SEASONAL AND DIURNAL VARIATION OF NITRATE CONCENTRATIONS WITHIN THE HYPORHEIC ZONE OF A LOW-GRADIENT, THRID-ORDER STREAM IN CENTRAL ILLINOIS

Kelly M. Hayden

74 Pages December 2012

This research examines the variation of nitrate concentrations deep within the hyporheic zone of a headwater stream to observe variation at a seasonal and diurnal scale.

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Seasonal variations in nitrate have been observed from September 2011 to October 2012. Concentrations in nitrate ranged from 0.07 to 0.43 mg/L, with the lowest concentrations occurring January, and the highest in September and May. The data show a steady decrease in

nitrate concentrations from September to January, following January, a steady increase was observed until May. Concentrations remained consistent until a decrease was observed in September. These variations were to be expected because of variations in precipitation, fertilizer application, and amount of organic matter entering the system. Diurnal patterns of increasing and decreasing nitrate concentrations were observed during the 24-hour sampling periods, though the observed variations did not correspond to changes in light availability. Both the seasonal and diurnal studies of nitrate concentrations provide insight into the ability for the hyporheic zone to remove nutrients from a stream system and the controls of the reduction processes.

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K.M.H.

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INTRODUCTION

Since the 1960s, application of fertilizers to agricultural fields has increased significantly, and as a result, the average concentration of nitrate in Midwestern streams has doubled (Dagg and Breed, 2003). Nitrate is able to enter stream systems through a variety of pathways, including: nitrification, erosion of soil from fertilized fields, surface runoff, leaching to groundwater, and agricultural tile drainage systems. Once in the waterways, nitrate may be removed by microbial processes in the streambed sediment and by plant uptake, or stored within the stream system (Buyck, 2005; Findlay et al., 1993; Schaller et al., 2004). However approximately 25% of the nitrate in the stream system will remain mobile and continue to the Mississippi River, where it is eventually discharged into the Gulf of Mexico (Dagg and Breed, 2003; Puckett et al., 2008). The increased amount of nitrate entering the Gulf has resulted in a growing zone of hypoxia that has been observed to affect an area as large as 20,000 km^2 (Dagg and Breed, 2003). Hypoxia occurs when excess nitrate in water causes an algal bloom to form, which decreases the dissolved oxygen concentration once the algal mass decays. The resulting low oxygen environment is detrimental to aquatic life, creating a large dead zone (Arango et al., 2007). Though hypoxia is a natural phenomenon, the magnitude and frequency of occurrence has increased significantly since the 1960s and coincides with the increase in fertilizer application (Dagg and Breed, 2003). The urgency of the condition in the Gulf has resulted in increased research examining the sources of the excess nitrate, its pathways to the streams, and the processes that remove the nitrate once it has entered the waterways. Much of the research focuses on surface waters and the top few centimeters of the hyporheic zone (Alexander et al., 2007; Alexander et al., 2000; Arango et al., 2007; Christensen et al., 1990; Dagg and Breed, 2003; Goodale et al., 2005; Mulholland et al., 2008; Scavia et al., 2004), but little has been conducted deep within the hyporheic zone of streams.

Nitrate and the Hyporheic zone

The hyporheic zone is a zone of mixing beneath a stream channel where stream water and groundwater interact (Figure 1.1) (Findlay et al., 1993).

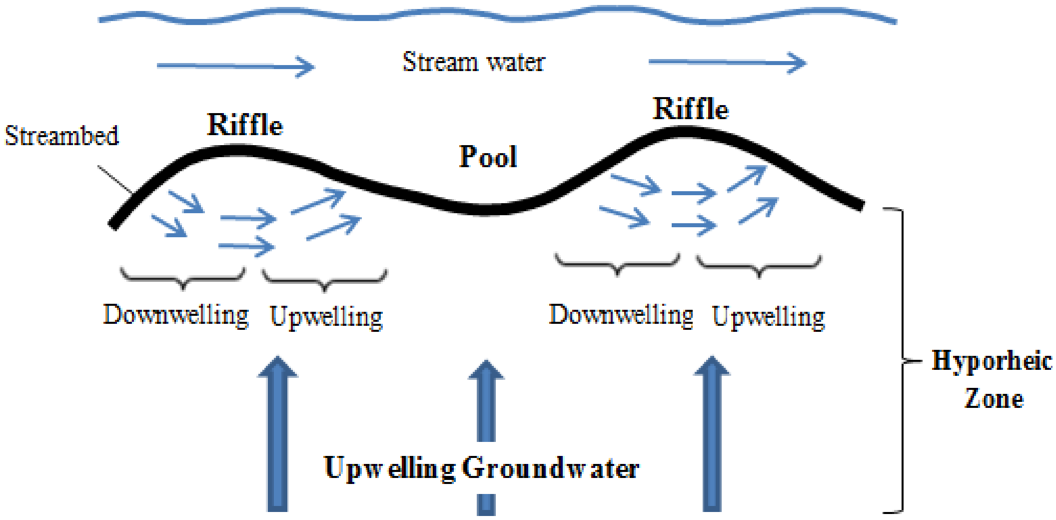

Figure 1.1: Diagram of the hyporheic zone of a stream. The hyporheic zone is where groundwater and surface water interact. Pools and riffles on the streambed surface may create localized zones of upwelling interstitial and downwelling stream water.

Triska *et al.* (1989) determined that the hyporheic zone could be divided into two layers. The upper layer contains from 98 to 100% stream water, and possesses similar chemical characteristics as the surface waters. The lower section of the hyporheic zone contains between 98 and 10% stream water and is characterized by chemical gradients. With increasing depth, the water chemistry changes with respect to concentrations of nutrients, organic carbon, dissolved oxygen, and carbon dioxide. Once the interstitial fluid is comprised of less than 10% stream water, it is no longer considered part of the hyporheic zone. Instead, it is a groundwater zone that is not influenced chemically by the stream water. Because the hyporheic zone is defined by a stream water to ground water ratio, the size and shape of the zone varies seasonally based on surface water and groundwater flow conditions (Storey et al., 2004).

Exchange of water within the hyporheic zone is largely controlled by hydraulic head of the channel and sediment permeability; however, topography of the streambed may influence the exchange of nutrients between the hyporheic zone and surface water (Figure 1) (Brunke and Gonser, 1997; Hester and Gooseff, 2010). Pools and riffles that make up the streambed create zones of upwelling interstitial water and downwelling stream water. Downwelling occurs at the ends of the pools where a high pressure zone exists due to the increased water depth. The ends of riffles cause the stream depth to increase, resulting in a low pressure zone that allows upwelling to occur (Brunke and Gonser, 1997). Downwelling of stream water into the hyporheic zone increases dissolved oxygen (DO) levels of the interstitial fluids, whereas water that is upwelled introduces nutrients from the groundwater to the stream (Boulton et al., 1998). Chemical gradients observed within the hyporheic zone are the result of microbial processes and are related to the amount of dissolved oxygen available (Boulton et al., 1998). Where high concentrations of DO are available, aerobic microbial processes occur in abundance. DO concentrations generally decrease with depth in the hyporheic zone as it is utilized by the microbes and anoxic groundwater is upwelled (Brunke and Gonser, 1997). However, pockets of anoxic water can exist surrounded by zones high in DO due to the heterogeneity of sediments (Boulton et al., 1998). These pockets of low DO allow anoxic microbial processes to occur even at shallow depths within the hyporheic zone. Anoxic conditions allow redox reactions to occur, specifically denitrification. Denitrification is a microbial process of the nitrate cycle that removes nitrate from the stream system, thus reducing the stream's overall nitrate concentration (Arango et al., 2007).

Nitrate Cycling

The nitrogen cycle uses bacteria and plants to convert nitrogen into a more useful form. The addition of nitrogen to a stream system is the beginning of the nitrogen cycling processes in streams. Atmospheric nitrogen (N_2) contributes small amounts of nitrogen to streams and adds to the overall concentration of nitrate in the hyporheic zone (Alexander et al., 2008). Nitrogen that enters the stream as dissolved organic nitrogen (DON) from biodegraded organic materials are thought to make up half of the amount of nitrogen in streams. Once in the stream, DON is mineralized to form bioavailable inorganic nitrogen (Van der Hoven, et al., 2008). The nitrogen from both the atmosphere and biodegradation are then fixed by bacteria to form ammonium, which is then nitrified by nitrifying bacteria. Ammonium may also enter the stream directly through surface runoff during periods of increased precipitation (Kemp and Dodds, 2002a).

Nitrification

Nitrification is an aerobic microbial process that oxidizes ammonium to form nitrate (Brunke and Gonser, 1997). Nitrification is responsible for converting between 9 and 15% of the ammonium that enters the stream system to nitrate (Kemp and Dodds, 2002b). This process increases the concentration of nitrate in the hyporheic zone and exhibits a downward gradient due to the decrease in availability of nitrifying microbes, dissolved oxygen, and organic carbon (Brunke and Gonser, 1997; Kemp and Dodds, 2002a). Once the ammonium has been converted to nitrate, it can be removed from the system through plant uptake or denitrified by microbes in the sediment.

Denitrification

Nitrate reduction deep within the hyporheic zone can be largely attributed to denitrification (Buyck, 2005; Findlay et al., 1993). Denitrification is an anoxic microbial redox reaction that uses nitrate as an electron acceptor and organic carbon to convert nitrate into atmospheric nitrogen (N_2). This process is responsible for between 37-76% of nitrate removal in streams; the other removal processes are plant uptake and nitrate storage (Fischer et al., 2005; Gu et al., 2007; Mulholland et al., 2008). Denitrification occurs within the hyporheic zone, where the microbes are able to colonize instead of in the water column where the they would be washed away (Gu et al., 2007). Because this process occurs in the hyporheic zone, grain size and retention time play a major role in the amount of nitrate the denitrifying microbes are able to remove (Puckett et al., 2008). When the retention time of the interstitial water is increased within the hyporheic zone, the prolonged contact with denitrifying microbes allows more nitrate to be removed from the system (Opdyke et al., 2006).

The denitrifying microbes require organic carbon and anoxic conditions to effectively convert the nitrate to nitrogen gas. This means that denitrification rates will be negligible until the oxygen in the sediment has been used by other microbial processes. Therefore, denitrification is most productive deeper in the hyporheic zone, however, the increased depth may result in organic matter becoming a limiting factor (Stelzer et al., 2011). Anoxic conditions can exist at shallow depths within the hyporheic zone where heterogeneity of sediments creates pockets of low DO (Boulton et al., 1998). These pockets of anoxic water allow both nitrification and denitrification to occur at the same depth. Aerobic nitrification and anaerobic denitrification are processes that are commonly coupled within the hyporheic zone (Kemp and Dodds, 2002a). Coupling occurs when nitrification in aerobic interstitial waters increases the concentration of nitrate in the water, allowing for denitrification to occur in anaerobic pockets within the hyporheic zone (Triska et al., 1993).

Variations in Nitrate Concentrations

Diurnal variations in nitrate concentrations within the streambed and hyporheic zone may be a result of changes in DO concentrations. As photosynthesis occurs in the sediment during daylight, the process causes concentrations of DO in the interstitial water to increases. Photosynthesis of algae in the streambed increases the amount of oxygen and reduces the size of the anoxic environment needed by the denitrifying microbes (Cleven and Meyer, 2003). Christensen *et al.* (1990) observed microbes were able to denitrify up to twice as much in the dark as in the light. The effects of photosynthesis have the potential to be observed deeper within the hyporheic zone despite the process itself occurring within the top few centimeters of the streambed. This is because water in the upper section of the streambed may be downwelled deeper into the hyporheic zone, effecting concentrations of dissolved oxygen in deeper interstitial waters (Boulton et al., 1998).

In addition to diurnal variations, seasonal variation in nitrate concentrations are common in Midwestern streams (Royer et al., 2004). Surface water variations in nitrate concentrates may be attributed to precipitation, fertilizer application, rate of stream water discharge, and the concentration of dissolved organic carbon in the streambed (Alexander et al., 2007; Arango et al., 2007; Dagg and Breed, 2003). Precipitation has the potential to increase the nitrate entering stream by increasing the amount of surface runoff and tile drainage during rain events. Nitrate concentrations tend to be higher during the early spring when increased precipitation increases runoff and fertilizers are applied to the fields, conversely, nitrate concentrations are typically lowest during the summer when there is increased plant uptake from growing crops (Pind et al., 1997; Royer et al., 2004).

In addition to surface processes, there are several seasonal factors that can affect nitrate concentrations within the hyporheic zone. These fluctuations can be attributed to changes in the amount of denitrification occurring. Denitrification rates tend to be lowest during the winter months (November to March) and highest in early spring and summer (April- July) (Chavan et al., 2008; Pind et al., 1997). The amount of denitrification that occurs is based on several environmental factors including: the availability of nitrate, organic carbon content, dissolved oxygen concentrations, pH, and temperature. Chavan *et al.* (2008) attributed the decrease in denitrification observed during winter months to a decrease in temperature. A positive correlation between denitrification and temperature was observed on a seasonal scale, indicating that the lower temperatures result in decreased microbial activity. No seasonal correlation was observed between denitrification and dissolved oxygen or pH, making temperature a significant control on the microbial environment. The increase in denitrification during spring and summer may be attributed to increased nitrate entering the system as plants no longer utilize the nitrate, and increased amounts of decaying foliage entering the stream providing more organic carbon for the denitrifying microbes. Stream discharge may also affect denitrification by altering retention time of the interstitial water within the hyporheic zone. High discharge may reduce denitrification by disturbing the streambed sediment, removing the denitrifying microbes (Pind et al., 1997). High discharge also reduces the water's retention time within the system, thus limiting the interaction between denitrifying microbes and the nutrients in the interstitial water (Opdyke et al., 2006). High discharge further disrupts the hyporheic zone by increasing downwelling of oxygenated water into the hyporheic zone, which reduces the anoxic environment required for denitrification (Baker and Vervier, 2004).

Objectives

The purpose of this study is to examine seasonal and diurnal variations of nitrate concentrations deep within the hyporheic zone. Changes in the nitrate concentrations observed in the hyporheic zone are caused by fluctuations in microbial processes, plant uptake, surface runoff, and spring fertilizer application to fields within the watershed (Alexander et al., 2007; Arango et al., 2007; Dagg and Breed, 2003).

The leading question of my research is "How do nitrate concentrations vary within the hyporheic zone of a low gradient, third-order stream." To answer this question, several sub-questions need to be examined: 1) How do nitrate concentrations vary seasonally; 2) How do nitrate concentrations vary diurnally; 3) How do nitrate concentrations vary with increasing depth? Researching nitrate concentration variation over several periods of time will serve to examine the potential for the hyporheic zone to reduce the nitrate concentration of a stream system, and provide insight into the role of denitrification. Understanding the potential for nitrate to be removed in the hyporheic zone is crucial in understanding the nutrient removal capacity of streams. Currently, the majority of the research conducted in the hyporheic zone focuses on the top five centimeters, despite studies indicating significant microbial processes occur up to several meters below the streambed (Stelzer et al., 2011). This research will examine nitrate concentrations from 30 to 150 cm below the streambed surface to gain insight into chemical gradients within the hyporheic zone.

It is important to understand the processes occurring in the hyporheic zone of Little Kickapoo Creek (LKC) because it is analogous to many low gradient streams throughout Illinois and the Midwest that receive agricultural surface runoff and tile drainage.

Hypothesis

It is expected that seasonal fluctuation in nitrate concentrations will be observed, with peaks occurring in the early spring and fall. Based on the typically seasonal increase in denitrification during the summer months, nitrate concentrations should be at their lowest during the summer. This expected pattern of variation is based on the timetable of fertilizer application, plant growth, precipitation, and amount of carbon available for denitrification.
It is also expected that the nitrate concentration will vary on a diurnal scale. Nitrate concentrations are expected to be lowest at night when there is no sunlight to stimulate photosynthesis that would oxygenate the interstitial water and hinder denitrification.

In addition to temporal variation, it is expected that nitrate concentrations will vary with depth. Concentrations are expected to decrease with depth because dissolved oxygen concentrations typically decrease with increasing depth. Therefore, interstitial water should be anoxic or near anoxic deeper within the hyporheic zone and provide the necessary environment for denitrification.

Site description

The study site for this research is located 10 kilometers south of Bloomington Illinois in a meander of Little Kickapoo Creek (LKC) on 700 N Road off US Highway 51 in an outwash valley just south of the Bloomington Moraine (Figure 1.2).

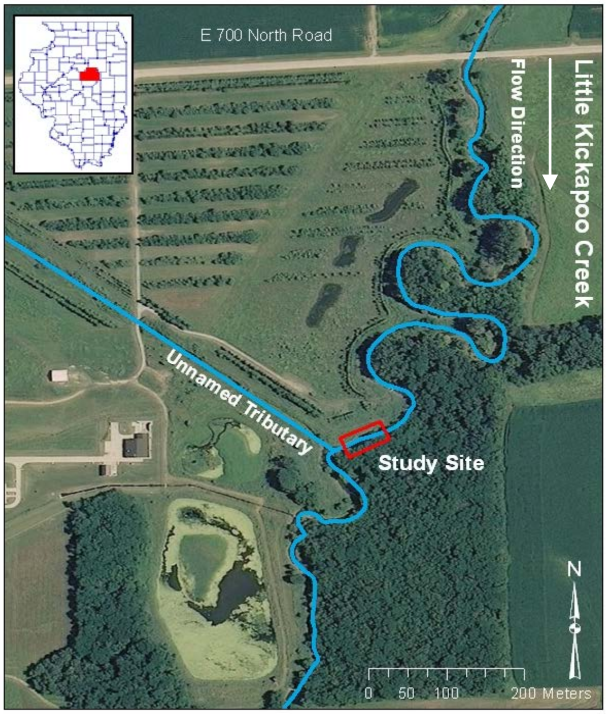

Figure 1.2: The study site for this research is located along a meander of Little Kickapoo Creek in central Illinois.

This location is adjacent to the Illinois State University Instructional Well Field and is owned by the Bloomington-Normal Water Reclamation District. LKC is roughly 11 km in length and flows south, south-west from Bloomington Illinois to the town of Randolph where it discharges into Kickapoo Creek (Figure 1.3).

