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# Examining Denitrification in Agricultural Ditch Sediments Vegetated with Rice Cutgrass (Leersia oryzoides): Modeling Seasonal Variation Across Increasing Levels of Nitrate Loading and Model Application to Pre-Existing Datasets

Shannon Lee Speir
*University of Arkansas, Fayetteville*

Examining Denitrification in Agricultural Ditch Sediments Vegetated with Rice Cutgrass
(*Leersia oryzoides*): Modeling Seasonal Variation Across Increasing Levels of Nitrate Loading
and Model Application to Pre-Existing Datasets


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Shannon L. Speir
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_____
J. Thad Scott
Thesis Director


_____                    _____
Jason M. Taylor                                     Lisa S. Wood
Committee Member                                    Committee Member


_____
Mike B. Daniels
Committee Member

**ABSTRACT**

Nitrogen (N) derived from fertilizer application in agricultural systems may contribute to significant environmental impacts, including eutrophication of fresh and coastal waters. Rice cutgrass (*Leersia oryzoides*) can significantly enhance denitrification potential in agricultural ditch sediments, but relationships with known drivers are not well understood. To address this, I examined effects of nitrate ($NO_3^-$) availability on dinitrogen gas ($N_2$) and $NO_3^-$ fluxes seasonally in Chapter 2. Denitrification rates were measured as $N_2$ flux from intact vegetated sediment cores using Membrane Inlet Mass Spectrometry (MIMS). Michaelis-Menten models were developed from observations to mathematically describe $N_2$ fluxes across the spring, summer, and fall seasons. Summer $N_2$ models exhibited the highest $V_{max}$ and $K$, with $N_2$ fluxes peaking near 20 mg $m^{-2}$ $h^{-1}$. In all seasons, percent $NO_3^-$ retention peaked at 1 mg $L^{-1}$, before decreasing with increasing $NO_3^-$ concentrations, except summer where maximum retention was maintained from 1-5 mg $L^{-1}$ before declining at higher concentrations. Denitrification rates were strongly correlated with $NO_3^-$ uptake rates by vegetated sediments in spring ($r^2 = 0.94$; $p < 0.0001$) and summer ($r^2 = 0.97$; $p < 0.0001$), but low $NO_3^-$ uptake resulted in virtually no net denitrification in fall and winter. Sediments vegetated with cutgrass immobilized a significant fraction of $NO_3^-$ entering them and permanently removed up to 30-40% of immobilized $NO_3^-$ through denitrification during the growing season. I then applied models developed in Chapter 2 to existing datasets from experiments conducted at two different scales: mesocosms and experimental ditches (Chapter 3). Both models estimated similar peaks in net $N_2$ fluxes from mesocosm data. Additionally, estimates of areal $N_2$ production from the mesocosm study were similar to those predicted via mass balance in a previous study. Model application to the experimental ditch study highlighted differences between weired and non-weired ditches;

however, estimates from linear regression model did not reflect trends previously reported in the literature. Further exploration into model application is necessary to determine the utility of both models, but both models may be useful in informing more complex models of N movement in agricultural watersheds to help land managers quantify the benefits of BMP implementation.

**ACKNOWLEDGEMENTS**

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## 1. INTRODUCTION

Humans increasingly intervene with natural ecosystem processes via current agricultural practices and urban expansion. Such practices compromise the quality of water bodies across the globe on the scale of the world's largest river basins to the smallest coastal watersheds (Turner and Rabalais, 2003). Many environmental threats, such as climate change, biodiversity loss, and degradation of land and freshwater, result from practices associated with human intervention, especially the growing demand for inexpensive and efficiently-produced agricultural products (Foley et al., 2011). Specifically, nitrogen (N) from crop fertilization is of major concern. Between 1800 and 2011, the world's population increased seven-fold (Lee, 2011), which has greatly increased the demand for agricultural products, especially food, across the globe. Nitrogen fertilizers produced industrially via the Haber-Bosch process will be increasingly relied upon for increased agriculture production, with global agricultural demand for industrial N fixation expected to reach up to 172 Tg N $yr^{-1}$ by 2100, approximately twice the fixation rate for 2000 (Winiwarter et al., 2013).

Fertilizers are sometimes, if not often, over-applied to fields (Prakasa Rao and Puttanna, 2000) and move easily from cropland into our waterways, resulting in the degradation of downstream ecosystems (Galloway et al., 2008). A far-reaching consequence of the over-application of fertilizer is eutrophication, the over-enrichment of aquatic ecosystems with nutrients or organic matter (Carpenter et al., 2011). Excessive inputs of nutrients from agricultural sources and associated eutrophication is one of the most common impairment of surface waters in the United States (U.S. EPA, 1990; Carpenter and Caraco, 1998). One major impact of eutrophication on our waterways is harmful algal blooms (HABs; Glibert et al. 2014). Harmful algal blooms can lead to hypoxia and anoxia (low to no dissolved oxygen, respectively),

summer fish kills, foul odors, and unpalatable drinking water, as well as the formation of carcinogenic trihalomethanes during water chlorination in treatment plants (Carpenter and Caraco, 1998). Harmful algal blooms are especially prevalent in the Gulf of Mexico due to high levels of nutrients draining into the Mississippi River from the USA's agricultural regions (Alexander et al., 2008). In the Gulf of Mexico, an increased occurrence of seasonal hypoxia has been attributed to the rise in riverine N and phosphorus (P) flux over the past few decades (Alexander et al., 2008) though the observed declines in dissolved oxygen have lagged ~10 years behind the increased use of fertilizers (Diaz and Rosenberg, 2008). The senescence of HABs can exacerbate seasonal hypoxia along the coast of Louisiana and Texas (Glibert et al., 2014). In the Gulf of Mexico, nutrient loading can also be indirectly connected to increased turbidity, loss of habitat, decreased marine biodiversity, and alterations in ecosystem structure and function (Rabalais et al., 2002). Anthropogenically-driven environmental changes, especially those related to intensive agriculture, are quickly driving the environment beyond its "planetary boundaries" (Rokstrom et al., 2009), highlighting the critical need for advances in best management practices (BMPs) that can reduce nutrient transport via runoff and leaching to imperiled ecosystems and combat this extensive environmental issue.

## 1.1 Nitrogen Movement in Watersheds

### 1.1.1 *Major N Forms and Mobility*

Nitrogen is a unique element in that it is found in diverse forms throughout the biosphere and can have cascading effects within an ecosystem (Galloway, 1998). Nitrogen gas ($N_2$) makes up approximately 78% of the atmosphere (Schlesinger and Bernhardt, 2013); however, it is biologically unavailable and must be transformed into a reactive form prior to biological assimilation.  Nitrogen gas is converted to more reactive N forms via biological N fixation

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(BNF) to create ammonia ($NH_3$), which can readily be transformed and utilized by the biota in both terrestrial and aquatic ecosystems. In most aquatic ecosystems, $NH_3$ is found in its ionized form, ammonium ($NH_4^+$). The balance between $NH_3$ and $NH_4^+$ in aquatic ecosystems is largely determined by pH; $NH_4^+$ predominates when pH is below 8 (Suzuki and Kwok, 1974). Due to the difficulty of breaking the N-N triple bond in an $N_2$ atom, only microbes possessing the enzyme nitrogenase can carry out BNF in nature (Howarth et al., 1988). Reactive N ($N_r$) can also be supplied to aquatic and terrestrial environments by anthropogenic sources such as fossil fuel combustion and industrial N fixation via the Haber-Bosch process (Glibert et al., 2014). The $NH_3$ produced via industrial N fixation enters the biosphere through fertilizer application, where it can undergo transformations into other N forms. Once in the landscape, biologically available N can then be immobilized by the biota, stored in organic matter, and transferred to higher trophic levels.

Nitrogen incorporated into biomass via assimilation can undergo mineralization via two processes: regeneration and ammonification. Regeneration is the mineralization of N found in detrital proteins to $NH_4^+$ by bacteria, fungi, and other organisms (Kirchman, 2012). Ammonification includes all the reactions that produce $NH_4^+$ from other detrital organic nitrogenous compounds (Kirchman, 2012); however, these reactions are not as well understood as regeneration. Nitrogen mineralization can be influenced by temperature (MacDonald et al., 1995; Rustad et al., 2001), soil moisture (Pastor and Post, 1986; Sierra, 1997), and oxygen concentrations (Updegraff et al., 1995; Bridgham et al., 1998).

Ammonium serves as the substrate for nitrification, a two-step process carried out by chemolithotrophic bacteria (Zumft, 1997). First, $NH_4^+$ is oxidized to nitrite ($NO_2^-$; Cavari, 1977). This transformation is usually considered the rate limiting step for nitrification (Kirchman 2012).

Then the $NO_2^-$ is then converted to nitrate ($NO_3^-$; Cavari, 1977). The two main steps of nitrification were previously thought to be carried out by only two groups of bacteria: *Nitrosomonas* and *Nitrobacter*, respectively (Schlesinger and Bernhardt, 2013). More recently, nitrifiers have been identified as a much more diverse group of microorganisms than previously thought (Koops and Pommerening-Röser, 2001; Könneke et al., 2005; Hayatsu et al., 2008). The conversion of $NH_4^+$ to $NO_3^-$ can become an issue in agricultural systems as $NO_3^-$ is a particularly mobile species (Turner and Rabalais, 2003). For example, the ecological processes that keep $NO_3^-$ bound in the soil and organic matter may be altered if the soil is sufficiently disturbed by farming practices, causing stored $NO_3^-$ to be released (Turner and Rabalais, 2003). Once released, $NO_3^-$ travels readily through the soil carried by shallow, subsurface flow or in deeper groundwater into nearby waterways (Lowrance, 1992).

### 1.1.2  *Nitrate Processing in Aquatic Environments*

Nitrate can undergo three microbially-mediated transformations in aquatic environments: denitrification (DNF), dissimilatory $NO_3^-$ reduction to ammonium (DNRA), and anaerobic ammonium oxidation (anammox). Denitrification is carried out by heterotrophic, facultatively anaerobic bacteria that utilize organic carbon (C) as an electron donor and $NO_2^-$ or $NO_3^-$ as a terminal electron acceptor to produce $N_2$ gas under reducing conditions (Payne, 1973; Seitzinger, 1988). The basic reaction proceeds as follows:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$
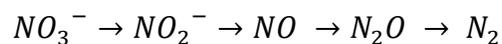
Denitrification rates are controlled by a variety of environmental variables, such as oxygen concentrations, the quality and quantity of organic C available, HRT, and the availability of $NO_3^-$ as a substrate. It is often called a "leaky" process as DNF does not always go to

completion, releasing nitric oxide (NO) or nitrous oxide ($N_2O$) into the environment. Nitrous oxide is a potent greenhouse gas, and stream and river DNF may contribute up to 10% of the global anthropogenic $N_2O$ emission rate (Beaulieu et al., 2011). Denitrification can occur coupled to nitrification, where $NO_3^-$ produced via nitrification acts as the substrate for DNF (Kirchman, 2012). When DNF goes to completion, it is considered a permanent removal mechanism for $NO_3^-$ in aquatic ecosystems because $N_2$ gas is an unreactive compound and readily diffuses back into the atmosphere.

Microbes use $NO_3^-$ to carry out DNRA, producing $NH_4^+$ as the final product (Burgin and Hamilton, 2007). Dissimilatory $NO_3^-$ reduction to ammonium is a catabolic process meaning DNRA generates energy for the bacteria rather than generating biomass. The end product is more biologically available and may be incorporated into biomass or converted back to $NO_3^-$ via nitrification. DNRA can either be linked to a fermentative pathway (Tiedje, 1988) or coupled to sulfur oxidation (Brunet and Garcia-Gil, 1996). Anammox is a process discovered relatively recently (1990's) that produces $N_2$ gas via the combination of $NH_4^+$ and $NO_2^-$. It is a chemolithoautotrophic transformation (Burgin and Hamilton, 2007), meaning it is carried out by autotrophic microorganisms that obtain energy by oxidizing inorganic compounds. The process must occur under anaerobic conditions with an ample supply of $NH_4^+$ and $NO_2^-$. Annamox can be inhibited by simple organic compounds, including pyruvate, ethanol, and glucose (Jetten et al., 1998). The $NO_2^-$ needed for anammox can potentially be derived from reduction of $NO_3^-$ by denitrifiers.

### 1.1.3   *Controls on Denitrification in Freshwater Environments*

In stream ecosystems, many physical variables and characteristics can affect the rates and efficiency of DNF.  As mentioned previously, denitrifying bacteria are facultative anaerobes,

meaning they only denitrify at low oxygen concentrations. In both marine and freshwater ecosystems, an oxygen concentration less than ~0.2 mg $L^{-1}$ is required for DNF to occur in water and sediments (Seitzinger, 1988). Denitrification can take place in reduced microzones in the aerobic surface layer of the sediments (Sorensen, 1978). These anoxic microzones allow DNF to be coupled to nitrification, an aerobic process. Nitrification may provide the substrate ($NO_3^-$) for DNF when these processes are separated vertically in the water column or sediments as a result of their different oxygen requirements (Billen, 1978). Therefore, a high availability of anoxic zones promotes high rates of DNF across varying substrata in lotic systems (Kemp and Dodds, 2002).

Temperature affects DNF rates. In general, increasing temperatures correspond to increasing DNF rates (Seitzinger, 1988). However, biological N removal, including $NO_3^-$, was found to be most efficient between 20°C to 25°C in wetlands (Spieles and Mitsch, 2000). A decrease in temperature from 22°C to 4°C resulted in a 77% decrease in potential DNF rates, suggesting lower temperatures may effectively suppress DNF and leave higher concentrations of $NO_3^-$ in the overlying water (Pfenning and Mcmahon, 1996). Additionally, studies have shown the lowest DNF enzyme activity occurs in the winter at temperatures below 11°C (Richardson et al., 2004). One study reported the highest DNF rates at winter temperatures in reservoir systems (Grantz et al., 2012); however, a cross-system meta-analysis of seasonal DNF rates showed the highest DNF rates generally occur in the warm summer months in aquatic ecosystems including rivers, lakes, coastal ecosystems, and estuaries (Piña-Ochoa and Álvarez-Cobelas, 2006). In temperate locales with a high degree of seasonality, temperature fluctuations throughout the year may play a significant role in regulating $NO_3^-$ removal from streams and rivers via DNF.

The concentration of $NO_3^-$ in the water above the sediments affects DNF in lotic systems. Bernot and Dodds (2005) found the most retention at low levels of N loading, while at moderate N loading levels, the capacity for DNF can become saturated and DNF rates will level off. However, contrary to these findings, Inwood et al. (2007) found a linear relationship between water $NO_3^-$ concentration (up to ~ 5 mg $L^{-1}$) and sediment DNF rates. When examining the effects of $NO_3^-$ loading across the USA, Mulholland et al. (2008) also found that as $NO_3^-$ loading in streams increased, the DNF rates also increased. Additionally, $NO_3^-$ uptake rates increased with increases in $NO_3^-$ in the overlying water (Dodds et al., 2002). It is also important to note that as the level of $NO_3^-$ loading increases, the efficiency of DNF, or the percent of $NO_3^-$ in the overlying water that is converted to $N_2$, decreases (Mulholland et al., 2008, 2009). This suggests that downstream N export will increase with more $NO_3^-$ in the water, especially when chronic N loading occurs (Bernot and Dodds, 2005). Thus, the availability of $NO_3^-$ has the potential to influence DNF rates and efficiency, especially at high levels of N loading.

Many studies have found that the quality and quantity of organic C can exert control over DNF rates. Carbon serves as the electron donor in DNF; therefore, C supply can influence denitrifying bacteria directly by providing energy (Pfenning and Mcmahon 1996) and indirectly through the consumption of $O_2$ by heterotrophic microbes, creating the ideal anaerobic conditions required for denitrifiers to convert $NO_3^-$ to $N_2$ (Hanson et al., 1994; Hill et al., 2000). Additionally, as the C:N ratio of an ecosystem's compartments increases, N turnover rates are greater (Dodds et al., 2004). Nitrogen processing was also found to be significantly influenced by particulate organic C (POC; Stelzer et al., 2014). POC exerts control over the redox conditions in sediments by affecting biological $O_2$ demand (Duff et al., 2007). Dissolved organic C (DOC) limitation can influence DNF in sediments or the water column (Seitzinger, 1988;

Groffman et al., 2002). An inverse relationship exists between oxidized forms of N ($NO_3^-$ and $N_2O$) and DOC, suggesting that oxidized N forms may only accumulate in areas with low quantities of DOC (Hedin et al., 1998), and the availability of ample DOC can stimulate DNF (Martin et al., 2001).

Finally, stream geomorphology, including width and channel depth, control the retention of $NO_3^-$ in streams (Royer et al., 2004). Smaller streams are often shallower, narrower, and have a longer hydraulic residence time (HRT), allowing the water carrying excess nutrients to have more contact with the substrata (Ranalli and Macalady, 2010). The HRT of a stream governs the exposure time of stream water N to microbial processing via DNF, and allows for the settling of particulate organic N and $NO_3^-$ diffusion to the benthic sediment (Alexander et al., 2000). As stream order increases, there is a sharp decline in likelihood of $NO_3^-$ being transformed by denitrifying bacteria (Howarth et al., 1996). The increase in both velocity and depth of the water with increased stream order results in the decrease in stream N loss per unit channel length (Alexander et al., 2000, 2008), with DNF playing a lesser role in N removal as stream size increases (Bernot and Dodds, 2005). In support of these findings, headwater streams have been identified as major sinks for N via DNF due to their small size and shallow depth (Mulholland et al., 2008). As much as 45.5% of the watershed N load may be retained in headwater streams (Alexander et al., 2000). In the Mississippi River Basin, a large, systematic decline in the rate of N removal has been observed when moving from small streams to large rivers (Alexander et al., 2000), and nutrient delivery percentages to downstream ecosystems generally increase with stream size (Alexander et al., 2008).

### 1.1.4 *The Importance of Headwater Streams in N processing*

Much of the work on N cycling in aquatic ecosystems has been conducted in headwaters streams. The Lotic Intersite Nitrogen Experiments (LINX I and II) identified small streams as critical sites for N transformations, including DNF, especially as their cumulative length is great. Headwater streams typically represent 60 to 80% of the total stream length within a catchment (Schumm, 1956; Shreve, 1969). Smaller streams have a large capacity to remove instream N loads, and DNF serves as a central N loss mechanism (Bernot and Dodds, 2005). In a tropical headwater streams, DNF can account for 35% or more of $NO_3^-$ uptake (Potter et al., 2010). Another study found DNF to account for 16% of $NO_3^-$ uptake in a small stream with low ambient $NO_3^-$ concentrations (Mulholland et al., 2004). The surrounding land use was found to have an impact on $NO_3^-$ concentrations and DNF in headwater streams as well (Inwood et al., 2005; Bernot et al., 2006; Arango and Tank, 2008). Sediment DNF was highest in agriculturally influenced headwater streams (Inwood et al., 2005), as was biological activity (Bernot et al., 2006).

Agricultural ditches are channelized equivalents of headwater streams that come into direct contact with $NO_3^-$ rich water in landscapes dominated by farming. However, N cycling dynamics in agricultural ditches were not included in the LINX studies, although these studies did examine agriculturally influenced streams. This identifies a critical gap in knowledge as to how N cycles in agriculturally influence landscapes. Trends in agricultural ditches should be similar to those observed in headwater streams, except ditch management practices often result in a loss of habitat complexity and sinuousity, as well as a decreased residence time. If ditch environments can be managed to promote DNF, ditch channels may have a large DNF capacity and serve as an effective sink for excess N. Agricultural ditches represent a viable management

target that can help improve nutrient best management practices (BMPs) and alleviate downstream impacts of nutrient loading.

