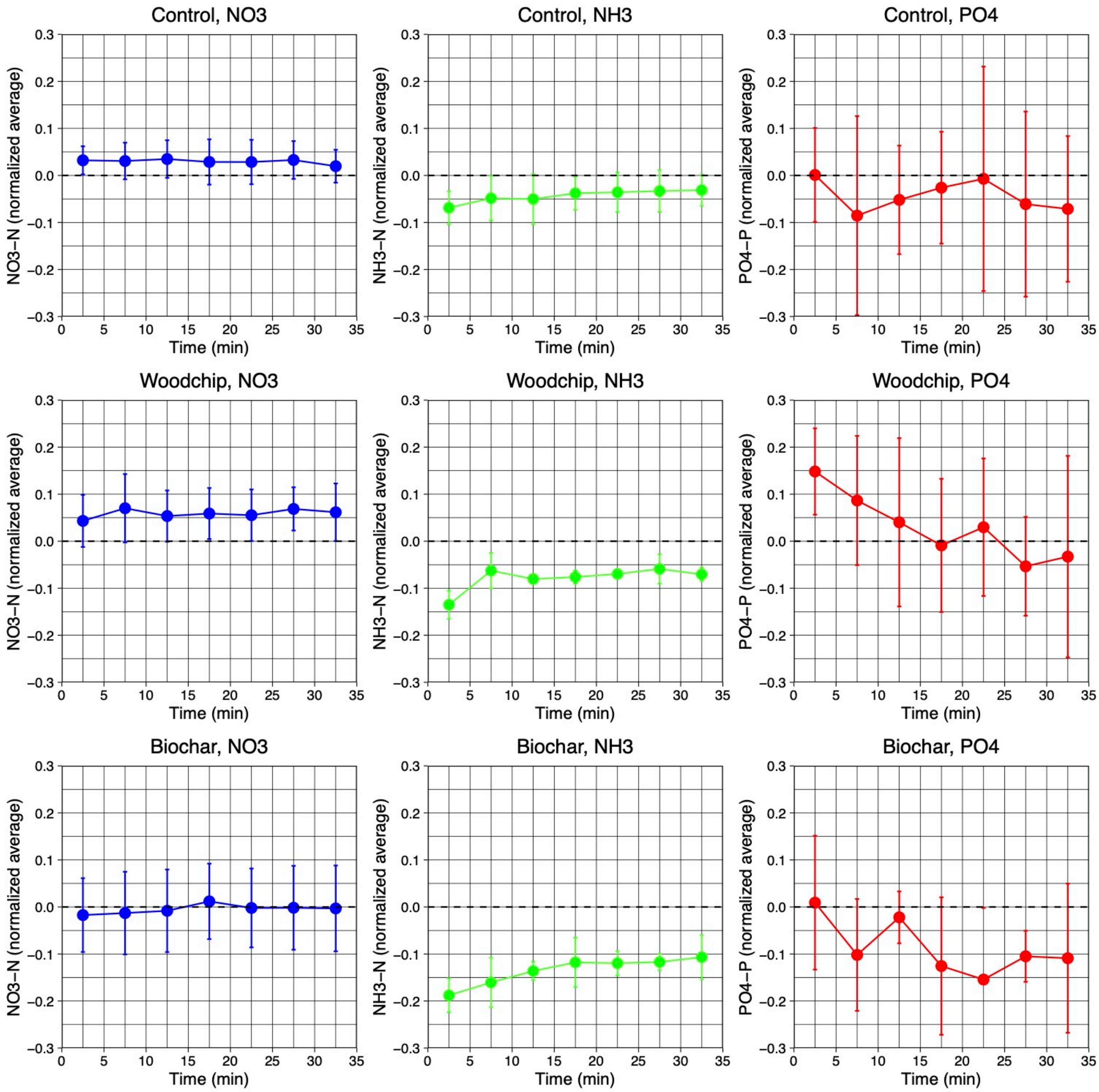

Figure 4.2. Normalized and averaged nutrient concentrations for all treatments.