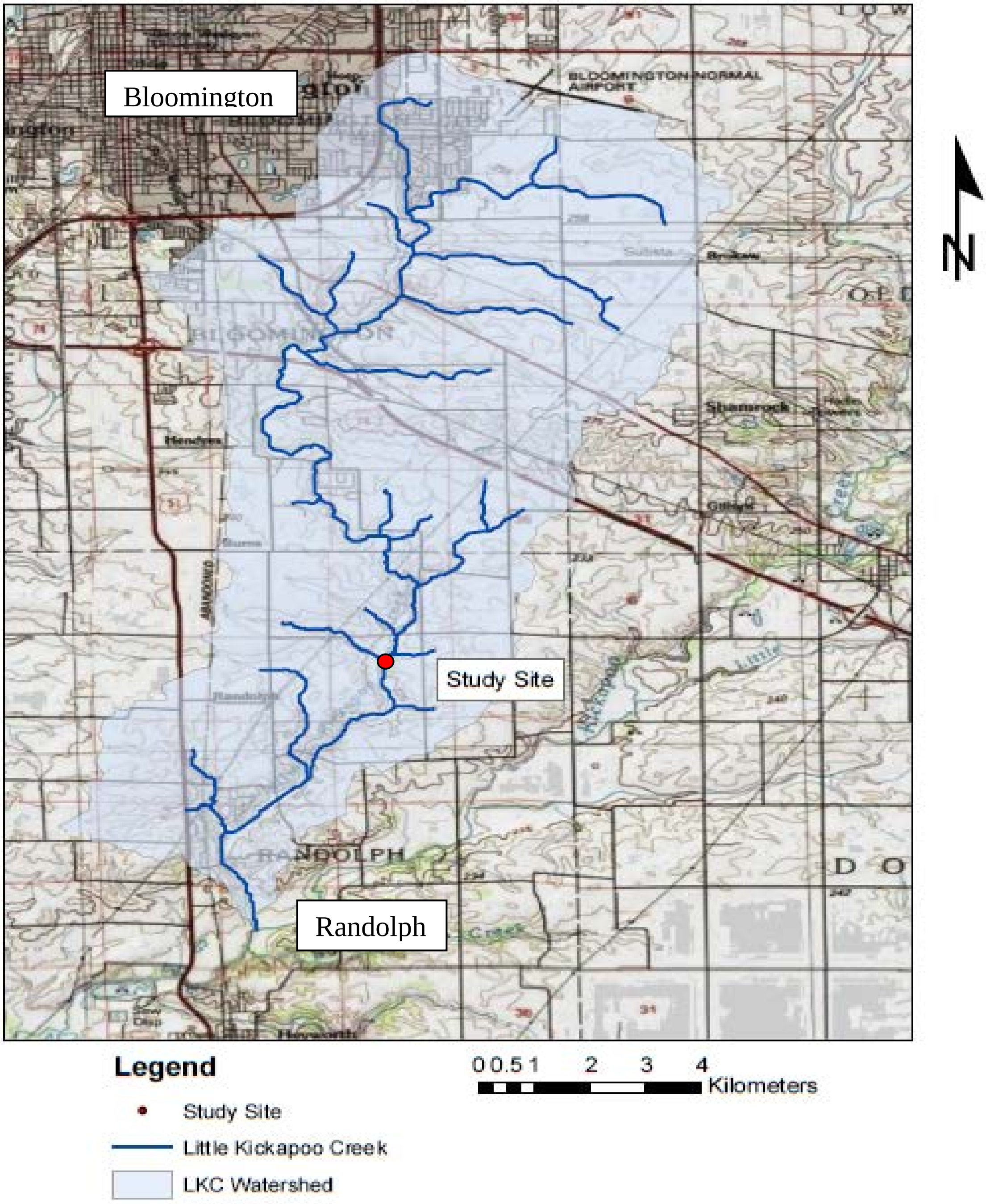

Figure 1.3: Little Kickapoo Creek extends 11 km from Bloomington Illinois to the town of Randolph where it discharges into Kickapoo Creek.

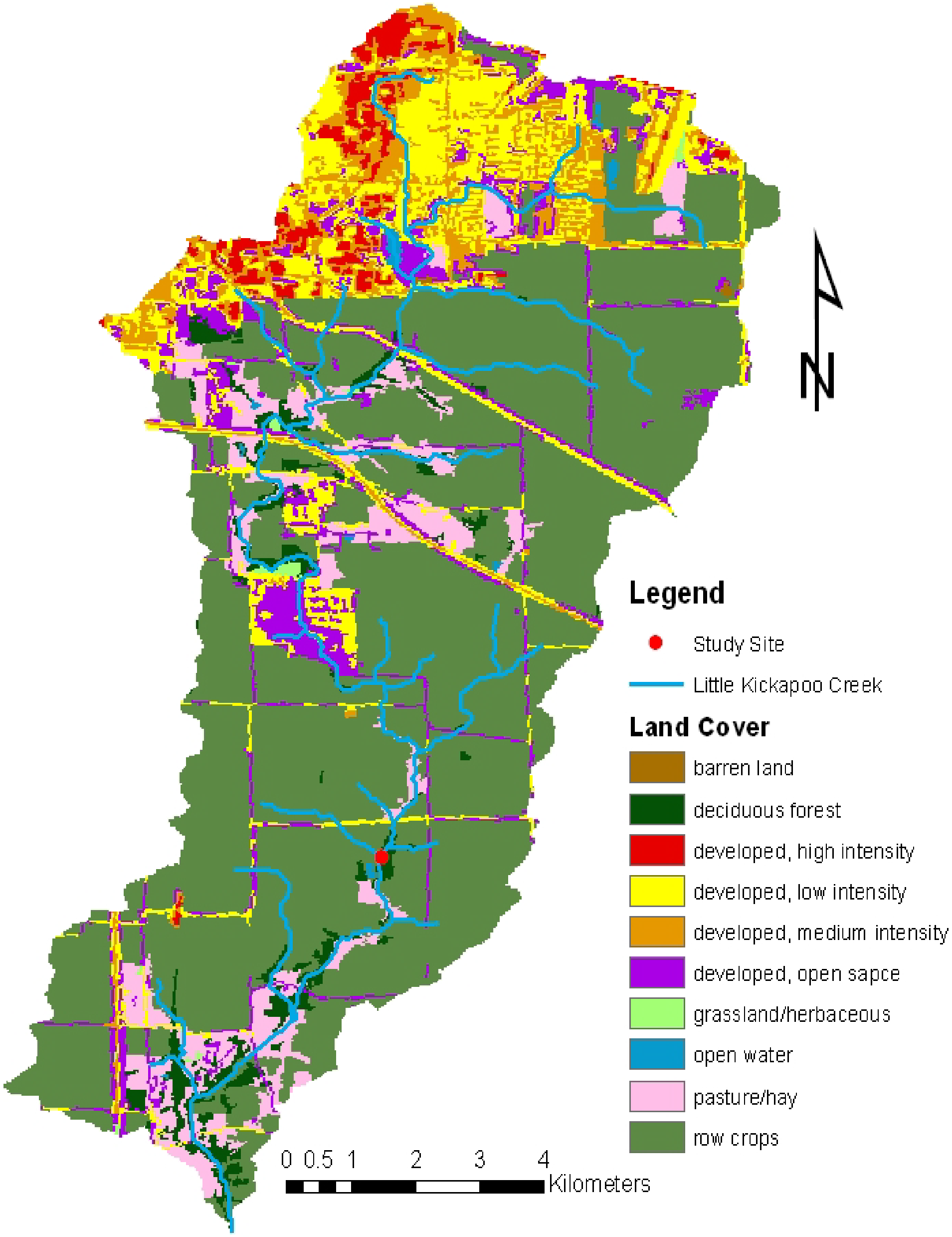

Figure 1.4: The northern section of the LKC watershed is urban and developed land. The majority of the watershed (60%) is row corps of corn and soybeans.

The LKC watershed covers 76 km^2 of primarily agricultural land of corn and soybeans with the stream's headwaters located in a moderately developed urban area (Figure 1.4). The stream is a gaining stream that receives water from groundwater, surface runoff, and tile drainage. The stream base flow ranges from 0.1 m^3/s to 4 m^3/s as the amount of runoff and discharge from tile drainage changes throughout the year. The stream has a maximum depth of 1.3 m and an average flow velocity of 0.25 m/s (Peterson and Sickbert, 2006).

This location was chosen for study because other research has been conducted in this section of Little Kickapoo Creek (Bastola, 2011; Beach, 2008; Buyck, 2005; Oware, 2010; Peterson and Benning, 2012; Peterson and Sickbert, 2006; Peterson et al., 2008; Van der Hoven et al., 2008); therefore this research will contribute to an ongoing study of the area. Previous research determined that denitrification was the main source of nitrate removal in the hyporheic zone of LKC. Plant uptake was found to affect the top ten centimeters of the hyporheic zone, and only played a major role during periods of intense plant growth (Buyck, 2005). Though denitrification is not being measured directly in this study, variations in the nitrate concentrations will allow the amount of denitrification occurring to be inferred. Peterson *et al.* (2008) determined that baseflow conditions at LKC entrain sediment ranging in diameter from 3 to 18 mm. At bankfull conditions, sediment up to 51 mm may be entrained and disturbance of the top 30 cm of sediment may occur. Disturbance of this top layer of the hyporheic zone occurs several times a year and results in a fluctuation in the abundance of denitrifying microbes and the residence time of the interstitial water.

Geology and Hydrology

Previous research conducted at Little Kickapoo Creek and field observations have detailed the geology and hydrology of the area (Peterson and Sickbert, 2006; Peterson et al., 2008; Van der Hoven et al., 2008); the following section describes the characteristics of the study area that are relevant to this research.

The southern portion of Bloomington, Illinois contains three geologic units that affect Little Kickapoo Creek (Figure 1.5). The lower two units are glacial deposits from the Wisconsin Glaciation, and the top unit is a Holocene alluvium that is still being deposited by the stream. Little Kickapoo Creek itself flows within the alluvium and outwash at the alluvium-outwash boundary.

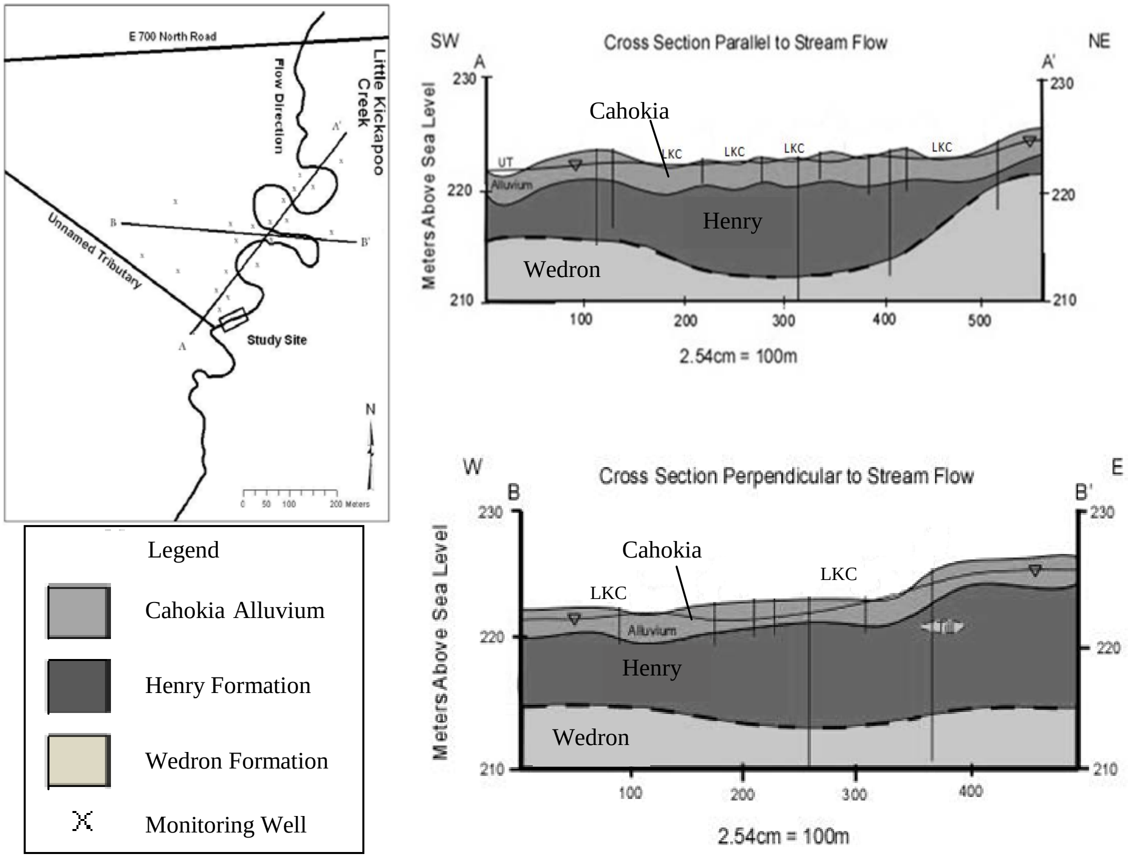

Figure 1.5: There are three geologic formations at the Little Kickapoo Creek study site. Cross sections A-A' and B-B' display the three units.

The upper most geologic unit for the area is the Cahokia Alluvium. This material is a Holocene deposit of clay, silt and sand. The alluvium varies in thickness across the site location, but along the banks of Little Kickapoo Creek the depth of material extends 2.5 meters below ground surface. The top of the water table is located within the alluvium.

The Henry Formation is a glacial outwash deposited during the Wisconsin glaciation. The deposit is dominated by gravel with some coarse-grained sand, and pinches out at the western boundary of the site. The thickness of the outwash deposit is variable, and has been observed at depths between 4 and 12 meters below the ground surface.

Below the glacial outwash is the Wedron Formation till. The till is predominantly clay with some silt and fine grained sand. The small grain size of this material allows the formations to act as a confining unit for the glacial outwash, meaning that the groundwater flowing into Little Kickapoo Creek is flowing through the outwash and not the till.

The water table in the area is generally located 1.5 to 2 meters below the ground surface and has a gentle north-south gradient. This creates a hydraulic gradient that allows groundwater to enter the stream, making it a gaining stream. The inflow of groundwater into the stream makes upwelling the dominant process, though small pools and riffles on the streambed surface create localized sections of downwelling.

CHAPTER II

SEASONAL AND DIURNAL VARIATION OF NITRATE CONCENTRATIONS WITHIN THE HYPORHEIC ZONE OF A LOW-GRADIENT, THRID-ORDER STREAM IN CENTRAL ILLINOIS

Abstract

Increased nutrient loading of Midwestern streams has generated a demand for research regarding the sources of the nutrients and removal processes that occur once the nutrients have entered a stream system. The purpose of this research is to examine the seasonal and diurnal changes in nitrate concentrations in the hyporheic zone of a third order, low-gradient stream to further understand the ability of a stream system to remove excess nutrients. The study area for this research is located in a meander of Little Kickapoo Creek, 11 kilometers south of Bloomington Illinois. Water samples were collected from five wells at 30, 60, 90, and 150 cm beneath the streambed surface, the stream, and groundwater. All water samples were refrigerated and analyzed for nitrate concentrations using an ion chromatography system. 24-hour sampling periods were also conducted where water samples were collected every hour for 24 hours to observe any diurnal variations.

Seasonal variations in nitrate have been observed from September 2011 to October 2012. Concentrations in nitrate ranged from 0.07 to 0.43 mg/L, with the lowest concentrations occurring January, and the highest in September and May. The data show a steady decrease in

nitrate concentrations from September to January, following January, a steady increase was observed until May. Concentrations remained consistent until a decrease was observed in September. These variations were to be expected because of variations in precipitation, fertilizer application, and amount of organic matter entering the system. Diurnal patterns of increasing and decreasing nitrate concentrations were observed during the 24-hour sampling periods, though the observed variations did not correspond to changes in light availability. Both the seasonal and diurnal studies of nitrate concentrations provide insight into the ability for the hyporheic zone to remove nutrients from a stream system and the controls of the reduction processes.

Introduction

Nutrient loading of Midwestern streams has degraded water quality not only in the streams themselves, but also in the Gulf of Mexico (Dagg and Breed, 2003). Since the 1960s, application of fertilizers to agricultural fields has increased significantly, and as a result, the average concentration of nitrate in Midwestern streams has doubled, and zones of hypoxia in the Gulf have grown both in frequency and magnitude (Arango et al., 2007; Dagg and Breed, 2003). Currently, extensive research is being conducted to determine how the excess nitrate affects stream systems, and how nitrate is removed from the system (Alexander et al., 2007; Alexander et al., 2000; Arango et al., 2007; Christensen et al., 1990; Dagg and Breed, 2003; Goodale et al., 2005; Mulholland et al., 2008; Scavia et al., 2004). Much of the research concentrates on the stream water and the upper few centimeters of the hyporheic zone (Boulton et al., 1998; Gu et al., 2007; Kasahara and Hill, 2007; Opdyke et al., 2006; Puckett et al., 2008), however, little research has focused on nitrate concentrations deep within the hyporheic zone.

Nitrate and the Hyporheic Zone

The hyporheic zone is a zone beneath a stream channel that contains between 100 and 10% stream water and exhibits chemical gradients (Findlay et al., 1993; Triska et al., 1989). Chemical gradients observed in the hyporheic zone are the results of microbial redox reactions related to the concentration of DO in the interstitial water and the heterogeneity of sediment (Boulton et al., 1998; Hlavacova et al., 2005). DO concentrations generally decrease with depth in the hyporheic zone as it is utilized by the microbes and as anoxic groundwater is upwelled (Brunke and Gonser, 1997). Anoxic redox reactions may occur at shallow depths within the hyporheic zone despite the abundance of DO; for heterogeneous sediments, may create pockets of anoxic water surrounded by zones high in DO (Boulton et al., 1998; Hlavacova et al., 2005). The heterogeneity of the hyporheic zone is not only important for redox reactions, but also for the processes involved in nitrate cycling.

O'Brien et al. (2007) determined that the rate and efficiency of nitrate cycling in streams may vary depending on the amount of nitrogen available. Streams with a larger concentration of nitrate showed an increase in the rate of nitrate cycling. These streams were shown to follow the Efficiency Loss model, which states that the increase in available nitrogen will increase rates of nitrate cycling but with reduced efficiency. Efficiency is lost because the increase in cycling occurs at a slower rate than the rate of the nitrogen increase.