## 1.2 Looking to the Future: Expanding on Current Management Practices in Agriculture

Agricultural ditches have recently been recognized for their potential in mitigating contaminants running off agricultural fields, including pesticides and nutrients (Moore et al., 2001; Cooper et al., 2004; Kröger et al., 2014). Current ditch management practices focus on rapid drainage, which is not conducive to the retention of agrochemicals. In the US, 25% of agricultural soils are artificially drained[1] with typical systems consisting of field drains, field ditches, and an outlet (Herzon and Helenius, 2008). Standard field ditch and surface and tile drainage systems can stimulate N losses from the soil, contributing to downstream pollution (Turner and Rabalais, 2003). Various management techniques have been adopted to reduce the transport of nutrients into surface waters, including maintenance of riparian zones and buffer strips, use of conservation and contour tillage, terracing, utilization of cover crops, and retention ponds (Carpenter and Caraco, 1998); however, the management of agricultural ditches for nutrient mitigation specifically has been a development of the 21st century (Moore et al., 2001). Given that agricultural ditches make up a significant length of fluvial waterways across the world, the implementation of BMPs that promote enhanced nutrient removal within the ditch channel has the potential to greatly reduce nutrient loads to downstream ecosystems.

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[1] http://www.pca.state.mn.us/index.php/water/water-types-and-programs/minnesotas-impaired-waters-and-tmdls/tmdl-projects/lower-mississippi-river-basin-tmdl/project-lake-pepin-watershed-stakeholder-meeting-presentation-archive.html

### 1.2.1  *Ditch Management: Low-Grade Weirs*

One potential BMP being explored is the addition of low-grade weirs to agricultural ditches. Weirs are essentially small dams placed in the ditch that act as an alternative drainage strategy in surface drainage ditches. They increase the hydroperiod and reduce the ephemeral nature of the drainage ditch system (Usborne et al., 2013). However, it is also important that the installation of weirs does not compromise the primary function of the ditch, that of drainage. Weirs do not increase flooding potential with correct installation, but simply hold water in ditches longer by slowing the return to pre-storm event levels (Prince Czarnecki et al., 2014).

Weirs reduce nutrient loading to downstream ecosystems. Increasing the hydraulic residence time can allow for increased microbial processing of nutrients in the overlying water. One study suggested that the presence of weirs improved conditions for P retention (Usborne et al., 2013). It has been acknowledged there are a lack of studies on N dynamics in weired ditches found in the literature (Littlejohn et al., 2014). However, reductions in $NO_3^-$ over time are higher in weired ditches as compared to ditches without weirs (Kröger et al., 2012). Weirs also enhance DNF (Kröger et al., 2014), which may result in the permanent removal of excess N from the watershed.

### 1.2.2  *Ditch Management: Two-Stage Ditches*

The two-stage is a ditch BMP that acts as an alternative to the traditional trapezoidal ditch channels. The two-stage ditch consists of a ditch with restored floodplains alongside the stream channel (Powell et al., 2007). They sustain original drainage function, increase channel stability, attenuate peak flooding, produce a self-flushing and self-sustaining system, and do not interrupt in-stream biota (Kallio et al., 2010). This BMP is most common in agricultural systems

with subsurface tile drains with drain outlets flowing directly onto the restored floodplains. During storm flow, the floodplains typically become inundated (Landwehr and Rhoads, 2003).

The two-stage ditch may increase both short-term and long-term retention of nutrients. The two-stage ditch has been observed to increase assimilatory uptake of nutrients into stream biota (Roley et al., 2014). They also increase the HRT and provide additional bioreactive surface area for transformations of N, including DNF (Roley et al., 2012b). Denitrification was observed to be higher at reach scale in two-stage ditches (Roley et al., 2012a; b). Under storm flow conditions, two-stage ditch restoration contributes significantly to $NO_3^-$ removal via DNF (Roley et al., 2012a). However, $NO_3^-$ concentrations are often so high in tile drain water that a significant fraction of the load is not likely removed (<10%; Roley et al., 2012a). Additionally, reach-scale N-removal increased 3-24 times during inundation due to increased bioreactive surface area and high DNF rates on the floodplain (Mahl et al., 2015). Despite evidence for higher reach-scale N removal via DNF, one study suggests that the two-stage ditch is insufficient as a stand-alone BMP to reduce $NO_3^-$ loads when concentrations are greater than 1 mg $L^{-1}$ (Davis et al., 2015). This highlights the need for additional N management practices that reduce N inputs to streams from the surrounding watershed in combination with establishment of two-stage ditches.

### 1.2.3 *Ditch Management: Vegetated Ditch Channels*

The maintenance of wetland vegetation in ditch channels may serve to reduce nutrient loading to downstream ecosystems as wetlands can be hotspots for N transformations (Ingersoll and Baker, 1998; Scott et al., 2008). Vegetated agricultural drainage ditches offer farmers and landowners a low-cost, environmentally beneficial BMP alternative (Cooper et al., 2002). This BMP has been successful in mitigating pollution from pesticides in agricultural runoff (Cooper et

al., 2004; Bennett et al., 2005; Moore et al., 2008). Vegetation serves as an important site for pesticide sorption (Moore et al., 2001) and can retain up to 99% of pesticides entering the ditch channel (Cooper et al., 2004).

Vegetated ditches can also be effective in reducing nutrient loads to downstream ecosystems as well. Vegetation within the channel exerts drag and friction on the flowing water, increasing the HRT of the ditch, and in turn increasing its chemical residence time (CRT; Kröger et al., 2009). The measured CRT of a vegetated drainage ditch was observed to be at least twice that of a non-vegetated ditch, resulting in greater potential for microbial transformation, adsorption, and biological assimilation of excess nutrients (Kröger et al., 2009). Vegetated ditches have been shown to significantly reduced the nutrient load reaching downstream aquatic receiving systems (Moore et al., 2010). Additionally, DNF potentials of vegetated ditches in the Mississippi Delta were 1.3 times higher than non-vegetated ditches (Ullah and Faulkner, 2006).

Species of ditch vegetation can also influence the amount of N removal in vegetated ditch channels. Cutgrass (*Leersia oryzoides*) and cattail (*Typha latifolia*) lowered $NO_3^-$ concentrations by 67% and 64% respectively and absorbed N more rapidly as compared to bur-reed (*Sparangium americanum*; Tyler et al., 2012). Another study compared the ability of unvegetated, cattail, and cutgrass ditch environments to denitrify (Taylor et al., 2015). They found that ditch sediments planted with cutgrass had the largest $N_2$ flux out of the system via DNF as compared to the other treatments. In general, ditch channel vegetation has the capacity to significantly reduce pesticide and nutrient movement to downstream ecosystems in a cost-effective manner if implemented at a larger scale.

### 1.2.4 *Study Objectives*

Understanding the role of headwater stream equivalents, such as drainage ditches, in N cycling is necessary for developing and assessing the utility of nutrient BMPs in the agricultural landscape. The study of N processing in ditches, especially within vegetated ditches, is still in its infancy. Current published work conducted on ditch BMPs for nutrient remediation generally lack temporal resolution as experiments are often conducted in the summer. Additionally, a wide range of $NO_3^-$ loads has not been explored. This identifies a critical need for studies addressing seasonal variation and runoff N load impacts on the nutrient mitigation properties of agricultural ditches.

My thesis expands on a previous study conducted by Taylor et al. (2015); however, my work focused on cutgrass ditch sediments specifically. The primary objective of the first study was to determine the influence of seasonal temperature variation and $NO_3^-$ loading on DNF in ditch sediments vegetated with cutgrass (Figure 1) with a series of intact sediment core incubations. The secondary objective of the first study was to mathematically describe measured data from the core incubations using to develop Michaelis-Menten (Figure 2) and linear regression models to predict net $N_2$ fluxes out of sediments vegetated with cutgrass. The objective of the second study was to assess the validity of models developed from the first study to describe pre-existing cutgrass data from two independent experiments. The models set the stage for building and refining tools agricultural land managers and those that serve them, such as crop consultants, county agents, and National Resources Conservation Service (NRCS) personnel, can use to predict the water quality benefits offered by the implementation of vegetated ditch BMPs. Understanding and modeling the seasonal effects of $NO_3^-$ levels on DNF

in sediments vegetated with cutgrass is essential to understanding the utility of this potential

BMP in reducing N loads to sensitive downstream ecosystems.

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## 1.4    Figure Legends

Figure 1. Mesocosms containing sediments vegetated with cutgrass from which samples were obtained. Photograph by Shannon Speir (author).

Figure 2. Michaelis-Menten relationship characterizing changes in denitrification rate ($V$) with an increase in $NO_3^-$ concentration ($[NO_3^-]$). $V_{max}$ represents the maximum denitrification rate, and $K$ is the $NO_3^-$ concentration at which $V$ is ½ of $V_{max}$.
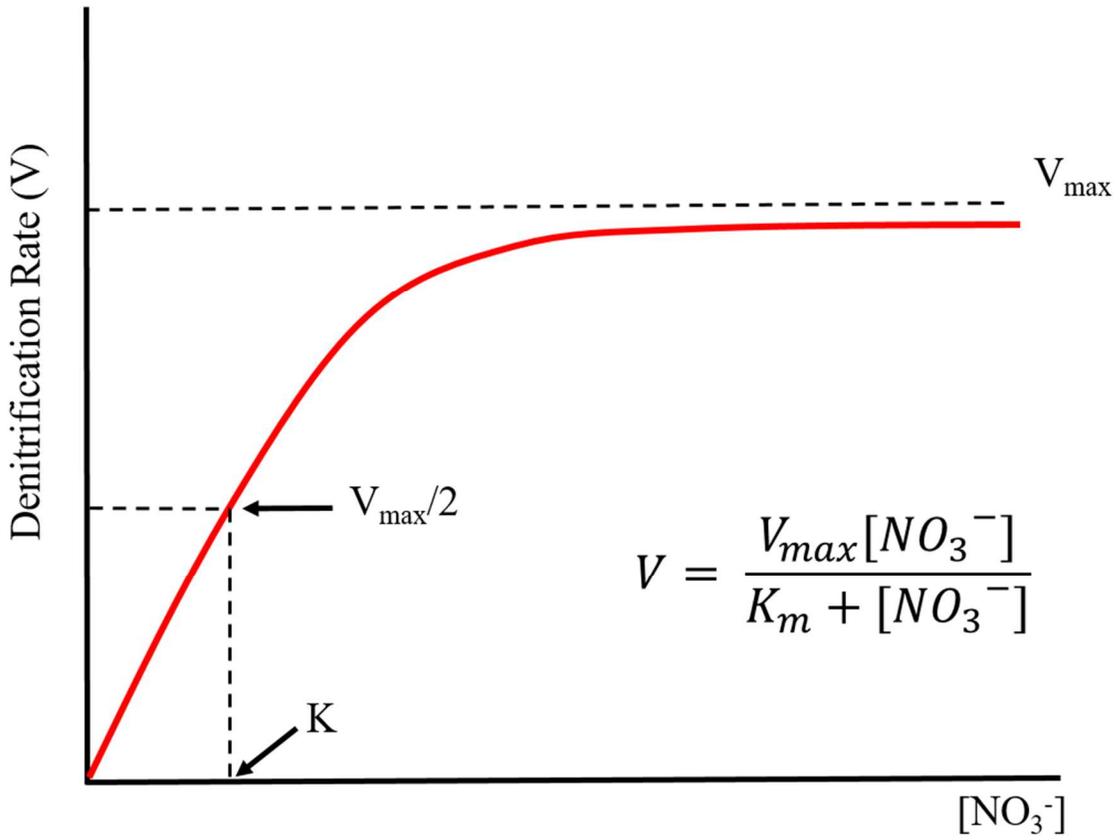
## 1.5 Figures



Figure 1

Figure 2

# 2. SEASONAL VARIATION IN DENITRIFICATION IN DITCH SEDIMENTS VEGETATED WITH RICE CUTGRASS (*LEERSIA ORYZOIDES*) ACROSS A NITRATE GRADIENT

## 2.1 Introduction

Demand for agricultural products continues to increase in response to a growing global population that is expected to reach 9.6 to 12.3 billion by 2100 (Foley et al., 2011; Gerland et al., 2014). Increased demand for food and fiber will require crop yields to be maximized in part by the use of nitrogen (N) fertilizers produced via industrial N fixation. Future rates of N fertilizer production are estimated to reach up to 172 Tg N yr$^{-1}$ by 2100, approximately twice the fixation rate of 2000 (Winiwarter et al., 2013). The addition of significant amounts of N to global biogeochemical cycles impacts both agricultural and natural ecosystems across the globe. Excess nutrients derived from fertilizer in the landscape can move readily from cropland to adjacent waterways, resulting in the degradation of downstream aquatic environments (Prakasa Rao and Puttanna, 2000; Galloway et al., 2008). Impacts of excess N on aquatic ecosystems include biodiversity losses, eutrophication, harmful algal blooms, and widespread coastal hypoxia (Carpenter and Caraco, 1998; Carpenter et al., 2011; Glibert et al., 2014). Landscape-scale models suggest agricultural sources contribute more than 70% of N delivered to streams and rivers in the Mississippi River Basin (Alexander et al., 2008); however, only 20-25% of this N is actually exported from rivers to oceans or inland basins (Van Breemen et al., 2002). This suggests substantial sinks for N exist in the landscape, with one such sink being denitrification (DNF; Mulholland et al., 2008; Aquilina et al., 2012).

Denitrification is carried out by heterotrophic, facultatively anaerobic bacteria that use nitrite ($NO_2^-$) or nitrate ($NO_3^-$) as a terminal electron acceptor in anaerobic respiration to produce

nitrogen gas ($N_2$; Payne, 1973; Seitzinger, 1988). It is a permanent removal mechanism for excess N as $N_2$ gas is an unreactive compound that readily diffuses out of freshwater into the atmosphere. Denitrification in aquatic environments is controlled by a variety of environmental variables, including organic carbon (C) availability (Duff et al., 2007; Fork and Heffernan, 2014), discharge and hydraulic residence time (HRT; Alexander et al., 2000; Royer et al., 2004; Ranalli and Macalady, 2010), oxygen concentrations (Seitzinger, 1988; Kemp and Dodds, 2002), and the availability of $NO_3^-$ in the overlying water column and sediments (Dodds et al., 2002; Inwood et al., 2007; Mulholland et al., 2008).

Headwater streams are important terrestrial-surface water interfaces in watersheds (Meyer et al., 1988) and have been identified as important sites for the natural processing of N via DNF (Alexander et al., 2000; Royer et al., 2004; Bernot and Dodds, 2005). Headwater streams are generally shallow and narrow, with lower water velocities as compared to higher order streams. This increases the amount of time water containing excess N is exposed to microbial processing and allows for more particulate organic N to settle out of the water column (Hill et al., 2000; Ranalli and Macalady, 2010). Agricultural ditches represent channelized equivalents of headwater streams and may have an equally important role in reducing N loading to downstream ecosystems. Current ditch management practices prevent efficient N processing because management goals are focused on moving runoff away from the fields as quickly as possible (Turner and Rabalais, 2003; Herzon and Helenius, 2008). However, if physical and biological conditions can be manipulated to create conditions that favor DNF, it may be possible to enhance N removal from the ditch environment and prevent continued impacts to downstream ecosystems.

Recently, new ditch management approaches have been explored, including the addition of low-grade weirs to ditches (Kröger et al., 2008, 2011, 2014), implementation of two-stage ditches (Roley et al., 2012b; Davis et al., 2015; Mahl et al., 2015), and the maintenance of vegetation within the ditch channel (Moore et al., 2001; Kröger et al., 2009). These ditch best management practices (BMPs) can facilitate nutrient removal by increasing the HRT of ditches, creating reducing conditions within the ditch, adding quality organic matter to ditches to enhance microbial processing, and providing additional binding sites for agrochemicals. A recent study compared the ability of unvegetated, cattail (*Typha latifolia*), and cutgrass (*Leersia oryzoides*) ditch sediments to denitrify excess N from fertilizers (Taylor et al., 2015). Experimental mesocosms planted with cutgrass, a common wetland plant, had the greatest percent reduction in runoff N load and the most $N_2$ flux out of the system via DNF as compared to the unvegetated and cattail treatments. Planting cutgrass in ditches may enhance DNF by adding quality organic C to the ditch and increasing the HRT of the ditch (Kröger et al., 2009; Taylor et al., 2015). However, a better understanding of how the presence of cutgrass influences DNF rates across a range of $NO_3^-$ concentrations year-round is critical to developing ditch BMPs that include vegetating the ditch channel with cutgrass.

I expanded on previous work on DNF in sediments planted with cutgrass with the objective of examining the influence of seasonal temperature change and level of $NO_3^-$ loading on DNF in cutgrass ditch environments. This study was designed to answer three questions:

(1) Do $N_2$ fluxes exhibit Michaelis-Menten kinetics across a $NO_3^-$ gradient in cutgrass ditch sediments seasonally?

(2) What direct and indirect effects might temperature have on seasonal variation in Michaelis-Menten kinetics in a cutgrass ditch system?

(3) How does DNF efficiency vary seasonally in cutgrass ditch sediments?

To address these questions, I conducted a series of 4 experiments consisting of 17 intact sediment cores per experiment over four seasons with 10 varying $NO_3^-$ concentrations in the overlying water as the experimental manipulation.

## 2.2    Materials and methods

### 2.2.1    *Pre-Incubation Preparation*

Continuous flow-through experiments with intact sediment cores (Scott et al., 2008; Grantz et al., 2012) were used to quantify sediment $N_2$ and $NO_3^-$ fluxes across a $NO_3^-$ gradient in cutgrass monocultures. One day prior to collecting sediment, I prepared 100 L of incubation water in two 50 L batches. A solution of deionized water amended with trace metal and mineral solutions (Table 1) and calcium carbonate was created to approximate background groundwater composition from the Mississippi alluvial aquifer. I tested the pH of the incubation water using a Fisher Scientific Accumet Basic pH meter with a target range of pH 7-8 and adjusted the pH if necessary. I distributed 5 L of incubation water to 9 carboys to be placed in the incubator. I then added varying amounts of sodium nitrate to each carboy to yield a gradient of $NO_3^-$ treatments: 0 mg N $L^{-1}$, 0.1 mg N $L^{-1}$, 0.5 mg N $L^{-1}$, 1.0 mg N $L^{-1}$, 2.5 mg N $L^{-1}$, 5.0 mg N $L^{-1}$, 7.5 mg N $L^{-1}$, and 10.0 N mg $L^{-1}$. The incubation water was aerated overnight for approximately 12 hours to equilibrate dissolved gases prior to being used in the continuous flow-through experiment.

### 2.2.2  *Mesocosm Sampling*

I collected intact sediment cores from previously constructed mesocosms at the US

Department of Agriculture (USDA)-Agricultural Research Service (ARS), National

Sedimentation Laboratory (NSL) in Oxford, MS. Mesocosms were created by filling each tub

with 22 cm of sand and placing 16 cm of sediment (type: Lexington silt loam) over the sand,

then planting with cutgrass. Both sediments and plant stocks were obtained from the University

of Mississippi Field Station in Abbeville, MS. Mesocosms were planted in April 2014 to allow

for plant communities, as well as detrital and microbial resources within the benthos, to establish

prior to beginning the experiment. I chose these mesocosms for sampling as they were well-

established cutgrass monocultures that could provide a homogenous sample site for core

extraction.

I destructively sampled mesocosms four times – June (spring), August (summer), and

October (fall) 2015; January (winter) 2016 –  by collecting 16 intact sediment cores from a single

mesocosm. Only one mesocosm was used per season because I knew removing cores would alter

the mesocosms between events. I used clear PVC (surface area $= 40.6$ cm$^2$, height $= 22.86$ cm) to

collect cores with an average of 12.5 cm of overlying water from each mesocosm. I manually

pushed cores approximately 10-15 cm into the sediment of the selected mesocosm at haphazard

locations. I removed cores by hand including sediment, trimmed vegetation, and rhizomes. Upon

removal, I capped the cores on both ends to be transported to the adjacent laboratory.

### 2.2.3  *Laboratory Core Incubations*

In the laboratory, I removed the upper core caps and resealed the cores with airtight

rubber stoppers. Rubber stoppers were outfitted with two pieces of Teflon™ tubing through each

stopper to provide inflow (AWG 20, 0.86 mm) and outflow (AWG 14, 1.63 mm) paths for the incubation water. The inflow tubing extended just above the sediment-water interface in the water column of the core. The outflow tubing was flush with the stopper on the interior of the core. Each previously prepared $NO_3^-$ treatment level was used to dose 2 cores (2 cores x 8 concentrations = 16 total cores); I randomly assigned cores to a treatment.

