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REDUCING NITRATE POLLUTION BY REDIRECTING FARM RUNOFF INTO THE SUBSURFACE OF AN HERBACEOUS RIPARIAN BUFFER ZONE

Tamru G. Taye

71 Pages

In areas dominated by agricultural land use, excess nitrate is one of the leading contributors to water pollution. Tile drains, installed to drain crop root zones for maximum yield, fast track the introduction of nitrate-laden water into a nearby stream. Due to the risks elevated levels of nitrate pose to an ecosystem and its inhabitants, the following project explores a method of reducing nitrate pollution. One that can do it as close to the source of the pollution as possible.

A tile draining a 60acre farm in central Illinois was redirected to discharge its contents through subsurface flow into an herbaceous riparian buffer zone (RBZ) located immediately below the farm. A transect of nested observation wells were installed across the riparian buffer intersecting the redirected subsurface tiles. A study on the groundwater and soils was performed to determine the ability the RBZ has for nitrate reduction/removal. Prior to redirecting tile water into the RBZ, chemical analysis of the RBZ water showed low concentrations of $\text{NO}_3\text{--N}$ ($\leq 5\text{mg/L}$). The conditions necessary for the reduction/removal of nitrate, such as low levels of dissolved oxygen, dissolved organic carbon, and organic matter in the soils, and groundwater temperature were also

investigated. A statistical comparison of the concentration of $\text{NO}_3\text{--N}$ at the wells (effluent) vs. the diversion box (influent) shows that the waters are significantly different ($p < 0.0001$). Concentrations measured in the diversion box leading to the riparian buffer averaged 13.6 mg/L. Once this water had entered the riparian buffer however, the average concentration registered was lower than 1mg/L; a 94% reduction in the RBZ. The factors responsible for this reduction/removal such as dilution and denitrification are explored as well as any seasonal trends in nitrate removal.

KEYWORDS: Denitrification, Dilution, Nitrate, Nitrate Removal, Riparian Buffer Zone

REDUCING NITRATE POLLUTION BY REDIRECTING FARM RUNOFF INTO
THE SUBSURFACE OF AN HERBACEOUS RIPARIAN BUFFER ZONE

TAMRU G. TAYE

A Thesis Submitted in Partial
Fulfillment of the Requirements
for the Degree of

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REDUCING NITRATE POLLUTION BY REDIRECTING FARM RUNOFF INTO
THE SUBSURFACE OF AN HERBACEOUS RIPARIAN BUFFER ZONE

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T. G. T

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CHAPTER I

INTRODUCTION

Nitrate in the Environment

Nonpoint source pollution of surface and subsurface waters is a global issue that has compromised countless aquatic ecosystems (Mayer et al., 2007). In areas dominated by agricultural land use, excess nitrate is one of the leading causes of surface water pollution (Anderson et al., 2014; Galloway et al., 2003; USEPA, 2009). As farmers scramble to meet global food demand, the amount of available nitrogen (N) in the terrestrial N cycle has doubled with agriculture contributing the most to this increase (Galloway et al., 2003; Anderson et al., 2014; David et al., 2010). Nitrogen in the form of nitrate is essential to plant growth. When it is present in excess amounts however, it can contaminate surface and subsurface waters, negatively impacting the quality of aquatic life. The Mississippi River Basin contains some of the highest concentrations of nonpoint source nitrate in the United States (USEPA, 2009). According to Keeney and Hatfield (2010) the Mississippi River receives over 19% of this nitrate from Illinois alone. Much of the nitrate, which is often above the EPA limit of 10mg/L NO₃--N, is then offloaded into the coastal zones of the Gulf of Mexico and is the leading causes of hypoxia, eutrophication, and biodiversity changes in this area (Dale et al., 2010; Galloway et al., 2003; USEPA, 2007)

The upper Mississippi River Basin, which includes Illinois, has very fertile soils and is subjected to intensive agricultural practices. To maximize yield and to prevent crops from drowning, the hydrology of the sub-watersheds in this basin is often modified by intensively tiling farmlands to drain the groundwater (David et al., 2010). Large scale channelization and installation of subsurface tile drains throughout Illinois and the upper Midwest have allowed farmers to maximize their yields whilst avoiding risks of excess water stress on crops (Sands et al., 2008, David et al., 2010). Headwater streams, which play a primary role in the mitigation of nitrate, are subjected to such modification (Alexander et al., 2000). Tiles allow waters and dissolved solutes to bypass the natural process involved in nutrient reduction within water moving through the soils as shown in Figure 1. Tiles draining farms instead discharge this nutrient-rich farm water into nearby surface waters (Royer et al., 2004). In the 17.4 million hectare of artificially drained farms in the Midwestern US, much of the nitrate riddled water is directly discharged into surface waters (Jayens et al., 2014). In fact, over 52% of nitrogen entering the Gulf of Mexico is directly related to the contribution of tile drains from corn and soybean production (Alexander et al., 2008). The excess amount of nitrate entering surface waters is therefore more than the headwater streams can process out by themselves.

Figure 1. The Nitrogen Cycle.

Within the soil, processes such as ammonification, immobilization, nitrification, and denitrification can transform nitrogen into different and often times harmless forms of nitrogen (Sylvia et al., 1998). Tiles short-circuit the role of soils in the nitrogen cycle and contribute to pollutants in surface streams. With that, the potential for removal of nitrate in the soils is lost. Due to their integral role in crop yields, removing tile drains to protect water bodies from pollution of nitrates is an impractical solution. So a compromise must be made to lessen the effects of tile drains and work around them.

Riparian Buffer Zones

Riparian buffer zones (RBZs) can potentially attend to the disconnect that occurs in a system as a result of tiling. A riparian buffer zone is land that is situated between an adjacent stream and an upland ecosystem (Gregory et al., 1991). More specifically, an herbaceous riparian buffer zone is a zone with land cover composed of grass, forbs and other herbaceous vegetation. Riparian buffer zones have been studied and shown to have

great potential for removing substantial amounts of pollutants, such as nitrates, from shallow groundwater (Hill, 1996, Forshay et al., 2005, Mayer et al., 2007). A meta-analysis of published riparian buffer studies by Mayer (2007) even suggested that the removal of nitrate did not depend on width or vegetation types of the riparian zone.

Since the majority of nitrate-laden farm water is discharged straight into surface waters by tiles, the benefits of riparian buffers can be better reaped when these tiles are reconnected with the subsurface of the riparian buffer zones. One way such a task can be undertaken is by intercepting and redirecting a tile drain to flow through a riparian buffer zone rather than directly discharging into a stream (Jaynes et al., 2014). Some of the processes by which riparian buffer zones can attenuate nitrate are by plant uptake, denitrification, dilution, and to a lesser extent microbial immobilization and plant uptake (Groffman et al., 1992; Jaynes et al., 2014).

Removal/Reduction of Nitrate

One mechanism by which nitrate is lost in the shallow groundwater zone and vadose zone is through plant uptake. Nitrogen is a primary nutrient required for plant growth, and when it is not utilized by the crops, it can infiltrate through the soil and make its way to the water table (Ranalli et al., 2010). This water is then carried away by subsurface tiles and can be introduced into a riparian buffer zone where plants assimilate the nitrate into their system. On the farm, before this nitrate can either be assimilated by plants, denitrified, or reduced to ammonia, it is transported away by tiles. This is where the potential to reduce the nitrate concentration right on the farm is lost. Therefore introducing this water into a nearby riparian buffer is the next best option to reestablish connection to the soil. In the process of being assimilated by the plant, the water soluble

nitrogen is converted to an organic N then to ammonium via ammonification and then to nitrate via nitrification (Figure 1). Nitrate assimilation by plants is most common in temperate climates like that of Illinois (Fennessy et al., 1997). It is worth noting, however, that plants usually act as temporary vessels for nitrate until they die and decompose, allowing some of the nitrate to be reintroduced back into the system (Ranalli et al., 2010). Moreover, in colder months the lack of available plant matter to absorb nitrate from the groundwater can be viewed as a limiting factor for a riparian zone's potential for nitrate removal.