Nitrate reduction within the hyporheic zone can be largely attributed to denitrification (Buyck, 2005; Findlay et al., 1993). Denitrification is an anoxic microbial redox reaction that uses nitrate as an electron acceptor and organic carbon to convert nitrate into atmospheric nitrogen (N_2). The nitrogen is then released into the air or reenters the cycle after being mineralized to ammonium. Denitrification is responsible for between 37-76% of nitrate removal in streams; the other removal processes are plant uptake and nitrate storage (Fischer et al., 2005; Gu et al., 2007; Mulholland et al., 2008; O'Brien et al., 2007). Denitrifying microbes require organic carbon and anoxic conditions to effectively convert the nitrate to nitrogen gas. Therefore, denitrification is most productive deeper in the hyporheic zone; however, the increased depth may result in organic matter becoming a limiting factor (Stelzer et al., 2011). Anoxic conditions can exist at shallow depths within the hyporheic zone where heterogeneity of sediments creates pockets of low DO (Boulton et al., 1998). Coupling of nitrification and denitrification is common within the hyporheic zone as interstitial water flows through areas of high and low oxygen concentrations (Storey et al., 1999).

Variations in Nitrate Concentrations

Diurnal variations in nitrate concentrations within the streambed and hyporheic zone are often observed when photosynthesis causes fluctuations in the amount of DO in the interstitial water. Photosynthesis of algae in the streambed increases the amount of dissolved oxygen in the interstitial water, reducing the size of the anoxic environment needed by denitrifying microbes (Cleven and Meyer, 2003). Christensen *et al.* (1990) observed microbes were able to denitrify up to twice as much in the dark as in the light. The effects of photosynthesis have the potential to be observed deeper within the hyporheic zone despite the process itself occurring within the top few centimeters of the streambed when downwelling introduces water with elevated DO concentrations into the hyporheic zone (Boulton et al., 1998).

In addition to diurnal variations, seasonal variation in nitrate concentrations are common in Midwestern streams (Royer et al., 2004). Surface water variations in nitrate concentrations may be attributed to precipitation, fertilizer application, rate of stream water discharge, and the concentration of dissolved organic carbon in the streambed (Alexander et al., 2007; Arango et al., 2007; Dagg and Breed, 2003). Precipitation has the potential to increase the nitrate entering stream by increasing the amount of surface runoff and tile drainage during rain events. Nitrate concentrations tend to be higher during the early spring when increased precipitation increases runoff and fertilizers are applied to the fields, conversely, nitrate concentrations are typically lowest during the summer when there is increased plant uptake from growing crops (Pind et al., 1997; Royer et al., 2004).

In addition to surface processes, there are several seasonal factors that can affect nitrate concentrations within the hyporheic zone. These fluctuations can be attributed to changes in the amount of denitrification occurring. Denitrification rates tend to be lowest during the winter months (November to March) and highest in early spring and summer (April- July) (Chavan et al., 2008; Pind et al., 1997). The amount of denitrification that occurs is based on several environmental factors including: the availability of nitrate, organic carbon content, dissolved oxygen concentrations, pH, and temperature. Chavan *et al.* (2008) attributed the decrease in denitrification during winter months to the low temperatures. A positive correlation between denitrification and temperature was observed on a seasonal scale, indicating that the lower temperatures result in decreased microbial activity. No seasonal correlation was observed between denitrification and dissolved oxygen or pH, making temperature a significant control on the microbial environment. The increase in denitrification during spring and summer may be attributed to increased nitrate entering the system as plants no longer utilize the nitrate, and increased amounts of decaying foliage entering the stream providing more organic carbon for the denitrifying microbes. Stream discharge may also affect denitrification by altering retention time of the interstitial water within the hyporheic zone. Times of high discharge have the potential to disturb the streambed sediment by removing the denitrifying microbes and reduce the water's retention time within the system (Pind et al., 1997). The shortened retention time reduces the duration of contact the interstitial water has with the denitrifying microbes and therefore, reduces the amount of nitrate removed. High discharge also increases the concentration of DO in the interstitial water because of increased turbulence and downwelling of oxygenated water into the hyporheic zone, which reduces the anoxic environment required for denitrification (Baker and Vervier, 2004).

Objectives

The purpose of this study at Little Kickapoo Creek (LKC) in Bloomington Illinois is to examine nitrate concentrations deep within the hyporheic zone and observe any diurnal and seasonal variations. Changes in the nitrate concentrations observed in the hyporheic zone are caused by fluctuations in microbial processes, plant uptake, surface runoff, and spring fertilizer application to fields within the watershed (Alexander et al., 2007; Arango et al., 2007; Dagg and Breed, 2003). Previous research conducted at LKC determined that denitrification was the main source of nitrate removal in the hyporheic zone. Plant uptake was found to affect the top ten centimeters of the hyporheic zone, and only played a major role during periods of intense plant growth (Buyck, 2005). Though denitrification is not being measured directly, variations in the nitrate concentrations will allow the amount of denitrification occurring to be inferred. Peterson *et al.* (2008) determined that baseflow conditions at LKC entrain sediment ranging in diameter from 3 to 18 mm. At bankfull conditions, sediment up to 51 mm may be entrained and disturbance of the top 30 cm of sediment may occur. Disturbance of this top layer of the hyporheic zone occurs several times a year and results in a fluctuation in the abundance of denitrifying microbes and the residence time of the interstitial water.

The leading question of my research is “How do nitrate concentrations vary within the hyporheic zone of a low gradient, third-order stream.” To answer this question, several sub-questions need to be examined: 1) How do nitrate concentrations vary seasonally; 2) How do nitrate concentrations vary diurnally; 3) How do nitrate concentrations vary with increasing depth? Researching nitrate concentration variation over several periods of time will serve to examine the potential for the hyporheic zone to reduce the nitrate concentration of a stream system, and provide insight into the role of denitrification. Understanding the potential for nitrate to be removed once it enters a stream is crucial in understanding the nutrient removal capacity of streams. Currently, the majority of the research conducted in the hyporheic zone focuses on the top five centimeters, despite several studies indicating significant microbial processes occur up to several meters below the streambed (Stelzer et al., 2011). This research will examine nitrate concentrations from 30 to 150 cm below the streambed surface to gain insight into chemical gradients within the hyporheic zone.

Hypothesis

It is expected that seasonal fluctuation in nitrate concentrations will be observed, with peaks occurring in the early spring and fall. Based on the typically seasonal increase in denitrification during the summer months, nitrate concentrations should be at their lowest during the summer. This expected pattern of variation is based on the timetable of fertilizer application, plant growth, precipitation, and amount of carbon available for denitrification.
It is also expected that the nitrate concentration will vary on a diurnal scale. Nitrate concentrations are expected to be lowest at night when there is no sunlight to stimulate photosynthesis that would oxygenate the interstitial water and hinder denitrification.

In addition to temporal variation, it is expected that nitrate concentrations will vary with depth. Concentrations are expected to decrease with depth because dissolved oxygen concentrations typically decrease with increasing depth. Therefore, interstitial water should be anoxic or near anoxic deeper within the hyporheic zone and provide the necessary environment for denitrification.

Site description

The study site for this research is located off of E 700 North Road on US Highway 51 in McLean County Illinois, about 10 kilometers south of the city of Bloomington (Figure 2.1). The research is conducted in a meander of Little Kickapoo Creek (LKC) situated in an outwash valley just south of the Bloomington Moraine.

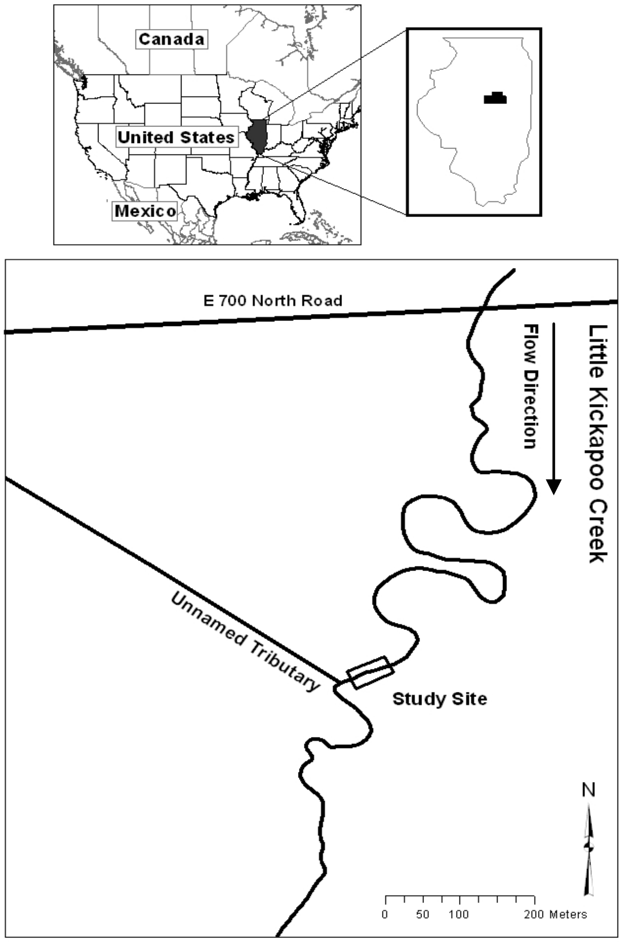

Figure 2.1: Little Kickapoo Creek (LKC) is located in McLean County Illinois. The research is being conducted along a meander of the stream located 10 km south of Bloomington, IL.

The stream is a gaining stream that receives water from groundwater, surface runoff, and tile drainage. The LKC watershed covers 76 km^2 of primarily row crops of corn and soybeans with the stream’s headwaters located at the southern edge of Bloomington. The stream is roughly 11 km in length and flows south from Bloomington to the town of Randolph where it discharges into Kickapoo Creek.

This location was chosen for study because other research has been conducted in this section of Little Kickapoo Creek (Bastola, 2011; Beach, 2008; Buyck, 2005; Oware, 2010; Peterson and Benning, 2012; Peterson and Sickbert, 2006; Peterson et al., 2008; Van der Hoven et al., 2008); therefore this research will contribute to an ongoing study of the area. The area is located adjacent to the Illinois State University Instructional Well Field and is owned by the Bloomington-Normal Water Reclamation District. It is important to understand the processes of this stream because it is analogous to many low gradient streams throughout Illinois and the Midwest that receive agricultural surface runoff and tile drainage.

Geology and Hydrology

Previous research conducted at Little Kickapoo Creek and field observations have detailed the geology and hydrology of the area (Peterson and Sickbert, 2006; Peterson et al., 2008; Van der Hoven et al., 2008); the following section describes the characteristics of the study area that are relevant to this research.

Little Kickapoo Creek is a third order, low gradient, meandering stream that flows through a glacial valley of fine-grained till and end-moraines formed during the Wisconsinan Glaciation. The valley was partially filled with coarse-grained outwash 4 to 12 meters thick and pinches out to the west. The top unit is a 2-meter thick Holocene alluvial floodplain deposit of clay, silt, and sand. The section of stream in which the research was conducted has not been modified and has a Sinuosity Index of 1.8. The streambed is positioned just below the top of the outwash, with banks that are sharply incised into the alluvium. The sediment in the channel ranges in size from gravel to coarse sand with some silt. Low-relief point bars have grain sizes ranging from gravel to mud.

The stream base flow ranges from 0.1 m^3/s to 4 m^3/s as the amount of runoff and discharge from tile drainage changes throughout the year. The stream has a maximum depth of 1.3 m and an average flow velocity of 0.25 m/s.

The water table in the area is generally located 1.5 to 2 meters below the ground surface and has a gentle north-south gradient. LKC is a gaining stream, making upwelling groundwater dominant over downwelling surface water, except where small-scale pools and riffles create localized sections of shallow downwelling.

Methods

To observe nitrate concentrations in the hyporheic zone, water samples were collected from five wells installed in the thalwag of LKC (Figure 2.2).

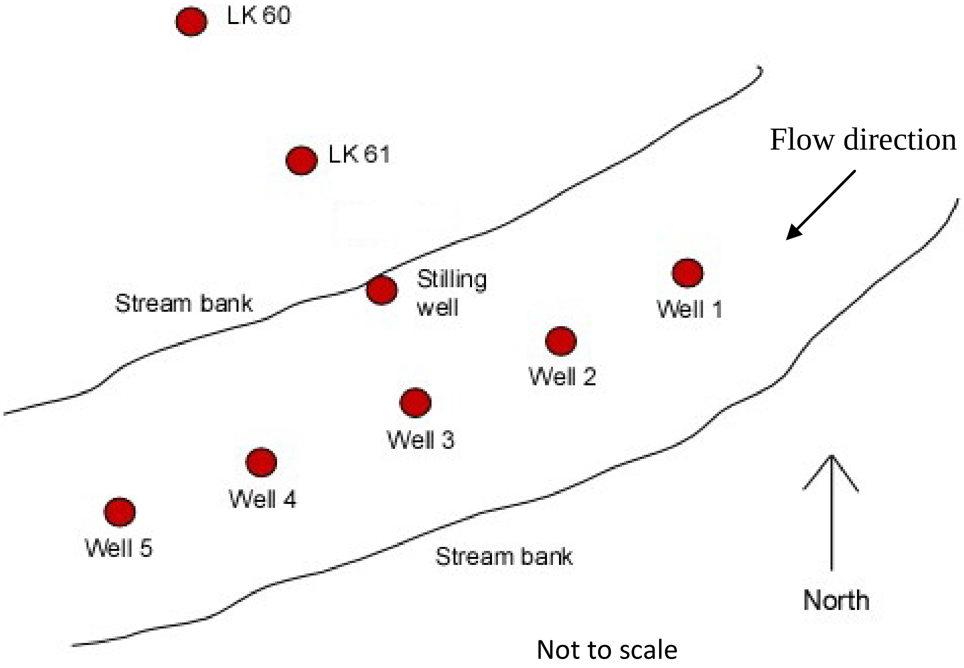

Figure 2.2: Water samples were taken from wells 1-5, LK 60, LK 61 and the stream.

Each well extends to a depth of 150 cm beneath the streambed surface with sections at 30 cm, 60 cm, 90 cm, and 150 cm (Figure 2.3) that are separated by foam insulation. Water inlets in each section allow water to enter each section within the well. Plastic tubing extends to each of the four sections to prevent mixing during extraction of the water.

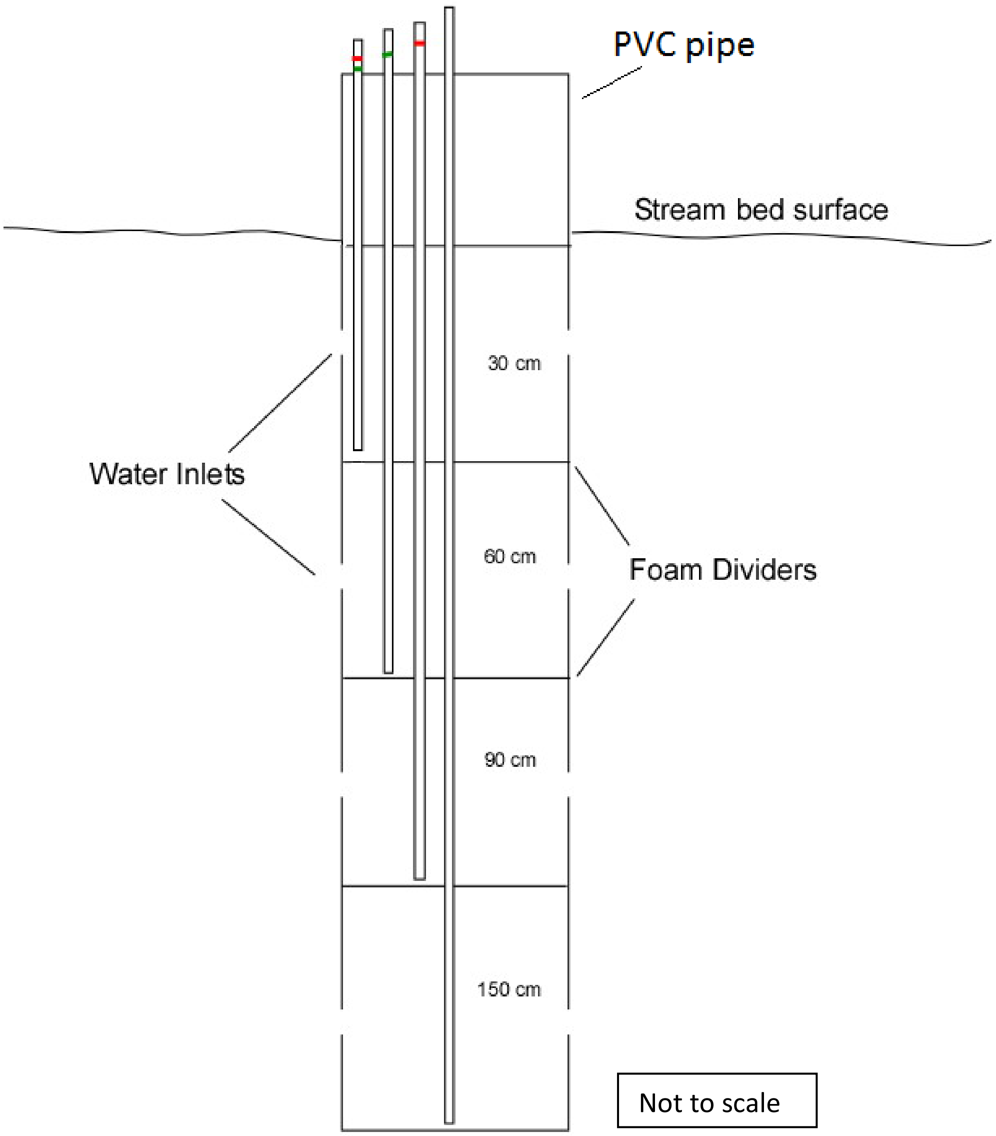

Figure 2.3: Diagram of PVC well installed in the stream bed. The well is divided at four depths to prevent mixing.

Water samples were extracted from the wells using a MasterFlex peristaltic pump, and refrigerated in plastic bottles until they were analyzed. The water extracted from each depth was analyzed in the field for dissolved oxygen (DO), specific conductance, conductivity, and temperature using a YSI-85. The pH was also measured in the field using an Oakton phTestr pH meter. Once collected, the samples were analyzed for nitrate concentrations using a DIONEX ICS-1100 ion chromatography system owned by Illinois State University.