I incubated cores within a Powers Scientific™ diurnal growth chamber (Model # DS33SD; Pipersville, PA) set at average ambient temperature for the study location for each season (25°C, 30°C, 20°C, 10°C for spring, summer, fall, and winter respectively). All incubations were conducted in the dark to prevent photosynthesis and the production of $O_2$ bubbles, which can confound dissolved $N_2$ gas measurements in closed-core systems (Kana et al., 1994; Gardner et al., 2006). Incubation water was pumped into the cores at an average rate of 0.71 mL min$^{-1}$ using an ISMATEC™ MV peristaltic pump (Model # 7332-00). During each incubation, I set up one control core (a 10 cm core lacking sediment) to account for potential physical effects related to a reaction with the core chamber materials.

I allowed cores to flow continuously for approximately 24 hours prior to sampling influent from each carboy and effluent from each core chamber. I collected 5 sample sets for analysis from each core over the 3-day incubation period; each sample set was taken approximately 12 hours apart. I collected dissolved gas samples in 20 mL glass vials capped with ground glass stoppers. Glass vials were filled to overflowing from the bottom to reduce gas exchange with the atmosphere and were immediately preserved by adding 260 µL of 50% w:v $ZnCl_2$. I wrapped the ground glass stoppers in Parafilm® and placed the vials inside 1 L Nalgene® dark bottles filled with water to prevent additional gas exchange. Bottles were refrigerated until the time of gas analysis. I collected nutrient samples in 50 mL plastic centrifuge

tubes. Centrifuge tubes were filled to ~35 mL for $NO_3^-$ analysis and immediately frozen after collection was complete for the given sample set. I transported dissolved gas and nutrient samples on ice to the University of Arkansas in Fayetteville, AR for analysis.

### 2.2.4 *Dissolved Gas and Nutrient Analyses*

I analyzed dissolved gas samples for their $N_2$ gas to argon ratios ($N_2$:Ar) using a Membrane Inlet Mass Spectrometer (MIMS) equipped with a Pfeiffer Prisma mass spectrometer and a Bay Instruments membrane inlet (S-25-75). Kana et al. (1994) describes the full MIMS set-up in detail. Potential instrument specific $O_2$ interference in $N_2$:Ar determination was previously ruled out on the MIMS by comparing the $N_2$ concentration of replicate (oxic) samples measured both with and without $O_2$ removal using a copper reduction column heated to 600°C (Eyre et al., 2002). Prior to being run on the MIMS, I allowed samples to equilibrate to the incubation temperature for the given season and adjusted the MIMS standard solution to match sample incubation temperatures prior to analysis.

The MIMS method assumes 100% Ar saturation, which varies due to temperature and salinity, but not due to biological production or consumption. Thus, biological effects on the dissolved $N_2$ in my samples can be separated from physical effects using the Ar signal. I converted sample $N_2$:Ar ratios for each sample to $N_2$ gas concentrations based on the following equation (Grantz et al., 2012):

$$[N_2]_{sample} = (N_2{:}Ar_{sample} \times [Ar]_{exp})\left(\frac{[N_2]{:}[Ar]_{exp}}{N_2{:}Ar_{standard}}\right)$$

Equation 1

where $N_2{:}Ar_{sample}$ is the measured $N_2$ gas signal of the sample and $N_2{:}Ar_{standard}$ is the measured $N_2$ gas signal for the standard, which is well-mixed deionized water open to the atmosphere that is

held at the same temperature as the samples. The terms *[Ar]* $_{exp}$ and *[N$_2$]:[Ar]* $_{exp}$ are the theoretical saturated concentration and ratio, respectively, calculated for each in situ sample temperature using gas solubility tables (Weiss, 1970). This calculation yields the concentration of N$_2$ gas, *[N$_2$]* $_{sample}$ in μmol L$^{-1}$ and was then converted to mg L$^{-1}$. I measured NO$_3^-$ colorimetrically using the cadmium reduction method. Nutrient analysis was carried out on a Turner Designs Trilogy Lab Fluorometer, with a spectrophotometer adapter containing a 510-nm filter cell for NO$_3^-$ analysis.

### 2.2.5   *Flux and Percent Nitrate Uptake Calculations*

To calculate areal dissolved gas and nutrient fluxes (mg m$^{-2}$ h$^{-1}$), I used the following equation:

$$Areal\ Flux = \frac{([Core]_{out} - [Core]_{in}) \times Q_{core} - ([Ctrl]_{out} - [Ctrl]_{in}) \times Q_{control}}{A}$$

Equation 2

where *[Core]* $_{out}$ and *[Core]* $_{in}$ are the experimental core chamber outflow and inflow dissolved gas or nutrient concentrations (in mg L$^{-1}$). *[Ctrl]* $_{out}$ and *[Ctrl]* $_{in}$ are the control core chamber outflow and inflow N$_2$ or NO$_3^-$ concentrations (in mg L$^{-1}$), respectively. *Q$_{core}$* and *Q$_{control}$* are the measured flow rates through the experimental core and control core chambers (in L h$^{-1}$), respectively, and *A* is the core surface area (in m$^2$). The solution to this equation yields an areal flux estimate for dissolved N$_2$ or NO$_3^-$ (in mg m$^{-2}$ h$^{-1}$) for each independent intact core. A positive flux indicates production of N$_2$ or NO$_3^-$, while a negative flux indicates consumption of N$_2$ or NO$_3^-$. I considered a positive net N$_2$ flux to represent DNF and a negative net N$_2$ flux to represent N$_2$ fixation. Negative flux values cannot be used in Michaelis-Menten models. Thus I

calculated potential DNF for use in developing these models. Potential DNF assumes zero is the lowest possible $N_2$ flux and was determined by correcting the lowest $N_2$ flux value for the season in question to zero and offsetting all other data points by the same value using the following equation:

$$DNF_{pot} = [N_2\ Flux] + (- [N_2\ Flux]_{min})$$

Equation 3

where *[N2 flux]* is an $N_2$ flux measured from my cores in a given season (in mg m$^{-2}$ h$^{-1}$), *[N2 Flux]min* is the minimum flux for a given season (in mg m$^{-2}$ h$^{-1}$), and *DNFpot* is the resulting potential $N_2$ flux (in mg m$^{-2}$ h$^{-1}$). Hereafter, potential DNF will refer to the *DNFpot* values used in model development. Net $N_2$ flux will be used to describe actual measured $N_2$ fluxes that Michaelis-Menten models were back-corrected to reflect. DNF will refer to positive net $N_2$ fluxes, and $N_2$ fixation will refer to negative net $N_2$ fluxes.

To calculate percent $NO_3^-$ uptake, I used the following equation:

$$\%\ NO_3^-\ Uptake = \frac{[NO_3]_{in} - [NO_3]_{out}}{[NO_3]_{in}} * 100$$

Equation 4

where *[NO3]in* is the concentration of $NO_3^-$ in the inflow water (in mg L$^{-1}$), *[NO3]out* is the concentration of $NO_3^-$ in the outflow water (in mg L$^{-1}$), and *% NO3 uptake* is the percent of $NO_3^-$ in the inflow water that is retained by the core.

### 2.2.6 *Statistical Analyses*

I developed seasonal Michaelis-Menten models to predict net $N_2$ fluxes across the experimental gradient of $NO_3^-$ inputs. Models were developed using potential DNF and then

back-corrected to reflect measured net $N_2$ fluxes. The Michaelis-Menten equation structure for this experiment was:

$$Flux = \frac{V_{max} * [NO_3 \; treatment]}{K + [NO_3 \; treatment]}$$

Equation 5

where $V_{max}$ is the maximum amount of net $N_2$ flux, *[NO3 treatment]* is the concentration of $NO_3^-$ in the overlying water (in mg $L^{-1}$), $K$ is the concentration of $NO_3^-$ in the overlying water at which the net $N_2$ flux is half of $V_{max}$, and *Flux* is the amount of $N_2$ flux produced at a given $NO_3^-$ concentration (in mg $m^{-2}$ $h^{-1}$). I used non-linear regression mixed effects models based on the Michaelis-Menten equation to estimate $V_{max}$ and $K$ for each season. I included a random effect in my models to account for nested samples ($\sim V_{max}$ | time) (Ritz and Streibig, 2008). When residuals indicated that variance in my $N_2$ data increased with increasing $NO_3^-$ treatment, I used an exponential variance structure (varExp) to improve heterogeneity of residuals and verified by examining plots of the normalized residuals and residual q-q plots (Zurr et al., 2009). Model improvement was also assessed by evaluating Akaike information criterion (AIC) scores for all model iterations (Quinn and Keough, 2002). Due to a deviation from the observed increasing monotonic pattern in net $N_2$ fluxes, I created two versions of the Michaelis-Menten models, one which excluded the 10 mg $L^{-1}$ from model development and one that included the 10 mg $L^{-1}$. Michaelis-Menten models were developed in the *nlme* package (Pinheiro and Bates, 2000) in R (version 3.2.3; R Development Core Team, Vienna, Austria). All models were then back-corrected from potential DNF to reflect my measured net $N_2$ fluxes by subtracting *[N2 Flux]min* from the overall model. (Table 2). R code for Michaelis-Menten non-linear regression mixed effects model development can be referenced in Appendix A.

Denitrification efficiencies (DNF per unit $NO_3^-$ uptake) were estimated using linear regression models in R (version 3.2.3; R Development Core Team, Vienna, Austria) with seasonal $NO_3^-$ uptake as the explanatory variable and $N_2$ flux as the response variable. Nitrate uptake was computed by taking the inverse of all measured $NO_3^-$ fluxes. The slope of the seasonal linear regressions provided estimates for the percent of $NO_3^-$ taken up that was denitrified, or DNF efficiency. Prior to running the seasonal linear regression models, I tested my data for violations of the assumptions of linear regression. Linearity, homogeneity of variance, and outside values were evaluated using scatterplots. Normality was examined using boxplots. After developing the linear regression models, residuals were examined using the autoplot function in *ggfortify* in R.

## 2.3    Results

### 2.3.1    *Modeling seasonal patterns in nitrogen fluxes*

In the spring, summer, and fall, net $N_2$ fluxes followed Michaelis-Menten saturation trends (Table 2). The greatest net $N_2$ fluxes were observed in the summer. The summer model developed excluding the 10 mg L$^{-1}$ treatment had a $V_{max}$ estimate of $43.74 \pm 6.46$ ($p \leq 0.0001$) and $K$ estimate of $4.27 \pm 1.13$ ($p = 0.0003$). The summer model developed including the 10 mg L$^{-1}$ treatment had a $V_{max}$ estimate of $31.83 \pm 3.61$ ($p \leq 0.0001$) and $K$ estimate of $2.45 \pm 0.60$ ($p \leq 0.0001$). The spring model developed excluding the 10 mg L$^{-1}$ treatment had a $V_{max}$ estimate of $19.94 \pm 2.68$ ($p \leq 0.0001$) and a $K$ estimate of $1.44 \pm 0.46$ ($p = 0.0027$). The spring model developed including the 10 mg L$^{-1}$ treatment had a $V_{max}$ estimate of $18.30 \pm 1.92$ ($p \leq 0.0001$) and a $K$ estimate of $1.20 \pm 0.35$ ($p = 0.001$). In both the spring and summer, net $N_2$ fluxes

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followed similar patterns. At the lowest $NO_3^-$ treatments, net $N_2$ fluxes were slightly negative. At 1 mg $L^{-1}$, net $N_2$ fluxes became positive and reached a maximum near 12 mg $m^{-2}$ $h^{-1}$ in the spring and 20 mg $m^{-2}$ $h^{-1}$ in the summer at the 7.5 mg $L^{-1}$ treatment (Figure 1A, B). However, the maximum net $N_2$ flux was greater in the summer than in the spring. A decrease in net $N_2$ flux was observed at the 10 mg $L^{-1}$ treatment in both seasons as well.

Fall net $N_2$ fluxes most strongly exhibited the characteristic Michaelis-Menten saturation curve (Figure 1C), with a $V_{max}$ estimate of $25.70 \pm 1.28$ ($p \leq 0.0001$) and a $K$ estimate of $0.27 \pm 0.07$ ($p \leq 0.0006$) for the fall model developed excluding the 10 mg $L^{-1}$ treatment. The fall model developed including the 10 mg $L^{-1}$ treatment a $V_{max}$ estimate of $27.61 \pm 1.12$ ($p \leq 0.0001$) and a $K$ estimate of $0.40 \pm 0.09$ ($p \leq 0.0001$) were calculated. Net $N_2$ fluxes were extremely negative at the lowest treatments in the fall, with fluxes steadily increasing toward zero as $NO_3^-$ treatment level increased. Additionally, the fall net $N_2$ fluxes peaked at a much lower measured maximum than was observed in the spring and summer. For the winter model developed excluding the 10 mg $L^{-1}$ treatment, the model estimate of $6.45 \pm 0.73$ for $V_{max}$ was significant ($p \leq 0.0001$), but the model estimate of $-0.02 \pm 0.02$ for $K$ was not ($p = 0.172$). For the winter model developed including the 10 mg $L^{-1}$ treatment, the model estimate of $7.36 \pm 0.63$ for $V_{max}$ was significant ($p \leq 0.0001$), but the model estimate of $-0.01 \pm 0.02$ for $K$ was not ($p = 0.555$). Net $N_2$ fluxes hovered around zero across all treatments in the winter (Figure 1D).

A saturation trend was observed in $NO_3^-$ fluxes across all seasons as well. All fluxes were negative indicating $NO_3^-$ uptake was occurring in the cores. Spring and summer $NO_3^-$ fluxes showed a similar saturation pattern as observed in the net $N_2$ fluxes during these seasons (Figure 2A, B). The most negative $NO_3^-$ flux occurred at the 7.5 mg $L^{-1}$ treatment in both seasons, with an increase to a more positive $NO_3^-$ flux observed at the 10 mg $L^{-1}$ treatment. Fall and winter

$NO_3^-$ fluxes remained near zero up to the 1 mg $L^{-1}$ treatment, then decreased slightly from the 2.5-10 mg $L^{-1}$ treatments (Figure 2C, D). The fall and winter seasons had low $NO_3^-$ uptake across treatments compared to spring and summer.

### 2.3.2  *Nitrate uptake and denitrification efficiencies*

I estimated how efficiently cores immobilize $NO_3^-$ in the inflow water across the range of $NO_3^-$ treatments by calculating percent $NO_3^-$ uptake (Figure 3). Across all seasons, the percent of $NO_3^-$ immobilized by the cores changed as the concentration of $NO_3^-$ in the overlying water increased. Efficiency declined after 1 mg $L^{-1}$ in the spring, fall, and winter, with steeper declines observed in the fall and winter (Figure 3A, C, D). In contrast, maximum efficiency occurred across a broader range of $NO_3^-$ concentrations from 0.5 to 5 mg $L^{-1}$ during the summer (Figure 3B).

The slope of the linear regression models of net $N_2$ flux versus $NO_3^-$ uptake provided estimates of DNF efficiency in each season. Denitrification was strongly correlated to $NO_3^-$ uptake rates by vegetated sediments in spring (Figure 4A; $r^2 = 0.94$, $p < 0.0001$) and summer (Figure 4B; $r^2 = 0.97$, $p < 0.0001$), with DNF efficiency ranging from ~28 to 37%. Statistically, DNF was correlated to $NO_3^-$ uptake in the fall as well, with a predicted DNF efficiency of 72% (Figure 4C; $r^2 = 0.48$, $p = 0.0027$). However, it is unlikely this reflects the biology of the system as $NO_3^-$ uptake rates were much lower in the fall as compared to the spring or summer and nearly all net $N_2$ flux was negative in the fall. The relationship between net $N_2$ fluxes and $NO_3^-$ uptake was not significant during the winter (Figure 4D; $r^2 = 0.05$, $p = 0.5873$), reflecting the overall lack of $NO_3^-$ uptake and net $N_2$ flux in my cores during the winter.

## 2.4    Discussion

Enhancing environmental conditions that favor DNF may be an effective way to facilitate N removal from agricultural landscapes. My study demonstrated that increased $NO_3^-$ availability resulted in an increase net $N_2$ fluxes and $NO_3^-$ uptake from the overlying water across all seasons except winter in a simulated agricultural ditch environment vegetated with cutgrass. Additionally, net $N_2$ fluxes and $NO_3^-$ uptake experienced a monotonic increase up to a $NO_3^-$ concentration of 7.5 mg $L^{-1}$ in the overlying water, suggesting that DNF rates increase with increasing $NO_3^-$ levels, but not indeterminately (Mulholland et al., 2008). The most DNF occurred in the spring and summer when more $NO_3^-$ was immobilized from the overlying water in the cores and vegetation was flourishing. During the growing season, cutgrass's thick root mat may aid in creating anoxic conditions required for DNF at the sediment-water interface (Taylor et al., 2015), and it likely contributes additional high quality organic matter to the system to serve as an electron donor in DNF via root exudates (Christensen and Sorensen, 1986). Little DNF occurred in the fall and winter, likely resulting from cooler water temperatures that suppressed DNF during these seasons (Kadlec and Reddy, 2001).

### 2.4.1    *Do N₂ fluxes exhibit Michaelis-Menten kinetics across a NO₃⁻ gradient in cutgrass ditch sediments seasonally?*

Spring, summer, and fall net $N_2$ fluxes exhibited Michaelis-Menten kinetics, but patterns in $N_2$ fluxes varied by season. In the summer, net $N_2$ fluxes peaked near 20 mg $m^{-2}$ $h^{-1}$, which corresponds to the maximum range of reported DNF rates at which saturation occurs (Bernot and Dodds, 2005), whereas maximum spring net $N_2$ fluxes peaked at just over 10 mg $m^{-2}$ $h^{-1}$. Other studies have reported a linear relationship between DNF rates and $NO_3^-$ concentrations in the overlying water (Inwood et al., 2007; Zhong et al., 2010); however, the maximum $NO_3^-$

concentrations measured in these studies ranged from 2 to 5 mg $L^{-1}$. This suggests the $NO_3^-$ concentrations may not have been high enough in previous studies to detect a saturation effect in DNF rates. Measured DNF rates in the spring were less than summer rates because spring $NO_3^-$ uptake was lower than $NO_3^-$ uptake in the summer. Previous studies have observed higher summer DNF rates in a range of aquatic habitats, including lakes, rivers, estuaries, and coastal environments (Piña-Ochoa and Álvarez-Cobelas, 2006).

I observed a decrease in net $N_2$ flux (i.e., less $N_2$ produced) and a corresponding increase in $NO_3^-$ flux (i.e., less $NO_3^-$ uptake) at the 10 mg $L^{-1}$ treatment in spring and summer. To avoid impacts of unmeasured competing microbial processes at the 10 mg $L^{-1}$ treatment on my Michaelis-Menten models, I excluded net $N_2$ fluxes estimates at the 10 mg $L^{-1}$ treatment from model development. A change in denitrifier activity was the most likely cause of the changes in net $N_2$ fluxes at the 10 mg $L^{-1}$ treatment as a decrease in plant $NO_3^-$ uptake would not have resulted in the observed decrease in net $N_2$ fluxes as well. It is unlikely that a competing process, such as dissimilatory $NO_3^-$ reduction to ammonia (DNRA) or anaerobic ammonium oxidation (anammox), was responsible for the observed trends as well. If DNRA was occurring, A decrease in $N_2$ production would have been observed, but no significant decrease in $NO_3^-$ uptake as $NO_3^-$ is one of the main substrates in DNRA (Koike and Hattori 1978). Due to the decrease in $N_2$ production as well, it is improbable that anammox was responsible for the observed trends in net $N_2$ fluxes as $N_2$ is a product of anammox (Burgin and Hamilton, 2007). It is possible a change in reducing conditions at the 10 mg $L^{-1}$ treatment in the spring and summer may have resulted in a relief of anoxic conditions favoring DNF (Speir, unpublished data).

While the fall net $N_2$ fluxes exhibited Michaelis-Menten kinetics, the negative fluxes suggest DNF was not occurring. It is likely that $N_2$ fixation is responsible for the consumption of

$N_2$ within my cores during the fall. However, I did not directly quantify $N_2$ fixation rates. In the winter, net $N_2$ fluxes remained near zero across all treatments. Denitrifier affinity for $NO_3^-$ is reduced as temperatures decrease (Nedwell, 1999), which may explain the lack net $N_2$ flux and $NO_3^-$ uptake in the winter. The lack of microbial activity during the winter season did not allow for the development of a predictive model for net $N_2$ fluxes in cutgrass ditch sediments during this season.