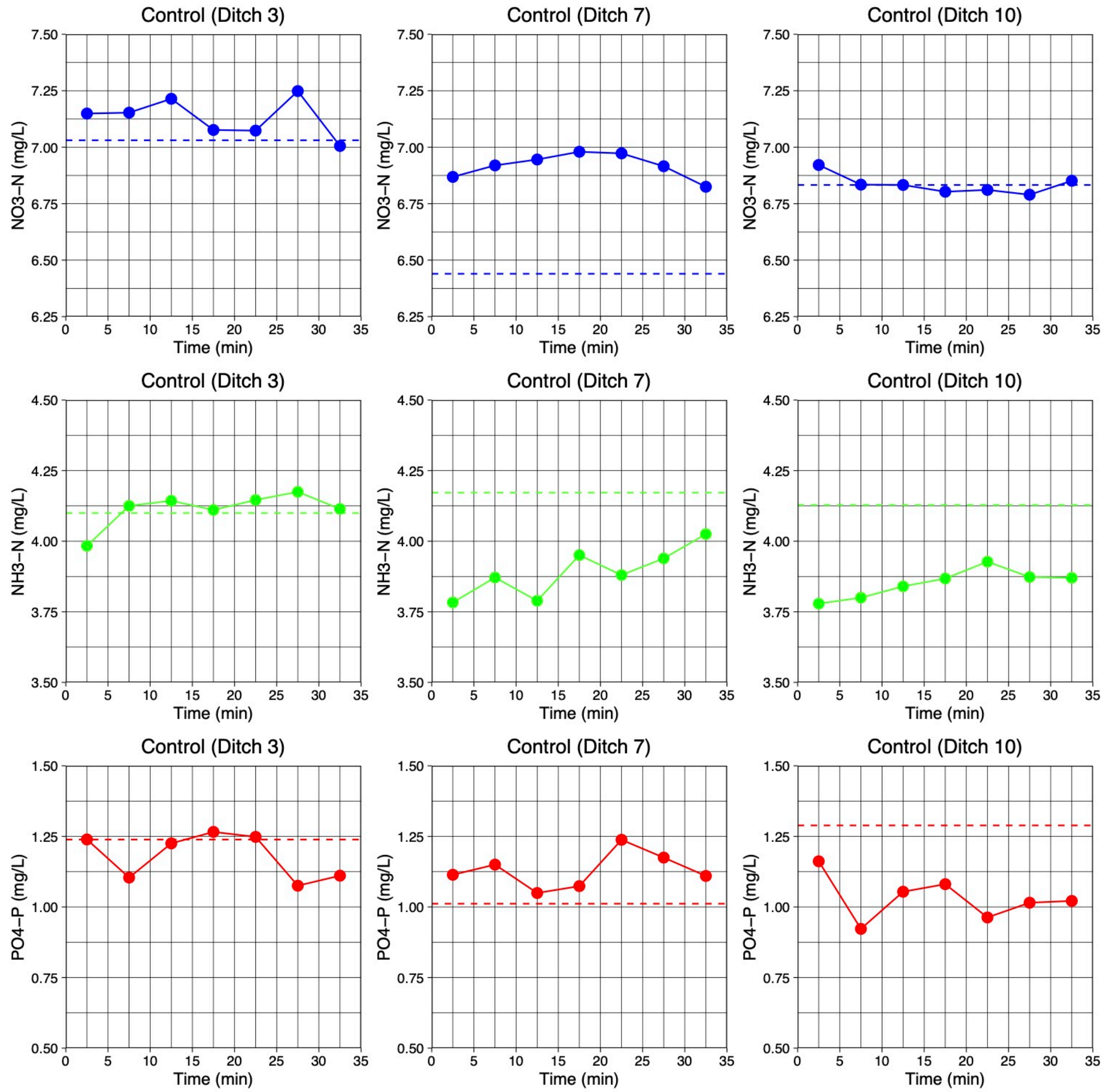

Figure 4.3. Raw nutrient data for control ditches. The dashed horizontal line represents the influent concentration.