To best observe seasonal changes in nitrate concentrations, samples were taken biweekly from September 2011 to September 2012. Additional samples were taken from the stream and surrounding monitoring wells. The stream sample was taken upstream near well number 3; the groundwater samples were taken from wells LK60 and LK61 located near the stream to observe the nitrate concentrations of the groundwater before it mixes in the hyporheic zone. These wells extend to a depth of about 1.5 meters, making the bottom of the wells even with the level of the streambed. To supply further detail regarding the stream and infer discharge, the stream stage was monitored continuously. A stilling well installed near well 3 was outfitted with a pressure transducer that recorded the stream stage at 15 minute intervals. The pressure transducer measured stream stage relative to a zero point that was established upon installation. As a result of this, after the pressure transducer was replaced after being removed to download data, the zero point was reset. This resulted in two sets of stream stage data that showed relative stream stage but did not share a common zero elevation.

To observe daily variations in nitrate concentrations, three 24-hour sampling sessions were conducted. Water samples were collected every hour for 24 hours. The first 24-hour sampling period was conducted September 30, 2011, the second 24-hour sampling period was on June 16, 2012, and the final sampling period occurred on September 8, 2012.

The availability of organic carbon is a limiting factor for denitrification; therefore the streambed sediment at LKC was analyzed for its organic carbon content. This allows for any relationship between nitrate fluctuation and organic carbon content to be observed. Sediment samples of the top four inches of the streambed were collected adjacent to wells 1, 3, and 5. Samples were taken once a month from May to October 2012 to observe any changes in organic content over time. Once collected, the samples were transported in plastic bags and air dried immediately. Samples were then transferred to glass containers and dried in an oven at approximately 100˚ C for 24 hours to remove any remaining moisture. The samples were then weighed and placed in an oven at 500˚C for four hours to burn off any organic matter. The samples were then weighed again; any difference between the two weight measurements represents the mass of the organic material in the sample.

Chloride concentrations were also analyzed from the collected water samples using the ion chromatograph. Chloride concentrations allow for the application of equations (1) and (2) derived from Triska *et al*. (1989). Equation (1) calculates the ratio of groundwater to stream water in the hyporheic wells using chloride as a conservative tracer.

$$\frac{Q_s}{Q_w} = \frac{(Cl_{HZ} - Cl_g)}{(Cl_s - Cl_g)} \qquad (1)$$

where:

$\frac{Q_s}{Q_w}$ is the ratio of stream water to well water

Cl_{HZ} is the average chloride concentration in the hyporheic zone

Cl_g is the average chloride concentration in the groundwater

Cl_s is the average chloride concentration in the stream water

Equation (2) derived in Triska *et al*. (1989) was applied using the results from the above calculation to calculate the concentration of nitrate present in the hyporheic zone assuming no biotic uptake or regeneration. These calculations are based on the concentrations of nitrate in the groundwater and stream water.

$$NO_3 - N = \frac{Q_s}{Q_w} * \left(N_s - N_g\right) + N_g \qquad (2)$$

where:

NO_3-N is the calculated concentration of nitrate in the hyporheic zone

N_s is the nitrate concentration of the stream water

N_g is the nitrate concentration of the groundwater

The calculated nitrate concentrations were then subtracted from the observed concentrations. A positive value indicates that nitrate is being added in the hyporheic zone and provides evidence for the occurrence of nitrification. A negative value indicates that nitrate was being removed from the system and provides evidence of denitrification.

Results

Seasonal data

Nitrate

Water samples were collected bi-weekly from September 2011 until mid-October 2012. Samples were not taken in November or December 2011 due to freezing temperatures. Samples were analyzed for nitrate and chloride concentrations and compiled to analyze seasonal trends. It should be noted that wells 1 and 2 were damaged and not operational from September 2011 until July 2012. Wells 1-3 were replaced on July 10, 2012 after a severe storm on June 16, 2012 damaged several wells.

Water samples taken from wells 1-5 at 30, 60, 90, and 150 cm beneath the streambed surface provided an observation of nitrate concentrations within the hyporheic zone at LKC. Nitrate concentrations in the hyporheic zone ranged from 0.02 – 0.72 mg/L, in the groundwater the range of nitrate was 0.02 – 0.46 mg/L, and the stream ranged from 0.15 – 4.69 mg/L. A two-factor ANOVA performed on the seasonal data showed that the nitrate concentrations varied over the duration of the period of observation (F(3,16)=15.23, p=<0.01), and there was no variation in nitrate concentration between the four depths (30, 60, 90, and 150 cm) (F(3,16)=0.63, p=0.60).

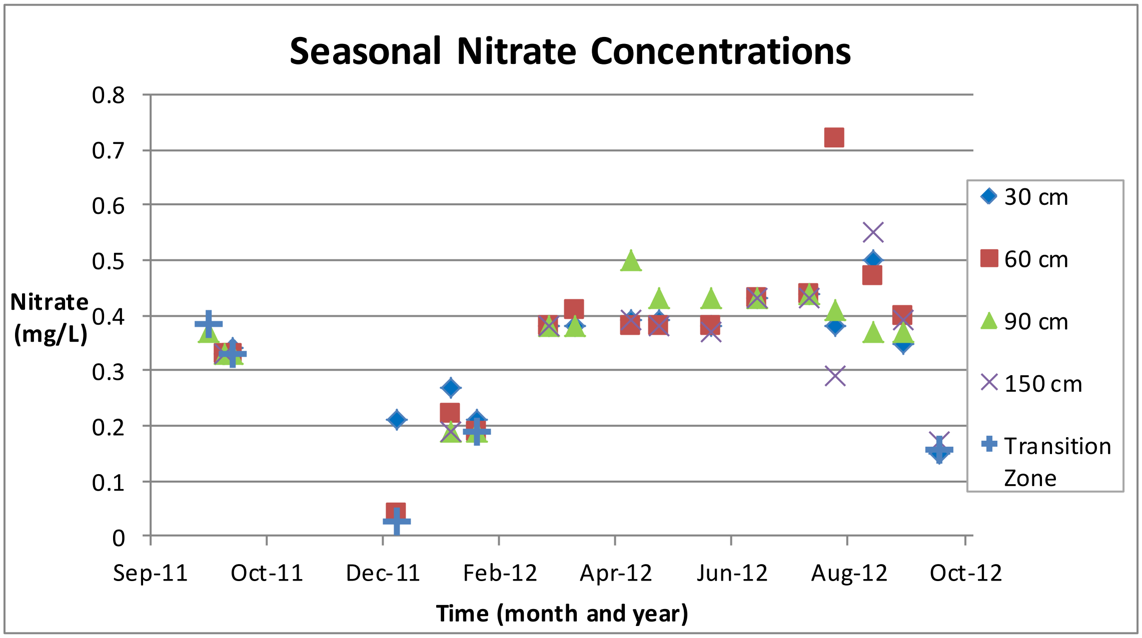

Figure 2.4: Seasonal nitrate concentrations in the hyporheic zone and transition zone. Stream and groundwater data were excluded in this figure to better show the variation in concentrations.

Samples collected from in the hyporheic zone (Figure 2.4) showed that the average nitrate concentration decreased 0.30 mg/L from September 2011 to January 2012 where nitrate concentrations reached their lowest. Some samples collected from beneath the streambed at 150 cm contained less than 10% stream water. This was determined by applying equation (1) to the nitrate and chloride data and multiplying the results by 100 to get the percent stream water present in the well. These samples are not considered to be a part of the hyporheic zone; instead they are a part of a groundwater transition zone. Samples from this transition zone contained nitrate concentrations similar to those found within the hyporheic zone (30, 60, 90 cm), and slightly elevated when compared to groundwater concentrations. These samples indicate that the hyporheic zone extends to a depth between 90 and 150 cm from September 2011 to February 2012 and again in October 2012 (Figure 2.4). From March until September 2012 the hyporheic zone extended deeper than 150 cm. The size hyporheic zone often changes seasonally (Storey et al., 2004) based on precipitation, groundwater flow, and surface water discharge.

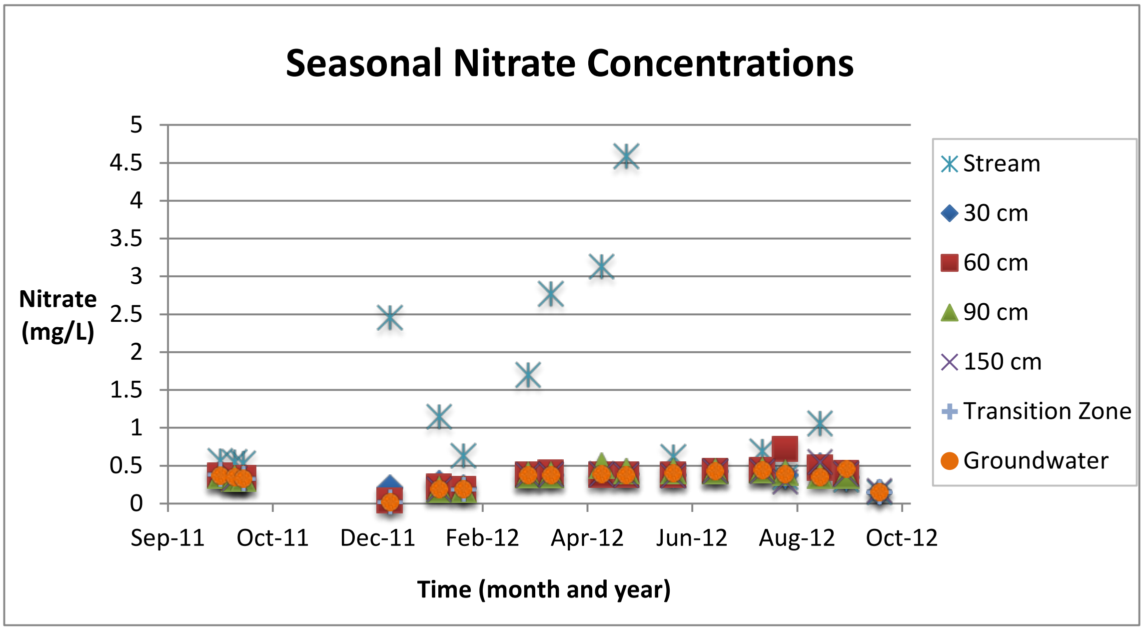

Figure 2.5: Seasonal nitrate concentrations. Groundwater and hyporheic concentrations of nitrate are consistent through the sampling period. However, the concentrations of nitrate in the stream have greater variance.

Nitrate concentrations of the stream were constantly higher than both the groundwater and the hyporheic zone. The seasonal distribution of the data did not follow the same pattern as the hyporheic zone and the groundwater (Figure 2.5). Stream nitrate concentrations were consistent until January where a sharp increase of 1.93 mg/L was observed. Concentration decreased from January until February, reaching concentrations consistent with those observed before the January increase. A second increase was then observed of 3.44 mg/L from March until May when nitrate concentrations reached their peak. Concentrations decreased by 3.97 mg/L in June and fluctuated only slightly until sampling ceased mid-October 2012.

From September 2011 to January 2012 the stream stage fluctuated between an elevated stage and a low stage (Figure 2.6). From September to November the stream stage increased due to autumn storms, the stream stage then decreased from November to January. Average nitrate increased 0.20 mg/L from February to May of 2012, where concentrations were the highest and plateaued until September. February to May also saw increased precipitation from spring storms causing a general increase in stream stage, however a summer drought between the months of May to August led to a seasonal low in stream stage (Figure 2.7). Nitrate concentrations then decreased in September by 0.28 mg/L until sampling ceased in mid-October of 2012. This increase in nitrate occurred during a general increase in stream stage due to autumn storms.

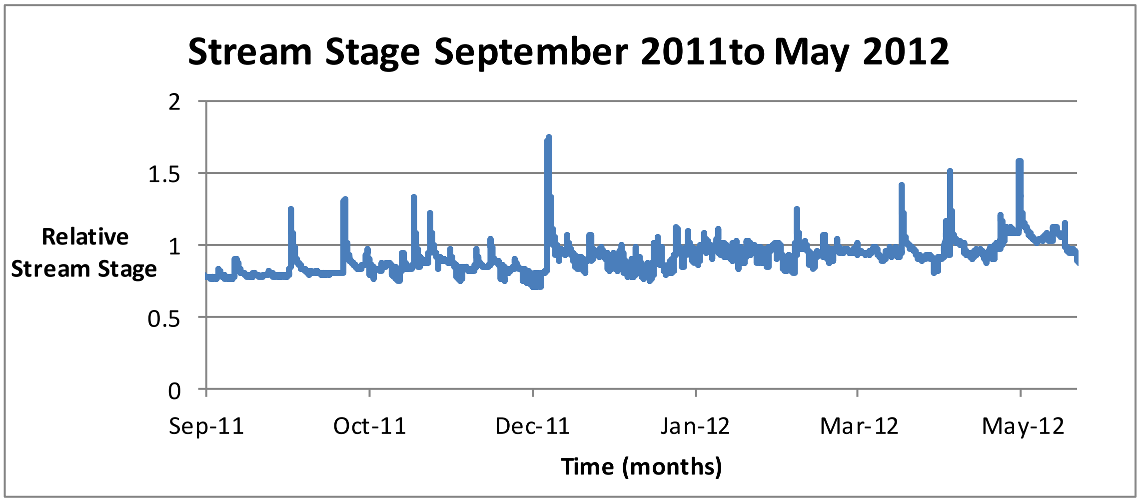

Figure 2.6: The stream stage at LKC was monitored using a pressure transducer recording in 15 minute intervals. The above graph shows the relative stream stage from the beginning of sampling in September 2011 until the end of May 2012.

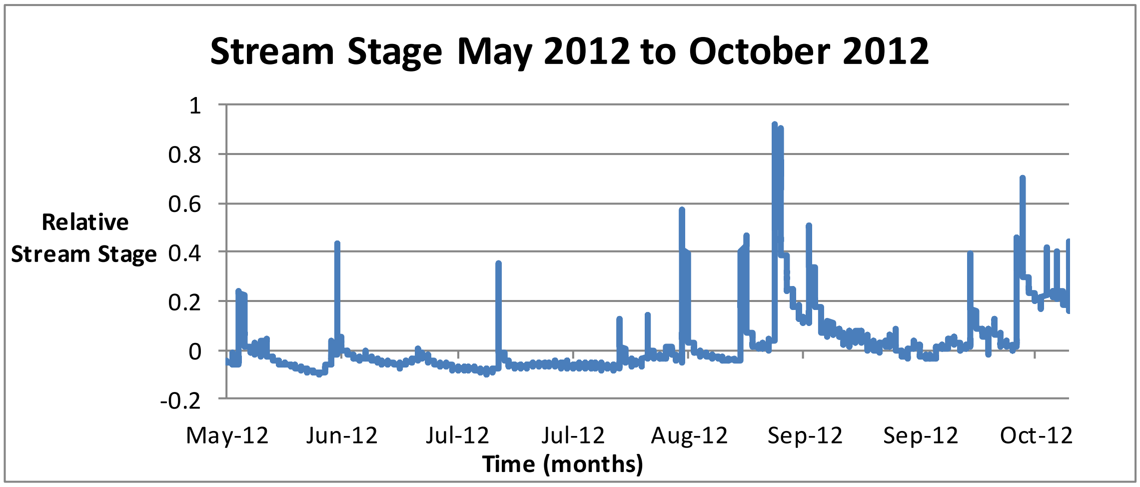

Figure 2.7: Streams stage measured using a pressure transducer from the end of May 2012 until October 2012.

The nitrate concentrations observed in the groundwater were similar to those observed in the hyporheic zone, and the data distribution followed the same seasonal pattern as the hyporheic zone. The data show that nitrate decreased 0.30 mg/L from September to January where nitrate concentrations reached their lowest. Nitrate increased 0.34 mg/L from February to May, where concentrations were the highest and plateaued until September. Concentrations then decreased by 0.28 mg/L until sampling ceased in mid-October.

Chloride

In addition to nitrate, water samples were also analyzed for concentrations of chloride for use as a conservative tracer. Chloride concentrations varied greatly between the hyporheic zone, groundwater, and the stream throughout the entire sampling period, though concentrations tended to be elevated during the winter months (Figure 2.8). Chloride concentrations in the hyporheic zone ranged from 8.94 to 100.52 mg/L. Stream concentrates of chloride were generally the highest and ranged from 19.45 to 263.53 mg/L. Groundwater concentrations were typically the lowest with concentrations ranging from 12.33 to 75.26 mg/L.

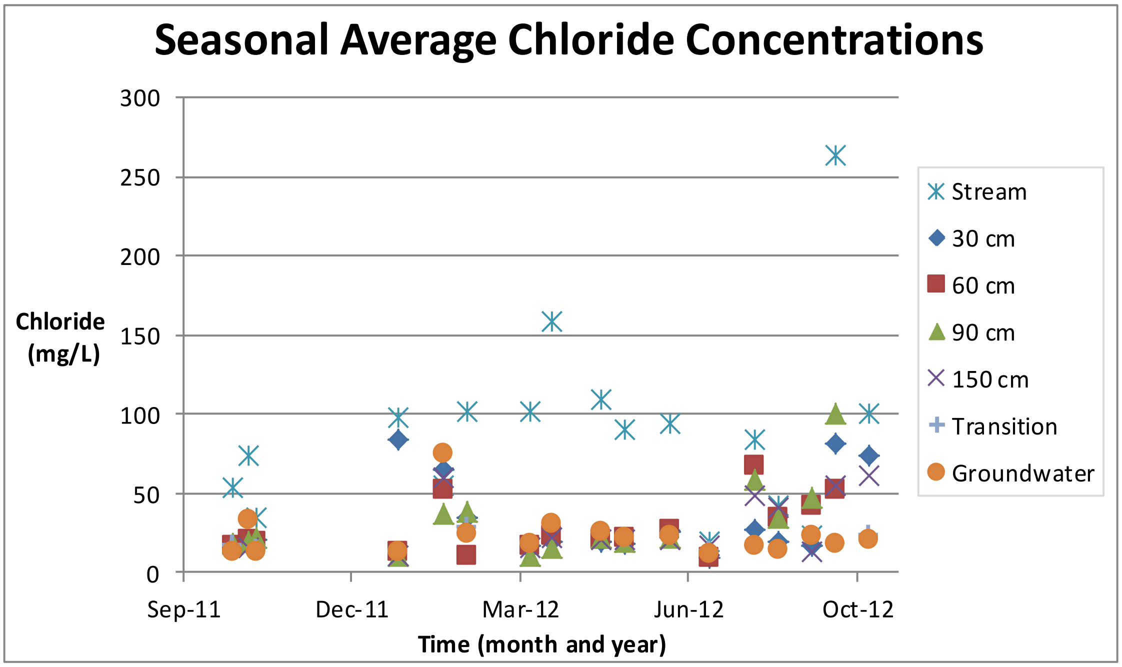

Figure 2.8: Water samples were analyzed for chloride concentrations. Concentrations were generally highest in the winter months and in the fall.