### 2.4.2 *What direct and indirect effects might temperature have on seasonal variation in Michaelis-Menten kinetics in a cutgrass ditch system?*

Seasonal temperature fluctuations can directly influence DNF rates throughout the year (Hanson et al., 1994), explaining some of the seasonal variation I observed in my Michaelis-Menten saturation curves. A negative linear relationship has been reported between the amount of $NO_3^-$ in the overlying water and increasing water temperatures (Pfenning and Mcmahon, 1996), and a positive linear relationship has been reported between DNF rates and increasing water temperatures (Wall et al., 2005). This suggests that lower temperatures may suppress $NO_3^-$ removal from the water column via DNF (Pfenning and Mcmahon, 1996). My findings corresponded to the findings of Pfenning and McMahon (1996) and Wall et al. (2005), as I observed the highest $NO_3^-$ uptake and the most DNF during spring and summer at the highest temperatures. Similar to the findings of Wall et al. (2005) in reservoir networks, the lack of $NO_3^-$ uptake across all treatments within cutgrass cores during winter incubations (10°C) suggests that if high $NO_3^-$ water is delivered to the ditches in winter, sediment DNF will remain low as long as temperatures remain low regardless of a relief of $NO_3^-$ limitation on DNF rates. Studies have also shown that the lowest DNF enzyme activity occurs in the winter at temperatures similar to those used in my winter incubation (0.2-11°C; Richardson et al. 2004). The minimal N removal from the cutgrass cores at fall and winter temperatures indicate other BMPs may be necessary to

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effectively control nutrients in runoff outside of the growing season. However, it is important to note that lower Mississippi River Basin water temperatures can vary considerably during the fall and winter, and warms spells during the winter may result in hot moments of DNF.

Temperature can also indirectly affect DNF by affecting C availability seasonally. A high availability of quality C in the summer when vegetation is flourishing may explain why summer had the greatest $V_{max}$ and net $N_2$ fluxes. Higher DNF activity in soil surrounding plant roots can be largely attributed to the deposition of root exudates (Philippot et al., 2009), and increases in temperature have been shown to have a stimulatory effect on the production of root exudates (Pramanik et al., 2000; Uselman et al., 2000). Warmer temperatures may have stimulated cutgrass to exude quality C into the rhizosphere, which in turn stimulated higher DNF rates in the summer. Additionally, cutgrass has been reported to have high biomass turnover rates (Farnsworth and Meyerson 2003), and warm summer water temperatures may have further increased breakdown of organic C into forms available to denitrifiers (Irons et al., 1994).

In the fall, net $N_2$ flux was extremely negative at the lowest treatments, indicating high rates of $N_2$ fixation were present across the lowest $NO_3^-$ treatments. The most negative net $N_2$ fluxes averaged approximately to -18 mg $N_2$ m$^{-2}$ h$^{-1}$. Negative net $N_2$ fluxes of this magnitude are often not expected in nature as $N_2$ fixation requires a high energetic input to break the triple bonds in an $N_2$ molecule (Hill, 1976). However, a number of studies have also reported negative net $N_2$ fluxes (Gardner et al., 2006; Scott et al., 2008; Grantz et al., 2012). Fulweiler et al. (2007) observed net $N_2$ fluxes in estuaries ranging from -7 to -18.2 mg $N_2$ m$^{-2}$ h$^{-1}$ at temperatures similar to the fall incubation temperature in the current study. Their most negative rates were within the range of the average lowest $N_2$ flux observed during my fall incubation. The greatest negative fluxes observed in the study done by Fulweiler et al. (2007) were measured in experimental cores

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that were not treated with an organic matter amendment. It is possible that the lack of organic C available as an electron donor in both the Fulweiler et al. (2007) experiment as well as in my cores in the fall may result in a greater proportion of $N_2$ fixation versus DNF occurring within the cores as DNF is likely $NO_3^-$ limited at low treatments and C limited across all treatments. As $NO_3^-$ limitation is relieved with increasing $NO_3^-$ treatments, the relative proportion of DNF compared to $N_2$ fixation increases, resulting in less negative net $N_2$ fluxes. Thus, cutgrass senescence in the fall may have decreased C availability (i.e., less quality root exudates available at fall temperatures) in my cores and resulted in high $N_2$ fixation rates. However, the exact mechanisms remain unclear and are deserving of further exploration. In the fall, it is also possible that a shift from N limitation at the low $NO_3^-$ treatments to C limitation at higher $NO_3^-$ treatments occurred (Inwood et al., 2007), explaining the lack of DNF observed in the fall in general. Low organic C availability in the winter may have also limited net $N_2$ fluxes across all levels of $NO_3^-$ loading (Groffman et al., 2002; Stelzer et al., 2014). As plants had fully senesced by winter, it is possible that the more labile C and nutrients had been leached from the organic matter remaining in the cores at this time (Stelzer et al., 2014), resulting in a lack of quality C for denitrifiers to use as an electron donor in the winter.

### 2.4.3   *How does DNF efficiency vary seasonally in cutgrass ditch sediments?*

Based on the linear regression models of net $N_2$ flux versus $NO_3^-$ uptake, I was able to estimate seasonal DNF efficiency. In the spring and summer, net $N_2$ flux was strongly correlated with $NO_3^-$ uptake rates by vegetated sediments, and DNF efficiency ranged from approximately 28-37% during the growing season. During the summer months, Taylor et al. (2015) observed DNF efficiencies > 50% in mesocosms planted with cutgrass based on mass balance estimates, which is even greater than the efficiencies observed in the current study. Thus, ditches vegetated

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with cutgrass may serve as effective sinks of $NO_3^-$ during the growing seasons when immediate field losses can be expected directly after fertilizer application.

While the relationship between net $N_2$ flux and $NO_3^-$ uptake was statistically significant in the fall, the trends in the data do not reflect a DNF relationship. Net $N_2$ fluxes were generally negative and $NO_3^-$ uptake was very low, suggesting that DNF was not occurring in the fall. It is likely that $N_2$ fixation rather than DNF was occurring as $N_2$ was being consumed within the core chambers; however, I did not directly quantify $N_2$ fixation rates in this study. The lack of correlation between $NO_3^-$ uptake and net $N_2$ fluxes in the winter can be attributed to the lack of microbial activity during this season (Richardson et al., 2004). Low DNF efficiencies have been observed in a constructed wastewater treatment plant wetland in the winter as well (Garcia-Lledo et al., 2011). Due to low uptake of $NO_3^-$ in the winter, net $N_2$ fluxes were very low regardless of the level of N loading.

Denitrification efficiency throughout the year may be affected by changes in $NO_3^-$ immobilization seasonally. Across all seasons, the percent of $NO_3^-$ immobilized by the core increased to a maximum at lower $NO_3^-$ concentrations, but decreased after peaking as $NO_3^-$ concentrations continued to rise. In general, $NO_3^-$ uptake velocity is known to decrease with an increase in levels of $NO_3^-$ in the overlying water (Mulholland et al., 2008, 2009). The decrease may reflect a switch to C limitation within cores at the high $NO_3^-$ treatments, as agricultural stream sediments have been shown to be limited by C availability rather than $NO_3^-$ availability (Inwood et al., 2007). More rapid decreases in percent $NO_3^-$ immobilized occurred in the fall and winter, which may result from a switch to C limitation at lower $NO_3^-$ concentrations than in spring or summer due to plant senescence and reduced C lability in the cooler seasons.

### 2.4.4 *Considerations for future exploration into DNF dynamics in cutgrass ditch systems*

While small-scale intact sediment core incubations are effective for measuring DNF, they also present limitations for elucidating how cutgrass fits into agricultural land management at a larger scale. For example, the cores have a longer HRT than a ditch, allowing more time for microbial processing to act on $NO_3^-$ in the incubation water, which may have resulted in an overestimation of how much DNF is occurring at a given $NO_3^-$ level. Water was continually supplied to the cores, yet ditches may go through a series of wetting and drying cycles, which may cause the system to reset its equilibrium periodically. The wetting and drying cycles can pose a risk for increased $N_2O$ production, which is a potent greenhouse gas. Drying may result in compromised anoxic conditions which can halt the conversion of $N_2O$ to $N_2$, but not prevent the production of $N_2O$ (Beaulieu et al., 2011). Further exploration into this potential risk is necessary to avoid the "swapping" of pollutants.

It would also be very difficult to mimic storm runoff with pulses of high $NO_3^-$ concentrations within a core system as water cannot be supplied to the cores in pulses that mimic storm conditions. Additional C sources from cropland must also be considered in future experiments. In the fall, both the breakdown of crop residues and fall tillage can release C into aquatic systems. I did not simulate these conditions within my cores; thus, the fall data may not be representative of what is occurring at ditch-scale and must be investigated further. The differences between intact sediment core experiments and ditch-scale experiments may also affect the utility of the Michaelis-Menten and linear regression models when scaling up to model application at the ditch or watershed level. Therefore, ditch-scale field experiments are needed to understand how ditches vegetated with cutgrass may function at a larger spatial scale within the agricultural landscape.

### 2.4.5 *Implications for ditch management in the agricultural landscape*

Agricultural ditches are the first point of contact for cropland runoff entering freshwater ecosystem networks, yet unvegetated ditches do not offer much reduction in nutrient loads because the hydrology of unvegetated ditches does not provide conditions that favor microbial processing (Taylor et al. 2015). However, sediments vegetated with cutgrass immobilize a significant fraction of $NO_3^-$ at relatively high rates of $NO_3^-$ loading (Taylor et al. 2015; the current study) and can permanently remove up to 40% of the $NO_3^-$ load via DNF during the growing season. Thus, establishing cutgrass in ditches can provide a long-term sink for excess $NO_3^-$ in the landscape and reduce nutrient pollution to downstream ecosystems. Reducing the impacts of agricultural practices and protecting our natural resources is becoming more critical as the world's population continues to grow rapidly. The results of the current study suggest that agricultural ditches vegetated with cutgrass have great potential as a nutrient management BMP, especially during the growing season. Additionally, the models I have developed for the spring, summer, and fall seasons may help refine landscape modeling tools and increase managers' ability to predict changes in nutrient loads from ditches planted with cutgrass and evaluate potential benefits at larger scales.

## 2.5    References

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## 2.6    Tables

Table 1. Ion concentrations used to make mineral and trace metal solutions to create a deionized water solution approximating Mississippi groundwater conditions.

| Compound | Concentration (g L$^{-1}$) |
|---|---|
| Mineral Solution | |
| NaCl | 80.0 |
| KCl | 10.0 |
| KH$_2$PO$_4$ | 31.8 |
| MgSO$_4$*7H$_2$O | 20.0 |
| CaCl$_2$ | 4.0 |
| | |
| Trace Metal Solution | |
| MnCl$_2$ | 0.74 |
| Fe(NH$_4$)$_2$(SO$_4$)$_2$*6H$_2$O | 0.80 |
| CoCl$_2$*6H$_2$O | 0.20 |
| ZnSO$_4$*7H$_2$O | 0.20 |
| CuCl$_2$*2H$_2$O | 0.02 |
| NaMoO$_4$*2H$_2$O | 0.02 |

Table 2. Seasonal incubation temperature (T), above- and belowground biomass, $N_2$ Michaelis-Menten model parameter estimates for $V_{max}$ and $K$ (± 1 SE), an estimated net $V_{max}$ based on model back-correction, and the back-corrected $N_2$ Michaelis-Menten (MM) models. Model parameters are included for both the models with and without the 10 mg $L^{-1}$ treatment.

| | Spring | Summer | Fall | Winter |
|---|---|---|---|---|
| T (°C) | 25 | 30 | 20 | 15 |
| AG Biomass (g) | 2.36 ± 0.25 | 3.60 ± 0.43 | 2.64 ± 0.38 | 2.47 ± 0.40 |
| BG Biomass (g) | 29.27 ± 3.52 | 38.88 ± 4.13 | 30.02 ± 3.38 | 32.02 ± 5.19 |

*Model excluding 10 mg $L^{-1}$*

| | Spring | Summer | Fall | Winter |
|---|---|---|---|---|
| $K$ (mg $L^{-1}$) | 1.44 ± 0.46*** | 4.27 ± 1.13*** | 0.27 ± 0.07*** | -0.02 ± 0.02 |
| $V_{max}$ (mg m$^{-2}$ h$^{-1}$) | 19.94 ± 2.68*** | 43.74 ± 6.46*** | 25.70 ± 1.28*** | 6.45 ± 0.73*** |
| Net $V_{max}$ (mg m$^{-2}$ h$^{-1}$) | 13.26 ± 2.68 | 35.27 ± 6.46 | 1.12 ± 1.28 | -1.82 ± 0.73 |
| Corrected MM Model | $Flux = \dfrac{19.94 * [NO_3]}{[NO_3] + 1.44} - 6.68$ | $Flux = \dfrac{43.74 * [NO_3]}{[NO_3] + 4.27} - 8.47$ | $Flux = \dfrac{25.70 * [NO_3]}{[NO_3] + 0.27} - 24.58$ | No Model |

*Model including 10 mg $L^{-1}$*

| | Spring | Summer | Fall | Winter |
|---|---|---|---|---|
| $K$ (mg $L^{-1}$) | 1.20 ± 0.35*** | 2.45 ± 0.59*** | 0.40 ± 0.09*** | -0.01 ±0.02 |
| $V_{max}$ (mg m$^{-2}$ h$^{-1}$) | 18.30 ± 1.92*** | 31.83 ± 3.61*** | 27.61 ± 1.12*** | 7.36 ± 0.63*** |
| Net $V_{max}$ (mg m$^{-2}$ h$^{-1}$) | 11.62 ± 1.92 | 23.36 ± 3.61 | 3.03 ± 1.12 | -0.91 ± 0.63 |
| Corrected MM Model | $Flux = \dfrac{18.30 * [NO_3]}{[NO_3] + 1.20} - 6.68$ | $Flux = \dfrac{31.83 * [NO_3]}{[NO_3] + 2.45} - 8.47$ | $Flux = \dfrac{27.61 * [NO_3]}{[NO_3] + 0.40} - 24.58$ | No Model |

†Significance levels: * ≤ 0.1, ** ≤ 0.05, *** ≤0.01

## 2.7 Figure Legends

Figure 1. Intact sediment core (A) and incubator set-up (B). Incubation water, located at the base of the incubator, is pumped upward to the cores. Water enters the cores just above the sediment water interface and is forced out of the outflow by pressure build up. Outflow water is routed into a collection tub located on the top shelf of the incubator.

Figure 2. Net $N_2$-N flux (mg $m^{-2}$ $h^{-1}$) as a function of $NO_3^-$-N treatment (mg $L^{-1}$) for the spring (A), summer (B), fall (C), and winter (D) seasons. Solid lines represent back-corrected Michaelis-Menten models excluding the 10 mg $L^{-1}$ treatment reflecting measured $N_2$-N fluxes. Dotted lines represent back-corrected Michaelis-Menten models including the 10 mg $L^{-1}$ treatment reflecting measured $N_2$-N fluxes.

Figure 3. Net $NO_3^-$-N flux (mg $m^{-2}$ $h^{-1}$) as a function of $NO_3^-$-N treatment (mg $L^{-1}$) for the spring (A), summer (B), fall (C), and winter (D) seasons.

Figure 4. Percent $NO_3^-$-N uptake as a function of $NO_3^-$-N treatment (mg $L^{-1}$) for the spring (A), summer (B), fall (C), and winter (D) seasons.

