IN SITU MEASUREMENT OF RIPARIAN GROUNDWATER DENITRIFICATION IN A SHORT-ROTATION PINE DOMINATED WATERSHED

by

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(Under the Direction of C. Rhett Jackson)

ABSTRACT

Here, we estimate denitrification in shallow groundwater to help determine if current streamside management zones (SMZs) can mitigate nitrate input from short-rotation pine management in the Upper Atlantic Coastal Plain. Water samples were collected monthly for a year and analyzed for nutrient (NO_3^-, NH_4^+, TN, DOC) and dissolved gas (N_2, Ar, N_2O) concentrations from the Fourmile Watershed at the Savannah River Site. Excess-N_2 produced by denitrification was measured using MIMS and N_2:Ar ratios. Nitrate concentrations decreased between the edge of the SMZ and the intermittent stream valley. N_2O was the dominant end product in the pine plantation. Median denitrification reaction progress ranged from 0.47 to 0.94. Environmental conditions, such as low pH, fluctuating water table, high concentrations of NO_3^-, and low levels of DOC, inhibited complete denitrification in the shallow groundwater. Denitrification in the SMZ mitigates the additional nitrogen inputs from short-rotation woody crop (SRWC) management.

INDEX WORDS: denitrification, nitrate, streamside management zone, short rotation woody crop, Savannah River Site, riparian zone, membrane inlet mass spectrometry, groundwater

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DEDICATION

This thesis is dedicated to my family and friends who have supported me throughout my graduate school journey, and to all the great teachers I have had along the way, from kindergarten to graduate school, both in the classroom and in life.

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TABLE OF CONTENTS

Page

CHAPTER

CHAPTER 1

INTRODUCTION

With increased potential for nitrate contamination in surface water due to anthropogenic inputs to the global nitrogen cycle, quantifying *in situ* denitrification as the natural process of nitrate attenuation in the environment has become a pressing issue for study. Although the majority of estimated denitrification happens in the soil of terrestrial environments, rates of denitrification in groundwater have been shown to be higher than any other freshwater system (Seitzinger et al., 2006). There have been extensive studies on denitrification in the terrestrial environment, but relatively few attempts to quantify groundwater denitrification on a watershed scale. The scientific research focused on assessing denitrification along shallow groundwater flow paths is limited to predominantly agricultural-dominated watersheds and methods for *in situ* measurements are complex (Groffman et al. 2006). Additional investigations on this nitrate removal process in groundwater are needed to understand the extent of this process in potential buffer areas and the environmental factors controlling the process.

Due to specific water chemistry parameters such as low dissolved oxygen (DO) and high dissolved organic carbon (DOC) required for the anaerobic microbial respiration process, subsurface water flow paths within riparian areas and hyporheic zones have the potential to be hotspots for denitrification, mitigating nitrate (NO_3^-) loading to surface waters. However, driving factors are complex and differ between sites (Rivett et al. 2008; Merrill and Tonjes 2014). Groundwater denitrification can mitigate up to 30% of applied nitrogen on agricultural fields (Jahangir et al. 2013). Other findings have suggested denitrification in groundwater may range from 4% to over 70% of applied nitrogen (Anderson et al. 2014). These wide-ranging results from previous studies suggest that the amount of NO_3^- removed from groundwater due to denitrification is site specific and difficult to predict without *in situ* measurements.

Denitrification has been considered one solution, of many, to the unbalanced equation of inflows and outflows of watershed nitrogen budgets (Fox et al. 2014). This phenomenon of "the missing N" (David and Gentry 2000) between hillslopes of headwater streams and catchment outflows has been observed across different landscapes, especially in agriculture or heavy fertilized systems (Fox et al 2014). A recent multi-year study at the Savannah River Site investigating the environmental effects of short-rotation woody crop (SRWC) production has observed its own missing N (Griffiths et al. 2017).

Monitoring and analysis of water quality within the Fourmile Watershed at the Savannah River Site (SRS) has shown that near-valley groundwater concentrations of NO_3^- within short-rotation pine plantation are higher than surface water concentrations of NO_3^- (Griffiths et al. 2017). Diminished NO_3^- concentrations in watershed outflows may be explained by vegetative uptake and denitrification in the riparian zones of headwater streams adjacent to a SRWC production plantation.

Quantifying *in situ* denitrification in shallow groundwater will close gaps in the nitrogen mass balance of the watershed and evaluate the efficacy of current forestry Best Management Practices (BMP) for the intensive silviculture of short-rotation pine production for bioenergy. These findings will be integrated into the ongoing experiment assessing the effect of intensively managed SRWC production on water quality and quantity.