September 2011 to January 2012 saw a decrease in nitrate concentrations, during this time chloride concentrations in the hyporheic zone ranged from 13.96 to 84.34 mg/L. Chloride in the stream ranged from 34.96-97.75 mg/L. Groundwater concentrations ranged from 12.33 to 75.26 mg/L.

The February to May increase in nitrate concentrations in the stream was accompanied by an overall increase in chloride concentrations with values ranging from 54.52 to 158.07 mg/L. Hyporheic zone chloride concentrations remained steady with ranges of 10.21 to 65.16 mg/L. Groundwater concentrations rose slightly with concentrations ranging from 18.43 to 75.26 mg/L.

From May to September nitrate concentrations plateaued in the hyporheic zone, chloride concentrations also remained nearly consistent with value ranging between 8.94 and 67.68 mg/L. Stream chloride concentrations began to decrease with ranges of 19.45 to 108.94 mg/L. groundwater chloride concentrations also experienced an overall decrease with concentrations ranging from 12.33 to 26.04 mg/L.

The sudden decrease in nitrate concentrations that occurred between the beginning of September and the end of October coincided with increases in chloride concentrations in both the hyporheic zone and the stream. Chloride in the hyporheic zone ranged from 12.99 to 100.52 mg/L and 23.71 to 263.53 mg/L in the stream. Groundwater concentrations of chloride did not change overall with values ranging from 18.76 to 22.74 mg/L.

Calculations

Chloride concentrations from each sample were used to calculate the concentration of nitrate that should be present in the hyporheic zone. Equations (1) and (2) derived in Triska *et al.* (1989) were applied to make the necessary calculations.

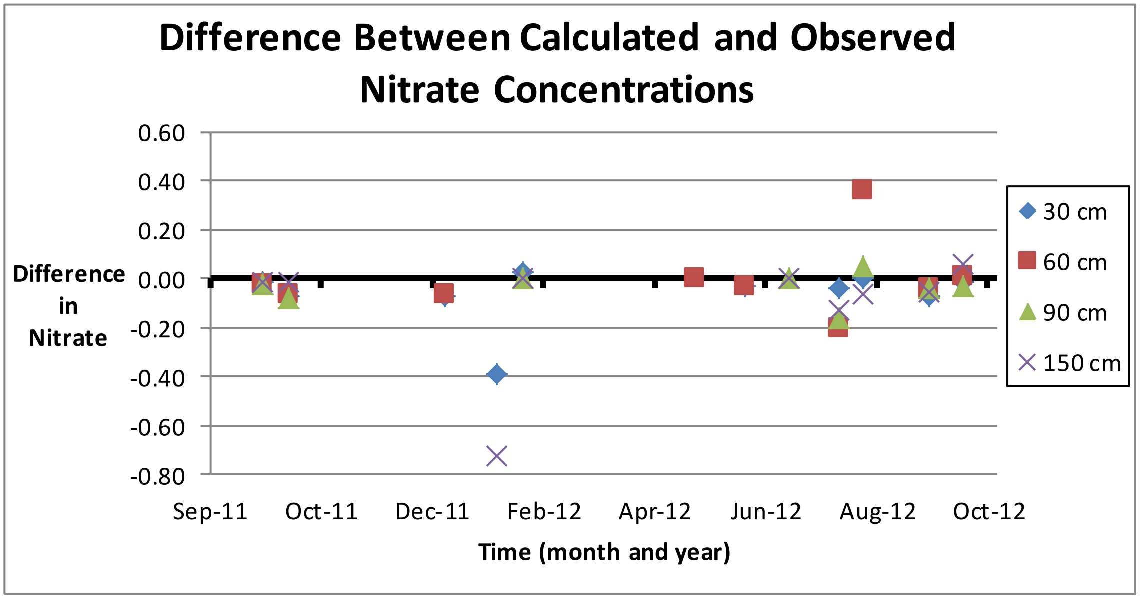

Figure 2.9: Difference between calculated and observed values for seasonal nitrate concentrations. Negative values indicate less nitrate was observed in the hyporheic zone than calculated, and positive value indicates more nitrate than calculated.

Once the concentration of nitrate was calculated, the calculated value was then subtracted from the observed concentration. A positive difference indicated that there was more nitrate in the hyporheic zone than there should be, and a negative difference indicated nitrate concentrations were lower than they should be. Due to the extensive use of salt as a road deicer in winter, the unsaturated zone near LKC acts as a source of chloride to the stream and groundwater (Lax and Peterson, 2009). Chloride stored in the unsaturated zone eventually discharges into the stream. Storm events have the potential to cause an increase in chloride concentrations when the stored chloride is flushed from the unsaturated zone into the stream and groundwater. This model was not able to be applied to all samples. Because this model uses chloride as a conservative tracer to calculate the ratio of stream water to groundwater in the hyporheic zone, the model cannot be applied when an excess of chloride is introduced. If the excess chloride elevates the chloride concentration in the hyporheic zone above the concentrations in stream and the groundwater, the ratio is distorted, generating results with negative values or values exceeding 100. Chloride concentrations in several water samples collected during the sampling period were excessively elevated due to the recent storm, making the model not applicable to every sample.

When the model was applicable, the results (Figure 2.9) indicated that nitrate concentrations were lower than the calculated values from September 2011 to February, June, early August, and September 2012 with the difference between observed and calculated concentrations ranging from -0.02 to -0.56 mg/L. A negative value indicates that nitrate is being removed from the hyporheic zone and suggests denitrification was occurring. The difference in concentrations between observed and calculated indicated an excess of nitrate in the hyporheic zone during late August and October 2012 with values ranging from 0.01 to 0.09 mg/L. A positive value indicates that nitrate is being added in the hyporheic zone, suggesting nitrification was occurring. The model indicated no difference in observed and calculated nitrate concentrations in May and July. The model was not able to be applied from March to April due to an excess of chloride in the hyporheic zone.

Dissolved oxygen

Dissolved oxygen concentrations varied seasonally and with depth (Figure 2.10). DO concentrations were elevated in September and October of 2011 and decreased during the winter months. DO increased overall from February to May, and concentrations decreased again from May until October 2012.

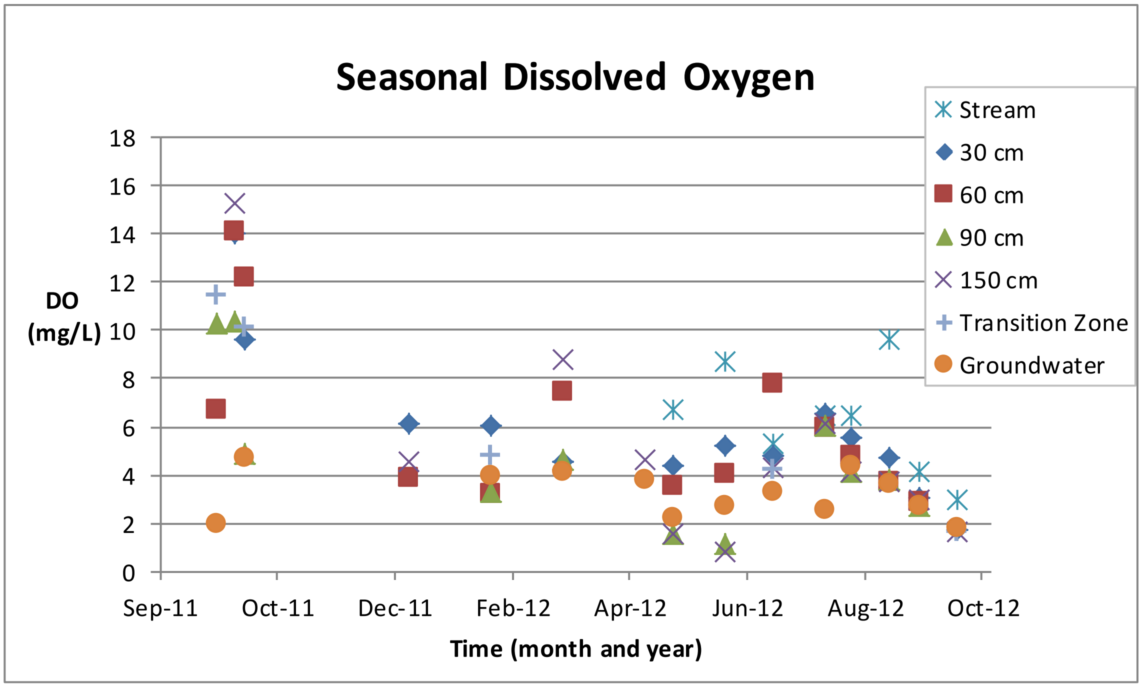

Figure 2.10: Dissolved oxygen concentrations varied greatly over the duration of sampling. Generally, groundwater had the lowest concentration of DO and the stream water had the highest concentrations.

The YSI-85 meter used to measure dissolved oxygen concentrations in the water samples was not calibrated until May of 2012. DO concentrations recorded in April and the beginning of May were inexplicably high and had to be discarded as error. Beginning in mid-May the YSI-85 meter was calibrated prior to each sampling collection, values for DO after calibration were lower and more consistent than data collected prior to calibration. Therefore samples analyzed for DO concentrations before May 20, 2012 may be elevated due to error.

Groundwater typically had the lowest dissolved oxygen concentrations with values ranging from 1.96 to 4.34 mg/L. Stream concentrations of dissolved oxygen were typically the highest with values ranging from 5.33 to 9.60 mg/L. The concentrations of dissolved oxygen varied throughout the hyporheic zone though the distribution of values at the various depths was not consistent across the data. Concentrations of dissolved oxygen in the hyporheic zone ranged from 0.82 to 15.01 mg/L.

Organic content

Sediment samples were taken once a month from May 2012 until October 2012 to observe changes in percent organic matter (% OM) in the top few centimeters of the streambed (Table 1). A sediment sample could not be taken in June due to an elevated stream stage when the sampling was to occur.

Date	% Organic Matter
5/20/2012	2.61%
7/10/2012	1.35%
8/19/2012	0.55%
9/23/2012	0.92%
10/13/2012	1.13%

Table 1: Percent organic matter was measured in the top few centimeters of streambed sediment.

The percent of organic matter in the samples were highest in May when sampling began. A decrease was observed from May until August when %OM reached its lowest. % OM increased from September until sampling stopped in October.

24-Hour Sampling Periods

September 30, 2011

Nitrate

The first of three 24-hour sampling periods began on September 30, 2011 at 12 pm with the intention of concluding the test at 12 pm on October 1, 2011. However, complications with the peristaltic pump did not allow sampling to occur between 3 am and 10 am on October 1.

Wells 1 and 2 were damaged and not able to be sampled during the 24 hour period. Overall, the stream stage was elevated in September, and on September 30 the stream stage had just decreased after a storm event that occurred on the 27th.

Samples that were collected and analyzed for nitrate concentrations showed ranges in values between 0.32 and 1.01 mg/L in the hyporheic zone, of 0.33 to 0.47 mg/L in the groundwater, and of 0.49 to 0.69 mg/L in the stream (Figure 2.11). During this time, the hyporheic zone generally extended to a depth between 90 and 150 cm. Samples collected at 150 cm contained less than 10% stream water, except at 1 and 10 am, and were therefore part of a groundwater transition zone and not the hyporheic zone. This is consistent with the extent of the hyporheic zone observed during seasonal observations.

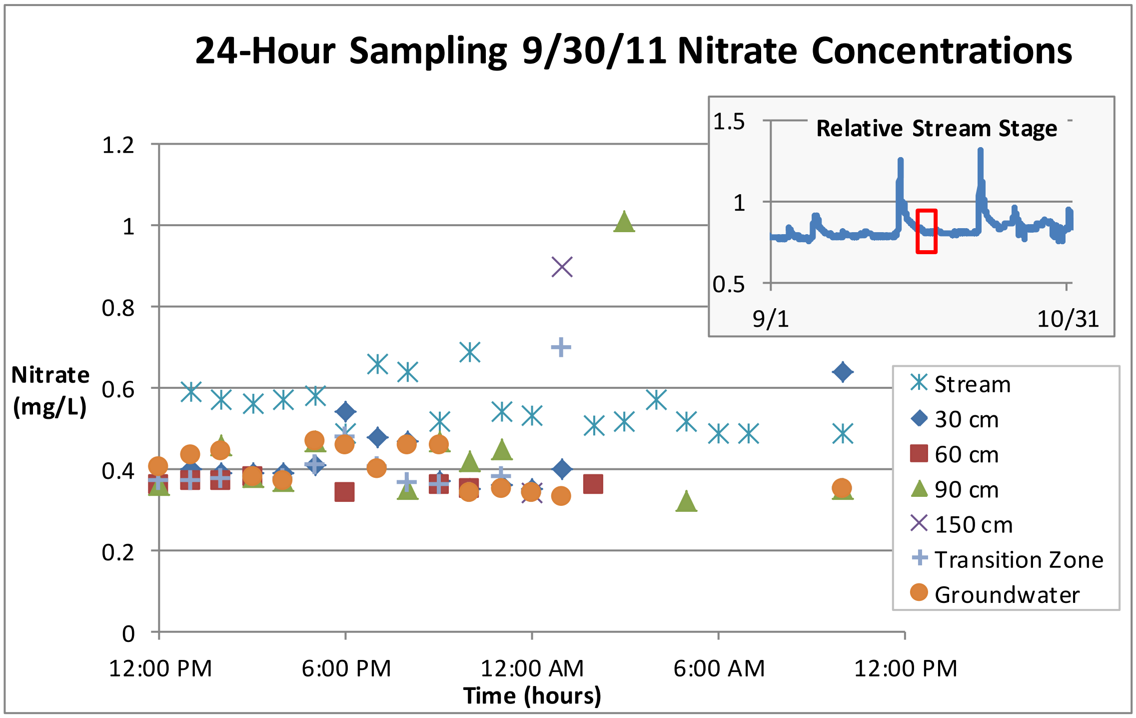

Figure 2.11: Samples were taken over a 24-hour period to observe diurnal variation in nitrate. The red box on the stream stage chart indicates the time period of the sampling.

Concentrations of nitrate in the transition zone ranged from 0.37 to 0.48 mg/L. Average nitrate concentrations in the hyporheic zone were at their lowest between the hours of 12 pm and 4 pm and then again between 9 pm and 12 am with values ranging between 0.37 and 0.43 mg/L. Concentrations reached their peaks from 5 pm to 8 pm and again at 1 am and 10 am with average values ranging from 0.39 to 1.01 mg/L.

A two-factor ANOVA performed on the data collected from the hyporheic zone showed that concentrations in nitrate varied over the duration of the test ($F(2,15)=3.22$, $p=<0.01$) but not between the four depths ($F(2,15)=2.17$, $p=0.13$). However, t-test using equal variance compared nitrate concentrations of samples taken during daylight hours (12 pm to 7 pm and 6 m to 10 am) to those collected after dark (8 pm to 6 am), the results showed no variation in the data between light and dark.

Chloride

Chloride concentrations did not vary greatly over the sampling period. Concentrations in the stream were significantly higher than concentrations in the groundwater and the hyporheic zone (Figure 2.12). Chloride in the stream ranged from 54.02 to 51.15 mg/L with the lowest concentrations occurring between 12 am and 10 am, and the highest between 4 pm and 7 pm. In the hyporheic zone concentrations ranged from 16.12 to 21.21 mg/L with the lowest concentrations occurring between 12 am to 10 am, and highest concentrations between 12 pm and 5 pm. Concentrations ranged from 11.89 to 13.99 mg/L in the groundwater with lowest concentrations occurring 1 pm and 3 pm, and concentrations were highest between 7 pm to 9 pm.

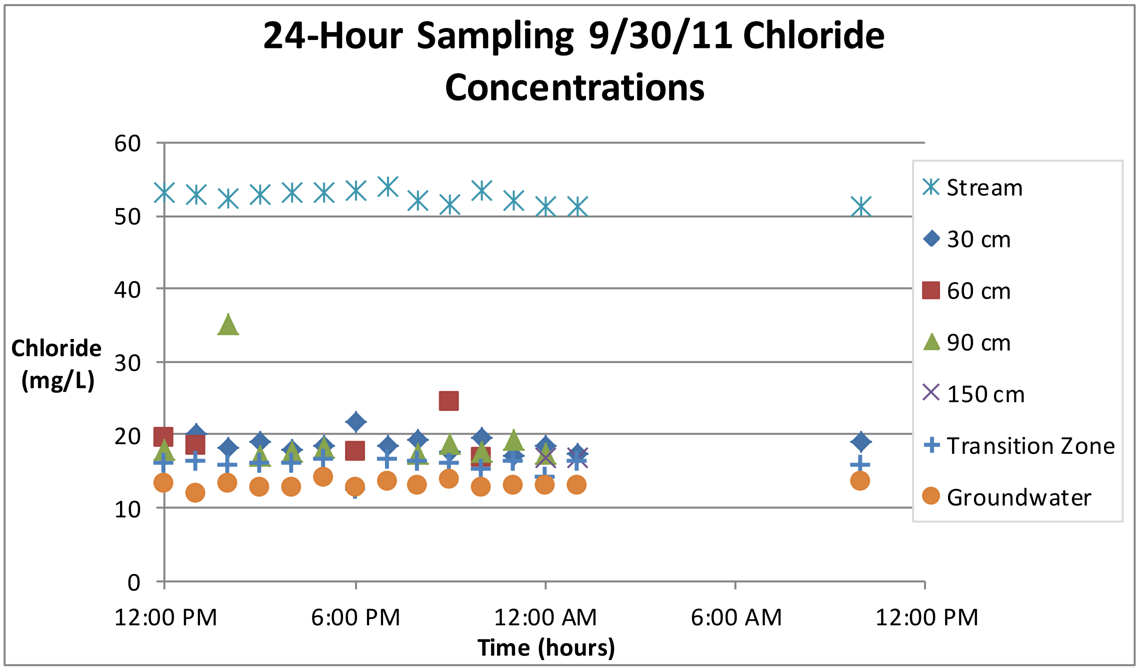

Figure 2.12: Samples were analyzed for chloride concentrations. Concentrations were consistently significantly higher in the stream and lowest in the groundwater. Concentrations did not show much variation over the sampling period.