Figure 5. Net $N_2$-N flux (mg $m^{-2}$ $h^{-1}$) as a function of net $NO_3^-$-N uptake (mg $m^{-2}$ $h^{-1}$) for the spring (A; $r^2 = 0.94$, $p < 0.0001$), summer (B; $r^2 = 0.97$, $p < 0.0001$), fall (C; $r^2 = 0.48$, $p < 0.0027$), and winter (D; $r^2 = 0.05$, $p < 0.5873$) seasons.
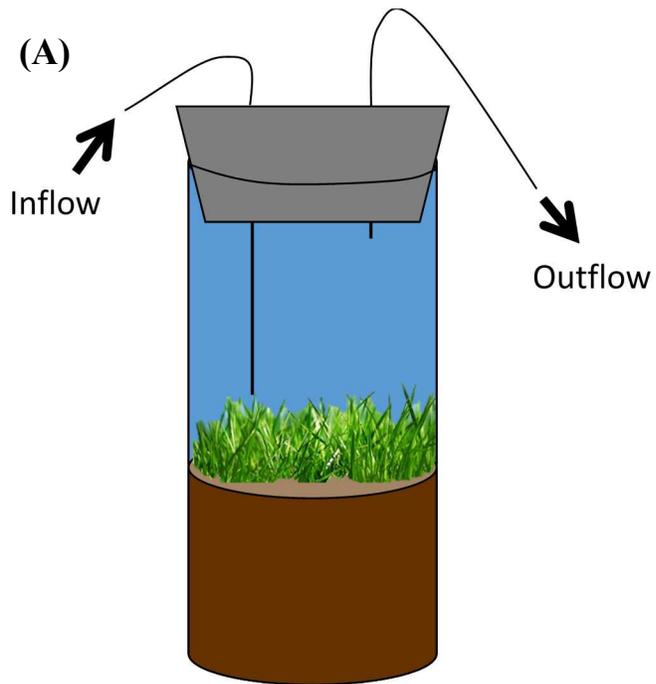
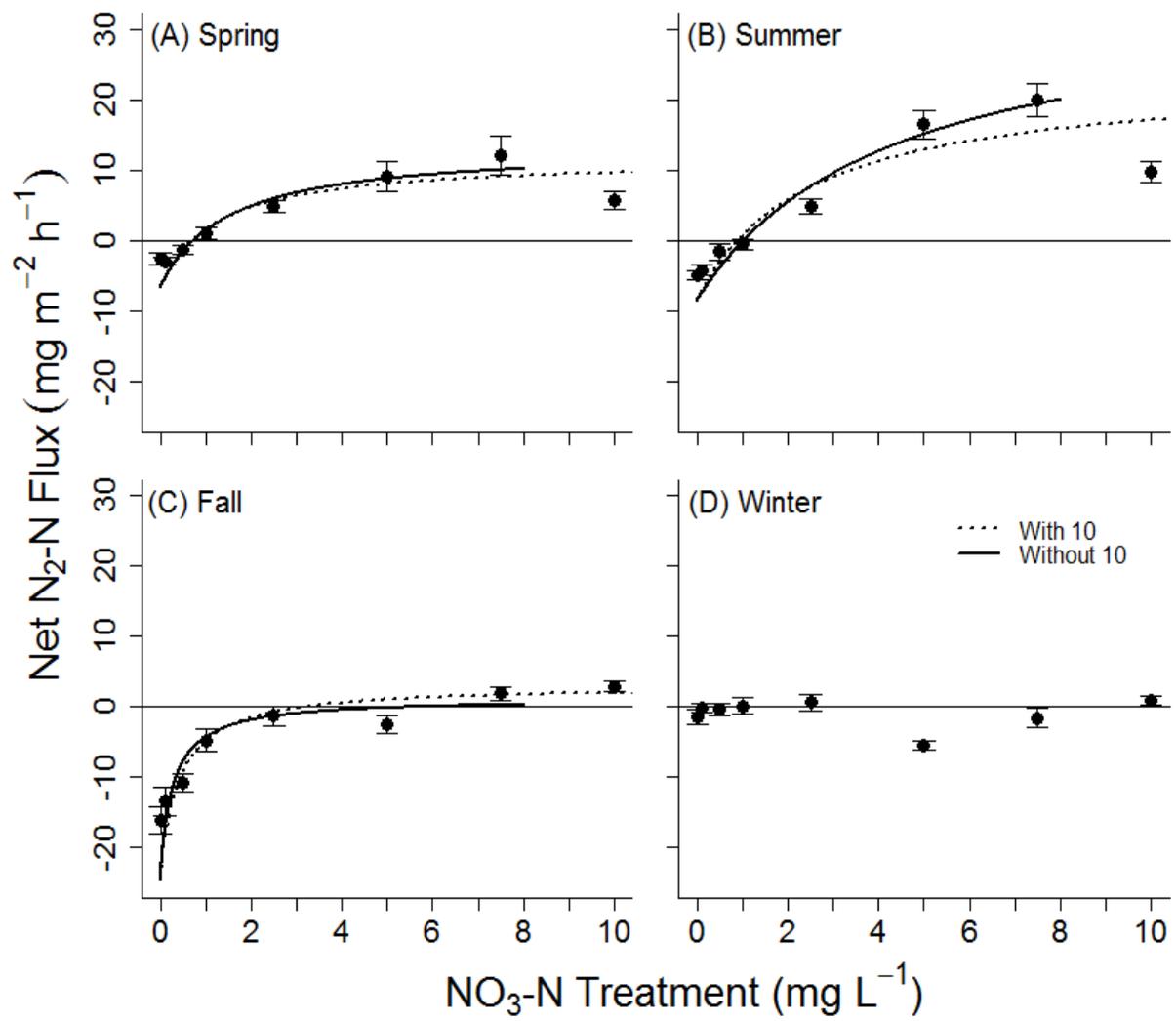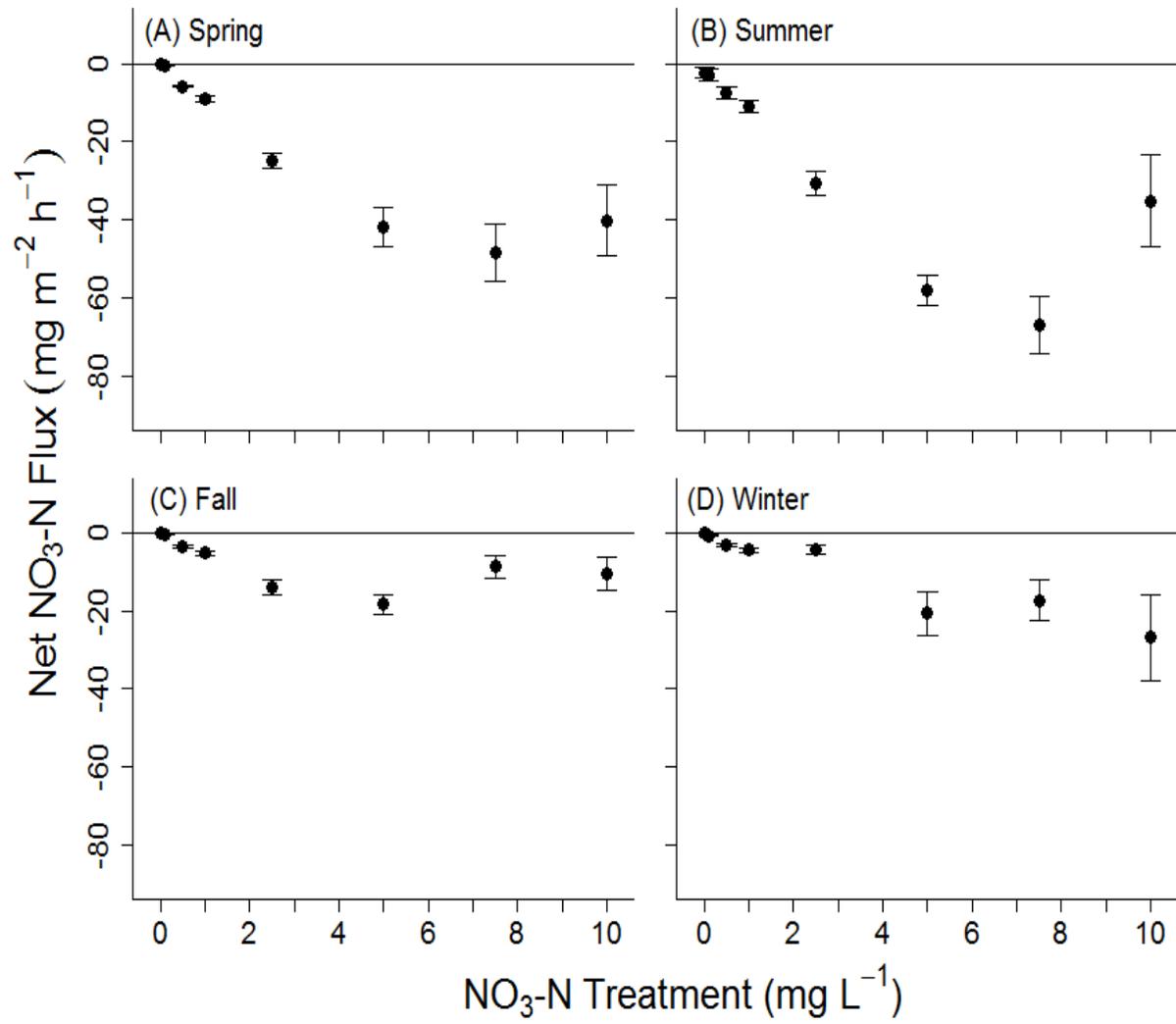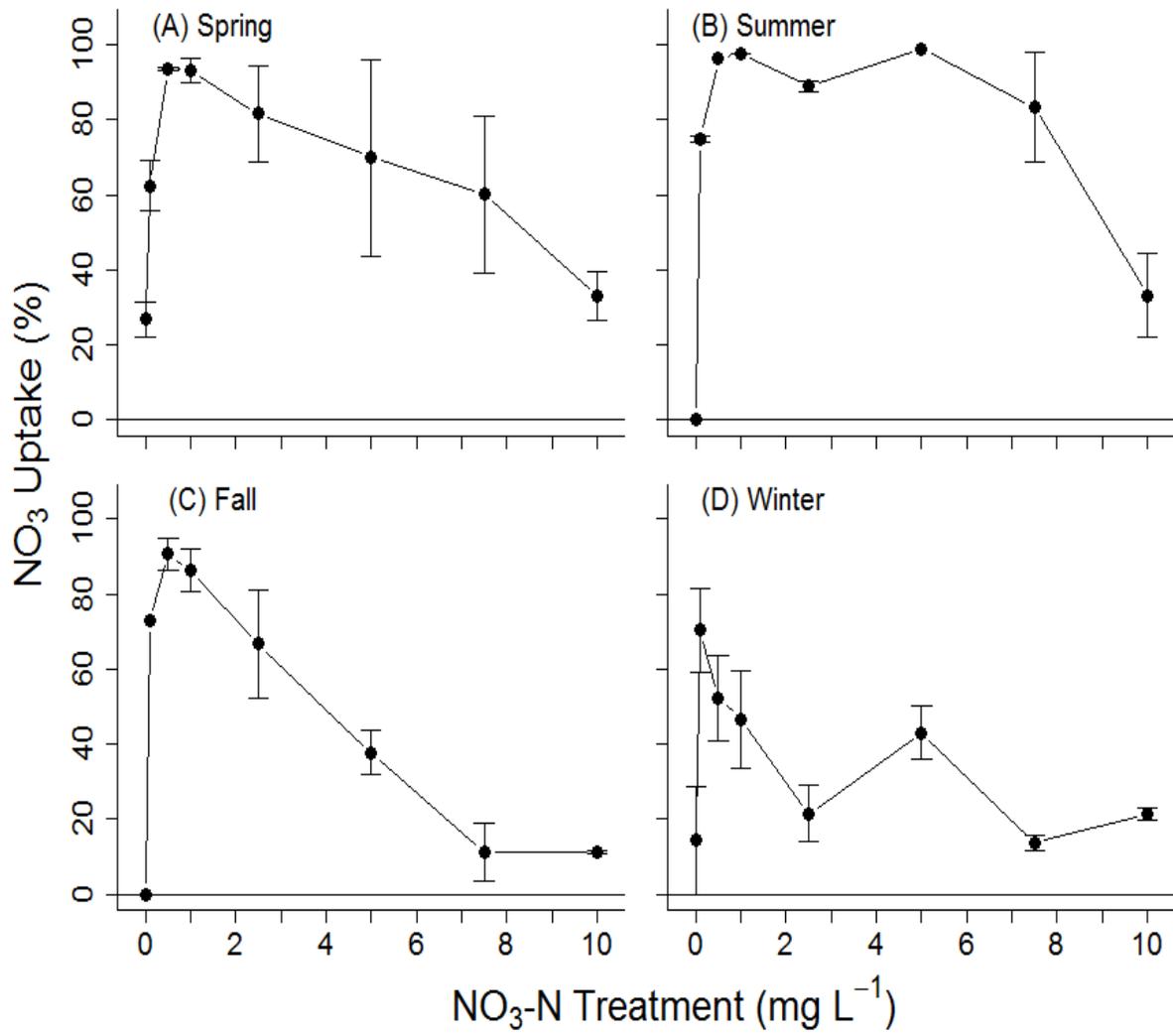## 2.8    Figures





Figure 1

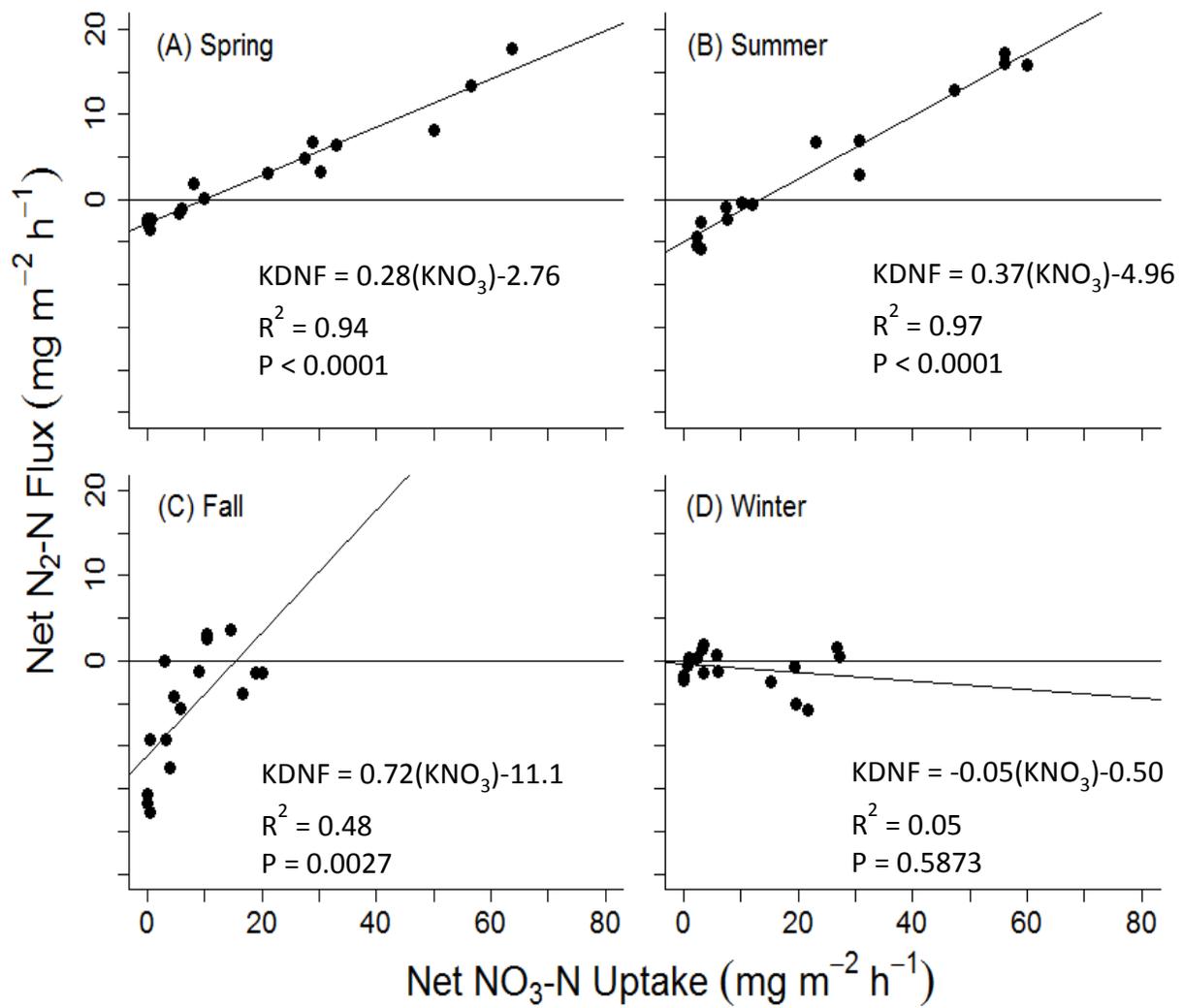Figure 2

Figure 3

Figure 4

Figure 5

**3.** **FROM THE CORE TO THE DITCH: THE INFLUENCE OF SCALE ON APPLICATION OF NITROGEN GAS FLUX MODELS TO ESTIMATE DENITRIFICATION RATES IN EXPERIMENTAL SYSTEMS VEGETATED WITH CUTGRASS (*LEERSIA ORYZOIDES*)**

**3.1** **Introduction**

Excessive inputs of nutrients, especially nitrogen (N), from agricultural sources to freshwater ecosystems contribute to the degradation of downstream water resources (Galloway et al., 2008). The resulting eutrophication of freshwater ecosystems is one of the most common impairments of surface waters in the United States (U.S. EPA, 1990; Carpenter and Caraco, 1998), and can lead to the development of harmful algal blooms in downstream ecosystems (Glibert et al., 2014). High levels of N loading into the Mississippi River Basin from major row crop agricultural regions in the United States result in  annual harmful algal blooms near the outlet of the Mississippi River in the Gulf of Mexico (Alexander et al., 2008). Harmful algal blooms can impact both marine and freshwaters, causing widespread hypoxia and summer fish kills (Carpenter and Caraco, 1998). Agricultural land mangers not only need best management practices (BMPs) that may reduce nutrient impacts, but also tools to predict water quality benefits provided by their implementation.

Wetlands can act as hot spots of N transformation processes and play a significant role in reducing pollution to downstream ecosystems by enhancing uptake and transformation of N (Mitsch and Gosselink, 2000), but are costly to construct and take valuable agricultural land out of production. If agricultural ditches can be managed to act as small wetland systems, they have the potential to mitigate N loads carried in agricultural runoff (Kröger et al., 2008; Moore et al., 2010; Mahl et al., 2015). The addition of low-grade weirs can increase the hydraulic residence time of ditches to allow for more microbial processing (Kröger et al., 2009), and maintenance of

vegetation in ditch channels can enhance nitrate ($NO_3^-$) removal (Tyler et al., 2012; Taylor et al., 2015). Ditch BMPs may be viable for large scale implementation because ditches make up a significant amount of fluvial waterways in agriculturally impacted areas (Herzon and Helenius, 2008). Ditches also serve as sentinels of downstream ecosystems in that the water quality exiting the ditch often impacts that of the entire watershed. As establishment and maintenance of BMPs can be costly (Gitau et al., 2004), tools that predict potential nutrient load reductions are necessary to evaluate the benefits of large-scale implementation of ditch BMPs.

A previous study demonstrated the presence of cutgrass (*Leersia oryzoides*) in ditch sediments resulted in significantly higher $N_2$ flux out of the system via denitrification (DNF) compared to unvegetated sediments or sediments planted with cattail (*Typha latifolia*; Taylor et al. 2015). In a follow-up study, I explored variation in DNF across a gradient of $NO_3^-$ loading levels as well as across a seasonal temperature gradient to better understand environmental controls that drive DNF in sediments planted with cutgrass (Chapter 2). Net $N_2$ fluxes exhibited Michaelis-Menten kinetics across a $NO_3^-$ gradient in the spring, summer, and fall, allowing for models to be developed describing the relationship between $NO_3^-$ concentrations in the overlying water and the amount of net $N_2$ flux produced. Additionally, a strong linear relationship between $NO_3^-$ uptake and net $N_2$ fluxes was also apparent in the spring, summer, and fall. Both the Michaelis-Menten and linear regression models developed based on intact sediment core incubations have the potential to be used as predictive tools for estimating DNF rates in larger scale ditch environments planted with cutgrass.

The objective of this study was to apply models developed in Chapter 2 to cutgrass data collected from two independent studies conducted at two different scales. The first study was a published mesocosm runoff experiment ("Study 1," Taylor et al. 2015). The second study

examined the movement of a $NO_3^-$ pulse through experimental ditches with and without weirs containing stands of vegetation dominated by cutgrass ("Study 2"; Iseyemi et al., unpublished). I chose to focus on three research questions when assessing model application and validity:

(1) Do both models reasonably estimate $N_2$ fluxes from cutgrass ditch sediments?

(2) Does scale affect model application?

(3) What constraints of applying models at larger scales affect development of tools for land managers?

Evaluating model application across scales can shed light on which model may function better in predicting DNF at landscape level and provide useful predictions to land managers when making decisions on which BMPs to implement on their land.

## 3.2    Materials and Methods

### 3.2.1    *Data Sources*

Data for Study 1 are from a simulated runoff event conducted in ditch mesocosms during early June 2014 in Oxford, Mississippi (Taylor et al., 2015). My goal was to apply the Michaelis-Menten model and the linear regression model developed in Chapter 2 to the time series $NO_3^-$ concentrations and computed $NO_3^-$ uptake rates from this experiment to derive more accurate estimates of DNF across the duration of the experiment. Mesocosms were used to mimic ditches with three varying vegetation treatments: unvegetated, cattail, and cutgrass. Nitrate concentrations and net $N_2$ fluxes were measured for all treatments at the first time effluent was released from the mesocosms in varied intervals from t = 0 to 168 h.  I only applied my models

to data collected in the three mesocosms planted with cutgrass treatments. This was done to compare total system DNF estimates based on my models to those generated during the original study based on net $N_2$ fluxes from ditch sediments vegetated with cutgrass measured in core incubations conducted at $NO_3^-$ concentrations close to mean study values that were extrapolated to the 48-hour time period.

The data for Study 2 was obtained from experiments conducted in the spring of 2012, 2013, and 2014, in experimental ditches at Arkansas State University in Jonesboro, Arkansas (Iseyemi et al. unpublished). Eight ditches containing mixed stands of vegetation dominated by cutgrass were used in the study. Half of the ditches were mowed and half the ditches were not. Additionally, half of the ditches contained two weirs and half of the ditches contained no weirs. Treatments were interspersed in such a way that not all ditches with weirs had the same mowing treatment. I had no a-priori hypothesis about the effect of mowing on denitrification, thus my analysis focused on comparing $NO_3^-$ uptake and net $N_2$ fluxes between weired and unweired ditches. Ditches were 60 m long; in ditches with weirs, one weir was placed at 20 m and the other weir was located at 40 m along the length of the ditch.

Water was supplied to the ditches from a retention pond located upstream of the ditches which was filled by a groundwater source having low background nutrient concentrations. Regardless of the presence of weirs, all ditches were managed to have a hydraulic residence time of 2 hours. As a result, ditches with weirs had an average flow rate of 58 L min$^{-1}$ and ditches without weirs had an average flow rate of 11.8 L min$^{-1}$ to ensure ditches had an equivalent residence time. Simulated runoff events were conducted in ditches during the spring of each year. Prior to exposure, ditches were allowed to flow for 1 week to allow for saturation of the ditch sediments.

Nutrient slugs, including $NO_3^-$ added as sodium nitrate, were mixed in large troughs containing 121 L of water. Average concentrations of $NO_3^-$ in the mixing chambers were 22.0 mg $L^{-1}$, 12.6 mg $L^{-1}$, and 18.3 mg $L^{-1}$ for 2012, 2013, and 2014 respectively. To begin an event, nutrient slugs were added to the ditch in one pulse ($t = 0$). In 2012, the mixing chamber for ditch 8 was knocked over prior to the event, allowing the nutrient slug to begin moving down the ditch before sampling could begin. During the $NO_3^-$ addition, samples for $NO_3^-$ were taken at 0, 20, 40, and 60 m along the ditch over a 24-hour period at varying time intervals. The full sampling scheme through time can be found in Table 1. Samples were transported on ice to the laboratory, where they were frozen until the time of analysis. Nitrate concentrations were analyzed using a Lachat QuickChem 8599 autoanalyzer (Lachat Instruments) automated ion analyzer using the cadmium reduction method. All duplicate $NO_3^-$ sample concentrations were averaged for use in data analysis.