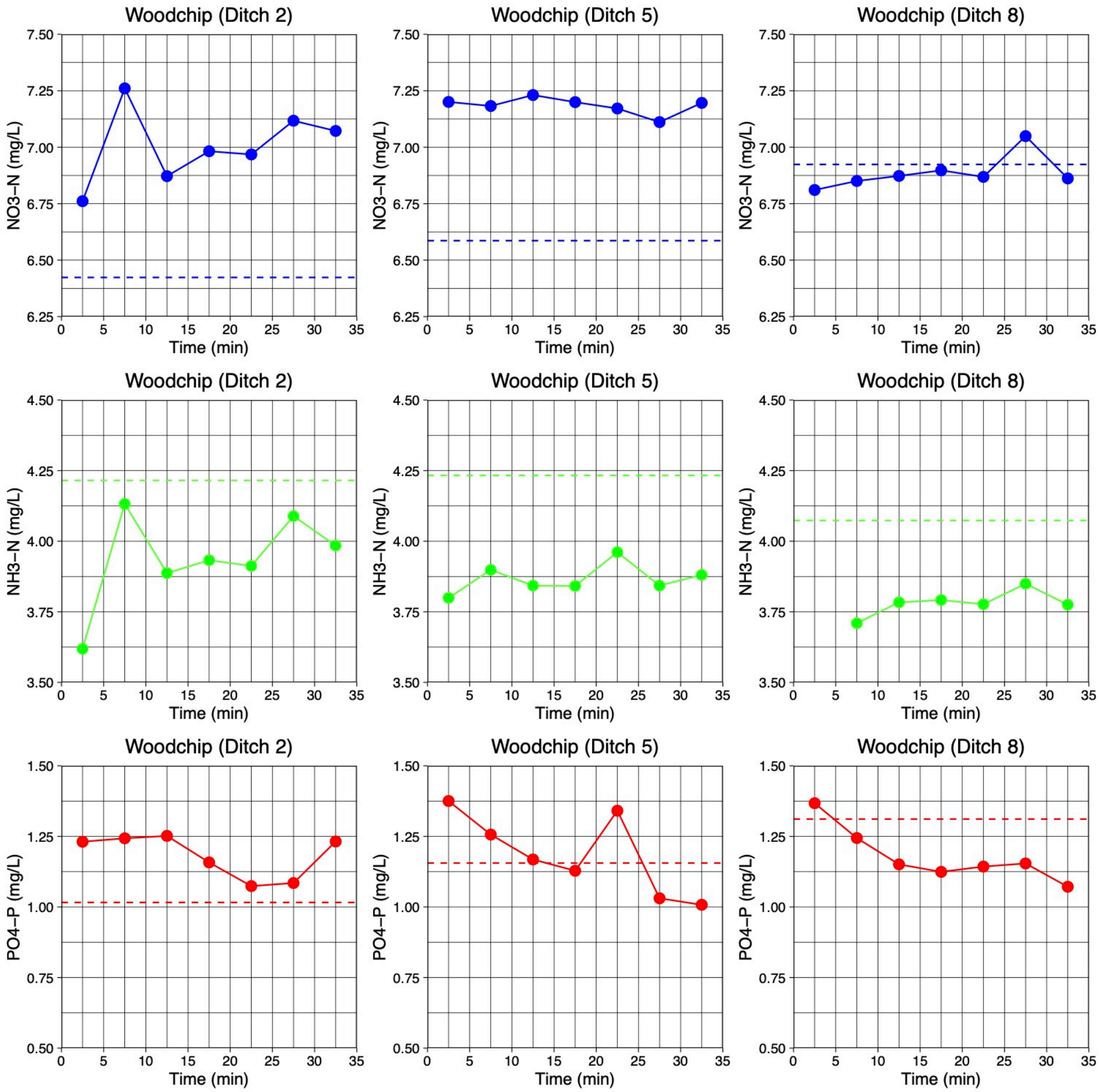

Figure 4.4. Raw concentration data for woodchip bioreactor ditches. The dashed horizontal line represents the influent concentration.

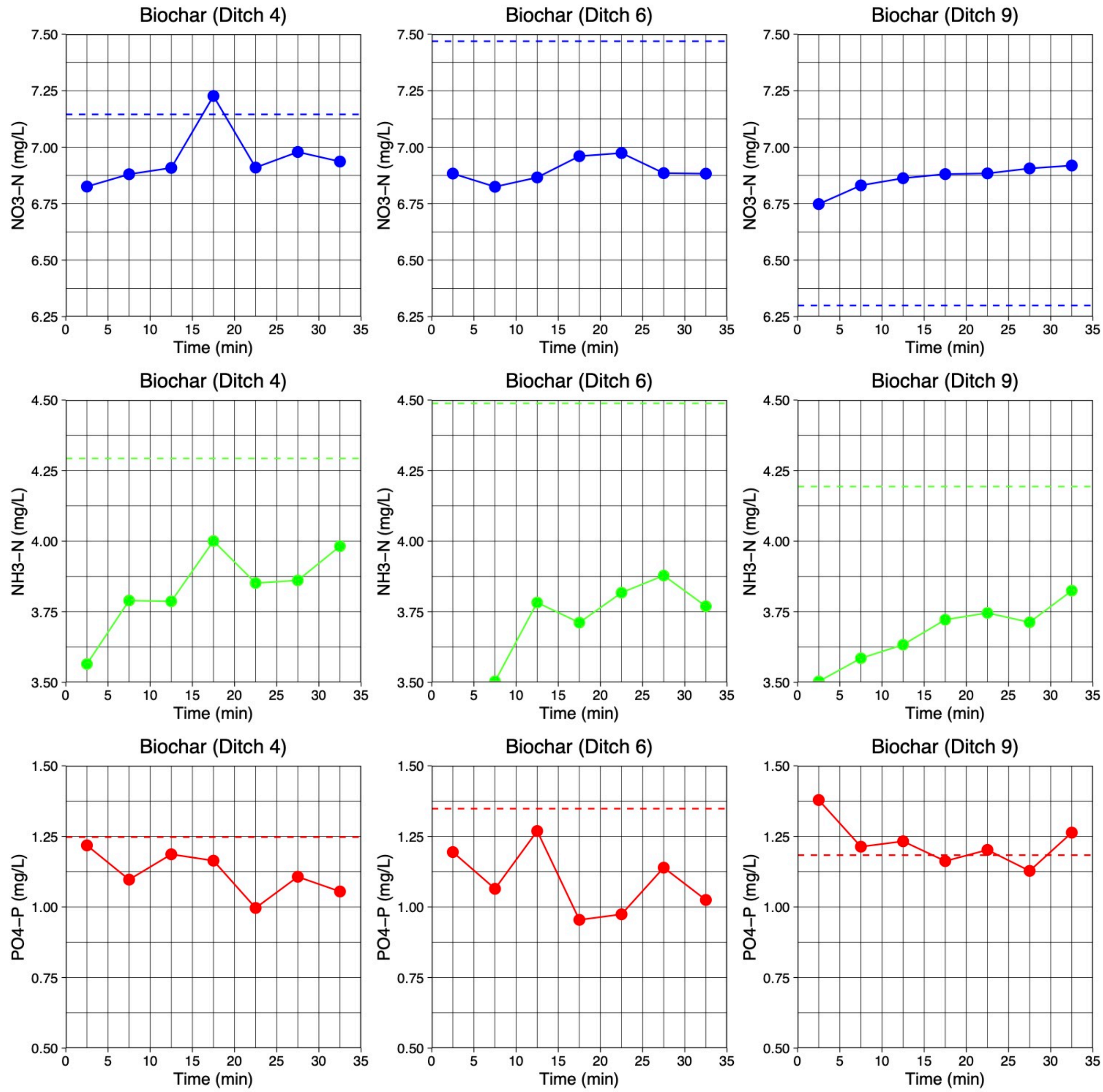

Figure 4.5. Raw nutrient data for woodchip and biochar bioreactor ditches. The dashed horizontal line represents the influent concentration.