Calculations

Further analysis of the data included using nitrate and chloride concentrations to apply equations (1) and (2) to the data (Figure 2.13). The results indicated less nitrate present than predicted in the hyporheic zone on September 30, 2011 from 12 pm to 5 pm and from 8 pm to 10 pm with the difference in nitrate ranging from -0.07 to -0.01 mg/L. this suggests that denitrification was occurring during these times. There was more nitrate in the hyporheic zone than calculated at 6 pm to 7 pm on September 30, 2011 and again at 11 pm until the test concluded at 10 am on October 1, 2011 with differences in nitrate ranging from 0.03 to 0.33 mg/L. The addition of nitrate was likely due to nitrification occurring within the hyporheic zone.

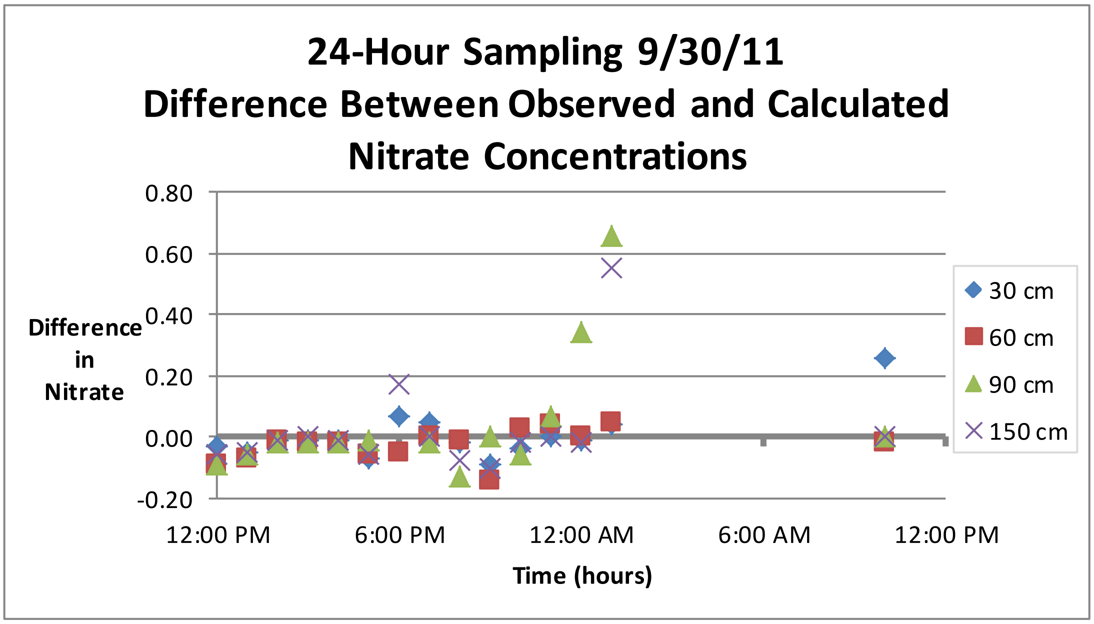

Figure 2.13: The results of equations (1) and (2) indicated that nitrate was being removed from 12 pm to 5 pm and from 8 pm to 10 pm. Nitrate was being added from 6 pm to 7 pm, 11 pm, and at the conclusion of the test at 10 am.

Dissolved oxygen

Concentrations of dissolved oxygen decreased in the hyporheic zone from 10.13 mg/L at 12 pm September 30, 3011 to 3.38 mg/L at 1 pm (Figure 2.14). Groundwater concentrations of dissolved oxygen were consistently the lowest values with ranges from 1.12 to 3.11 mg/L. Dissolved oxygen in the hyporheic zone stayed nearly constant between 1 pm and 1 am when sampling was stopped. The range of dissolved concentrations during this time was 1.60 to 3.77 mg/L. Dissolved oxygen concentrations increased by 1.76 mg/L when a round of samples was taken at 10 am.

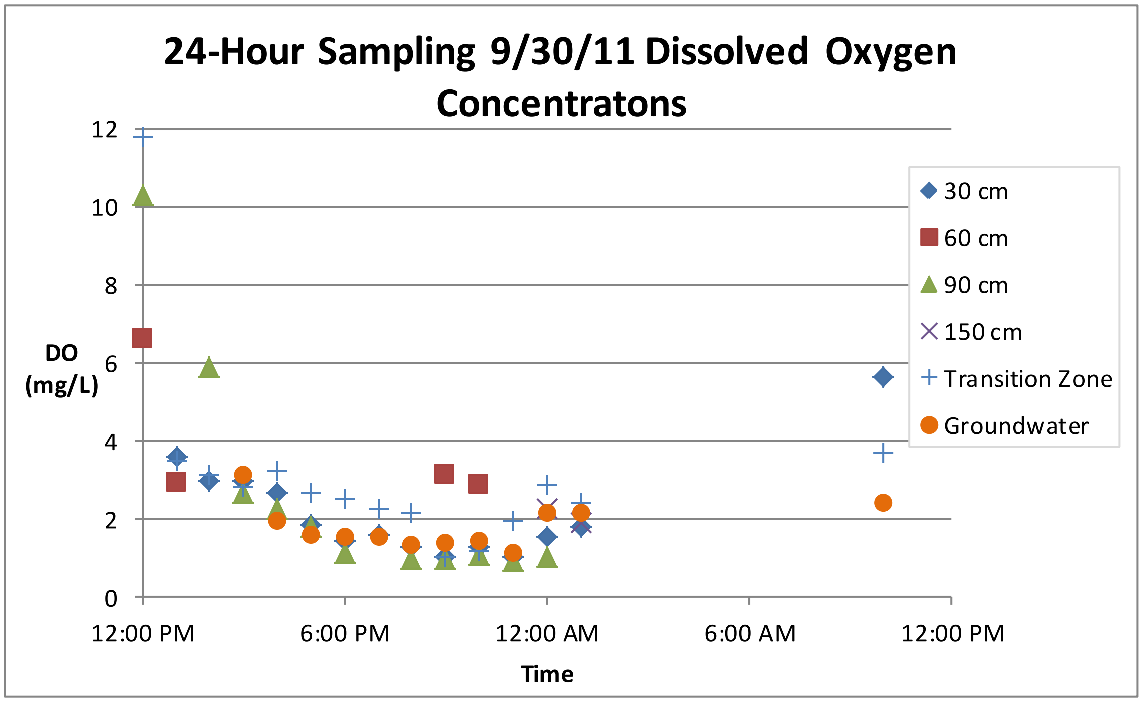

Figure 2.14: Concentrations of dissolved oxygen decreased in the hyporheic zone from 12 to 1 pm. Groundwater concentrations of dissolved oxygen were consistently the lowest. Dissolved oxygen in the hyporheic zone stayed nearly constant between 1 pm and 1 am when sampling was stopped.

June 16, 2012

Nitrate

The second of three 24-hour sampling periods began on June 16 2012 at 12 pm with the intention of concluding the test at 12 pm on June 17, 2012. However, a strong storm system moved into the area causing sampling to stop from 9 pm to 3 am. Sampling resumed at 3 am continued until 6 am where debris in the stream damaged wells 1-3. At the beginning of the sampling period, the stream stage was low due to drought conditions; however during the storm the stream stage rapidly increased to near bankfull conditions and remained elevated until the sampling period concluded. The stream stage rose above the tops of the remaining wells, making it impossible to continue sampling, thus concluding the test at 5 am on June 17, 2012. Samples that were collected and analyzed for nitrate concentrations showed ranges in values between 0.35 and 0.56 mg/L in the hyporheic zone, variations of 0.30 to 0.44 mg/L in the groundwater, and 0.3 to 1.02 mg/L in the stream (Figure 2.15).

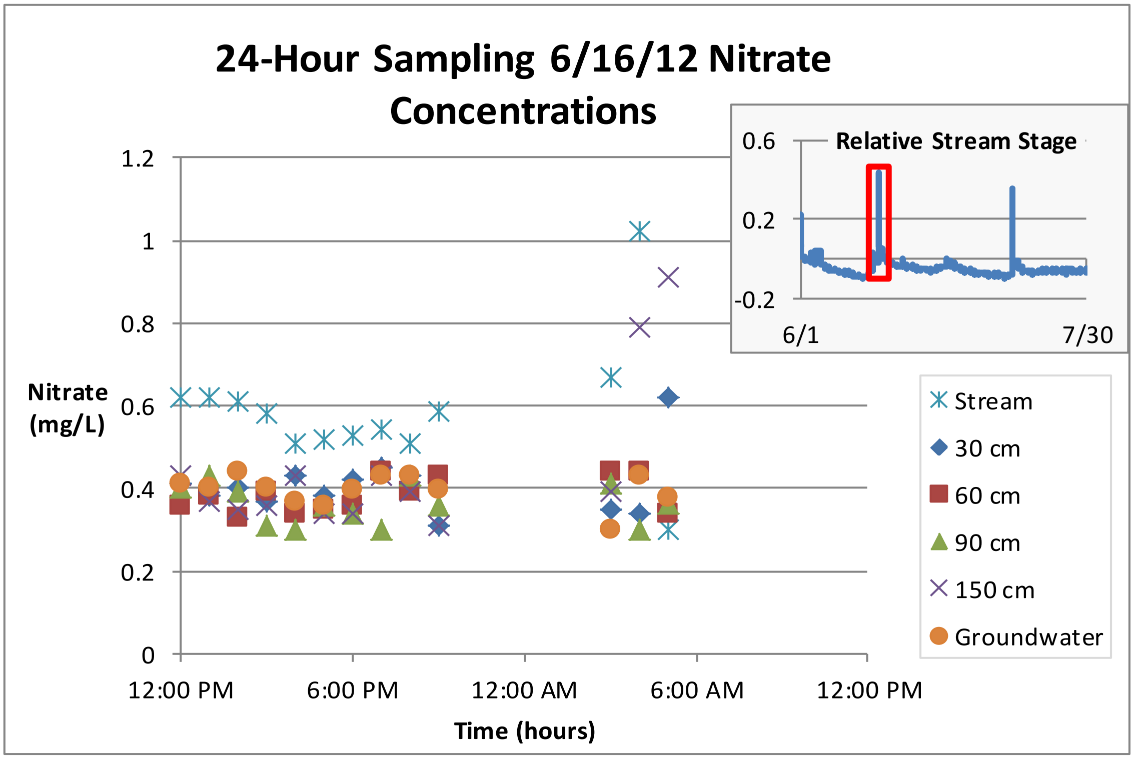

Figure 2.15: Sampling begin at 12 pm 6/16/12 and conclude at 5 am. The stream water had consistently the highest concentrations of nitrate. The red box on the stream stage chart indicates the time period of the sampling. The stage was became elevated at 4 am due to a storm.

A two-factor ANOVA of the data collected from the hyporheic zone showed that concentrations in nitrate did not vary over the duration of the test (F(2,120=0.81, p=0.64) or at depth within the hyporheic zone (F(2,12)=2.03, p=0.15). A t-test using equal variance compared nitrate concentrations of samples taken during daylight hours (12 pm to 8 pm and 5 am) to those collected after dark (9 pm and 3 am to 4 am), the results showed no variation in the data between light and dark.

Chloride

Chloride concentrations ranged greatly over the course of the sampling period. Concentrations in the stream ranged from 76.79 to 217.7 mg/L, concentrations in the hyporheic zone range from 23.12 to 46.36 mg/L, and 22.8 to 27.33 mg/L in the groundwater. Overall, chloride concentrations were highest after the storm began at 9 pm (Figure 2.16). It is likely that the rainfall caused chloride stored in the unsaturated zone to be flushed into the stream and led to the increase in average chloride in the stream system.

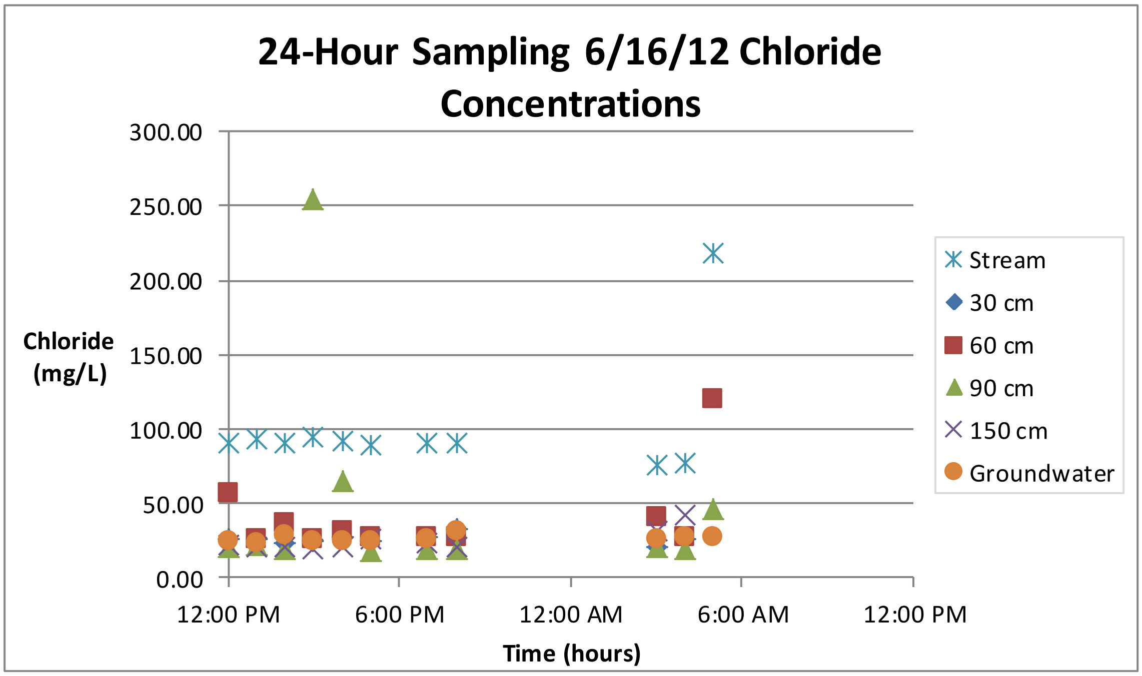

Figure 2.16: Chloride concentrations ranged greatly over the course of the sampling period with the highest average concentrations occurring after the storm at 9 pm.

Calculations

Further analysis of the data included using nitrate and chloride concentrations to apply equation (1) and (2) to the data (Figure 2.17). The equations could not be applied to all collected samples, for the equations only apply when concentrations of chloride in the hyporheic zone are between concentrations observed in the groundwater and the stream. Due to limitations of the calculations, the percent stream water was not able to be calculated for the majority of the samples and therefore the extent of the hyporheic zone could not be determined. For samples taken between 30 and 150 cm that allowed for the application of the equations, the percent stream water was above 10%, indicating that they were a part of the hyporheic zone. The results indicated less nitrate present than predicted in the hyporheic zone on June 16 from 12 pm to 4 pm, 7 pm to 8 pm, and again at 4 am on June 17, with the difference in nitrate ranging from -0.16 to -0.01 mg/L. There was more nitrate in the hyporheic zone than calculated at 6 pm and 9 pm on June 16 and again at 5 am on June 17 when the test concluded, with differences in nitrate ranging from 0.02 to 0.08 mg/L. Calculated Nitrate concentrations were the same as those observed at 5 pm on June 16.

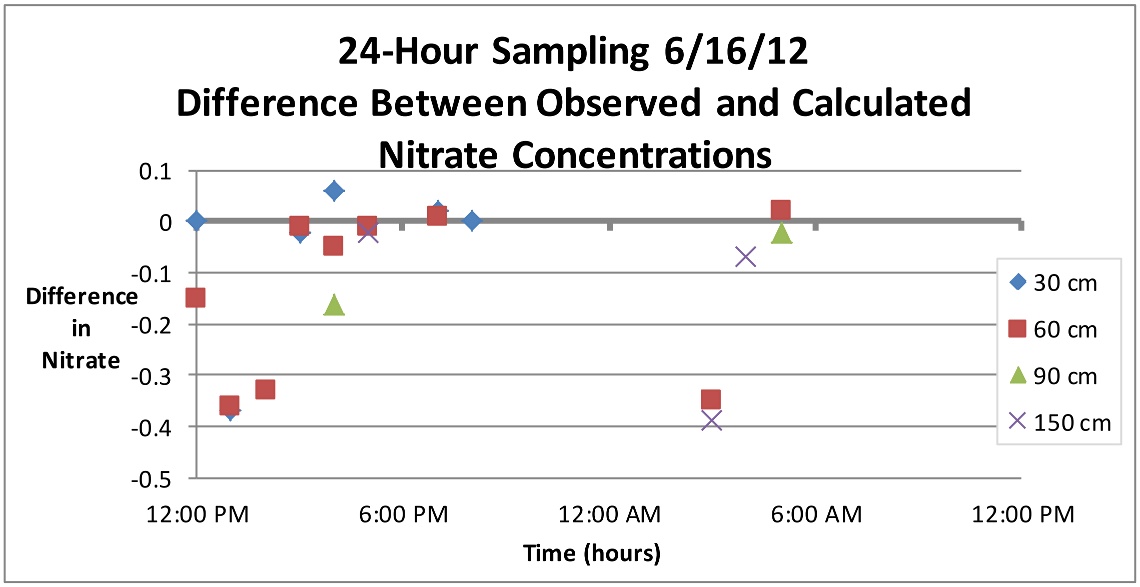

Figure 2.17: The results of equation (1) and (2) indicated nitrate was being removed from the hyporheic zone from 12 pm to 4 pm, 7 pm to 8 pm, and again at 4 am. Nitrate was being added at 6 pm and 9 pm and again at 5 am when the test concluded.