Another major way nitrate is removed from the system is via denitrification. Denitrification is a biochemical reaction that permanently removes nitrate from a system, reducing it to Nitrogen Monoxide (NO), Nitrous Oxide (N₂O) and Nitrogen gas (N₂) (Galloway et al., 2003; Hill, 1996). Therefore, maximizing denitrification in the riparian buffer can improve the quality of water downstream. In order for denitrification to occur though, there are certain conditions that must be met. Some of these conditions are the presence of nitrate, low levels of dissolved oxygen (D.O.) in anoxic environments, available organic carbon (O.C.), and dissolved organic carbon (D.O.C) (Arango et al., 2007; Mulholland et al., 2009). Wu (2012) found that the rate of denitrification was strongly dependent on the availability of organic carbon. Plants and dead roots contribute to the upper layers of the soil by providing labile organic matter. Among other properties of the buffer, Jaynes (2014) strongly recommends studying the soil within which the redirected tile water flows and denitrification occurs.

Equation (1) shows the series of steps nitrate undertakes during denitrification, equation (2) shows the overall stoichiometry of the reaction of nitrate reduction and

equation (3) specifically shows the process of nitrate reduction by aid of organic matter. Oxidized nitrate (and possibly nitrite as well) is converted to N₂ gas along with the oxidation of the organic matter.

The bacteria that denitrify the system only use nitrate as the electron acceptor in the absence of oxygen, making it an example of an anaerobic respiration (Hill, 1996; Sylvia et al., 1998). Therefore, denitrification is conducted by anaerobic bacteria when the concentration of dissolved oxygen is low (Wu et al., 2012; Rivett, 2008). Denitrifying bacteria make up a large portion of soil bacteria, possibly because they turn to nitrate as an electron donor in the absence of oxygen. So anaerobic conditions are among the major limiting factor of how well soils can denitrify. The availability of nitrate and organic carbon are also as among the limiting factors. The majority of nitrate is removed from a riparian buffer zone when the groundwater table saturates the organic rich layer of the soil allowing for high respiratory activity by bacteria (Hill, 1996; Ranalli et al., 2010; Sylvia et al., 1998). This will likely be the case as redirected tile water will temporarily raise the riparian buffer water table, exposing the water to the organic layer in the soil.

Microbial immobilization of nitrate presents yet another mechanism by which nitrate is removed out of the system or temporarily halted. Microbial immobilization of Nitrogen involves soil microorganisms using the available labile carbon resources (Davidson et al., 2003). Both nitrate and ammonium are equally subject to uptake by plants. However, microbial immobilization of nitrate is dependent on the microbial

immobilization of ammonium (Lowrance et al., 2000). A study conducted by Recouse (1990) aimed at defining the conditions and the extent at which nitrate immobilization occurs in the soils found that microbial bacteria tend to first or exclusively deplete the ammonia pool by immobilizing it before moving onto nitrate.

Streams, such as those in the Midwest, possess the conditions required for substantial denitrification to occur in the hyporheic zone and remove the pollutants themselves (Hill, 1996). But they are often saturated with nitrate concentrations greater than they are able to remove from tile discharge (Roley et al., 2012). So the concentration of nitrates needs to be lowered if not completely removed before it reaches said streams. Riparian buffer zones are such a medium where this removal could take place.

If the nitrate introduced to the buffer is not removed via plant uptake, denitrification or immobilized by microbial bacteria, there is always the potential for dilution with groundwater. While dilution does not remove nitrate out of the system, it can greatly reduce the concentration by mixing it with groundwater. Dilution is another way the effects of nitrate on the environment can be lessened in the short term. Continuous dilution however is not a sustainable long term solution.

This study will investigate if nitrate concentrations in tile drained waters can be reduced or removed by reintroducing the water into the subsurface underlying an herbaceous riparian buffer zone. The results of this study will inform us on whether or not reintroduction of tile water into the RBZ subsurface is a viable option for nutrient mitigation. The results can also be used to help modify best management practices to combat excessive nutrient concentrations in surface waters. This natural remediation

process has the potential to save on costs by tackling the issue very close to the source rather than later in the streams and other water bodies.

Research Questions and Hypothesis

In cases where water pollution is concerned, if prevention fails, the pollution should be contained as close to the source as possible. Perhaps even remove the pollutant near the source. In this particular case, an herbaceous riparian buffer zone presents us with an opportunity to do just that. In investigating the suitability of the riparian buffer zone, this study will ask the following questions:

1. Does the riparian buffer zone significantly reduce/remove nitrate concentrations from the redirected tile?

Riparian buffer zones have shown great potential in attaining non-point source nitrate. I hypothesize that this riparian buffer zone will aid in significant reduction in nitrate concentrations.

2. Is there a seasonal difference in nitrate at the influent and effluent?

Nitrate concentrations introduced into the RBZ may differ throughout the year but the RBZ can still significantly reduce them.

3. What factors, if any, are responsible for the reduction in nitrate concentrations in the RBZ?

In this RBZ, I expect denitrification to play the biggest role in nitrate removal.

There may also be dilution with the RBZ groundwater.

Study Site

T3 is a low order stream that is adjacent to a grassy riparian buffer zone with agricultural farm upland from that buffer (Figure 2). The stream drains in to Evergreen Lake northwest of Normal, Illinois which serves as a source of drinking water as well as a recreational area to the City of Bloomington. The farm sitting upslope of the riparian buffer currently has an artificial tile drain installed that drains directly into T3. To reduce the concentration of nitrate that drains directly into T3 and ultimately into the Evergreen Lake, the tile has been intercepted, redirected and forced to flow through the subsurface of the herbaceous riparian buffer zone. This redirected tile lies approximately 1m (3.3ft) deep in the buffer. Using GIS, topographic analysis suggests a gentle 7% down slope from the farm to the buffer and then to the stream. So local groundwater flow, which would likely closely follow surface topography, is moving west away from the farm and towards the stream.

Figure 2. Aerial shot of the study site. With diversion box (white), diversion wells (blue 2, 4, 6, 8, 10, 12), reference wells (C1, C2, C3), and redirected subsurface tiles (red)

The soils of the study site belong to the Strawn-Mayville-Birkbeck association. This association has a Loess parent material less than 10 inches thick which is underlain by loamy glacial till (Windhorn, 1990). The soils are well drained with moderate permeability in the upper part and moderately slow in the underlying material. The seasonal high water table can reach up to 0.6m from the surface (Windhorn, 1990). There is a moderate amount of organic content in these soils with a moderate potential for frost action (Sanks et al., 2015; Windhorn et al., 1990). The surface layer (0 – 0.5m) is dark brown and dark black clay with high organic content. Below that (0.5 – 1.5m) is a dark yellowish brown and yellowish brown firm clay loam with sand and gravel present. This layer is where the water table is detected. The substratum (>1.5m) is grayish tight clay that is much drier than the layer above it; the glacial till (Figure 3).

Figure 3. Cross-section of the soil profile at the study site. Included with the nested wells (Peterson et al., 2015)

The study site consists of two main transects of observation wells, some of which are nested (Figure 2). Wells 2, 4, 6, 8 and 10 are nested wells at each have four wells at screened at different depths. These wells are approximately screened at 4.5m, 3m, 2.3m,

and 1.5m and are labeled A, B, C, and D respectively. Figure 4 gives a visual description of the wells screened at different depths. This transect of nested wells intersects the redirected subsurface tiles as seen in Figure 2. The other transect with no nested wells (C1, C2, C3) is the reference well site. Situated on the same riparian buffer zone, these three wells are used to study the chemistry of the groundwater without tile influence.

Figure 4. Nested well setup (left) and general well setup (right)

CHAPTER II

METHODS

Nitrate Sample Collection and Analysis

Nitrate sampling events were undertaken once every two weeks from June to December 2015. Sampling of groundwater in the RBZ begun on 11th of June, 2015. Three sampling events were undertaken before diversion of tile water to the riparian buffer begun. This way, a comparison of the pre and post diversion chemistry of the groundwater of the RBZ can be made. Diversion of tile discharge into the RBZ begun on 17th of July, 2015 and has been in place since. In contrast, there were eleven sampling events undertaken since diversion to the RBZ begun making a total of fourteen sampling events.

In the laboratory, 60mL sample bottles are triple rinsed with DI water, acid washed and then triple rinsed again with DI water. Similarly, sampling syringes and filter tips are thoroughly cleaned under DI water and acid washed if necessary. The filter tips, which attach to the syringe, are equipped with 0.6 μ m-pore size filter paper to prevent any solid material getting into the pre labeled 60mL sample bottle.

Once in the field, a Solinist model 101 water level meter was used to determine water table height. This is recorded and used to calculate the volume of water present inside each well. The calculated volume of water in the well is then tripled and that amount of water is purged out of the well using a 1L bailer. In this way, we're assured to get the fresh

groundwater. The same bailer is also used to draw out a sample from the stream T3 and the diversion box that redirects tile water to the RBZ. Some wells do not recharge quickly enough to have triple the volume of water removed from them. In this case, the wells are bailed until muddy or there is little to no water left in the well.