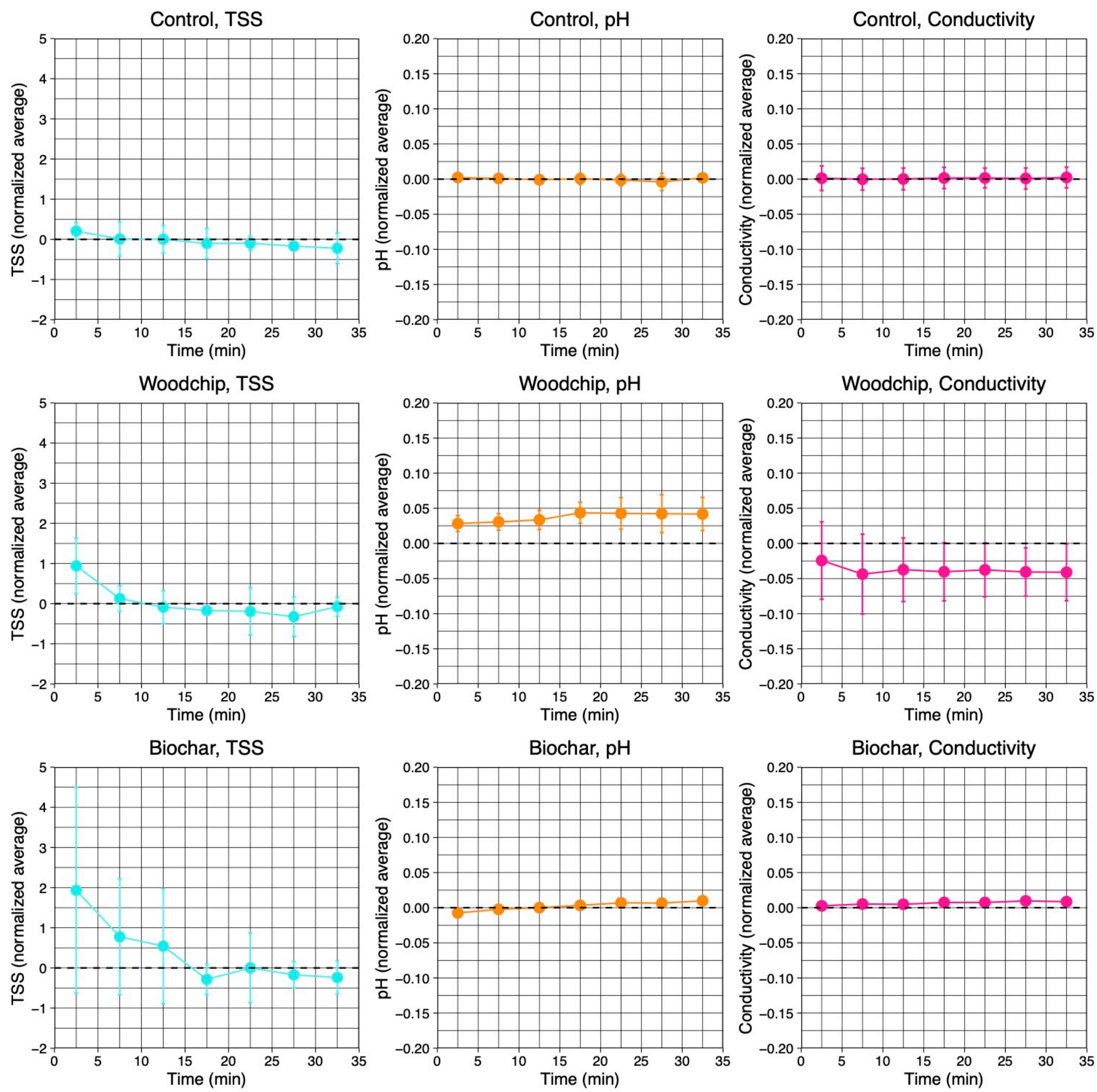

Figure 4.6. Normalized and averaged water quality parameters for all treatments.

Figure 4.7. BT samples prior to filtering. Clear color differences can be seen between samples taken at the beginning versus the end of the experimental run.

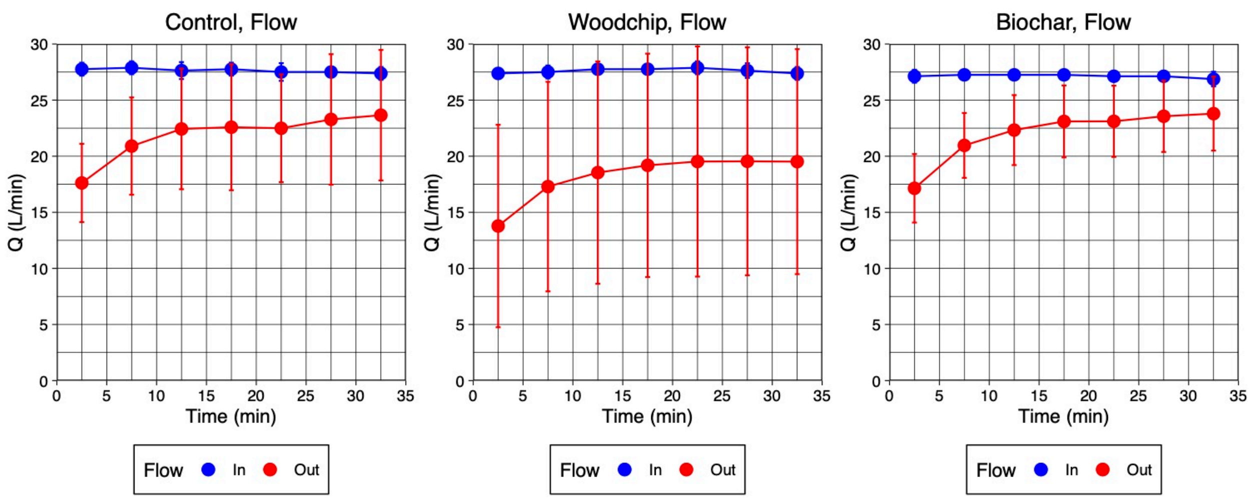

Figure 4.8. Flow rates averaged across triplicates of each treatment.