### 3.2.2 *Model Application: Spring Michaelis-Menten Model*

I applied the spring Michaelis-Menten model developed in Chapter 2 to both Study 1 and Study 2. The final model is as follows:

$$Net\ N_{2-mm}\ Flux = \frac{19.94 * [NO_3]}{1.44 + [NO_3]} - 6.68$$

Equation 1

where *[NO3]* is the concentration of $NO_3^-$ in the overlying water (in mg $L^{-1}$), the value subtracted from the characteristic Michaelis-Menten model is back-correction carried out so model predictions reflect measured fluxes, and *net N2-mm flux* is the $N_2$ flux produced at that given $NO_3^-$ concentration (in mg $m^{-2}$ $h^{-1}$).

For Study 1, measured $NO_3^-$ concentrations at each sampling time were used to estimate the amount of net $N_{2-mm}$ flux based on Michaelis-Menten kinetics at each time point (t= 0 to 168 h). I then plotted all net $N_{2-mm}$ flux outputs from 0 to 48 h only versus time and integrated the area under the resulting curve. This provided an estimate of the total mass of $N_2$ produced over the course of the first 48 hours of the experiment for comparison to estimates from a previous study (Taylor et al., 2015). I used the spline integration method in the *MESS* package in R (version 3.2.3; R Development Core Team, Vienna, Austria) to conduct this analysis.

For Study 2, I used the maximum $NO_3^-$ concentrations measured at each sampling point along the length of the ditch to give us an estimate of instantaneous DNF while tracking the movement of the pulse past each sampling location along the ditch. I chose to only use maximum concentrations to constrain the analysis to the movement of the nutrient pulse through the ditch system. To obtain an areal mass estimate of $N_2$ produced in each ditch, I plotted the maximum net $N_{2-mm}$ flux outputs at each sampling location along the ditch versus time for all ditches and integrated the area under the resulting curve. Integration was carried out using the spline integration method with the *MESS* package in R (version 3.2.3; R Development Core Team, Vienna, Austria). The area under the curve provides an estimate of $N_2$ produced via DNF per $m^2$ for the nutrient pulse.

### 3.2.3  *Model Application: Spring Linear Regression Model*

I applied the spring linear regression model from Chapter 2 to both Study 1 and 2 as well. The final spring linear regression model is as follows:

$$Net\ N_{2-lr}\ Flux = 0.28(NO_3\ Uptake) - 2.76$$

Equation 2

where *NO₃ uptake* is $NO_3^-$ uptake (in mg m$^{-2}$ h$^{-1}$) and *net N$_{2-lr}$ flux* is the resulting $N_2$

produced (in mg m$^{-2}$ h$^{-1}$). For Study 1, I first had to calculate $NO_3^-$ uptake in the mesocosm for

given time intervals throughout the stagnant phase (t=6 to 168 h) using the following equation:

$$NO_3 \; uptake = \frac{[(NO_3)_{T2} - (NO_3)_{T1}] * (volume/t)}{SA}$$

Equation 3

where *(NO₃)$_{T2}$* is the $NO_3^-$ concentration in the effluent at time period 2 (T2; in mg L$^{-1}$), *(NO₃)$_{T1}$*

is the peak $NO_3^-$ concentration in the effluent at time period 1 (T1; in mg L$^{-1}$), *volume* is the

volume of the mesocosm (in L), *t* is the amount of time between T1 and T2 (in h), *SA* is the

surface area of the mesocosm (in m$^2$), and *NO₃ uptake* is the $NO_3^-$ uptake rate in the mesocosms

for the whole time interval (in mg m$^{-2}$ h$^{-1}$). Then the calculated $NO_3^-$ uptake rates for the entire

mesocosm were used in the linear regression model I developed (Eq. 2) to obtain a total $N_2$ flux

for that time period in each mesocosm.

In order to apply the linear regression to Study 2, I first had to calculate $NO_3^-$

uptake over the course of the ditch during the pulse with the following equation:

$$NO_3 \; Uptake = \frac{([NO_3]_{in} - [NO_3]_{out}) * Flow_{pulse}}{SA}$$

Equation 4

where *[NO₃]$_{in}$* and *[NO₃]$_{out}$* are the peak $NO_3^-$ concentrations measured at 0 m and 60 m,

respectively, (in mg L$^{-1}$), *Flow$_{pulse}$* is the flow rate of the pulse through the ditch (in L h$^{-1}$), *SA* is

the surface area of the ditch (in m$^2$), and *NO₃ Uptake* is the total amount of $NO_3^-$ taken up from

the pulse across the length of the ditch (in mg m$^{-2}$ h$^{-1}$). Then the calculated $NO_3^-$ uptake rates

were applied to the linear regression model I developed (Eq. 2) to obtain an estimate of $N_2$ flux along the length of the ditch during the pulse.

### 3.2.4 *Statistical Analyses*

To analyze the data statistically, I carried out a one-way analysis of variance (ANOVA) in R (version 3.2.3; R Development Core Team, Vienna, Austria) on the Study 1 and Study 2 data. For Study 1, my goal was to evaluate the differences between Michaelis-Menten and linear regression model estimates from the current study. For Study 2, my goal was to evaluate the effect of weir presence on $NO_3^-$ concentrations and net $N_{2\text{-mm}}$ fluxes for the Michaelis-Menten model, as well as $NO_3^-$ uptake and net $N_{2\text{-lr}}$ fluxes for the linear regression model. The 2012 ditch 8 data from the accidental premature nutrient release did not appear to be outliers so I did not exclude the data from the statistical analyses. I also carried out a two-way ANOVA on the areal $N_2$ flux estimates from Study 2 to evaluate in the effect of year and weir. ANOVAs assume that both observations and errors are normally distributed, variances for group response and residuals are homoscedastic, and the observations and errors are independent. I used graphical examination in R to test the assumptions of both statistical tests prior to carrying out the analyses and to examine residuals after conducting the analyses.

### 3.3 Results

### 3.3.1 *Model Application to Mesocosm Experiments (Study 1)*

During the 6 h runoff period, $NO_3^-$ concentrations rose from background levels (~0.03 mg $L^{-1}$) as enriched water replaced unenriched water in mesocosms. Nitrate concentrations reached a maximum of approximately 4 mg $L^{-1}$ during the stagnant phase of the experiment 9 h

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after initiation of the runoff event (Figure 1A). After peaking, rapid uptake of $NO_3^-$ was evident during the stagnant period with concentrations declining to approximately 0.30 mg $L^{-1}$ by 48 h (Figure 1A). There was no evidence of excess $NO_3^-$ in the water column after 72 h. Predicted mesocosm net $N_{2-mm}$ fluxes followed a similar trend (Figure 1B). At 0 h, net $N_{2-mm}$ fluxes were predicted to be negative. As $NO_3^-$ concentrations began to rise, predicted net $N_{2-mm}$ fluxes became positive. Like $NO_3^-$ concentrations, I predicted a peak at 9 h of 7.91 mg $m^{-2}$ $h^{-1}$ in net $N_{2-mm}$ flux. From 48 to 168 h, predicted net $N_{2-mm}$ fluxes decreased as $NO_3^-$ concentrations decreased as well. Over a 48 h period, I predicted that $310.80 \pm 5.03$ mg of N was denitrified, greater than the estimate of $284.48 \pm 29.69$ calculated by applying measured DNF rates to a mass balance from the same dataset (Taylor et al., 2015).

I observed a positive peak in $NO_3^-$ uptake rates ($\sim$40 mg $m^{-2}$ $h^{-1}$) between 9 and 12 h followed by a rapid decline in $NO_3^-$ uptake between 12 and 24 h as $NO_3^-$ was removed from the system (Figure 2A). Uptake rates were slower and continued to decrease from 24 to 72 h. Between 72 and 168 h, I did not observe measurable $NO_3^-$ uptake. Predicted net $N_{2-lr}$ fluxes from the mesocosms followed a similar pattern as $NO_3^-$ uptake (Figure 2B). Between 6 and 9 h, predicted net $N_{2-lr}$ fluxes were negative when $NO_3^-$ concentrations were still climbing to a maximum. Between 9 and 12 h, predicted net $N_{2-lr}$ fluxes were greatest at 7.62 mg $m^{-2}$ $h^{-1}$. Predicted net $N_{2-lr}$ fluxes tracked changes in $NO_3^-$ uptake and declined rapidly from 12 to 48 h. My regression model predicted negative net $N_{2-lr}$ fluxes from 24 to 48 h, once $NO_3^-$ concentrations were expected to limit $N_2$ production based on my previous study (Chapter 2). After 48 h, net $N_{2-lr}$ fluxes were predicted to be negative, indicating DNF is likely limited by background $NO_3^-$ concentrations in the mesocosms. The peak net $N_2$ fluxes were not significantly different between the two models (ANOVA, $F = 1.797$, $p = 0.251$)

### 3.3.2  *Model Application to Experimental Ditches (Study 2)*

I observed a nonlinear decline in peak $NO_3^-$ concentrations over the length of the experimental ditches, indicating that rapid uptake occurred in the first 20 m of the ditch (Figure 3). This pattern was "stronger" and consistent between years in the weired ditches (Fig. 3A), whereas a similar nonlinear pattern was only observed in 2012 for unweired ditches (Fig. 3B). Overall, differences in $NO_3^-$ concentrations were not statistically significant between weired and unweired ditches (Table 2). Trends were not significantly different across years in the weired and unweired ditches as well (Table 2).

Patterns in predicted net $N_{2-mm}$ fluxes also demonstrated potential differences in DNF between weired and unweired ditches (Figure 4). I observed linear declines in net $N_{2-mm}$ fluxes with steeper slopes over the length of weired ditches compared to unweired ditches. However, predicted net $N_{2-mm}$ fluxes exhibited considerably more variability across weired ditches compared to the unweired ditches (Fig. 4). The difference in predicted net $N_{2-mm}$ flux between weired and unweired ditches was statistically significant (Table 2). Trends were not significantly different across years in either type of ditch (Table 2).  Predicted net $N_{2-mm}$ fluxes integrated over time were not significantly different between weired and unweired ditches each year the experiment was carried out (Figure 5; Two-Way ANOVA, $p = 0.993$). Calculated $NO_3^-$ uptake was significantly different between weired and unweired ditches (Figure 6A; Table 2), but there was no difference between years (Table 2). When applied to the linear regression model, the results suggested unweired ditches also had significantly greater overall net $N_2$ fluxes out of the ditch (Figure 6B; Table 2), which is contrary to what was observed as the overall result of the Michaelis-Menten model estimates for net $N_{2-mm}$ flux. Again, the difference in net $N_{2-lr}$ flux between years was not significant (Table 2).

## 3.4 Discussion

Developing models agricultural land managers can use to evaluate the benefits of adopting ditch BMPs facilitates their widespread implementation. My study applied both Michaelis-Menten and linear regression models developed to predict net $N_2$ fluxes from cutgrass ditch sediments from two independent datasets to validate both models. I demonstrated both models generally predicted net $N_2$ fluxes within a comparable range from cutgrass ditch sediments when applied at the mesocosm level. Additionally, the predicted areal mass of $N_2$ flux from the mesocosms were comparable to estimates derived via application of measured DNF rates to mass balance (Taylor et al., 2015). I was able to examine how scale affects model application by including both mesocosm and experimental ditch data in model validation. My results suggest the Michaelis-Menten model may be more suitable across scales, especially when comparing experimental treatments with different spatial parameters.

### 3.4.1 *Do both models reasonably estimate $N_2$ fluxes from cutgrass ditch sediments?*

For Study 1, both models resulted in similar estimates of maximum predicted net $N_2$ fluxes, indicating estimates based on more detailed models were comparable to estimates based on more simplified models. The estimates of maximum net $N_2$ fluxes are similar to the maximum DNF rates observed in a wetland in during the same time of year Studies 1 and 2 were conducted (Poe et al., 2003). When comparing the mean of areal mass estimates of net $N_2$ flux, the estimate derived from the predicted net $N_{2\text{-mm}}$ fluxes is greater than that reported from a 48-hour mass balance applied to measured $N_2$ fluxes out of the same system (Taylor et al., 2015). The approach used in Taylor et al. (2015) may underestimate areal $N_2$ fluxes in the mesocosms as the measured DNF rates from intact cores applied to the mass balance approach were assumed to represent the average DNF rates for the entire mesocosm for the duration of the study. The

extrapolation of measured DNF rates to the entire mesocosm may present a scaling issue in the original study and does not account for variation in DNF rates as $NO_3^-$ concentrations changed in the mesocosms over time for this specific application. In contrast, my Michaelis-Menten model is able to account for the variation in DNF rates with $NO_3^-$ concentrations through time. However, predicted areal $N_2$ fluxes from the current study were within one standard error of the areal mass estimates predicted via the mass balance approach, suggesting a mass balance of measured DNF rates and integration of predicted net $N_{2\text{-mm}}$ fluxes may still be comparable methods for estimating areal mass of net $N_2$ flux produced in cutgrass systems.

For Study 2, $NO_3^-$ concentrations and predicted net $N_2$ fluxes decreased along the length of the ditch, suggesting $NO_3^-$ was being taken up and denitrified. The highest predicted net $N_{2\text{-mm}}$ fluxes were between 10 and 15 mg m$^{-2}$ h$^{-1}$, which was within the range of maximum reported net $N_2$ fluxes observed in cutgrass environments during the spring and summer (Chapter 2). The net $N_{2\text{-lr}}$ fluxes in weired and unweired ditches were also generally within the range reported in Chapter 2. Positive net $N_2$ fluxes corresponded to the highest $NO_3^-$ concentrations, indicating DNF is occurring when $NO_3^-$ availability does not limit these systems. The predicted net $N_2$ fluxes in experimental ditches are also similar to those observed in other aquatic ecosystems, including rivers, lakes, estuaries, and coastal environments (Piña-Ochoa and Álvarez-Cobelas, 2006). This indicates both models estimate reasonable net $N_2$ fluxes in experimental ditches as well as mesocosms.

### 3.4.2  *Does scale affect model application?*

My results suggest scale may have an effect on the application of both models to environments containing sediments vegetated with cutgrass. Both models resulted in comparable predicted $N_2$ fluxes when applying them to the mesocosm data from Study 1. The maximum

predicted $N_2$ fluxes from Study 1 ranged from 7.62 to 7.92 mg m$^{-2}$ h$^{-1}$. These estimates fall within the range of reported DNF rates for ditch sediments vegetated with cutgrass (Taylor et al. 2015; Chapter 2) and are similar to reported annual DNF rates in equivalent systems (Seitzinger, 1988; Piña-Ochoa and Álvarez-Cobelas, 2006). This suggests my models are valid at the mesocosm scale.

In contrast, the application of my models to larger scale experimental ditch data from Study 2 highlighted the difficulty in applying lab based quantitative DNF relationships to field-scale data. In the ditches without weirs, I predicted greater instantaneous net $N_{2-mm}$ flux at each point along the length in the ditch; however, this would indicate $NO_3^-$ concentrations were actually greater across the length of the ditch and less $NO_3^-$ overall was removed in the unweired ditches via DNF. In weired ditches, less instantaneous net $N_{2-mm}$ flux at the 60 m sampling location suggests $NO_3^-$ concentrations are lower at the outflow point than in unweired ditches.

Additionally, in Study 2, the linear regression model predicted unweired ditches to have greater predicted net $N_{2-lr}$ fluxes than weired ditches, which contrasts directly with what has been documented in the literature (Kröger et al., 2014). This can be attributed to the fact that the weired ditches included in the study had a greater surface area during runoff events. This resulted in lesser estimates of $NO_3^-$ uptake as uptake is normalized by area, which suggests even if the actual mass of $NO_3^-$ taken up across the length of the ditch was similar in both types of ditches, it would be masked due to differences in surface area. This would give rise to net $N_{2-lr}$ flux patterns that do not reflect what is actually occurring within the system. As with the Michaelis-Menten models, these results contrast with trends reported in the literature. Discharge has been shown to affect the percentage of N loading retained (Saunders and Kalff, 2001), and with lesser discharge is associated with increased DNF (Alexander et al., 2000; Kröger et al., 2012). Weired ditches

generally have greater hydraulic and chemical residence times (Kröger et al., 2009) and greater DNF potential than unweired ditches (Kröger et al., 2014); however, application of the linear regression model does not reflect these trends. Therefore, the linear regression model may not be useful in comparing environments with different spatial parameters. At larger scales, I suggest the Michaelis-Menten model may be more suitable for comparing data as the formula does not take surface area of the experimental unit into account.

### 3.4.3 *What constraints of applying models at larger scales affect development of tools for land managers?*

I identified several constraints when applying my sediment core models to large scale systems, especially whole-ditch systems. In general, it is important to consider the hydrology of the system when validating both models. The ditches used in the experimental ditch study had a reduced residence, or flushing time, as compared to my cores used in the model development experiment. A longer residence time in the cores may have allowed more time for denitrifying bacteria to act on $NO_3^-$ being supplied in the overlying water, resulting in higher rates of DNF (Royer et al., 2004; Inwood et al., 2005). Thus, application of my models may result in an overestimate of predicted net $N_2$ fluxes. Additionally, as cores were dosed with $NO_3^-$ continuously, the models may not be well-suited for nutrient pulse experiments, such as Study 2. As natural runoff events will include a rise and fall of $NO_3^-$ concentrations, like nutrient pulse experiments, my models may not accurately represent the nutrient dynamics at the ditch-scale.

The Michaelis-Menten model may also produce estimates of net $N_2$ fluxes that do not reflect the biology of the system. Firstly, it estimates instantaneous net $N_2$ fluxes, or how much DNF may be occurring at an exact moment. This can result in misleading patterns in data as seen in the application of the Michaelis-Menten model to Study 2, where unweired ditches were

predicted to have more net $N_2$ flux at each point along the ditch. The experimental units (i.e., weired and unweired ditches) for which $NO_3^-$ uptake is calculated may also result in trends in net $N_2$ fluxes predicted by the linear regression model that are a result of model application rather than biological significance due to differences in spatial scale.

The range of input values is also an important consideration when applying both the Michaelis-Menten and linear regression models. The Michaelis-Menten model was developed using $NO_3^-$ concentrations of up to 7.5 mg $L^{-1}$ in the overlying water. Therefore, I would not recommend using $NO_3^-$ concentrations significantly greater than 7.5 mg $L^{-1}$ as inputs to the Michaelis-Menten model. If extrapolated to much higher $NO_3^-$ concentrations, a saturation effect in net $N_2$ fluxes may be observed which does not truly exist in the experimental system due to the nature of the Michaelis-Menten relationship. However, DNF has been shown to become saturated anywhere from 2 to 7.5 m $L^{-1}$ (Inwood et al. 2007, Zhong et al. 2010; Chapter 2), so it is unlikely this would present an issue at marginally higher $NO_3^-$ concentrations. Linear regression model development was based on $NO_3^-$ uptakes rates up to about 80 mg $m^{-2}$ $h^{-1}$; therefore, if $NO_3^-$ uptake rates exceed this maximum, I would advise the model not be applied to the given dataset in question or those data points be excluded from the analysis.

It is also critical $NO_3^-$ uptake is calculated reliably. For example, the experimental ditch study did not utilize a conservative tracer when employing the nutrient pulses, making calculating $NO_3^-$ uptake along the ditch more difficult and perhaps resulting in over- or underestimations of net $N_2$ fluxes out of the ditch system when applying the linear regression model. Reliable ways of calculating nutrient uptake include solute injections (Davis and Minshall, 1999), short-term nutrient additions with a conservative tracer (Stream Solute Workshop 1990, Bernot et al. 2006), stable isotope additions (Hamilton et al., 2001; Ashkenas et

al., 2004; Sobota et al., 2012), and mass balance (Molot and Dillon, 1993; Dodds et al., 2000; McMillan et al., 2010). These methods should be considered when designing experiments to which the linear regression model will be applied.

### 3.4.4   *Conclusions*

I suggest further exploration into the application of both models to assess their usefulness. More comparisons of model estimates with cutgrass environment mass balances as well as comparisons with measurements of DNF rates are necessary to refine the Michaelis-Menten and linear regression models and accurately predict net $N_2$ fluxes from cutgrass systems. Once both models have been fully assessed, they may be used to inform more complex landscape-scale models, such as the Soil and Water Assessment Test (SWAT) and Agricultural Non-Point Source (AGNPS) Pollution models. If a ditch module can be created for the SWAT or AGNPS pollution models, the effects of different ditch management practices, including planting ditch sediments with cutgrass, could be evaluated more easily at the watershed scale to determine their costs and benefits. This would allow land managers to fully understand the implications of putting vegetated ditch BMPs in place on their cropland by considering the influence of other environmental variables and determining whether if vegetated ditches are an effective economical means of reducing nutrient loads to sensitive downstream ecosystems.