Once all wells have undergone a round of purging, they are ready to be sampled. Just as in purging, a 1L bailer is used to draw water out of the wells. This water is transferred from the bailer into a clean 1L container for ease of use. A syringe is used to draw some of the water out of the 1L container. For each sample, the syringe is rinsed with the corresponding water before it is used to filter that water. Once the sample water is in the syringe, the filter tip with the filter is attached to the syringe. Slowly the water in the syringe is forced to pass through the filter and straight into a pre-labeled 60mL bottle. The 60mL bottle with the newly filtered sample is then quickly capped and put in to a cooler filled with ice. The same process is carried out in sampling the rest of the wells, the stream T3, and the diversion box.

Once sample collection in the field has concluded, the cooler containing all the samples is promptly transported back to the lab and the samples are stored in a freezer. Prior to analysis on the Ion Chromatograph (I.C.), the samples are allowed to thaw out. Then a 5mL aliquot is taken from each 60mL bottle and carefully put into a corresponding 5mL vial for analysis in the Dionex DX-120 Ion Chromatograph (I.C.). The I.C. is first calibrated with 3 known concentrations of standard solutions. Furthermore, as a way of making sure the I.C. is performing as expected and there is no drift in the machine, Quality Assurance (QA) and Quality Control (QC) were used by adding standards and blanks to

the queue. A standard and a blank are added to the queue after every 7 samples from the study. There were no replicates made for samples collected in the field and they were only analyzed once in the lab.

A one-way analysis of variance (ANOVA) ($\alpha = 0.05$) was performed to determine if statistical differences in nitrate concentrations are observed between the wells and the diversion box. If the ANOVA results indicated a statistical difference ($p < 0.05$), then a post-hoc Tukey's HSD ($\alpha = 0.05$) was conducted.

Dissolved Organic Carbon (D.O.C.) Sample Collection and Analysis

Samples for D.O.C. analysis were collected and stored in a slightly different manner than for nitrate analysis. In the laboratory, 40mL Borosilicate amber vials are acid washed and then placed in a 5000C oven for 1 hour to combust any organic material present. Before going in the oven, the amber vials are capped by a thin layer of aluminum foil to prevent dust entering the vials during combustion. This aluminum layer will act as a boundary between the water sample and the bottle cap during sampling, limiting contact and possible contamination from the plastic cap. 0.45 μ m and 0.6 μ m filters are also placed in the oven, wrapped in aluminum foil, for 1 hour at 500 0C. Once the amber vials are cooled, they are capped with a plastic cap with the aluminum foil layer in between. These bottles are labeled prior to being taken out to the field. The same type of syringe and filter tips used for nitrate analysis were also used here. These were triple rinsed with D.I. water, acid washed and then triple rinsed in D.I. water again.

In the field, a bailer is used to purge the selected wells to be tested for D.O.C. After purging is complete, the bailer is used to draw out water to be sampled. This water is placed

in a 1L container. From this container, the syringe is used to extract out some water. Unlike nitrate sample collection, the water for D.O.C. passes through two different filters. First through the 0.6 μ m and then through 0.45 μ m and straight into the 40mL amber vial. Both filters can be placed in the filter holder as long as they're in the proper order. Samples for D.O.C analysis are filtered through the 0.45 μ m to make sure that any particulate that may have gotten through the 0.6 μ m filter goes no further. Once in the syringe, careful steps are taken to make sure nothing enters the amber vials besides the filtered sample. Once the water is inside the amber vial, it is quickly wrapped with the aluminum foil and capped with the plastic cap. The vials are placed in a cooler and promptly transported to the lab and refrigerated until ready for analysis.

Samples were sent to the University of Michigan Biological station for analysis. In the lab, samples were run on an OI Analytical Aurora 1030 T.O.C analyzer. Dissolved inorganic carbon was removed by acidifying each sample with 5% H₃PO₄- in the reactor and flushing with He for 5 minutes. Evolved CO₂ was monitored using NDIR spectroscopy, and no residual CO₂ remained in any sample prior to D.O.C analysis. For D.O.C analysis, 10% sodium persulfate was injected, and the reaction chamber was pressurized with He and heated; evolved CO₂ was measured after 5 minutes using NDIR spectroscopy (van Geldern et al., 2013).

Measuring Dissolved Oxygen (D.O.)

Dissolved oxygen (D.O.) measurements were conducted at the same time and frequency as sample collection for nitrate analysis; once every two weeks. Measuring D.O. was done using the YSI ProPlus probe with a cord long enough to reach the water in deep wells. Once all the wells are purged and sampled, the probe is sent down into the well to record the D.O. (percent and mg/L), and other properties such as Specific Conductivity (SpC) and temperature.

The readings on the YSI probe, especially the D.O. figures, are allowed to stabilize before they are recorded into the field book. This stability is indicated when the flashing of the units on the screen ceases. The same procedure was carried out for all other wells and the diversion box. For the stream T3, the probe is tossed into the middle of the stream and the values are recorded.

Denitrification Assay

Nitrogen Purging/Flushing of Vials

Measurement of denitrification rate at the study site was conducted by following and slightly modifying the methods used by Grebliunas (2015). For denitrification assay, nitrogen gasses are trapped in a 10mL vials. Prior to injecting sample gasses into these 10mL vials for analysis, they need to be thoroughly rinsed with nitrogen. 10mL vials are taken to a nitrogen gas station in the laboratory. At this station, a nitrogen gas container is attached to a pipe with an in and out flow. Attached to this pipe is also a vacuum pump to help evacuate the gas. The pipe itself has 6 fingers with needle tips that inject the gas into the 10mL vials. Once the 10mL vials are attached to the nitrogen station, the nitrogen gas

is introduced into the pipe and the extending fingers and into the attached 10mL vials. Making sure all the valves are turned on, nitrogen gas is injected into the 10mL vials for approximately 10 seconds. Then all the valves allowing air into the 10mL vials are shut off as well as the main valve feeding in nitrogen. At this time, the vacuum pump is immediately turned on. This way, all the nitrogen that was used to flush the inside of the 10mL vials is evacuated by the vacuum pump. This process of flushing the 10mL vials with nitrogen and then evacuating the gas is repeated three times. When the last round of gas has been evacuated from the 10mL vials, the vials are removed from the pipe into a container of D.I. water so as to not allow outside air into the vials. Safety equipment such as goggles and a glove are required for this part.

Chloramphenicol Preparation

In the hood, 1g of chloramphenicol was weighed out on a weigh boat and transferred in to a 1L volumetric flask. This volumetric flask is triple rinsed with DI water prior to usage. DI water is used to dissolve the chloramphenicol in the weigh boat to make sure we got it all into the 1L volumetric flask. The rest of the 1L volumetric flask is then filled with DI or Nano-pure water until the 1L mark. This solution is swirled by hand to mix it. Later, a stir bar is added to the volumetric flask and is allowed to mix on a stir plate for another 5-10 minutes. Other than the mixing on the stir plate, all of the above needs to be performed in the hood.

Acetylene Preparation

Some calcium carbide rocks (5-6) are placed into a 125mL media bottle whilst in the hood. Two gas bags each labeled “acetylene” and “acetylene + N₂” are retrieved and

placed nearby. Any existing air in these gas bags is evacuated using a syringe with a needle tip. Some DI water was squirted into the 125mL media bottle containing the calcium carbide rocks. This is a very quick reaction which produces a lot of gas so the media bottle is capped quickly to trap that gas. A 60mL syringe with a needle attached to it is used to collect some of the gas that was produced as calcium carbide reacted with DI water. The syringe is inserted into the media bottle and quickly fills up with the gas. Caution must be used here as the syringe will fill up with the gas quickly and the top may even pop off due to high pressure. Once the syringe is full of air, it is removed from the media bottle and injected into the gas bag labeled “acetylene”. This process of collecting the gas from the calcium carbide reaction media bottle and injecting into the “acetylene” gas bag is repeated until the gas bags fills up. Should the media bottle containing the calcium carbide rocks run out gas, adding a few squirts of D.I. water into the media bottle will restart the reaction and more gas can be collected.