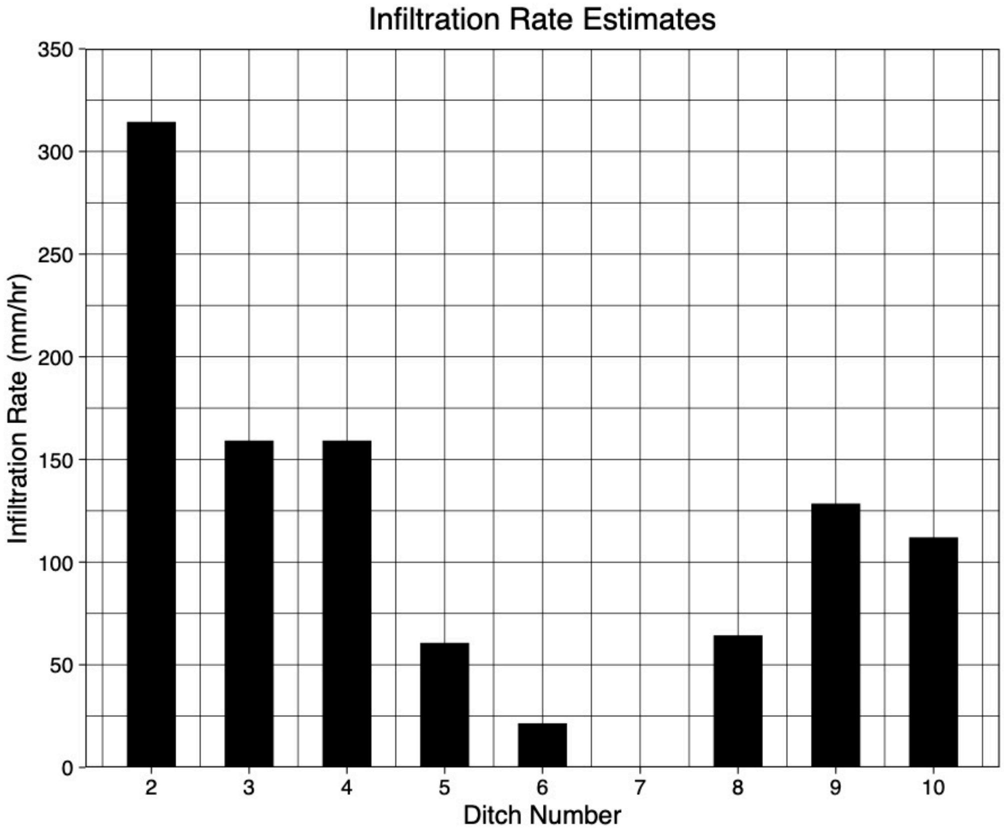

Figure 4.9. Mean infiltration rates calculated for each ditch.

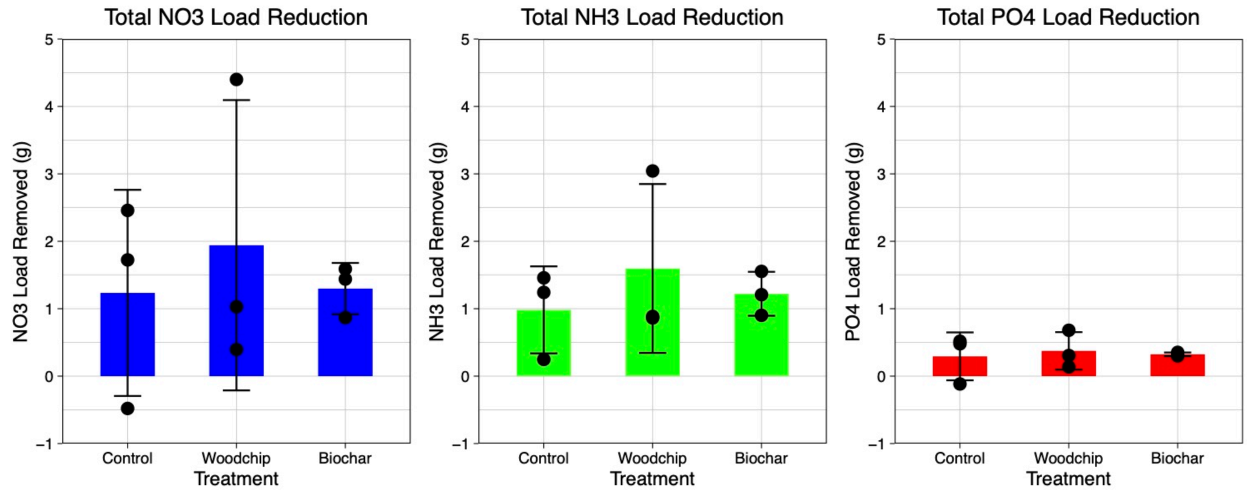

Figure 4.10. Load removed calculated for each ditch and then averaged across treatments. Black dots indicate load removed values for each of the three triplicates for each treatment. Error bar represents one standard deviation above and below the mean.