Dissolved oxygen

Concentrations of dissolved oxygen had a general trend of increasing over the duration of the test (Figure 2.18). Groundwater concentrations of dissolved oxygen were not consistently the lowest values, samples taken from 90 and 150 cm within the hyporheic zone tended to have the lowest concentrations. Groundwater DO levels ranged from 0.95 to 2.93 mg/L. DO in the upper two collection depths (30 and 60 cm) ranged from 2.23 to 6.00 mg/L. DO concentrations were generally lowest at 90 and 150 cm in the hyporheic zone, with concentrations ranging 0.82 to 3.79 mg/L. Stream concentrations of DO were the highest values until the storm surge, after the surge when sampling resumed at 3 am, DO concentrations were highest at 30 cm. Stream values ranged from 1.01 to 11.07 mg/L over the duration of the test.

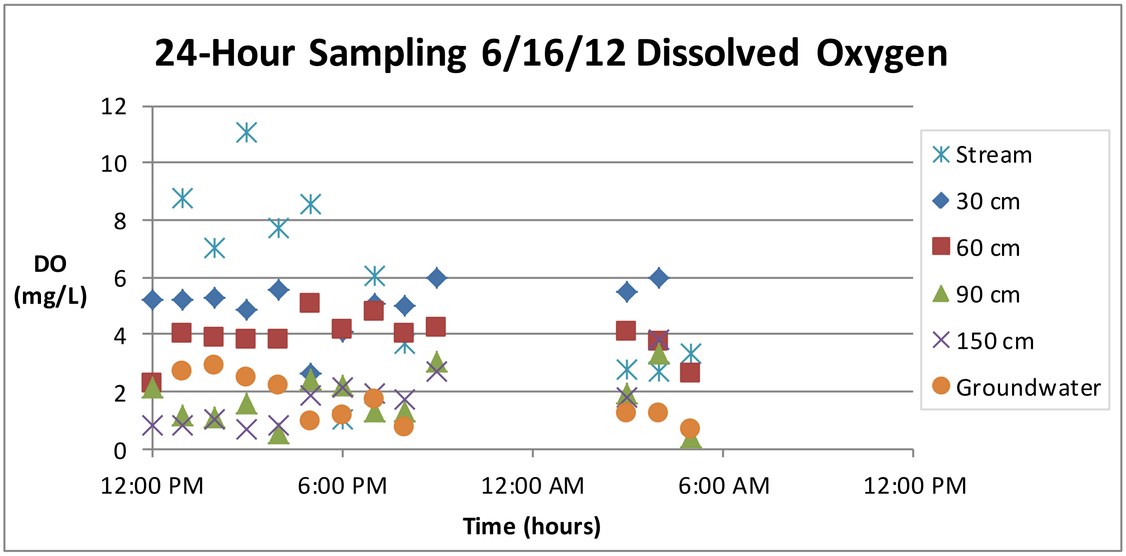

Figure 2.18: Groundwater concentrations of dissolved oxygen were not consistently the lowest values, samples taken from 90 and 150 cm within the hyporheic zone tended to have the lowest. Stream concentrations of DO were the highest values until the storm surge, after the surge when sampling resumed at 3 am, DO concentrations were highest at 30 cm.

September 8, 2012

Nitrate

The last of three 24-hour sampling periods began on September 8, 2012 at 10 am and concluded on September 9, 2012 at 10 am. Seasonally the stream stage was elevated, during this sampling period the stream stage was elevated above the seasonal average due to a recent storm. Samples that were collected and analyzed for nitrate concentrations showed ranges in values between 0.06 and 0.70 mg/L in the hyporheic zone, variations of 0.16 to 0.56 mg/L in the groundwater, and 0.53 to 1.06 mg/L in the stream (Figure 2.19).

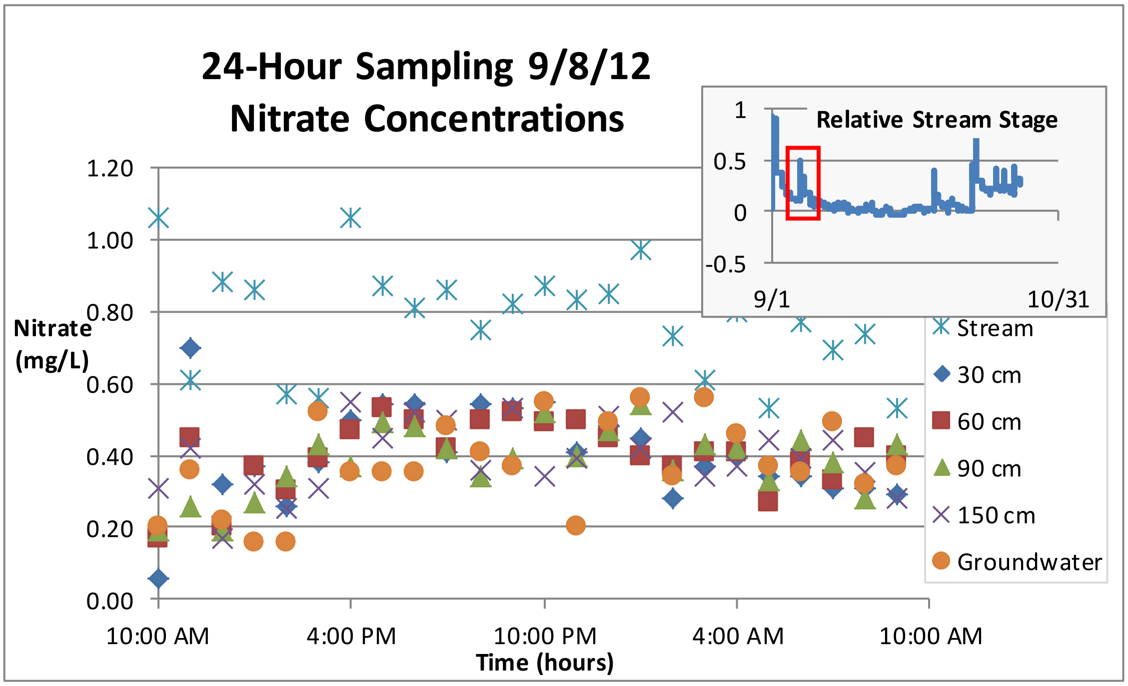

Figure 2.19: Sampling began at 10 am 9/8/12 and continued until 10 am 9/9/12. Stream water constantly had the highest concentrations of nitrate. On average, groundwater had the lowest concentrations, though variation in concentrations was observed. The red box on the stream stage chart indicates the time period of sampling.

A two-factor ANOVA of the data collected from the hyporheic zone showed that concentrations in nitrate varied over the duration of the test (F(2,230=4.13, p=<0.01) but not between the depths of the hyporheic zone (F(2,23)=0.61, p=0.55). However, t-test using equal variance compared nitrate concentrations of samples taken during daylight hours (10 pm to 7 pm and 6 am to 10 am) to those collected after dark (8 pm to 6 am), the results showed no variation in the data between light and dark. Nitrate concentrations in the hyporheic zone decreased from an average of 0.18 mg/L at the beginning of the test (10 am 6/16/2012) to an average of 0.51 mg/L at 6 pm. Nitrate concentrations then plateaued at an average of 0.44 mg/L from 7 pm until 1 am. A decrease in nitrate was then observed at 2 am with nitrate concentrations of 0.38 until the end of the test at 9 am where nitrate 0.35 mg/L.

Chloride

Chloride concentrations ranged greatly over the sampling period (Figure 2.20). Chloride concentrations in the stream ranged from 1.95 to 135.64 mg/L with the lowest concentrations occurring between 12 pm and 8 pm, and highest concentrations occurring between 2 am and 10 am. In the hyporheic zone, concentrations ranged from 18.85 to 60.48 with the lowest concentrations occurring between 10 am and 2 pm. Chloride in the groundwater showed the widest range in concentrations with values between 6.04 to 201.64 mg/L. Concentrations within the groundwater were highest between 3 pm and 8 pm.

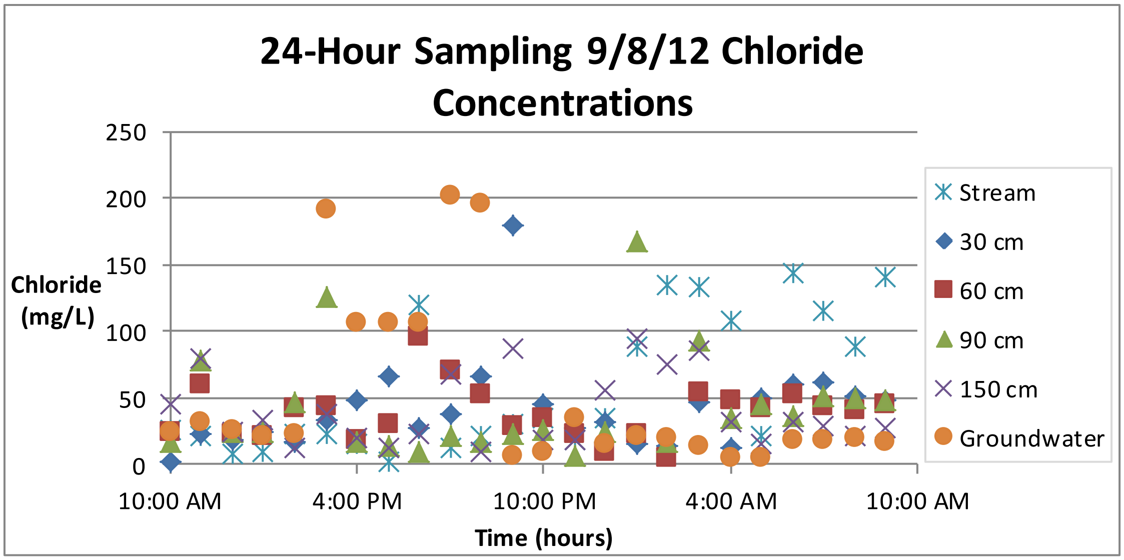

Figure 2.20: Chloride concentrations ranged greatly throughout the sampling period for all depths sampled. From 3 to 8 pm groundwater concentrations were at their peak, stream concentrations peaked between 2 and 9 am.

Calculations

Further analysis of the data included using nitrate and chloride concentrations to apply equations (1) and (2) to the data (Figure 2.21). The model was not able to be applied to all of the samples collected during the test, for the chloride concentrations in the hyporheic zone often exceeded the concentrations of the stream and groundwater. A storm surge had occurred in the days leading up to the 24-hour test, raising the stream stage and flushing chloride stored in the unsaturated zone into the stream system. Because of this limitation, the extent of the hyporheic zone was not determined. When the model was applied to samples taken between 30 and 150 cm, they were found to contain greater than 10% stream water, indicating they were a part of the hyporheic zone.

Though the model was not able to determine a groundwater transition zone, it was able to suggest periods where nitrate was being removed. The results indicated nitrate was being removed from the hyporheic zone for the entirety of the 24 hours with an exception being that at 2 am, when the calculated values and the observed values for nitrate were the same. The differences between the observed and calculated nitrate concentrations differed from -0.50 to -0.03 mg/L.

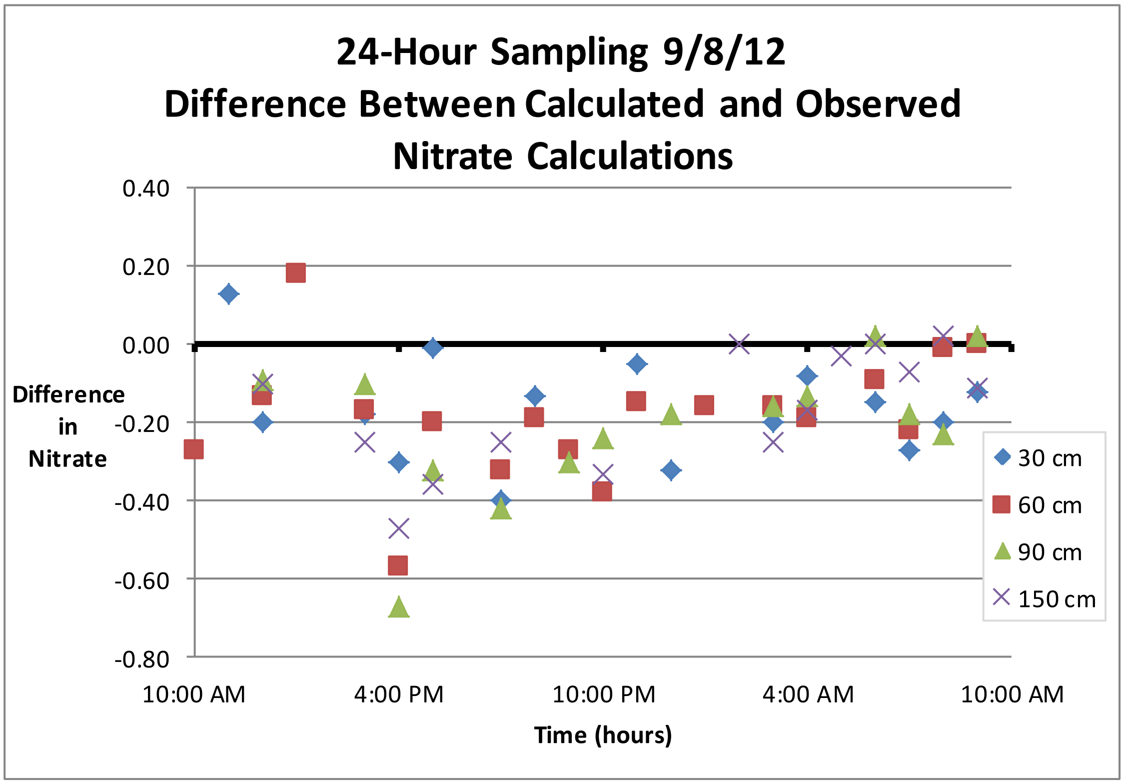

Figure 2.21: When chloride concentrations allowed the model to be applied, the results indicated less nitrate present than predicted in the hyporheic zone for the entirety of the 24 hours with an exception being that at 2 am the calculated values and the observed values for nitrate were the same.

Dissolved oxygen

Concentrations of dissolved oxygen in the hyporheic zone showed no pattern of distribution until 8 pm when DO levels decreased and there was no difference between the depths of the hyporheic zone (Figure 2.22). DO levels in the hyporheic zone ranged from 0.89 to 6.7 mg/L over the course of the test, with the highest levels occurring between 10 am and 8 pm and from 8 am to 10 am. Groundwater concentrations of dissolved oxygen were consistently the lowest values with ranges from 1.01 to 4.88 mg/L. Stream concentrations of DO were generally the highest values with a range of 1.80 to 9.60 mg/L.

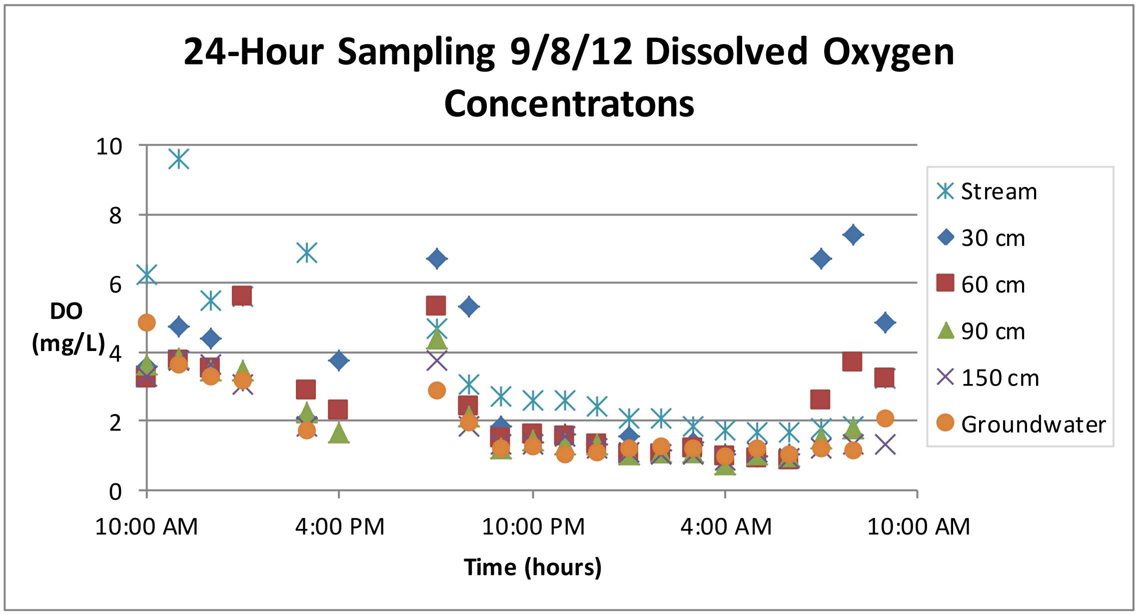

Figure 2.22: Concentrations of dissolved oxygen in the hyporheic zone showed no pattern of distribution until 8 pm when DO levels decreased. The highest DO occurred between 10 am and 8 pm and from 8 am to 10 am. Groundwater concentrations of dissolved oxygen were consistently the lowest, and stream concentrations were generally the highest.