A separate 125mL media bottle is filled up with N₂ gas at the nitrogen station, making sure to rinse the media bottle with N₂ gas at least three times before filling it up. Additionally, two separate 60mL syringes labeled “N₂” and “acetylene” are used to collect and transfer gasses to their respective gas bags. For example, the syringe labeled “N₂” will be transferring N₂ gas only. Using the syringes labeled “acetylene” and “N₂”, mix some acetylene and N₂ into the gas bag labeled “acetylene + N₂”. The ratio for the mixing of the gasses is such that for every 1 syringe full of acetylene gas, 9 syringes full of N₂ is added to the “acetylene + N₂” gas bag. So by the end, there should be a gas bag full of acetylene and N₂ with a 1:9 ratio.

Soil Collection and Processing

Soil samples were collected from 12 different locations at the riparian buffer zone (Figure 5). Four sites were upslope of the redirected tiles in the RBZ (sites 1 – 4), which will have little to no influence from the redirected tile water. Four sites were in between the redirected tiles (sites 5 – 8), where the redirected tiles will influence the surrounding chemistry. And three sites were below the bottom most tile (sites 9 – 12), which will also have influence from the redirected tiles. An auger of 0.6m (2in) width is used to dig up the soil samples. Soil samples were collected right around the water table; the zone of mixing. A homogenized soil sample, immediately above and below the water table, is collected at each of the 12 locations. This is the area where the newly redirected water is going to interact with the soils the most. The samples are bagged in a Ziploc container and refrigerated until ready for analysis. At the time of collection, there was no diversion event into the RBZ and as such the water table was relatively low.

Figure 5. Soil sampling sites (yellow marks) at the RBZ

Ash Free Dry Mass

The amount of organic matter in the soils is of interest to us. Before doing any kind of work on the soils, they are thoroughly homogenized. For every sample of soil collected at each sampling site, two replicates are made when undertaking ash free dry mass. For example, for the soil sample at site 1 that is above the water table, there are two replicates and another two for below the water table. The same thing is done for all the other twelve sampling sites. Each replicate is first packed into a syringe of 30 mL (ccs) so that the volume of soil analyzed is kept consistent. The soils are taken to a weighing station and each 30cc of soil is weighed on a tin pan. The samples are then placed in a 100 Celsius

oven over night to remove moisture. The next day, the dry samples are weighed again before being placed in a 550 Celsius oven to remove organic matter. They are once again weighed at the end after removing from the 500 0C oven.

Capturing the Gas

Once soil samples have been collected from the field, they must be analyzed for their nitrogen gas within 48 hours. In a 125mL media bottle, 10g of soil sample is weighed out for Site 1, above the water table. Sample replication is also carried out here. For example, soils from above the water table at site 1 have 2 replicates and another 2 replicates for below the water table at site 1. In the end, there are a total of 48 samples to be analyzed. Using a pipette, 45mL of water is added to each media bottle and their replicates. Furthermore, 0.7ml of 0.1M solution of KNO₃- is pipetted and added into the media bottles with the soil. Using the chloramphenicol prepared earlier, 5mL of it is pipetted into the media bottle with the soil samples. Once all of the above has been added to the media bottle with the soil, it is taken to the N₂ pump station. Here, each media bottle of soil samples is attached to the N₂ pump needles. Another needle is inserted into the same media bottle to act as a vent through which air can escape due to positive pressure build up. These bottles containing the soil samples are flushed with N₂ gas for 5 minutes. The bottles are swirled every minute for ideal mixing. After 5 minutes, the vent needle is removed first, to build a slight positive pressure in the media bottles. A separate and clean syringe is used to allow some of the air out of these sample bottles. A syringe is hollowed out by removing the inside part of it and instead adding about 2mL of water into it. A needle is attached to the bottom of this syringe. This syringe is taken and inserted into the bottles containing the soil

samples. When the syringe is inserted, the gas escaping the media bottles is seen bubbling the water in the syringe. This allows the media bottle to re-equilibrate to atmospheric pressure. When the water stops bubbling, the hollow syringe is removed from the bottles.

In the hood, 15mL of acetylene is drawn out from the gas bag with the appropriate syringe and is injected into the soil sample bottles. These bottles are shaken vigorously. Then each of the media bottles with the soil is allowed to rest for about 15 minutes. After 15 minutes, 15mL of gas is drawn from the head space of the media bottles and inject into an already prepared 10mL vial. Prior to capturing the gas from the head space, the syringe must be rinsed with the gas being captured. A volume of gas is drawn whilst the syringe is in the media bottle. The gas is then returned back into the media bottle. This is repeated three times to rinse out the syringe. This process of rinsing and drawing gas from the head space of the media bottle and injecting it into the vials every hour is repeated for all media bottles containing soil samples. By the end, there should be 5 vials for every media bottle of soil sample. So a total of 240 vials are analyzed accounting for all replicates. Every time gas is removed from the head space of the media bottle containing the sample, 15mL of previously made ‘acetylene + N₂’ should be returned into it.

Gas Chromatography – Soil Denitrification Assay

Gas samples collected from the head space of the media bottle and injected into the 10mL vials were analyzed for N₂O using a GC-2104 Gas Chromatograph (G.C.). Using the equation $M = C_g (V_g + V_l a)$, where C_g is the concentration of N₂O in the gas phase determined from the gas chromatograph, V_g and V_l are the volumes of the gas and liquid phases respectively and a is the Bunsen absorption coefficient for the temperature of the

assay. Denitrification rates are determined by regressing the amount of N_2O in the bottle against time. An excel spreadsheet prepared by Grebliunas (2015) is used to calculate the slope; i.e. rate of denitrification.

CHAPTER III

RESULTS

Hydrology

One of the many aspects of this study was to continuously monitor water table elevations. This method of nitrate reduction by letting redirected tile water flow through the RBZ subsurface rather than straight to the stream always presents a possibility of back-up in the tile, especially during high tile flow. This back-up of tile water could damage crop yield negating the purpose of tiling farms. So it is imperative that no back-flow occurs in the tile and that the redirected tile water instead moves down through the RBZ and downslope towards the stream. There are 6 nests of wells that intersect the redirected tiles in the RBZ and each nest has 4 wells screened at different depths (1.5m, 2.3m, 3.5m, and 4.5m). This will allow us to observe the groundwater table and chemistry with good vertical resolution. As is common in Illinois and much of the Midwest, the water table is likely to follow the surface topography. A GIS analysis shows that the riparian buffer has a gentle 7% gradient to the west and towards the stream. As such, I expected the water table to follow a similar path towards the stream T3. Below, figure 6 shows the mean water table pre and post diversion at each well nest and depth, throughout the study period using the water table tape.

Figure 6. Mean pre and post diversion water table elevations

All the different depth wells show that groundwater flow is downgradient away from the higher points (well 2) and towards the stream T3 as expected (Appendix H). The shallower wells (5' and 7.5'), which are representative of the true water table of the RBZ, suggest a gradual flow towards the stream throughout the entire study. From this we can deduce that there is has been danger of back-up caused by the diversion events.

The nested wells allow us to monitor the chemistry of the RBZ with fair vertical resolution. However, not all of the wells, especially the deeper ones, are hydrologically connected with the redirected tile drains and their discharge in the RBZ.

Rothschild et al. (2015) plotted the water table response as a result of the diversion for the shallowest wells equipped with decagon CTDs (which measure conductivity, temperature and depth to water). Figure 7 shows that the shallowest wells (1.5m) of nests 4, 6, 8, and 10 that were equipped with decagon CTDs reacted the most by raising the water table and eventually equilibrating. Our interpretation of this is that these wells responded most clearly to the diversion and are hydrologically well connected with the redirected tile water.

Using this information, only these diversion wells (effluent) were selected for analysis and comparison against the diversion box (influent) and T3 for nitrate comparison. More specifically, only the shallow wells fitted with CTDs. Nest 2 showed no response to diversions and is henceforth omitted from any analysis.

In the end, the following wells were selected for statistical analysis as they were thought to be the most representative of the fate of the redirected tile water in the RBZ.

Diversion wells (4D, 6C, 6D, 8C, 8D, 10C, and 10D) Reference wells (C1, C2, C3) as well as the diversion box and stream T3.

Figure 7. Change in water table due to diversion into the RBZ

Nitrate at the Influent vs. Effluent

A statistical comparison of the mean nitrate concentration at the diversion box, the diversion wells, stream T3, and reference wells showed that these four locations have significantly different concentrations ($P < 0.0001$), with the exception of the reference wells and diversion wells which show no statistical difference between each other (Figure 8). More importantly, the diversion wells (effluent) have a significantly lower nitrate concentration than the diversion box (influent). The mean concentration of nitrate at these four locations was as follows: diversion box was 13.6mg/L, T3 was 8.6mg/L, at the diversion wells was 0.7mg/L, and the reference wells was 0.5mg/L.