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**Tables**

Table 1. Nutrient concentration sampling time schematic along the experimental ditches for Study 2.

| Time (h) | Site A (0 m) | Site B (20 m) | Site C (40 m) | Site D (60 m) |
|---|---|---|---|---|
| 0 | X | X | X | X |
| 0.25 | | X | X | X |
| 0.5 | | X | X | X |
| 0.75 | | X | X | X |
| 1 | X | X | X | X |
| 1.25 | | X | X | X |
| 1.5 | | X | X | X |
| 2 | X | X | X | X |
| 2.5 | | X | X | X |
| 3 | | X | X | X |
| 3.5 | | X | X | X |
| 4 | X | X | X | X |
| 5 | | X | X | X |
| 6 | | X | X | X |
| 7 | | X | X | X |
| 8 | X | X | X | X |
| 10 | | X | X | X |
| 24 | X | X | X | X |

Table 2. Effects of weir and year on $NO_3^-$ concentrations, $NO_3^-$ uptake, and predicted net $N_2$ fluxes from both Michaelis-Menten (MM) and linear regression (LR) models for Study 2, with $F$ values and associated $p$ values based on a one-way analysis of variance.

| Response | Source of Variation | $F$ | $p$ |
|---|---|---|---|
| $NO_3^-$ concentrations | Weir | 2.75 | 0.101 |
| | Year | 0.89 | 0.413 |
| $NO_3^-$ uptake | Weir | 5.26 | 0.032 |
| | Year | 1.44 | 0.259 |
| Net $N_2$ fluxes (MM) | Weir | 8.58 | 0.004 |
| | Year | 0.53 | 0.59 |
| Net $N_2$ fluxes (LR) | Weir | 5.26 | 0.032 |
| | Year | 1.44 | 0.259 |

Table 3. Comparison of areal mass estimates of $N_2$ fluxes out of the cutgrass mesocosms over a 48-hour period for the mesocosm runoff experiment dataset based on two different methodologies.

| | $N_2$ Flux (mg m$^{-2}$) | Method | Source |
|---|---|---|---|
| Mesocosm A | 339.04 | Spline Integration | Current Study |
| Mesocosm B | 258.58 | Spline Integration | Current Study |
| Mesocosm C | 334.78 | Spline Integration | Current Study |
| Mesocosm Mean ± SE | 310.80 ± 5.03 | Spline Integration | Current Study |
| Mesocosm Mean ± SE | 284.48 ± 29.69 | Mass Balance* | Taylor et al. 2015 |

*Full Method: Measured DNF rates applied to a mass balance approach

**Figure Legends**

Figure 1. Measured $NO_3^-$ concentrations (mg $L^{-1}$) through time (A) and predicted net $N_{2-mm}$ fluxes (mg $m^{-2}$ $h^{-1}$) through time resulting from the application of the Michaelis-Menten model (B) for study 1.

Figure 2. Calculated $NO_3^-$ uptake (mg $m^{-2}$ $h^{-1}$) through time (A) and predicted net $N_{2-lr}$ fluxes (mg $m^{-2}$ $h^{-1}$) through time resulting from the application of the linear regression model (B) for study 1.

Figure 3. Peak $NO_3^-$ concentrations (mg $L^{-1}$) at each longitudinal sampling location along the ditch for both weired (A) and non-weired (B) ditches for study 2.

Figure 4. Maximum predicted net $N_{2-mm}$ fluxes (mg $m^{-2}$ $h^{-1}$) determined via application of the Michaelis-Menten models at each longitudinal sampling location along the ditch for both weired (A) and non-weired (B) ditches for study 2.

Figure 5. Areal mass estimates of $N_2$ removal (mg $m^{-2}$) throughout the nutrient pulse for both weired and non-weired ditches for study 2 by year.

Figure 6. Calculated $NO_3^-$ uptake (mg $m^{-2}$ $h^{-1}$) across years (A) and predicted net $N_{2-lr}$ fluxes (mg $m^{-2}$ $h^{-1}$) across years resulting from the application of the linear regression model (B) for both weired and non-weired ditches for study 2.
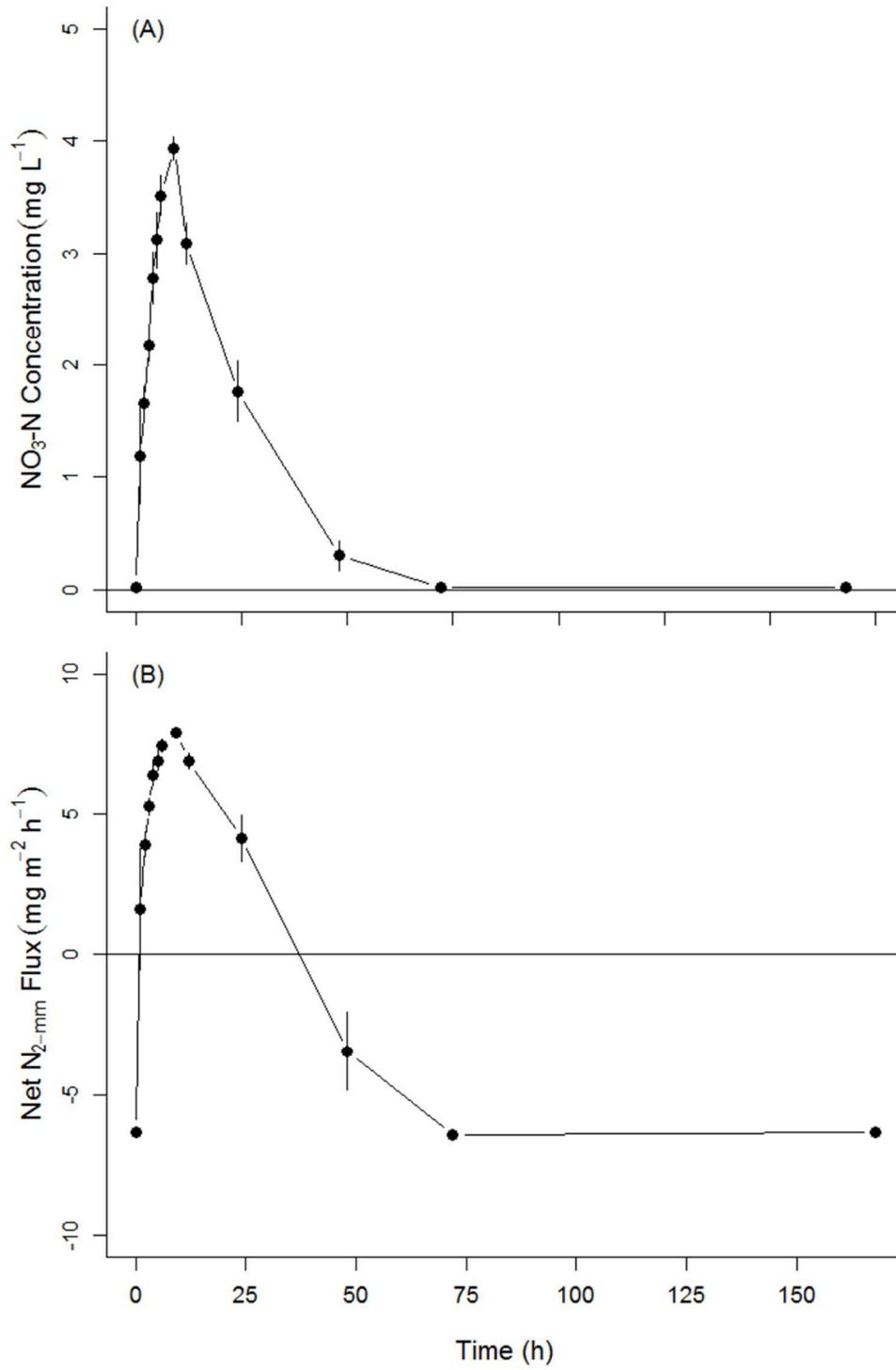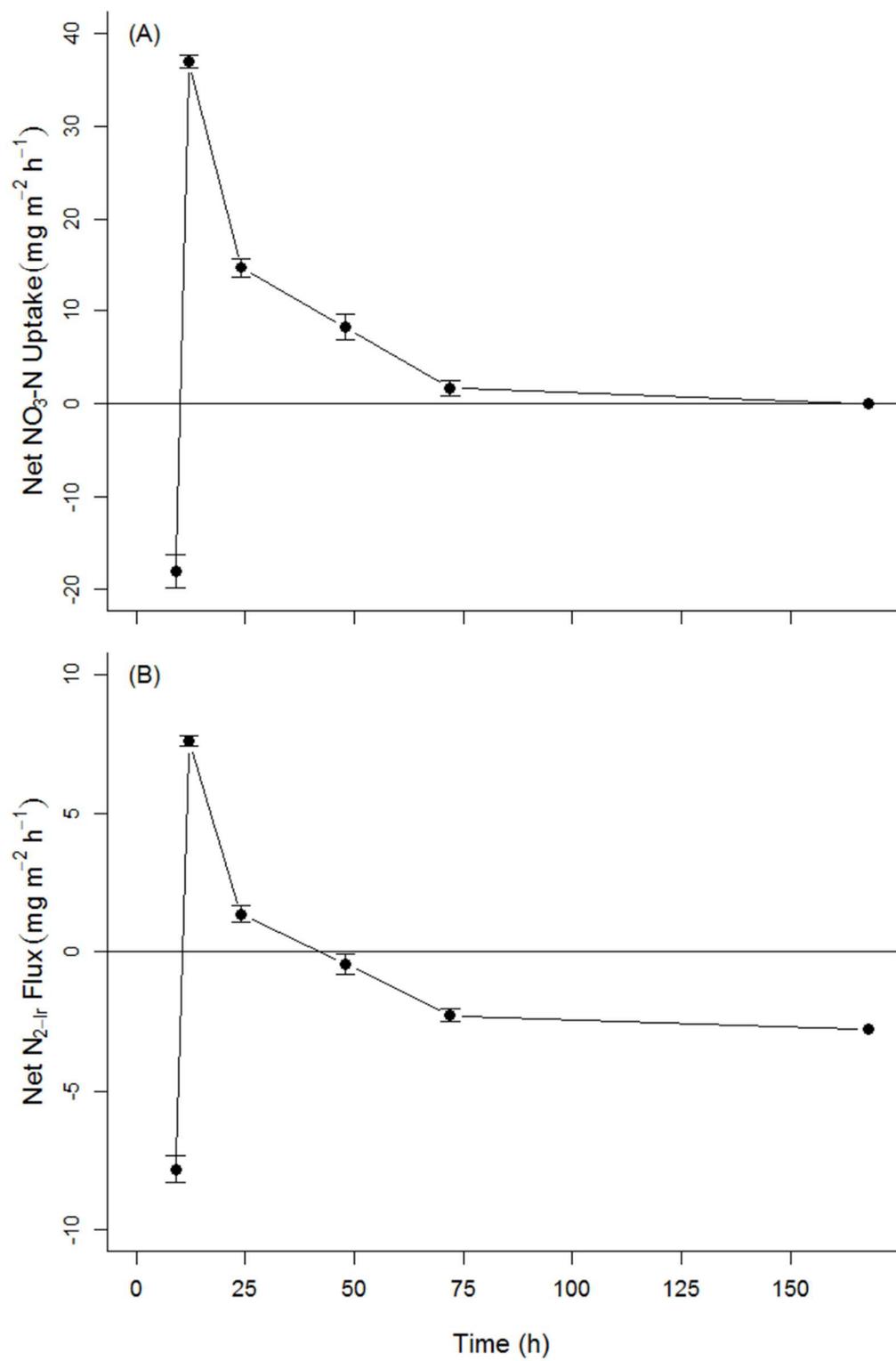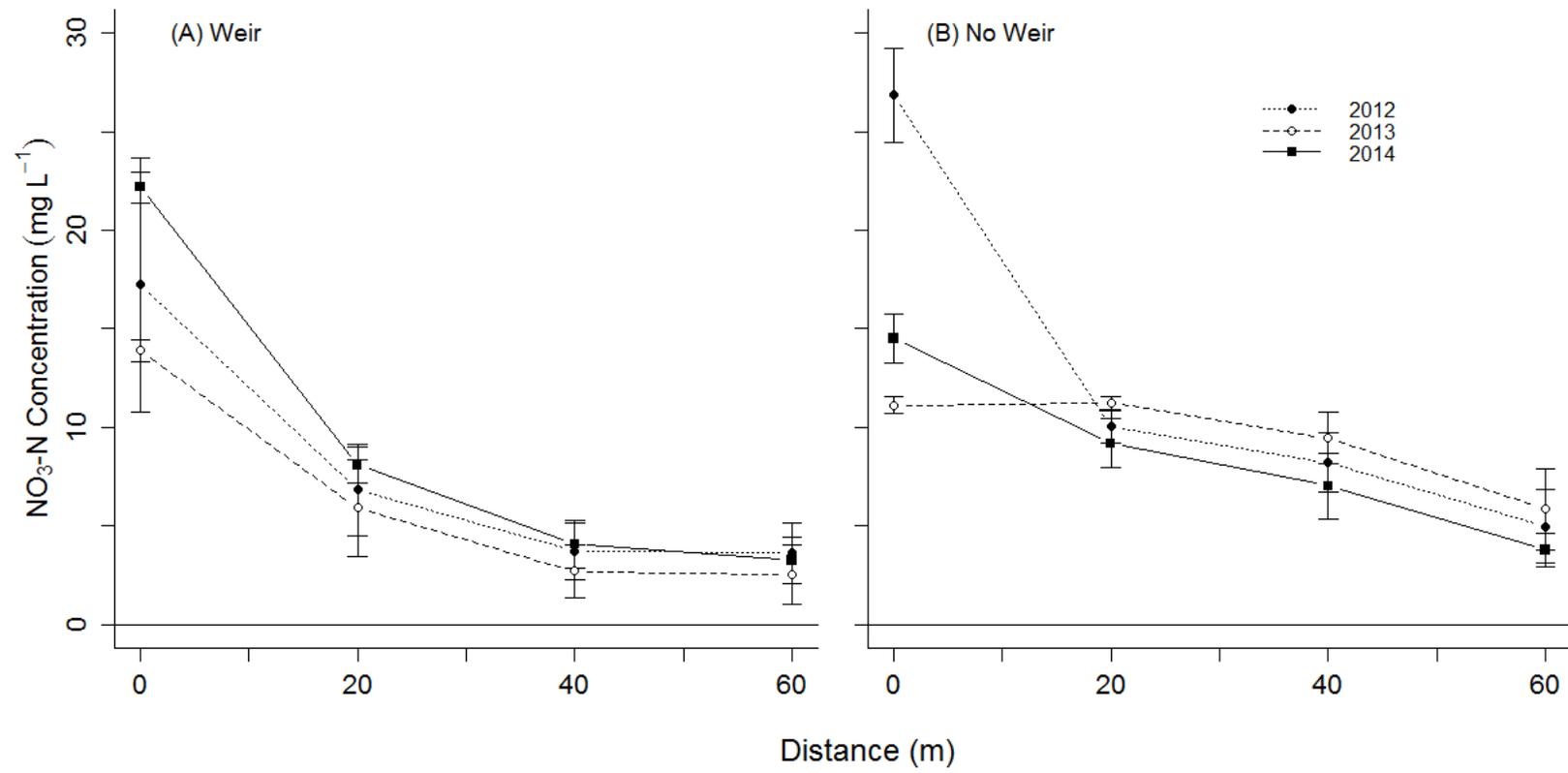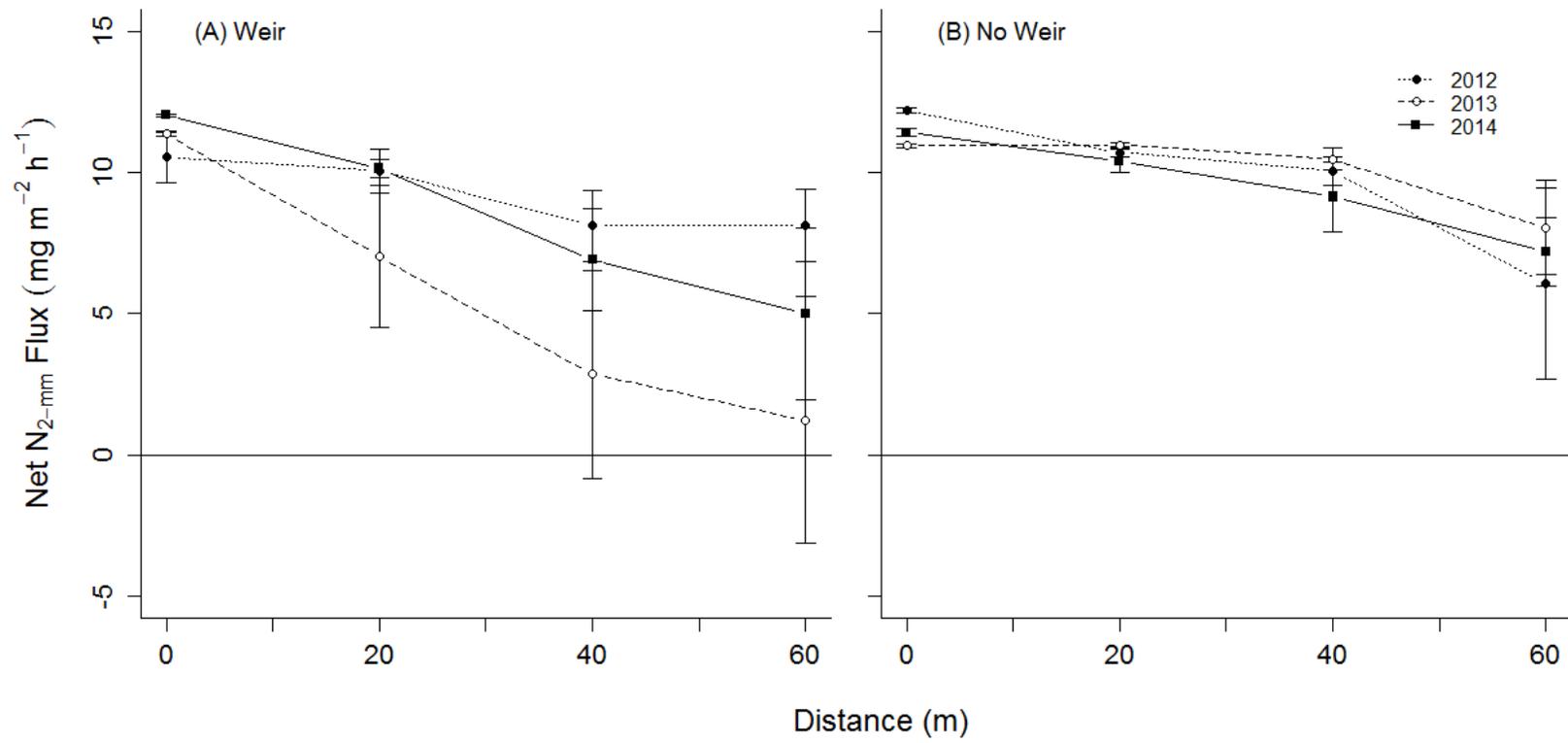
**Figures**