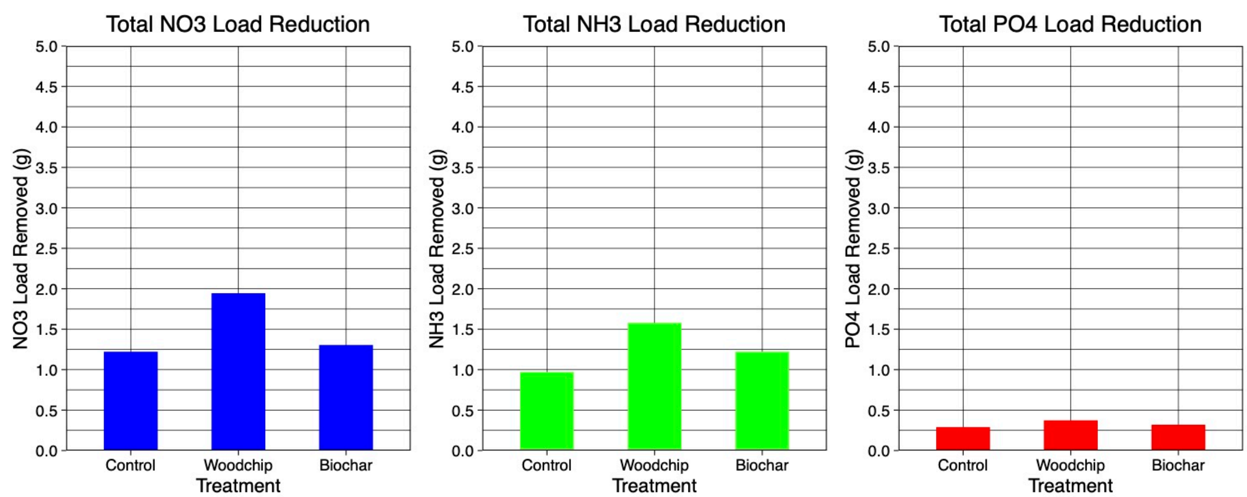

Figure 4.11. Load removed calculated from average nutrient concentrations and flows.

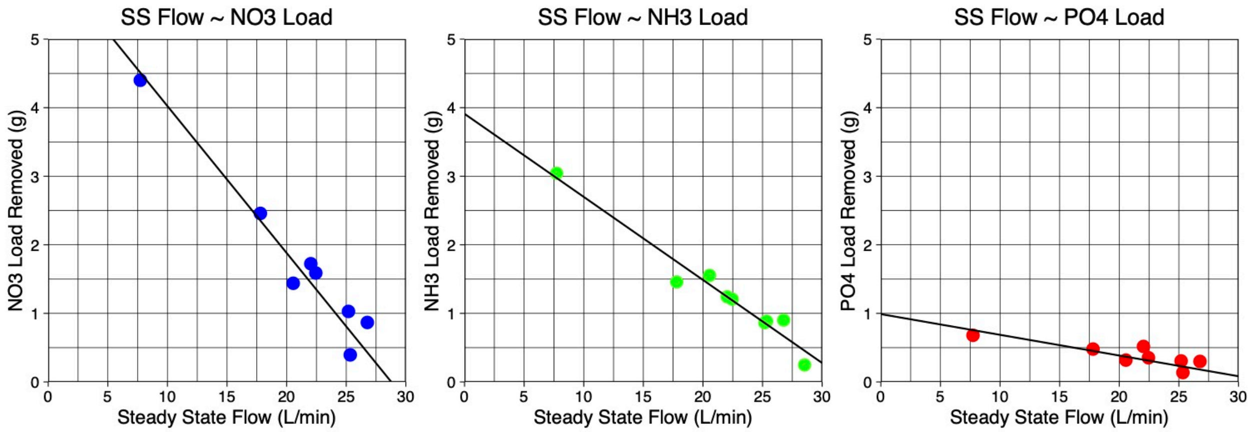

Figure 4.12. Linear regressions between quasi steady-state flow and load removed for each nutrient.