Figure 8. Mean nitrate concentrations. T3, diversion box, diversion wells, and reference wells.

The vast difference in nitrate concentrations between the diversion box and the diversion wells shows us that nitrate is significantly reduced once it enters the RBZ; by up to 94%. The nitrate concentrations registered at the diversion box are representative of what would have made it to stream T3. By diverting the tile water into the RBZ, we are already easing the burden on stream T3.

Seasonal Change in Nitrate Concentrations at the Influent vs. Effluent

Since the amount of nitrogen fertilizers applied over a calendar year varies, it is worth noting how nitrate concentrations in the riparian buffer, diversion box, and stream T3 would also differ. Since data collection began early summer (July) and concluded early winter (December), only summer and fall conditions could be compared with sufficient data.

Figure 9. Seasonal mean nitrate concentrations. T3, diversion box, diversion well, and reference well.

Nitrate concentrations at the diversion box (influent), separated into summer and fall, showed that there was significant difference in the concentrations of nitrate during the two seasons. There was a significant difference in nitrate concentrations between summer and fall at the diversion box which feeds the RBZ ($P=0.04$). The summer mean concentrations were more than double that of the fall possibly due to recent fertilizer application in early summer. In the summer, mean nitrate in the diversion box (input) is as high as 20mg/L compared to the fall where mean nitrate concentration drops to 8.5mg/L.

There was, however, no difference in nitrate concentrations at the diversion wells (effluent) during summer and fall. This is contrary to the fact that the diversion box was unloading double the nitrate concentrations into the RBZ in the summer than in the fall. So the concentration of nitrate at the diversion box was twice as high in the summer than in the fall but the concentrations at the diversion wells is $\leq 1\text{mg/L}$ both in the summer and fall.

In sum, nitrate concentrations at the diversion box are higher than the EPA permitted drinking water limit of 10mg/L. But once this water is introduced into the RBZ, the nitrate concentrations are reduced significantly to below 1mg/L. So much so that they show no significant difference in nitrate concentrations when compared to the reference wells, which have no tile influence. Similarly, post-diversion nitrate concentrations in the diversion wells do not appear to be different from pre-diversion nitrate. The nitrate concentrations in the RBZ have been significantly reduced to the point where they are indistinguishable from pre-diversion levels. Seasonally, mean nitrate concentrations at

the diversion box were more than double in the summer than in the fall. However, the RBZ does not appear to be affected by this. In fact, the RBZ had higher efficiency when there were higher nitrate concentrations presented to it in the summer.

Pre vs. Post Diversion Nitrate

Once the appropriately responding diversion wells were identified, I conducted a statistical analysis of the concentrations of nitrate as it enters the RBZ. To observe how nitrate concentrations have varied over the study period, it is useful to separate them out into three key events. The first one is pre diversion. This gives us an idea of the groundwater chemistry before we introduced tile water into the RBZ. The other two events were post diversion and they had to be separated themselves. Even though diversion to the riparian buffer begun on July 17th, there was not always flow in the tiles heading into the RBZ. Some days, the redirected tile was dry. So the post diversion event has been split into post diversion with flow and post diversion with no flow.

The pre diversion nitrate concentrations appeared to be higher than post diversion with flow. Though this difference is not significant, I expected to see the opposite (Figure 10).

I also expected post diversion with no flow to have the lowest concentrations. This is a period where the buffer does not receive any tile water and can instead process out any nitrate that may be present from pervious flow events.

Figure 10. Pre vs. post diversion nitrate.

Nitrate at Reference Wells vs. Diversion Wells

A comparison of nitrate between the reference and diversion wells showed that there is no significant difference between them ($P=0.2243$). The nitrate concentrations from the redirected flow appear to have been so efficiently reduced/removed out of the RBZ that the nitrate concentrations are not different from an area of the RBZ with no tile input (reference wells).

Figure 11. Mean nitrate concentrations at reference wells and diversion wells.

Dilution

One of the major factors that is potentially responsible for nitrate reduction in the RBZ is dilution. This is referring to the dilution of the tile water with the RBZ groundwater as a result of diversion. Whilst dilution of tile water with groundwater does not remove nitrate out of the system, it can potentially reduce the concentrations to below harmful levels.

Chloride is used as a conservative tracer to observe the role of denitrification. A box plot comparison of chloride, nitrate, and chloride/nitrate is made for all post-diversion sampling events. It appears that even though dilution is occurring between the diversion box and the RBZ, it is not responsible for significantly reducing the concentrations. Figure 12 shows the mean chloride, nitrate and chloride/nitrate concentrations in the wells and diversion box over 5.5 months' period. Wells 4, 6, 8, 10, and 12 are represented here with the diversion box and reference wells at each end. The mean chloride concentrations in the diversion wells showed no significant difference from the diversion box. Nitrate concentrations on the other hand were significantly lower in the diversion wells than the diversion box. This reduction in nitrate and not chloride is indicative of nitrate removal processes other than dilution was dominant. Otherwise, we should have seen Chloride decline in a relatively similar manner to nitrate once it enters the RBZ.

As a result of the difference and the decline of concentrations in nitrate, the Cl⁻: N ratio in the diversion wells (effluent) is higher than the diversion box (influent).

Figure 12. Dilution at T3. post diversion mean chloride, nitrate, and chloride/nitrate concentrations at the diversion box vs. diversion wells.

Zooming in on a few sampling dates out of the 5.5 months period reveals a similar trend. Sampling event on 12/1/2015 is chosen to observe this trend in dilution.

Additionally, at this sampling date, the diversion box was visibly diverting water into the RBZ.

On this day, both chloride and nitrate show decline in concentration compared to the diversion box. To assess the extent to which dilution is contributing to the reduction in nitrate, the chloride concentration at the influent (diversion box) is compared to the effluent (diversion wells).

Using the formula $(C_{Cl^- \text{ tile}} - C_{Cl^- \text{ well}}) / (C_{Cl^- \text{ tile}})$, tile driven dilution is calculated.

On 12/1/2015, there was a tile driven dilution observed at the wells. By the time the tile

water had reached the wells, it had lost up to 17% of its nutrients due to dilution (Appendix D).

The subsurface flow path in the RBZ was been determined to be away from the top most nest of wells (#2) and towards the stream T3 (Figure 6). Therefore, there is a possibility of dilution between wells as the ground water makes its way across the RBZ. This calculation was determined for 2 post diversion days that were not tile driven dilution events. This means that the diversion box was not operating and there was no flow into the RBZ. On 10/13/2015 and 11/17/2015 there was evidence of dilution as chloride concentrations declined across the RBZ towards the stream (Appendix D).

Denitrification

The other major factor that is possibly responsible for nitrate reduction/removal is denitrification. Unlike dilution, denitrification completely removes nitrate out of the system and is therefore the preferable path. For denitrification to efficiently remove nitrate out of the system, several conditions must be appropriate. These include:

Dissolved Organic Carbon (D.O.C)

A study of the level of dissolved organic carbon in the RBZ showed that there were generally low levels of D.O.C in the shallow wells in late September (fall) and early December (winter). Most of the D.O.C levels were below 3mg/L with the exception of well 2 in the fall at 14.5mg/L (Figure 13). A D.O.C :NO₃⁻-N ratio of 1:1 or greater is generally preferred for effective denitrification (Sobieszak and Szewczyk 2010).

Figure 13. Dissolved organic carbon in the shallow wells. On 9/29/15 (fall) and 12/1/15 (winter)

An analysis of the ratio of D.O.C: NO_3^- - N for the fall (red) is generally a 1:1 or greater. This ratio was generally lower in the winter (green). Figure 14 below shows this seasonal difference in D.O.C: NO_3^- - N ratio of the diversion wells

Figure 14. Dissolved organic carbon vs. nitrate. On 9/29/15 (fall) and 12/1/15 (winter)

Soil Organic Matter

A dry ash free mass analysis of the soils from the RBZ showed that the RBZ has low-moderate organic matter in the soils around the water table. On average, the % OM at the top most location of the RBZ is 4.30% (site 1 – 4), middle is 4.21% (site 5 – 8) and near the stream T3 is 4.57% (site 9 – 12) (Figure 5). Organic matter availability is expected to decline with depth and these soil samples were taken at depths ranging from 0.8m – 1.8m (2.5' – 5.9').