Discussion

Seasonal

Water samples were taken in the hyporheic zone from 30 to 150 cm to observe variation. The hyporheic zone, as defined by Triska *et al.* (1989), contains between 10 and 100% stream water. Some samples collected from at 150 cm beneath the streambed taken from September 2011 to October 2012 contained less than 10% stream water. This was determined by applying equation (1) to the data to calculate the ratio of stream water to groundwater within the five hyporheic zone sampling wells, then multiplying the results by 100. Samples that contained less than 10% surface water are not considered to be a part of the hyporheic zone; instead they are a part of a groundwater transition zone. These samples indicate that the hyporheic zone extends to a depth between 90 and 150 cm in the fall and winter. During the summer months, several samples taken at 90 cm contained less than 10% stream water, indicating that the hyporheic zone decreased to between 60 and 90 cm. This is likely due to the decreased precipitation and stream stage decreasing the downwelling of surface water into the subsurface. It should be noted that though downwelling may affect the extent of mixing in the hyporheic zone, overall LKC is a gaining stream, making upwelling of groundwater the dominant process throughout the year (Bastola, 2011; Oware, 2010). Upwelling of groundwater was also observed in several instances while collecting water samples. Water collected from the hyporheic zone continued to flow for a few seconds after the pump was disconnected. In the spring months, the extent of the hyporheic zone and the transition zone were not observed. The elevated chloride concentrations in the stream and groundwater during this time did not allow for the application of equation (1). Seasonal observation of nitrate show that concentrations varied throughout the year. The sudden rise in nitrate concentrations in the stream from February until May coincides with the application of fertilizers to agricultural fields within the LKC watershed (Figure 2.5). The increase in nitrate in the stream also corresponds to an increase in nitrate concentrations within the hyporheic zone and the groundwater. The month of May saw a percent organic matter in streambed sediment of 2.61%, which was the highest percentage of organic matter observed. Organic matter present in the streambed surface may act as a source of dissolved organic carbon for utilization by denitrifying microbes deeper within the hyporheic zone (Arango et al., 2007). Studies have shown that elevated concentrations of organic matter in the streambed can stimulate denitrification in the hyporheic zone when nitrate concentrations in the stream system are high (Arango et al., 2007; Boulton et al., 1998). This indicates that although nitrate concentrations rose from February to May, the available carbon may have increased denitrification. At this time, the results of equations (1) and (2) indicated that although denitrification in the hyporheic zone may have been increasing at this time, nitrate was being added. Equations (1) and (2) were applied to the seasonal nitrate data, though elevated concentrations of chloride in the hyporheic zone made the model not applicable for samples collected in March and April. The results of the calculations using February water samples indicated a possible trend of excess nitrate present in the hyporheic zone for the months of March and April. Ammonium being a common component in fertilizers makes nitrification of ammonium a likely source for the additional nitrate. Nitrification typically converts 9-15% of ammonium in streams into nitrate, therefore an increase in ammonium entering the stream would lead to an increase in overall nitrate concentrations (Kemp and Dodds, 2002b). The general increase of DO concentrations in the hyporheic zone from February to May is further evidence for an increase in nitrification, which is an oxic microbial process. Results of the calculations for samples collected in May showed that the theoretical concentrations of nitrate in the hyporheic zone matched observed values. This indicates that there is no net removal or addition of nitrate in the hyporheic zone. At this time a decrease in DO on the hyporheic zone was observed, fertilizer application had been reduced, and nitrate concentrations in the hyporheic zone began to plateau. This could indicate a shift from more oxic water and nitrification to a more anoxic environment and increased denitrification.

The increase in nitrate in the stream and subsurface from February to May also corresponds to an increase in stream stage as the result of increased precipitation. The increased stream stage could be the source of the elevated concentrations of DO in the hyporheic zone and the reduced efficiency of denitrifying microbes. Baker and Vervier (2004) found that increased discharge during the spring months increased downwelling of stream water into the hyporheic zone and therefore introduced oxygenated water into the subsurface that could hinder denitrification.

Nitrate concentrations in the stream rapidly decreased from its peak of 4.59 mg/L at the beginning of May to 0.62 mg/L in June, where concentrations plateaued until a decrease was observed in mid-September. Nitrate concentrations in the hyporheic zone and the groundwater stayed nearly constant from the beginning of May until September despite the rapid decrease in stream nitrate concentrations at the end of May. Though nitrate concentrations were elevated during the summer months, when equations (1) and (2) were applied to the data, the results show that nitrate was being removed from the hyporheic zone; this is likely due to denitrification. Seasonal studies of denitrification have reported the summer months as having the highest amount of denitrification occurring (Arango et al., 2007; Baker and Vervier, 2004; Stelzer et al., 2011). The summer months also saw a decrease in the percent of organic matter in the streambed. Organic matter may have been decreasing because it was being utilized by increased denitrification occurring in the streambed and subsurface.

Though denitrification appears to be occurring during this time period, nitrate concentrations are at their seasonal peak. This is likely because the efficiency of denitrification has not increased to meet the demand of the nitrate load. This is exhibited in the Efficiency Loss model, which states that the increase in available nitrogen will increase rates of nitrate cycling but with reduced efficiency (O'Brien et al., 2007). Efficiency is lost because the increase in cycling occurs at a slower rate than the rate of the nitrogen increase.

During these summer months the stream stage had also lowered as a result of decreased precipitation, and the DO concentration in the hyporheic zone displayed an overall decrease. The decrease in stream stage may cause nitrate removal from denitrification to increase because residence time of water within the hyporheic zone increases as discharge decreases (Pind et al., 1997). The decreased concentrations of DO in the subsurface may cause a reduction in the size of the oxic zone, allowing a larger environment for anoxic denitrifying microbes. The percent of organic matter in the streambed decreased from May until August, when the lowest percent of organic matter was observed at 0.55%. By the end of September, however, the percent organic matter had increased to 0.92%. The lower amounts of organic matter may have been caused by denitrification occurring in the streambed utilizing the carbon. the reduction in organic matter as it is used by denitrifying microbes can lead to an overall reduction in the amount of denitrification occurring as carbon becomes a limiting factor for denitrification (Arango et al., 2007).

Stream nitrate concentrations were lowest during the months of September and October in both 2011 and in 2012. These decreases in nitrate in the stream were reflected in both the hyporheic zone and the groundwater. Seasonal studies of denitrification have determined that denitrification rates are typically lowest in autumn and winter when both temperatures and available nitrogen are low (Arango et al., 2007; Baker and Vervier, 2004; Stelzer et al., 2011). The results of applying equations (1) and (2) to the data indicate that nitrate was being removed from the hyporheic zone despite the potential decrease in denitrification. The removal of nitrate from the system suggests denitrification may still have been occurring at significant rates during the autumn months. The discrepancy in the results may be related to the depth at which the hyporheic samples were taken. Baker and Vervier (2004) and Arango *et al.* (2007) both took their water samples from a depth of 5 cm. Denitrification occurring closer to the surface is likely to be more sensitive to changes in temperature than denitrification that occurs deeper beneath the streambed. Stelzer *et al*. (2011) collected samples from a range of 23 to 71 cm beneath the streambed, however the hyporheic zone of the stream in which the research was conducted only extended 5 cm into the subsurface. Stelzer et al. (2011) still observed the decrease in denitrification in the autumn months, but samples consisted of nearly 100% groundwater. Therefore unlike in this study, samples taken by Stelzer *et al.* (2011) were taken underneath the hyporheic zone and not influenced by stream water.

DO concentrations decreased along with nitrate concentrations in September and October of 2012. DO concentrations for September and October of 2011 were elevated; however, the data from this time period has an increased level of error associated with it as the result of questionable equipment calibration. The decrease in DO within the hyporheic zone is evidence for continued denitrification in the hyporheic zone. Though DO concentrations decreased from September to October, autumn months saw an increase in stream stage. Unlike previous periods of high stream stage, the DO concentrations in the hyporheic zone were not elevated. DO concentrations in the stream were at a seasonal low in September and October with concentrations near those present in the hyporheic zone. Therefore, additional stream water downwelling into the hyporheic zone as a result of the increased stream stage would not likely cause an increase in overall DO concentrations.

24-Hour Sampling Periods

Three 24-hour sampling periods conducted to observe diurnal variations in nitrate concentrations showed that the observed variation was correlated to stream nitrate concentrations and not to the availability of light. It is likely that the samples were taken too deep within the hyporheic zone to be affected by DO variations caused by photosynthesis at the surface. In general, equations (1) and (2) showed that for each of the three 24-hour sampling periods, denitrification was the primary process occurring for the duration of the sampling period. Variations observed in the amount of nitrate being removed also did not correspond to changes in the amount of available light. Variation in nitrate during the first two sampling periods was slight, however significant variation was observed during the last of the sampling periods. The stream stage during the last sampling period, September 8, 2012, was significantly higher than the stream stage during the previous two sampling periods. The increased stream stage led to an increase in downwelling of stream water into the hyporheic zone and resulted in increased variability of nitrate.

Nitrate concentrations observed during the first 24-hour sampling period in September 2011 were consistent with those observed during the second sampling period in June 2012. Concentrations were expected to be low in September due to the seasonal decrease of nitrate present in the stream. Concentrations were likely low in June because denitrification is elevated in the summer months. This is supported by the results of equations (1) and (2) which suggest that more nitrate was being removed during the June sampling period than during the September 2011 sampling period. Nitrate concentrations were elevated above the typical seasonal concentrations in September 2012. Concentrations were elevated in the stream and the hyporheic zone after a storm increased surface runoff, tile drainage, and stream stage days before the samples were taken.

September 30, 2011

The 24-hour sampling period that began on September 30, 2011 at 12 pm did not last the entire 24 hours due to equipment trouble. Samples that were collected varied only slightly in nitrate concentrations (Figure 2.11). The variations that were observed did not reflect the changing daylight as hypothesized. Sunset occurred at 7:30 pm with direct sunlight moving off of the stream at 7:00 pm. A t-test confirmed that there was no variation in nitrate concentrations of the stream, hyporheic zone, or groundwater between samples taken in the daylight and samples taken after dark.

From the beginning of the sampling at 12 pm until 4 pm nitrate concentrations in the hyporheic zone, the groundwater, and the stream were steady at a low concentration. Nitrate concentrations in all three locations increased and reached their peak from 4 pm until 8 pm. The increase in nitrate occurred partially during the transition from light to dark. Though it was hypothesized that denitrification would increase when photosynthesis stopped in the absence of daylight causing DO concentrations to decrease, nitrate concentrations continued to rise after sunlight was no longer in contact with the stream. DO concentrations in the hyporheic zone decreased from 12 pm until 4 pm, these changes in concentrations also did not correspond to changes in light. Calculations from equations (1) and (2) indicate that from 12 pm until 5 pm there was less nitrate present in the hyporheic zone than calculated. This is an indicator that denitrification removed nitrate during this time. The decrease in DO concentrations at this time is further evidence that conditions were appropriate for an increase in denitrification to occur. More nitrate was present from 6 pm until 7 pm, which suggests a reduction in denitrification and a possible increase in nitrification. Nitrification and denitrification are often coupled in the hyporheic zone (Kemp and Dodds, 2002a), making it possible for nitrification and denitrification to alter the net nitrate concentrations in the hyporheic zone throughout a 24 hour period. Nitrate concentrations decreased and returned to low concentrations from 9 pm to 12 am. Nitrate rose again at 12 am until sampling was stopped at 1 am. Concentrations remained elevated when a set of samples was taken in the daylight at 10 am. Equations (1) and (2) showed that there was less nitrate present in the hyporheic zone than calculated from 8 pm until 10 pm.

From 11 pm until 1 am there was more nitrate present in the hyporheic zone than calculated, more nitrate was also present at 10 am.

DO concentrations decreased from 12 pm until 4 pm where concentrations remained steady until sampling was stopped at 1 am. The decrease in DO concentrations in the hyporheic zone also did not correspond to the changes in the amount of daylight on the stream. The stream stage was low for the duration of the sampling period, therefore the amount of surface water downwelling into the hyporheic zone is likely to have been reduced (Baker and Vervier, 2004). Studies that have shown diurnal changes in denitrification based on the absence and presence of light were not conducted *in situ* (Brunke and Gonser, 1997; Christensen et al., 1990). The water samples from the hyporheic zone were taken at depths greater than 30 cm beneath the streambed surface. It is likely that the samples were taken at too great a depth to be affected by changes in DO concentrations caused by photosynthesis at the streambed surface.

June 16, 2012

Sampling did not occur during the entire 24-hour sampling period. A thunderstorm caused sampling to stop at 9:15 pm until 3 am. After sampling resumed at 3 am, debris from the storm damaged wells 1-3, and the stream stage rose above the tops of the remaining wells causing sampling to cease at 5 am.

A two-factor ANOVA without replication confirmed that concentrations in the stream, hyporheic zone, and the groundwater did not vary over the course of the sampling period. Concentrations of nitrate were slightly elevated from 3 am until sampling stopped at 6 am indicating that a trend of increasing nitrate might have been observed had sampling continued (Figure 2.15). Equations (1) and (2) indicated that nitrate concentrations in the hyporheic zone remained less than the theoretical values for the entirety of the sampling period. This indicates that nitrate was being removed from the hyporheic zone, likely due to denitrification, for the entire sampling period. This is not consistent with previous 24-hour sampling period that saw variation in excess and reduced nitrate in the hyporheic zone. Studies of seasonal denitrification trends have determined that denitrification is typically increased during the summer and reduced during the autumn months (Arango et al., 2007; Baker, 2003; Baker and Vervier, 2004; Pind et al., 1997). A seasonal increase in denitrification provides evidence that the removal of nitrate during the sampling period was from denitrification.

Sunset occurred at 8 pm and sunrise was at 5 am, however neither the results of the calculation nor the concentrations of nitrate themselves reflected the changes in light. Concentrations of DO remained consistent throughout the sampling period and also showed no response to the amount of light. However, after the storm and subsequent increase in stream stage, the stream no longer had the highest DO concentrations. The increased stream stage increased downwelling of nitrate-rich stream water into the hyporheic zone (Baker and Vervier, 2004), increasing the concentration of DO and nitrate in the subsurface. Though more nitrate was not observed at most depths within the hyporheic zone, the increase was observed at 30 and 150 cm beneath the streambed surface.

September 8, 2012

Sampling began at 10 am on September 8, 2012 and stopped after the full 24 hours at 10 am September 9. Variations in nitrate concentration in the stream, hyporheic zone, and groundwater were observed throughout the sampling period (Figure 2.19). Nitrate concentrations in the hyporheic zone and the groundwater increased from the start of sampling at 10 am until 5 pm. Concentrations plateaued from 6 pm until 1 am when concentrations began to decline. Concentrations plateaued after the decline from 3 am until sampling stopped at 10 am. Sunset occurred at 7:30 pm and sunrise was at 6 am, however the changes in nitrate do not reflect the changes in light. Concentrations of DO changed throughout the 24 hour period as well. Concentrations decreased from the start of sampling until 8 pm, and then concentrations remained steady until 7 am when they began to increase. The timing of the changes in DO concentrations suggests the variation may be a reflection of light availability. The stream stage during this sampling period was significantly elevated when compared to the previous two sampling periods. The difference in stream stage could account for the increase in variation of DO and nitrate concentrations. An elevated stream stage forces surface water into the hyporheic zone (Boulton et al., 1998), and the extreme conditions of the stream stage at the time of sampling may have allowed surface water to penetrate deep enough within the hyporheic zone to affect concentrations at the sampling depths. The variation in nitrate concentrations observed in the hyporheic zone are reflective of those seen in stream concentrations, providing evidence that increased stream water was downwelled into the hyporheic zone. Elevated stream stage and increased stream discharge are often correlated to increased concentrations of nitrate (Baker and Vervier, 2004; Royer et al., 2004).

The results of applying equations (1) and (2) to the data indicate that there was less nitrate in the hyporheic zone than calculated for the majority of the sampling period. This suggests that nitrate was being removed from the hyporheic zone and suggests that denitrification was occurring. There was excess nitrate in the hyporheic zone at 11 am, 1 pm, and 2 am. Excess nitrate could be present as nitrification converts ammonium in the hyporheic zone to nitrate. Coupling of nitrification and denitrification may have been occurring, causing net nitrate to increase and decrease throughout the sampling period (Kemp and Dodds, 2002a).

The results of the two equations showed that the amount of nitrate being removed throughout the sampling period did not reflect the changes observed in the concentrations of DO or nitrate. Instead, nitrate concentrations in the subsurface reflected the pattern of nitrate variation observed in the stream water.

Conclusion

Nitrate concentrations displayed seasonal variation in the hyporheic zone with peaks occurring in the spring and summer, and lows in the fall and winter months. The increase of nitrate during the spring coincides with the application of agricultural fertilizers, and concentrations remained elevated throughout the summer due to the abundance of nitrate in the system. Nitrate concentrations experienced a seasonal low when nitrate was not being added to the system.

Seasonal concentrations of nitrate in the hyporheic zone are correlated more strongly to concentrations in the stream, groundwater, level of stream stage, and DO concentrations than to periods of increased or decreased nitrate removal. Optimal conditions for denitrification such as anoxic water and the presence of organic carbon were never reached during the sampling year, indicating that processes such as plant uptake or nitrate adsorption may have been responsible for the observed variations in nitrate and not denitrification.

Data collected from the three 24-hour sampling periods showed that water deep within the hyporheic zone was not affected by diurnal changes in light availability. The majority of the data showed little to no variation in nitrate in the hyporheic zone; however samples collected during a period of elevated stream stage saw significantly more variation. It is likely that the samples were collected too deep within the hyporheic zone to be affected by changes in water chemistry due to photosynthesis at the surface.

CHAPTER III

CONCLUSION

Summary of the Results and Discussion

Nitrate concentrations displayed seasonal variation in the hyporheic zone with peaks occurring in the spring and summer, and lows in the fall and winter months. The increase of nitrate during the spring coincides with the application of agricultural fertilizers, and concentrations remained elevated throughout the summer due to the abundance of nitrate in the system. Nitrate concentrations experienced a seasonal low in fall and winter when nitrate was not being added to the system.

Seasonal concentrations of nitrate in the hyporheic zone were correlated more strongly to concentrations in the stream and groundwater, level of stream stage, and DO concentrations than to calculated periods of nitrate removal or addition. Optimal conditions for denitrification such as anoxic water and the presence of abundant organic carbon were never reached during the sampling year, indicating that processes such as plant uptake, conversion to organic nitrate, or nitrate adsorption may have been responsible for the observed variations in nitrate and not denitrification.

Data collected from the three 24-hour sampling periods showed that water deep within the hyporheic zone was not affected by diurnal changes in light availability. It is likely that the samples were collected too deep within the hyporheic zone to be affected by changes in water chemistry due to photosynthesis at the surface. The majority of the data showed little to no variation in nitrate in the hyporheic zone; however samples collected during a period of elevated stream stage saw significantly more variation.

Recommended Future Work

Further research at LKC is needed in order to fully understand the processes occurring in the hyporheic zone and the stream's capacity to remove and store nitrate. Proper conditions for denitrification were never observed during the course of this research. DO concentrations never reached anoxic levels and were therefore too high to allow for denitrification to occur. Examining dissolved organic carbon (DOC) content deep within the hyporheic zone in addition to DO concentrations may provide further insight into the controls on microbial processes at LKC. DOC often acts as a limiting factor for denitrification deep beneath the streambed in stream systems that have high levels of nitrate. Low concentrations of DOC in combination with oxic conditions may be restricting microbial processes.

Examining other nitrate removal and storage mechanisms would also provide valuable insight into the processes that are occurring deep within the hyporheic zone. Seasonal patterns in plant uptake, conversion of nitrate to organic nitrogen, and nitrate adsorption may be occurring in the hyporheic zone, resulting in the observed variations.

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