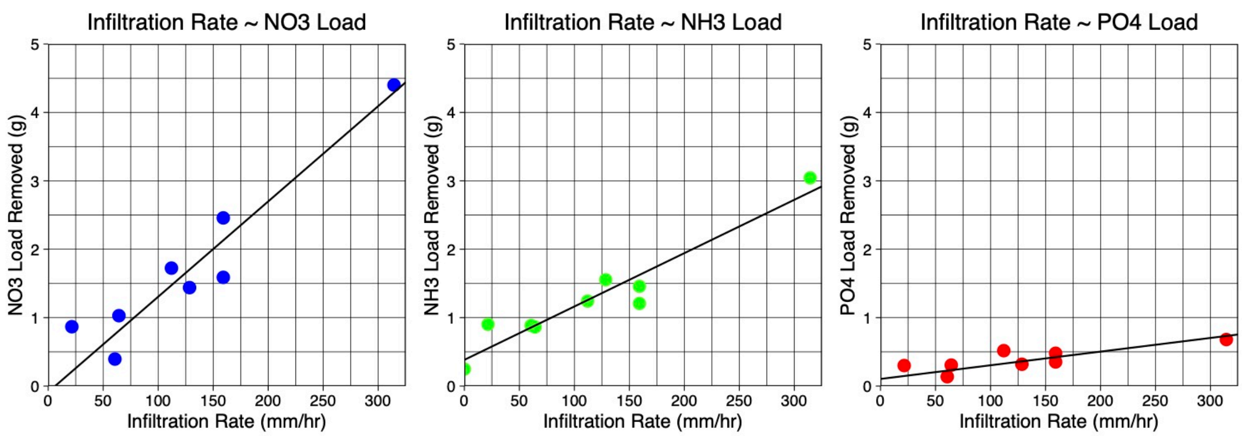

Figure 4.13. Linear regressions between infiltration rate and load removed for each nutrient.

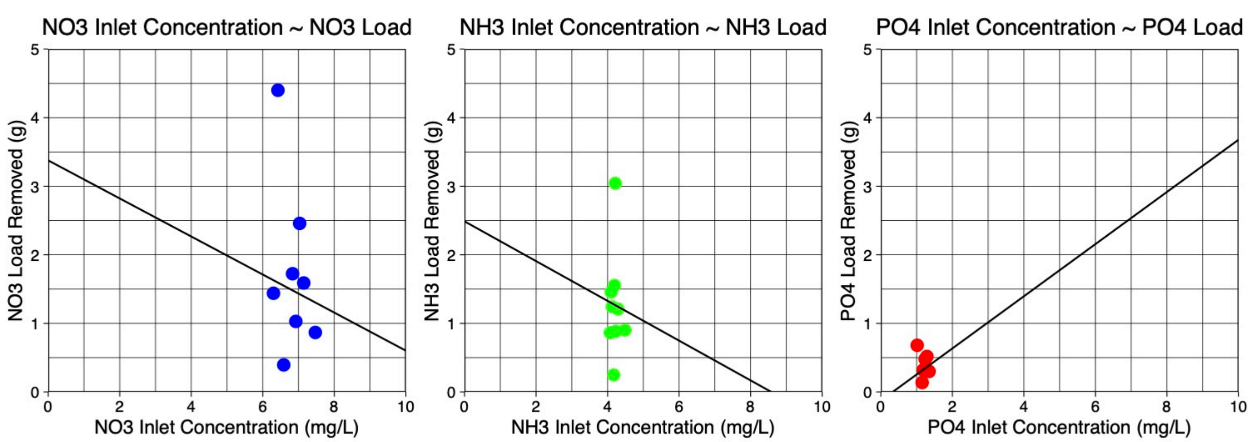

Figure 4.14. Linear regressions between inlet concentration and load removed for each nutrient.

Table 4.1. Inlet concentration summary for all experimental ditches.

Nutrient	Target Inlet Concentration (mg/L)	Mean Inlet Concentration (mg/L)	Standard Deviation
NO3-N	4.5	6.79	0.39
NH3-N	3.9	4.21	0.12
PO4-P	1.6	1.20	0.12

Table 4.2. Results of ANOVA on load removed for each of the three nutrients of interest.

Nutrient	Shapiro Test p-value	Bartlett Test p-value	ANOVA p-value
NO3-N	0.53	0.17	0.83
NH3-N	0.08	0.27	0.68
PO4-P	0.74	0.04	0.93

Table 4.3. Results of linear regression on load removed for each of the three nutrients.

Independent Variable	Dependent Variable	Slope	R^2 Value	p-value
Quasi-SS Flow	NO3 Load Removed	-0.214	0.93	1.64×10^{-5}
Quasi-SS Flow	NH3 Load Removed	-0.121	0.95	5.38×10^{-6}
Quasi-SS Flow	PO4 Load Removed	-0.030	0.63	0.00647
Infiltration Rate	NO3 Load Removed	0.0139	0.90	5.69×10^{-5}
Infiltration Rate	NH3 Load Removed	0.00780	0.90	5.96×10^{-5}
Infiltration Rate	PO4 Load Removed	0.00120	0.64	0.00615
NO3 Inlet Conc.	NO3 Load Removed	-0.280	-0.14	0.841
NH3 Inlet Conc.	NH3 Load Removed	-0.290	-0.14	0.905
PO4 Inlet Conc.	PO4 Load Removed	0.380	-0.096	0.603

Chapter 5. Conclusions and Recommendations

5.1 Conclusions

5.1.1 Hypothesis 1: The addition of in-ditch bioreactors results in greater N and P reductions than in the vegetated ditches alone.

No statistical differences were found for load removed of NO3-N, NH3-N or PO4-P between treatments. Thus, there were no significant increases in N and P reductions as a result of adding in-ditch bioreactors to vegetated ditches. Some differences between treatments were observed in plots of load removed (Fig. 4.10) and normalized nutrient concentration (Fig. 4.2). While these differences in load removed were small, there were some increases in removal in WT and BT across all nutrients when compared to CT. When comparing normalized nutrient concentrations, it appeared that the addition of biochar in BT increased NO3-N removal compared to WT and CT. Phosphorus load removal was slightly increased through the addition of both bioreactor types (WT and BT), but there was a significant amount of variability in P concentrations across the triplicates. There were some decreases in P concentration observed in CT with no bioreactor present, and a large increase in P occurred in WT before any removal began. These slight differences between treatments indicate that there may be a potential for similar technologies to have an effect, but would require additional design adjustments and further testing of replicates.