The box plot below also shows that there is a consistently similar amount of organic matter at above and below the water table around the RBZ.

Figure 15. Percent organic matter in the soils at the RBZ. Above (Abv.) and below (Blw.) the water table (4.2 %– 4.7%).

Dissolved Oxygen

There appears to be low enough dissolved oxygen in the riparian buffer zone for microbial bacteria to favor nitrate as their main electron acceptor. Figure 16 shows that the reference wells were found to have a significantly higher D.O. at 3.21mg/L than the diversion wells at 2.50mg/L ($P=0.0056$). Both transect of wells, diversion and reference wells, appear to have an anoxic environment suitable for denitrifying microbial bacteria.

Figure 16. Mean dissolved oxygen at the reference wells and diversion wells

Acetylene Inhibition Method

This method of measuring denitrification is based on the ability acetylene has to inhibit the reduction of N_2O to N_2 . N_2O accumulation in the head space of a media bottle containing the sediment is measured over incubation time ($t = 15\text{m}, 60\text{m}, 120\text{m}, 180\text{m},$ and 240m). If denitrification is occurring in the soil, the acetylene will make N_2O the terminal product of NO_3^- reduction. This way, the NO_3^- -N reduced to N_2O can be quantified. This informs us the potential the soil has to denitrify NO_3^- -N and remove it out of the system in the form of N_2O .

Soil samples were collected from 12 sites on the RBZ (Figure 5). At the time of soil collection, there was no diversion event into the RBZ occurring. So the groundwater, around which the soil samples were collected, is lower than what it would be during a diversion event. The depth the soils were collected from ranged $0.8\text{m} - 1.8\text{m}$ ($2.6' - 5.9'$). Well below the organic layer composition which is at $0\text{m} - 0.5\text{m}$

Of the 12 sites studied, only two showed signs of potential denitrification. Site 2 and Site 7 showed rates of denitrification at $42.5\mu\text{g}$ and $13.2\mu\text{g}$ per hour respectively. This means the soils collected from these sites have the potential to remove $42.5\mu\text{g}$ and $13.2\mu\text{g}$ of NO_3^- -N every hour (Figure 17). Each of these sites also had similar organic matter content of 4.5% (Figure 15). Since 30 grams of soil was used for each denitrification potential test, the rate of denitrification at the two sites is $1.4\mu\text{g NO}_3^- \text{N h}^{-1} \text{ g}^{-1}$ and $0.44\mu\text{g NO}_3^- \text{N h}^{-1} \text{ g}^{-1}$ respectively.

Figure 17. Rate of denitrification and percent organic matter.

Of the twelve sites tested, only two sites showed potential denitrification, albeit low rates. These are however more than half a meter deep in the subsurface where organic matter is declines and denitrifying bacteria can become scarce. Sanks et al. (2015) found that % organic matter is more than double in the upper parts of the soil profile (10cm – 20cm) than where I sampled from.

Furthermore, these soil samples were collected when there was no active diversion into the RBZ. An analysis of the water table changes due to diversion showed that the water table in the RBZ reaches and saturates the organic layer (Appendix A). This part of the profile, where % organic matter is also higher, is where we would expect to see the greatest amount of denitrification occurring in the RBZ (Hill, 1996; Ranalli et al., 2010; Sylvia et al., 1998).

CHAPTER IV

DISCUSSION

Nitrate Concentrations

Over a 7-month period, the groundwater chemistry of this 105m wide (East – West) and 240m long (North – South) herbaceous riparian buffer zone (RBZ) was studied with and without the influence of a redirected tile drain containing agricultural runoff. Diversion of tile water into the RBZ introduced mean nitrate concentrations of 13.6mg/L and even raised the water table in the RBZ by as much as 22cm (Figure 7). However, across all wells in the buffer, the mean nitrate concentrations dropped to 0.7mg/L. A 94% reduction in concentrations between the influent and effluent. With a full distance of 35.6 m from the top of the redirected tile to the bottom/stream bank of the RBZ (the active zone of the RBZ), the removal rate is $0.5 \text{ mg N L}^{-1} \text{ m}^{-1}$. This is half of the rate of removal found in a similar study by Jaynes et al. (2014) in Iowa. Over the shortest distance, from the redirected tile to nest 8, the RBZ has a removal rate of $2.66 \text{ mg N L}^{-1} \text{ m}^{-1}$. Due to the significant decline in nitrate once it was diverted into the buffer, it is fair to assume that most, if not all, the nitrate is removed in the buffer before reaching the stream T3 downgradient.

This rapid decrease in nitrate concentrations in the buffer is in akin to what has been found in other riparian buffer studies (Cooper, 1990; Ranali et al., 2010; Hill, 1996; Mayer, 2010). A meta-analysis of riparian buffer zones conducted by Mayer et al., (2010)

showed that riparian vegetation seldom mattered when it comes to how effective an RBZ can be in removing nitrate. A comparison of our rate of nitrate removal in the RBZ against this meta-analysis ranks this RBZ as one of the most efficient RBZs at 94%. Appendix I lists some examples of similar studies that looked at RBZ responses to nitrate. Similar to my study, all the case studies here looked at subsurface flow in an herbaceous riparian buffer zone. The results show this herbaceous RBZ to be among the most efficient ones.

A chemical comparison of the pre and post diversion events shows that these two sampling periods have very similar nitrate levels. I expected to see post diversion nitrate concentrations to be higher since we are introducing farm runoff high in nitrate. The buffer processes the nitrate out of the system so effectively, however, that post diversion nitrates are indistinguishable from pre diversion events. Similarly, the mean nitrate concentrations in the diversion wells were not significantly different from the nitrate concentrations in the reference wells ($P=0.2243$). Again the RBZ appears to be so efficient that an area of the buffer that receives tile nitrate is indistinguishable from an area on the same buffer with no tile influence.

Seasonally, mean nitrate at the diversion box was significantly higher in the summer than in the fall. This was to be expected since the recent application of nitrogen based fertilizers and precipitation events in the summer leach water soluble nitrogen. By fall, most of the fertilizer has either been taken up by crops or discharged into the tile. This seasonal difference in nitrate at the diversion box is however not seen at the RBZ. Once in the RBZ, the concentrations for both the summer and fall are very low ($\leq 1\text{mg/L}$). In fact, it appears that the RBZ had higher efficiency in the summer (98%) than in the fall (88%), even though there were higher concentrations presented to the buffer during the summer.

Warmer season seems to support higher rates of nitrate removal. Dhondt et al., (2001) found that nitrate concentrations after harvest (fall) peaked to 22mg/L NO_3^- - N but the RBZ significantly reduced concentrations to below 5mg/L. Whilst there was a difference in the time of year where influent nitrate was highest, Dhondt et al. (2001), and my study show that the RBZ significantly removed nitrate regardless of the season.

Processes Removing Nitrate

There are different processes that account for nitrate removal and/or retention in the RBZ as redirected nitrate-rich farm discharge flows through the subsurface of the RBZ. In this study, nitrate reduction/removal processes such as dilution and denitrification were given more attention than processes like plant uptake or microbial immobilization. Plant uptake and microbial immobilization are given little attention and are often times regarded as not playing as big a role as denitrification or dilution (Hedin et al., 1998). Biotic nitrogen pools can become saturated, and immobilized nitrogen can be re-released into solution by microbial immobilization. As such, they are thought to contribute very little to nitrate uptake in riparian buffers (Paterson and Schnoor, 1983; Pinay et al., 1995)

The contribution of dilution to reducing nitrate was found to be low at this RBZ. There were no significant changes in Cl^- concentrations between the influent (div box.) and effluent (div. wells). In contrast nitrate on the other hand was significantly reduced between the influent and effluent. The fact that we see a significant decline in concentrations of one anion (nitrate) and not the other anion (chloride) is a strong indication of absence of dilution. Low rates of dilution are actually beneficial for the long term life of the RBZ. Dilution only reduces nitrate concentrations and is a temporary solution. With continuous input of redirected farm discharge, the RBZ will inevitably accumulate nitrogen in the

groundwater if dilution was the primary process for nitrate removal. In a similar study conducted on Molenbeek River in Belgium, Dhondt et al., (2001) found that dilution did not contribute to the significant decline in nitrate concentrations. At best, they found that there was a 10% difference in chloride concentrations between the influent and effluent suggesting low rates of dilution.