Figure 1

Figure 2

Figure 3

Figure 4
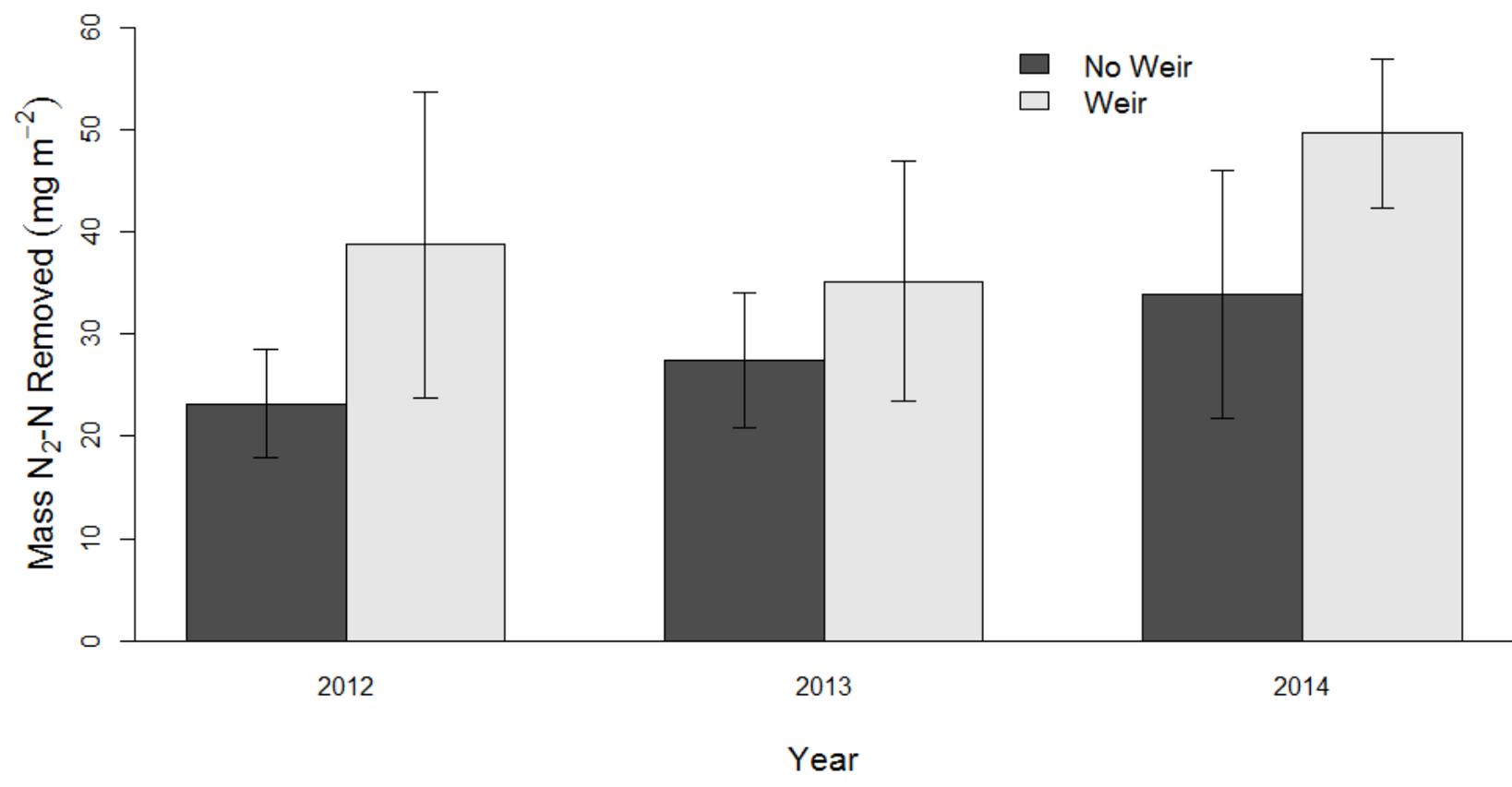
Figure 5

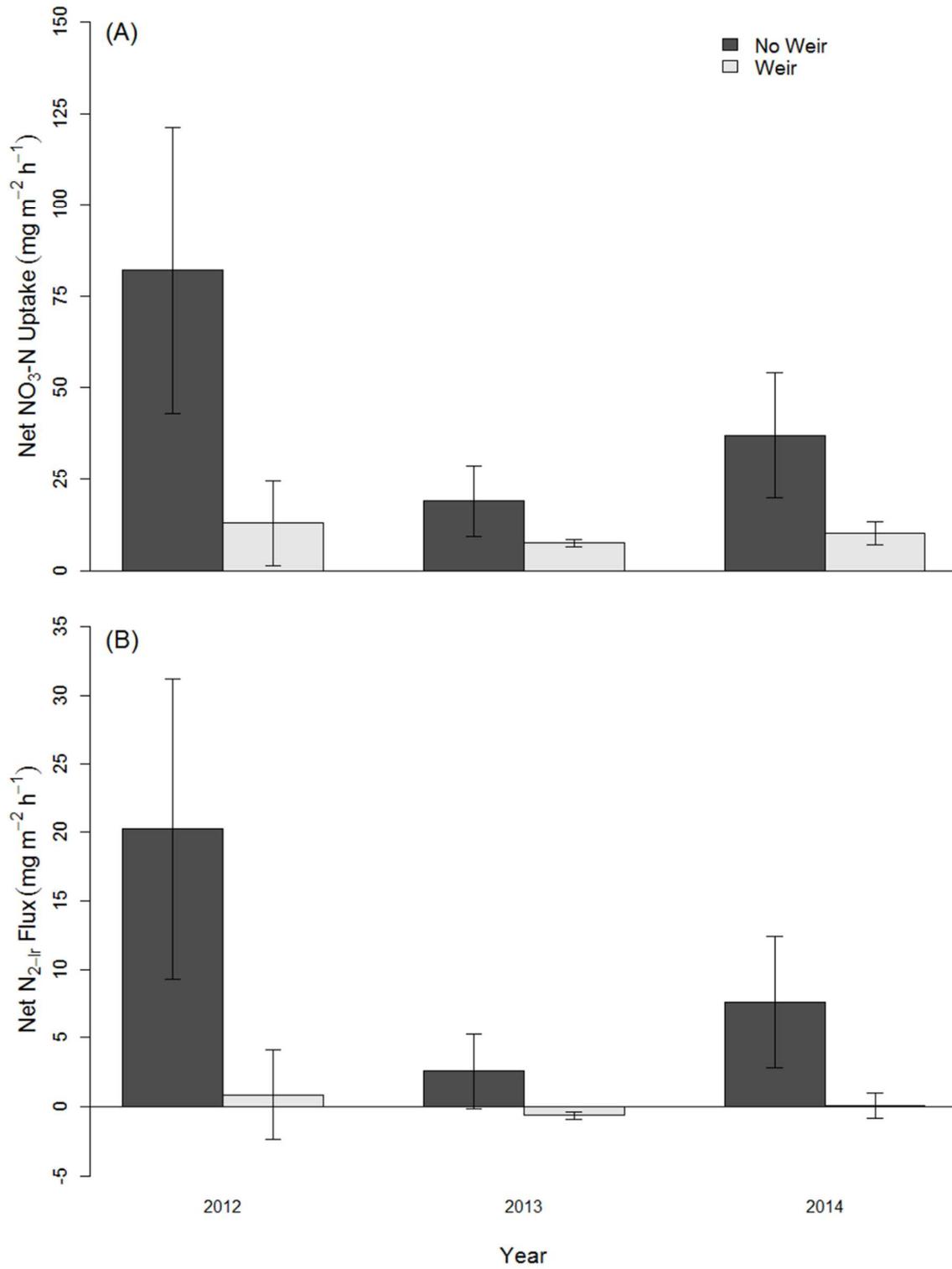Figure 6

## 4. CONCLUSION

The primary objectives of this study were to determine how nitrate ($NO_3^-$) concentrations influence denitrification (DNF) in ditch sediments vegetated with rice cutgrass (*Leersia oryzoides*) throughout the year, model net nitrogen gas ($N_2$) fluxes, and apply the models I developed to pre-existing datasets to assess their utility. Agricultural ditches have been increasingly acknowledged for their role in enhancing nutrient removal from cropland runoff (Cooper et al., 2004; Moore et al., 2010; Kröger et al., 2014), and the addition of vegetation to the ditch channel is a best management practice (BMP) that can control nutrient loading to downstream ecosystems (Kröger et al., 2009; Tyler et al., 2012; Taylor et al., 2015). However, most studies are restricted to the summer months, and only one study has explored how DNF is influenced by individual plant species commonly found in agricultural ditches (Taylor et al., 2015).

Cutgrass in particular can enhance nitrogen (N) removal and DNF in ditch sediments in comparison to unvegetated and cattail (*Typha latifolia)* ditch sediments during the early summer (Taylor et al., 2015). The results of the current study expanded on the findings of Taylor et al. (2015) by quantifying how varied $NO_3^-$ concentrations influence DNF in cutgrass ditch sediments throughout the year. Denitrification rates were greatest in the spring and summer in cutgrass ditch sediments, with both high net $N_2$ fluxes out of the system and high $NO_3^-$ uptake observed. The maximum net $N_2$ flux observed in the summer was nearly 20 mg m$^{-2}$ h$^{-1}$, corresponding to the upper range of reported values at which saturation of DNF rates occurs (Bernot and Dodds, 2005). In contrast, little DNF or $NO_3^-$ uptake was observed in the fall and winter. The percent of $NO_3^-$ retained in the intact sediment cores reached a maximum at low $NO_3^-$ concentrations and decreased as $NO_3^-$ concentrations in the overlying water continued to

rise. This suggests $NO_3^-$ uptake and DNF rates may become saturated at high levels of $NO_3^-$ loading (Mulholland et al., 2008, 2009). Denitrification efficiency, or the percent of $NO_3^-$ converted to $N_2$ by denitrifying bacteria, was greatest in the spring and summer, ranging from approximately 30-40% of total $NO_3^-$ uptake.

In the spring, summer, and fall, net $N_2$ fluxes exhibited characteristic Michaelis-Menten saturation curves, allowing for the development of models to predict net $N_2$ fluxes from cutgrass ditch sediments. Linear regression models were also developed to predict net $N_2$ fluxes based on $NO_3^-$ uptake in ditch sediments vegetated with cutgrass. Two pre-existing datasets were used to validate the models. Model application yielded similar estimates of net $N_2$ fluxes predicted by the two models at the mesocosm scale, suggesting the Michaelis-Menten and linear regression models predict comparable results. Integrating net $N_2$ fluxes predicted by the Michaelis-Menten model with respect to time resulted in a similar estimate of the areal mass of $N_2$ denitrified derived as compared to a mass balance approach used by Taylor et al. (2015). Model application also highlighted the enhanced N removal ability of ditches containing weirs as compared to conventional ditches. Ditches with weirs and vegetation had net $N_2$ fluxes up to 7 mg m$^{-2}$ h$^{-1}$, much greater than the reported fluxes in unweired ditches (1 mg m$^{-2}$ h$^{-1}$; Kröger et al., 2014). Thus, vegetation paired with weirs in agricultural ditches may be a powerful tool for enhancing DNF and reducing the downstream movement of excess N.

Collectively, these results suggest the addition of cutgrass to agricultural ditch channels may represent a viable BMP for reducing N loading from cropland to aquatic ecosystems. Cutgrass ditches can potentially remove up to 40% of the $NO_3^-$ load entering the ditch system during the growing season. Therefore, ditch sediments vegetated with cutgrass may be beneficial in mitigating immediate N losses after fertilizer application and potentially act as long-term sinks

for excess N in the landscape. However, additional management practices, such as cover crops, may be necessary to manage N in runoff outside of the growing season, as little DNF was observed in the fall and winter. The pairing of low-grade weirs with vegetation in the ditch channel may be necessary to optimize conditions for DNF (Kröger et al., 2014). The Michaelis-Menten and linear regression models can help agricultural land managers evaluate the permanent N removal capacity of implementing vegetated ditch BMPs at a larger scale. Future studies should be focused on how cutgrass functions to enhance N removal in the agricultural landscape at ditch- and watershed-scale and validating both the Michaelis-Menten and linear regression models. Improved temporal resolution throughout the year would also be beneficial in assessing how DNF in cutgrass ditch sediments is influenced by temperature at a finer level. Finally, it is important to explore the likelihood of cutgrass establishing in fields and affecting crop production and yields, as this may make vegetated ditch BMPs less attractive to farmers and land managers.

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## 5.    Appendix A

## 5.1    Michaelis-Menten Non-Linear Regression Mixed Effects Model Code in R

*#SET WORKING DIRECTORY*

```
setwd("C:\\Users\\shann\\Desktop\\thesis.data\\")
getwd()
```

*#IMPORT AND REVIEW DATA FOR POTENTIAL DNF W/O 10 mg/L*

```
nlme3<-read.csv("2seasonaln2gasdatawo10.csv",header=TRUE)
nlme3
head(nlme3)
summary(nlme3)
```

*#IMPORT AND REVIEW DATA FOR POTENTIAL DNF W/ 10 mg/L*

```
nlme4<-read.csv("2seasonaln2gasdata.csv",header=TRUE)
nlme4
head(nlme4)
summary(nlme4)
```

*#LOAD LIBRARIES*
```
library(nlme)
library(sciplot)
```

*#GENERAL MIC MEN FORMULA*

```
dnf.formula <- potkdnf ~ (Vm * NO3trmt)/(k + NO3trmt)
```

*#MODELS W/O 10 mg/L*

```
june.m1 <- nlme(dnf.formula, fixed = list(Vm~1, k~1), subset = season == "june", random = Vm
~ 1|time, start = c(15,0.3), weights=varExp(form=~ NO3trmt), data=nlme3)
summary(june.m1)
```

```
aug.m1 <- nlme(dnf.formula, fixed = list(Vm~1, k~1), subset = season == "august", random =
Vm ~ 1|time, start = c(25,1), weights=varExp(form=~NO3trmt), data=nlme3)
summary(aug.m1)
```

```
oct.m1 <- nlme(dnf.formula, fixed = list(Vm~1, k~1), subset = season == "october", random =
Vm ~ 1|time, start = c(26,0.3), weights=varExp(form=~NO3trmt), data=nlme3)
summary(oct.m1)


jan.m1 <- nlme(dnf.formula, fixed = list(Vm~1, k~1), subset = season == "january", random =
Vm ~ 1|time, start = c(8,0.1), weights=varExp(form=~NO3trmt), data=nlme3)
summary(jan.m1)
```

#MODELS W/ 10 mg/L

```
june.m2 <- nlme(dnf.formula, fixed = list(Vm~1, k~1), subset = season == "june", random = Vm
~ 1|time, start = c(15,0.3), weights=varExp(form=~ NO3trmt), data=nlme4)
summary(june.m2)


aug.m2 <- nlme(dnf.formula, fixed = list(Vm~1, k~1), subset = season == "august", random =
Vm ~ 1|time, start = c(25,1), weights=varExp(form=~NO3trmt), data=nlme4)
summary(aug.m2)


oct.m2 <- nlme(dnf.formula, fixed = list(Vm~1, k~1), subset = season == "october", random =
Vm ~ 1|time, start = c(26,0.3), weights=varExp(form=~NO3trmt), data=nlme4)
summary(oct.m2)


jan.m2 <- nlme(dnf.formula, fixed = list(Vm~1, k~1), subset = season == "january", random =
Vm ~ 1|time, start = c(8,0.1), weights=varExp(form=~NO3trmt), data=nlme4)
summary(jan.m2)


###
###
###
```

#IMPORT DATA TO CREATE FIGURE

```
data <- read.csv("dnffigs.csv", header=TRUE)
head(data)
```

*#concentration vectors for models up to 7.5 mg/L and 10 mg/L*

concVec7.5 <- seq(from = 0, to = 8, by = 0.0001)
concVec10 <- seq(from = 0, to = 11, by = 0.0001)

*#predicted model lines for 7.5 mg/L model(value subtracted at end is model back-correction)*

june.m1.p <- predict(june.m1,data.frame(NO3trmt=concVec7.5),level=0) - 6.683857378
aug.m1.p <- predict(aug.m1,data.frame(NO3trmt=concVec7.5),level=0) - 8.469756473
oct.m1.p <- predict(oct.m1,data.frame(NO3trmt=concVec7.5),level=0) - 24.57748961
jan.m1.p <- predict(jan.m1,data.frame(NO3trmt=concVec7.5),level=0) - 8.27486135

*#predicted model lines for 10 mg/L model (value subtracted at end is model back-correction)*

june.m2.p <- predict(june.m2,data.frame(NO3trmt=concVec10),level=0) - 6.683857378
aug.m2.p <- predict(aug.m2,data.frame(NO3trmt=concVec10),level=0) - 8.469756473
oct.m2.p <- predict(oct.m2,data.frame(NO3trmt=concVec10),level=0) - 24.57748961
jan.m2.p <- predict(jan.m2,data.frame(NO3trmt=concVec10),level=0) - 8.27486135

###
###
###

*#4 PANEL FIGURE*

par(mfrow=c(2,2), oma=c(4,4,1,1), mar=c(2,2,0,0))

*## JUNE*
*#POINTS WITH SCIPLOT*
lineplot.CI(no3[pot=="no"],june[pot=="no"], data = data, ylim=c(-25,30), xlim=c(0,10),
type="p", xaxt="n", yaxt="n", x.cont=TRUE, legend=FALSE, bty="l",cex=1.5)

*#MODELS W/O AND W/ 10 mg/l*
lines(concVec7.5, june.m1.p, lty=1, lwd=2)
lines(concVec10, june.m2.p, lty=3, lwd=2)

*#PANEL LABEL*
text(1.25,29,"(A) Spring",cex=1.5)

*#LINE THROUGH ZERO*
lines(c(-1,11),c(0,0),lty=1)

*#CREATE AXES*
axis(1,at=c(0,1,2,3,4,5,6,7,8,9,10),labels=c("","","","","","","","","","",""),cex.axis=1.6)
axis(2,at=c(-30,-20,-10,0,10,20,30),labels=c("-30","-20","-10","0","10","20","30"),cex.axis=1.6)


## AUGUST
lineplot.CI(no3[pot=="no"],aug[pot=="no"], data = data, ylim=c(-25,30), xlim=c(0,10),
type="p", xaxt="n", yaxt="n", x.cont=TRUE, legend=FALSE, bty="l",cex=1.5)
lines(concVec7.5, aug.m1.p, lty=1, lwd=2)
lines(concVec10, aug.m2.p, lty=3, lwd=2)
text(1.5,29,"(B) Summer",cex=1.5)
lines(c(-1,11),c(0,0),lty=1)
axis(1,at=c(0,1,2,3,4,5,6,7,8,9,10),labels=c("","","","","","","","","","",""),cex.axis=1.6)
axis(2,at=c(-30,-20,-10,0,10,20,30),labels=c("","","","","","",""),cex.axis=1.6)


## OCTOBER
lineplot.CI(no3[pot=="no"],oct[pot=="no"], data = data, ylim=c(-25,30), xlim=c(0,10), type="p",
xaxt="n", yaxt="n", x.cont=TRUE, legend=FALSE, bty="l",cex=1.5)
lines(concVec7.5, oct.m1.p, lty=1, lwd=2)
lines(concVec10, oct.m2.p, lty=3, lwd=2)
text(.8,29,"(C) Fall",cex=1.5)
lines(c(-1,11),c(0,0),lty=1)
axis(1,at=c(0,1,2,3,4,5,6,7,8,9,10),labels=c("0","","2","","4","","6","","8","","10"),cex.axis=1.6)
axis(2,at=c(-30,-20,-10,0,10,20,30),labels=c("-30","-20","-10","0","10","20","30"),cex.axis=1.6)


*#JANUARY - THIS PANEL HAS NO MODELS (NOT SIGNIFICANT)*
lineplot.CI(no3[pot=="no"],jan[pot=="no"], data = data, ylim=c(-25,30), xlim=c(0,10), type="p",
xaxt="n", yaxt="n", x.cont=TRUE, legend=FALSE, bty="l",cex=1.5)
text(1.3,29,"(D) Winter",cex=1.5)
lines(c(-1,11),c(0,0),lty=1)
axis(1,at=c(0,1,2,3,4,5,6,7,8,9,10),labels=c("0","","2","","4","","6","","8","","10"),cex.axis=1.6)
axis(2,at=c(-30,-20,-10,0,10,20,30),labels=c("","","","","","",""),cex.axis=1.6)


*#CREATE OVERALL AXIS LABELS*
par(mfrow=c(1,1))
mtext(expression(paste("Net N"[2]* "-N Flux ", ("mg m"^-2 * " h"^-

```
1))),side=2,line=3.5,cex=1.75)
mtext(expression(paste("NO"[3]* "-N Treatment (mg L"^-1 * ")")),side=1,line=4.3,cex=1.75)


#CREATE LEGEND FOR PREDICTED MODEL LINES
legend(locator(1),bg=NULL,c("With 10","Without 10"),lty=c(3,1),lwd=c(2,2),bty="n")
```