5.1.2 Hypothesis 2: In-ditch bioreactors containing both woodchips and biochar results in greater P removal than an in-ditch bioreactor with only woodchips.

No statistical difference was found for load removed of PO4-P between WT and BT treatments. The woodchip only bioreactor removed approximately 0.05 g of PO4-P more than the woodchip bioreactor with biochar (Fig. 4.10-11). Comparison of reductions in concentration, showed that WT leached a lot of P at first and then overtime reduced P below the input concentration (Fig. 4.2). However, in BT there was no pattern of leaching present and outlet P concentrations remained below inlet P concentrations throughout the entire experiment. Woodchips were present in both WT and BT, so there was some indication that the addition of biochar cancelled out the effects of the woodchips leaching biochar. Significant variability in P concentrations and lack of a statistical difference between WT and BT PO4-P loads lead to the conclusion that biochar did not increase P removal.

5.2 Recommendations

Recommendations related to the nutrient amended solution include: 1) accurately measure or standardize well water volume in the source tank; and 2) sample nutrient concentrations in the source tank throughout the experiment. While the mass of salt added remained constant, inlet nutrient concentrations were variable between ditches because the volume of well water in the tank was not well controlled or measured. This could have been avoided by using an accurate flow meter to measure volume added to the tank or, a slightly less accurate but simpler method, would be to fill to an existing line or mark a fill line on the tank. Sampling from the tank throughout the experiment would help to more clearly understand nutrient concentration changes through the ditch, ensure that the nutrient amended solution was well mixed, and allow calculating nutrient load removed more accurately. Sampling the inlet mixture would also help in understanding how much variability was in the measurements themselves, since the solution should be of a consistent composition.

Recommendations related to flow rate include: 1) calibrate the weir prior to simulated runoff events by timing how long it takes to collect water in a container of known volume; 2) replicate the weir across all nine ditches instead of using a single movable weir; and 3) pump unamended water through the ditch until steady-state is reached. No tests were done to confirm the accuracy of the weir prior to using it in the experiment, which forced reliance on published equations that may not have been well suited to the weir used in this study. A simple test of the weir could have been done by visually assessing when the flow had reached steady-state and timing how long it took to fill a container of known volume from the weir outlet. Doing this several times and comparing the calculated fill rates to the depth above the weir would provide a simple calibration of the weir and provide a baseline of what the flows should be when comparing flows calculated from published equations. Making nine identical weirs rather than using one and moving it between every experimental flow event would reduce the potential that the weir would be improperly placed. While care was taken to carefully install the weir each time, the installation required the earth and vegetation around the weir to be disturbed almost immediately before the flow event began. There was potential that the weir could be installed at an angle not exactly perpendicular to the flow or that during the flow event the moved soil would change the position of the weir as it became saturated with water. Putting the weirs in place for a few weeks prior to the experimental run would allow the soil and vegetation to settle and make it possible to confirm that the weirs were installed in a consistent manner across all the ditches. Finally, pumping unamended water through the ditches until steady-state conditions were reached and then pumping in the nutrient amended solution would help to reduce effects of flow variation and infiltration and might reduce some of the noise observed in both flow and nutrient concentration measurements. This would require a more complex pump and inlet hose set-up as well as some experimentation to determine a consist time that could be used across all ditches to reach steady-state. While reaching steady-state may help clean-up the results, it would also increase fuel consumption due to pumping, require more well water and trips on the trailer fill the tank and increase the time spent to conduct the experiment

Recommendations related to the bioreactors include: 1) pre-rinsing them with unamended well-water; and 2) adjusting their design to saturate more of the sock. Pre-rinsing the bioreactors would remove some of the excess sediment found on the woodchips. In the bioreactors amended with biochar, rinsing would likely remove some of the small particles of biochar and reduce the large spike in TSS at the beginning of the experimental flow events. Based on observations in the field, it was clear that only the very bottom of the sock was interacting with the water and a large portion of the substrate added to the bioreactors was not inundated with water. This was likely because the sock was highly porous, allowing water to flow right through it and not reducing flow. Creating a pool of water upstream of the sock would force the water to find other flow paths which ideally would have been through the upper portion of the sock. Saturation of the sock could be increased by locating a device below the sock to force pooling or by excavating in the ditch and placing the sock within the ditch bed rather than on top of the ground surface. It is also possible that the steep slope of the ditch reduced the potential for pooling. Design specifications for ditches or grassed waterways have a maximum slope of 4% which is significantly less than the 10% slope used in this study (VA DEQ, 2011). Adding a flow reducing device below the sock, digging the sock into the ditch, or lowering the slope of the ditch could all have helped to saturate the sock and create anoxic conditions within it.

Recommendations related to nutrient and water quality measurements include: 1) measuring total phosphorus (TP) as well as PO4-P; and 2) measuring DO within the ditch and the bioreactor. Measuring TP would provide an estimate of how much PO4-P adsorbed onto sediment particles and whether the high TSS in the biochar bioreactors was correlated with an increase in TP at the outlet. Having a DO measurement would allow more accurate claims to be made about whether or not anoxic conditions were created within the bioreactor sock. With the current observations and results, we can only guess that there was limited denitrification occurring due to a lack of NO3-N removal from the ditch. Adding an additional measurement would allow more robust claims about denitrification to be made.

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