Denitrification on the other hand completely removes nitrate out of the RBZ and is the preferred path of nitrate removal. Below is a table summarizing what I found about some of the key components that play a role in denitrification in the RBZ and how they are different seasonally. At the bottom the outcome of denitrification rates, or those I expect, are listed based on the conditions. These conditions put together can potentially explain the low different rates of denitrification in the winter.

Table 1

Preferred Conditions for Denitrification and the Conditions Found at the RBZ

Conditions	Preferred	Summer	Fall	Winter
<i>Dissolved Organic Carbon</i> (mg/L)	High[4, 14, 22, 32]	High*	Low (3-0.5)	Low (1.2-0.4)
<i>Percent Organic Matter</i>	High[14, 24, 34]	High*	Moderate*	Moderate (4.2-4.7)
<i>Dissolved Oxygen (mg/L)</i>	Low[19, 21, 25,40]	Low (3.1-3.3)	Low (1.6-1.8)	Low (0.6-4.1)
<i>Mean NO₃⁻ – N (mg/L)</i>	High[13,14, 32,34]	Low (0.3-0.2)	Low.(1.2-0.8)	Med. (8-1)
<i>Groundwater temp. (C)</i>	Warm[28]	Warm (22–10)	Warm(18–13)	Cold (13–9)
<i>Denitrification Rate</i>	High[14, 21,]	High*	Medium*	Low

Note. The numbers in square brackets refer to references in numerical order. The numbers in parenthesis are ranges measured at study site. * indicates that no information was available in literature so the conditions listed are general estimates

Denitrifying bacteria utilize dissolved organic carbon as an electron donor for metabolism. In this way, denitrification can be limited by availability of dissolved organic carbon in the RBZ. A D.O.C:NO₃—N ratio of 1:1 or greater is generally preferable for efficient denitrification (Sobieszuk and Szewczyk 2010). The RBZ showed dissolved organic carbon was adequate in the fall but not in the winter to match the 1:1 ratio (Table 1). The ratio of D.O.C:NO₃⁻ - N in the fall was 1:1 or greater. But this ratio was lower in the winter due to less D.O.C. Dry ash free mass analysis of the soil samples from the RBZ, tested for denitrification potential, also showed low-moderate percent organic matter.

Denitrification is also dependent on an anoxic environment (Mayer et al., 2010; Knowles, 1982; Seitzinger, 1988). In the absence of dissolved oxygen, microbial bacteria turn to nitrate as an electron acceptor. Dissolved oxygen was consistently low in the RBZ throughout the study with a mean 2.3mg/L creating an anoxic environment for the anaerobic bacteria (Table 1).

Peak denitrification is likely to occur in the presence of nitrate (Ryan et al., 1998; Hill, 1996). In this study, there was no supply of nitrate to the RBZ at the time of soil sampling. Figure 9 shows that in the summer higher concentrations of nitrate presented to the RBZ resulted in higher efficiency (98%). This efficiency was lower in the fall (88%) and even lower in the winter (85%).

Furthermore, denitrifying microbial bacteria tend to favor warmer conditions in the soil for efficient denitrification. Rate of denitrification can depend on how warm the soil is (Maggi et al., 2015). Microbial bacteria can remain dormant in colder seasons and become active in warmer ones accounting for the different rates of denitrification. Whilst soil temperature data is lacking, the groundwater temperature at the RBZ can reflect the

conditions well. As Table 1 and Appendix 7 show, temperature in the RBZ has significantly declined over the seasons changing from 22⁰C in the summer to 9⁰C in the winter.

The water table in the RBZ also saturated the organic layer when tile water was diverted into the RBZ. For two to four days, the organic layer remained saturated with some of the redirected tile water before the water table dropped (Appendix A). This part of the soil profile is where denitrification is likely highest as many authors have stressed. There is a consensus amongst researchers in this area that nitrate is effectively removed out of an RBZ when the nitrate laden water is exposed to the organic layer in the soil (Hill, 1996; Ranalli et al., 2010; Sylvia et al., 1998). Sanks (2015) found that organic matter in this top layer is double what I found in the sand and gravel layer just below.

Ryan et al. (1998) also reported on the relationship of denitrification rates with depth. Using the same technique of acetylene-block method, Ryan (1998) tested soil cores and their denitrification potential and found that 80% of denitrification happens in the top 0.1m of the soil (Ryan et al. 1998). Soil samples at this RBZ were collected from 0.7m and below.

In sum, there were several conditions at the RBZ that led to low rates of denitrification I found in the winter. There was no supply of nitrate at the exact time of sampling and denitrification is dependent on availability of nitrate. This also meant that the organic layer was not saturated with the nitrate laden water. D.O.C in the area was below desired levels with low to moderate organic matter. Groundwater temperatures were also much lower in the winter compared to the summer. Bacteria in the soil best denitrify in warmer conditions (Hill, 1996; Sylvia, 1998). The samples were also collected from depths where denitrification rate significantly decline (Ryan et al., 1998).

This low rate of denitrification in the winter is further supported by the observed efficiencies in the RBZ seasonally. Figure 9 shows how efficient the RBZ is when comparing the influent (div. Box) to the effluent (div. Wells). In the summer the RBZ is up to 98% efficient. In the fall however, this efficiency falls to 88%. In the winter it is down to 85%. These efficiencies still remove substantial amount of nitrate out of the RBZ but the decline in efficiency associated with season is a strong indication of why denitrification rates may be low in the winter.

Future Work

In the future, denitrification potential in the RBZ should be investigated immediately after a diversion event and in the upper section of the soil profile; i.e. the organic layer (O-layer). This section of the soil will almost likely show much higher rates of denitrification.

The fact that D.O.C was lower in the winter than the fall also merits its own investigation. Observing the seasonal trend in D.O.C may prove useful when considering at what time of the year the RBZ is at its most efficient. Lower concentrations of D.O.C, especially compared to nitrate concentrations, may result in low rates of denitrification. Grebliunas (2015) suggested that allochthonous D.O.C is always an option that can be applied to increase the D.O.C concentrations in the area and improve denitrification. If seasonal D.O.C differences appear negligible to the rate of denitrification, we can focus on the activity of microbial bacteria and the extent to which they affect denitrification.

Besides sampling the O-layer after a diversion event, we also need to study the D.O.C concentrations in the O-layer for appropriate comparison. What we currently have are D.O.C measurements in the sand/gravel layer.

A bromide tracer test performed at the RBZ could also provide us with a clearer picture of the flow speed and possibly the flow path in the RBZ. This could further shed light on the hydrologic connectivity of the RBZ and the redirected tile water. It can reveal if the flow paths in the RBZ favor microbial denitrification environments. In areas of the RBZ where nitrate reducing conditions may be limited, such as deep in the subsurface, long residence time may also permit slow nitrate removal reactions to be effective. If this is the case, a tracer test may reveal the pattern.

CHAPTER V

CONCLUSIONS

This method of diverting the tile drainage into the RBZ using a diversion box appears to be a practical method for reducing nitrate concentrations. Groundwater flow in the RBZ shows a downgradient flow towards the stream T3 (figure). This slight elevation relief of the RBZ ensures that there is no back-up caused in the diversion box and instead, the redirected tile water flows towards the stream T3. Nitrate concentrations were significantly reduced once in the RBZ. Whilst the RBZ showed low rates of denitrification potential in the winter, it appeared to have the necessary conditions for denitrification in the summer and fall. Slight improvements on when and where to measure rates of denitrification will have a better reflection on the RBZ. It is imperative that the redirected tiles discharge their contents in the section of the RBZ where nitrate reducing environments are present. This was the case in this study. The Organic layer is saturated with the redirected water and organic matter is adequately available. Since the nitrate concentrations introduced into the RBZ were significantly higher than what was already in the stream T3, this method is already easing the burden of nitrate on T3. In the long-term, this method of reducing nitrate concentrations by intercepting and diverting tile water into the RBZ can improve on T3's own capacity for nitrate removal.

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APPENDIX A

WATER TABLE SATURATION OF ORGANIC LAYER

Figure A-1. Organic layer saturation at well 4

Figure A-2. Organic layer saturation at well 6

Figure A-3. Organic layer saturation at well 8

Figure A-4. Organic layer saturation at well 8

APPENDIX B
PRE AND POST DIVERSION NITRATE

Table B-1

Pre Diversion Nitrate Concentrations

Pre Diversion						
Source	Source ID					
		Average	Max	Min	Plus	Minus
4	4C, 4D	2.570	3.270	1.680	0.700	0.890
6	6C, 6D	2.79333	3.990	1.694	1.197	1.099
8	8C, 8D	1.312667	1.941	0.542	0.628	0.771
10	10C, 10D	2.285	4.230	4.230	1.945	-1.945
12	12C, 12D	1.646	2.290	2.290	0.644	-0.644
13	13, 14, 15	0.7825	1.05	1.05	0.268	-0.268
15	Div. Box	18.629	24.104	24.104	5.475	-5.475
17	T3	12.02367	15.155	15.155	3.131	-3.131

Table B-2

Post Diversion with Flow Nitrate Concentrations

Prost Diversion with Flow						
Source	Source ID					
		Average	Max	Min	Plus	Minus
4	4C, 4D	1.617	3.731	0.197	2.114	1.420
6	6C, 6D	0.952225	3.477	0.0418	2.525	0.910
8	8C, 8D	0.35905	1.2	0.031	0.841	0.328
10	10C, 10D	2.0615	12.2	0.258	10.139	1.804
12	12C, 12D	3.110375	10.333	0.56	7.223	2.550
13	13, 14, 15	0.249308	0.983	0.01	0.734	0.239
15	Div. Box	15.82825	26.3	1.492	10.473	14.336
17	T3	11.78375	14.652	4.283	2.868	4.501

Table B-3

Post Diversion with Flow Nitrate Concentrations

Prost Diversion with No Flow						
Source	Source ID					
		Average	Max	Min	Plus	Minus
4	4C, 4D	2.397571	7.211	0.1	4.813429	2.297571
6	6C, 6D	0.579607	1.590	0.0785	1.010393	0.01107
8	8C, 8D	0.402308	1.578	0.0983	1.175692	0.304008
10	10C, 10D	0.448286	1.153	0.07	0.704714	0.378286
12	12C, 12D	1.683571	0.55	0.61	1.866429	1.073571
13	13, 14, 15	0.583619	1.289	0.178	0.705381	0.405619
15	Div. Box	12.42333	21.3	1.566	8.876667	10.85733
17	T3	7.637857	9.398	6.25	1.760143	1.387857

Figure B-1. Well nest 4 nitrate

Figure B-2. Well nest 6 nitrate

Figure B-3. Well nest 8 nitrate

Figure B-4. Well nest 10 nitrate

Figure B-5. Well nest 12 nitrate

APPENDIX C

MEAN NITRATE BY SOURCE AND BY SEASON

Table C-1

Mean Nitrate by Source

Source	N	Mean Nitrate (mg/L)	Std. Err. (mg/L)
Div. Box	10	13.648	3.177906
Ref	30	0.507257	0.06315
T3	10	8.5948	0.801916
Well	80	0.746408	0.131617

Table C-2

Mean Nitrate by Season

Season	Source	N	Mean NO ₃ ⁻ (mg/L)	N Std.Err. (mg/L)	Mean D.O (mg/L)	D.O. Std. Error (mg/L)
Fall	Div. Box	6	8.5664	4.321397	5.084	0.599038
Fall	Ref	18	0.656667	0.077124	2.835556	0.385541
Fall	T3	6	7.279667	0.452561	6.003333	0.699989
Fall	Well	48	1.004049	0.207931	1.76625	0.159395
Summer	Div. Box	4	20	2.293832	5.665	0.58848
Summer	Ref	12	0.283142	0.069893	3.905	0.419754
Summer	T3	4	10.5675	1.465955	6.39	0.348401
Summer	Well	32	0.367997	0.074913	3.22	0.188821

Table C-3

Mean Nitrate by Source

Source	N	Mean Nitrate (mg/L)	Std Err. (mg/L)
Ref	30	0.507257	0.06315
Well	80	0.746408	0.131617

APPENDIX D

DILUTION

Table D-1

Dilution

Date	Source	Chloride	% dilution (tile driven)	% dilution (well to well)
9/29/2015	Tile/Div	4.3387		
9/29/2015	4	10.16065	-134%	
9/29/2015	6	10.1302	-133%	
9/29/2015	8	6.22785	-44%	
9/29/2015	10	7.6834	-77%	
9/29/2015	12	6.1154	-41%	
Date	Source	Chloride	% dilution (tile driven)	% dilution (well to well)
10/13/2015	Tile/Div	5.1592		
10/13/2015	4	10.03935	-95%	4%
10/13/2015	6	9.66965	-87%	16%
10/13/2015	8	8.07445	-57%	-7%
10/13/2015	10	8.6324	-67%	39%
10/13/2015	12	5.2436	-2%	
Date	Source	Chloride	% dilution (tile driven)	% dilution (well to well)
11/17/2015	Tile/Div	6.427		
11/17/2015	4	11.6242	-81%	10%
11/17/2015	6	10.4325	-62%	8%

(Table Continues)

Date	Source	Chloride	% dilution (tile driven)	% dilution (well to well)
11/17/2015	8	9.5495	-49%	-7%
11/17/2015	10	10.23855	-59%	8%
11/17/2015	12	9.4699	-47%	
Date	Source	Chloride	% dilution (tile driven)	% dilution (well to well)
12/1/2015	Tile/Div	15.8214		
12/1/2015	4	14.1456	11%	
12/1/2015	6	17.1718	-9%	Avg.
12/1/2015	8	10.90085	31%	17%
12/1/2015	10	15.51635	2%	
12/1/2015	12	14.01985	11%	

APPENDIX E
DISSOLVED ORGANIC CARBON

Table E-1

Dissolved Organic Carbon

	29-Sep			1-Dec		
	Nitrate	D.O.C		Nitrate	D.O.C	
Well	(mg/L)	(mg/L)	Ratio	(mg/L)	(mg/L)	Ratio
4C	3.346			3.731	0.414	0.110962
4D	2.946	3.055	1.036999	0.962		
6B	0.551	0.98	1.778584	0.537	0.31	0.577281
6C	0.569	1.244	2.186292	3.477	1.235	0.355191
6D	0.427	0.88	2.06089	1.441		
8B	1.891	2.393	1.265468	3.126	26.843	8.587012
8C	BDL			1.2	0.996	0.83
8D	0.333	0.516	1.54955	0.981	1.577	1.607543
10C	0.581	1.384	2.3821	0.974	1.137	1.167351
10D	0.334	1.125	3.368263	12.218	1.095	0.089622
12B	0.360			7.204		
12C	BDL			10.333	1.657	0.16036
14(C2)	0.983	0.635	0.645982	1.469	0.97	0.660313

APPENDIX F
DISSOLVED OXYGEN

Table F-1

Dissolved Oxygen

Source	N	Mean D.O. (mg/L)	Std. Err. (mg/L)
Div. Box	10	5.342222	0.409654
Ref Wells	30	3.263333	0.297454
T3	10	6.158	0.428368
Div. Wells	80	2.34775	0.14521

APPENDIX G

GROUNDWATER TEMPERATURE AT T3 RBZ

Figure G-1. Well nest 12 nitrate

APPENDIX H WATER TABLE AT T3 USING MANUAL WATER TABLE TAPE

APPENDIX I

COMPARISON OF HERBACEOUS

RBZ EFFECTIVENESS

Table I-1

Comparison of Herbaceous RBZ Effectiveness

NO ₃ Concentration								
Vegetation Type	Flow Path	Buffer Width (m)	Mean influent (mg/L)	Mean Effluent (mg/L)	% Nitrogen Removal Effectiveness	NO ₃ Removal mgL ⁻¹ m ⁻¹	Study	Origin
Herb	Sub.	50	66	0.02	100	0.13	Martin et al. (1999)	Ontario, Canada
Herb	Sub.	30	44.7	0.45	99	1.48	Vidon and Hill (2004)	Ontario, Canada
Herb	Sub.	34	13.6	0.7	94	0.5	Taye et al. (2016)	Illinois, USA
Herb	Sub.	60	1.7	0.14	92	0.03	Sabater et al. (2003)	7 European Countries
Herb	Sub.	25	12.15	1.92	84	0.41	Hefing and de Klein (1998)	Netherlands
Herb	Sub.	39	16.5	3	82	0.35	Osborne and Kovacic (1993)	Illinois, USA
Herb	Sub.	25	15.5	6.2	60	0.37	Vidon and Hill (2004)	Ontario, Canada
Herb	Sub.	40	0.35	0.23	34	<0.01	Sabater et al. (2003)	7 European Countries