Objectives

The main goal of this mensurative study is to quantify gradients in nitrogen species in groundwater flow paths from an intensively managed pine stand through a forested riparian buffer adjacent to an intermittent stream and to estimate *in situ* denitrification in the shallow groundwater flow paths. Specific study objectives include:

1) Measure excess N_2 and N_2O in groundwater flow paths from an intensively managed, short-rotation pine stand through a streamside management zone,
2) Calculate groundwater denitrification from observed excess N_2 and N_2O measurements,
3) Monitor NO_3^- concentrations in groundwater from a short-rotation pine plantation, streamside management zone (SMZ), and hyporheic zone of an intermittent stream, and
4) Analyze relationships between denitrification measurements, groundwater chemistry parameters, and NO_3^- concentrations.

Predictions

We developed some expected predictions based on previous scientific studies regarding denitrification:

1) Due to the abundance of driving factors, concentrations of dissolved N_2 produced from denitrification will be higher in the SMZ compared to the pine plantation.
2) Concentrations of N_2O will be higher in subsurface flow paths in the pine plantation where observed nitrate concentrations are high.
3) Nitrate and total nitrogen concentrations will be lower within the SMZ than the pine plantation based on previous observations in other parts of the experimental watershed and fertilization of the pine plantation.
4) Groundwater denitrification estimates will be higher within the forested SMZ than the pine plantation based on a review of the literature and potential presence of driving factors of denitrification.
5) Concentrations of end products of denitrification, excess N_2 and N_2O, will be positively correlated with levels of DOC and negatively correlated with concentrations of DO and NO_3^- and oxidation-reduction potential (ORP).

CHAPTER 2

LITERATURE REVIEW

Short-Rotation Woody Crops: Why, What, and How

Timber and forest products are important industries in the United States, especially in the southern U.S. where timberlands account for over 80 million hectares and provide 60% of all national wood products (Wear and Greis 2013; Oswalt and Smith 2014). Largely driven by the passage of the Energy Independence and Security Act of 2007 (EISA), there has been an increased interest in converting marginal crop and timberlands to plantations of short rotation woody crops (SRWC) for bioenergy (Griffiths et al. 2018). As part of the EISA, the Renewable Fuels Standard (RFS) calls for the U.S. to produce 36 billion gallons of renewable fuels per year for domestic consumption by 2022 with a heavy emphasis on woody biomass-based biofuels (EISA 2007). The Southeastern U.S. contains 51% of all potential woody feedstocks for bioenergy, including SRWC (Joslin and Schoenholtz 1997; Stein et al. 2013; Forisk Consulting 2018). Short-rotation woody crops can be used for a variety of different bioenergy applications, such as the production of biofuels and production of wood chips or pellets for woody biomass energy facilities (Stein et al. 2013), increasing the appeal to convert cropland and timberlands to SRWC production.

Several different native and genetically modified species of trees can be managed as SRWC, but loblolly pine (*Pinus taeda*) has the highest potential for SRWC production for bioenergy in the southern United States because it currently compromises the majority of the timberlands in the southeast (Perlack et al. 2005; Dickmann 2006; Kline and Coleman 2010; Griffiths et al. 2018). Pine plantations used for SRWC are intensively managed with herbicide, pesticide, and fertilizer applications along with mechanical site prep to facilitate fast lignocellulosic growth rates and short harvesting rotations (8-12 years) (Griffiths et al. 2017). While short-rotation woody crops require less total amount of fertilizer compared to other bioenergy crops, such as corn or perennial grasses, fertilizer applications can still have detrimental effects on water quality (US Environmental Protection Agency 2011). Nitrate (NO_3^-) and phosphorus (P) from fertilizer applications can be transported to streams, causing eutrophication (Vitousek et al. 1997). Some early studies have shown that conversion from row-crop agriculture to SRWC plantations for bioenergy use in the southeastern U.S. improved water quality of surface water and groundwater flows even in early years of stand establishment (Joslin and Schoenholtz 1997; Thornton et al. 1998; Nyakatawa et al. 2006). A recent study provides evidence that forestry best management practices can protect water quality of adjacent aquatic ecosystems from the frequent application of fertilizers required for SRWC production (Griffiths et al. 2017).

Forestry Best Management Practices and Stream-Side Management Zones

Spurred by the amendments to the Federal Water Pollution Control Act in 1972, better known as the Clean Water Act (CWA), guidelines to reduce non-point sources of pollution in forestry were established and implemented (Shepard 2006). These guidelines, termed Best Management Practices (BMPs), provide landowners and industry professionals measures to reduce erosion, maintain site productivity, and protect water quality throughout all stages of timber harvesting (Secoges et al. 2013). These practices that are set by each state include guidelines for stream crossings, proper construction of forest roads, and leaving intact forested riparian zones, also known as stream-side management zones (SMZs) (South Carolina Forestry Commission 1998). SMZs protect riparian areas, land adjacent to surface water bodies, that can serve as a buffer and filtering zone between surface water bodies and silvicultural practices upslope (Pinho et al. 2008). Riparian areas are an important ecotone and serve important ecosystem functions such as near-stream habitat, flood control, bank stabilization, and protection of water quality (Fennessy and Cronk 1997; Wenger 1999). Vegetated riparian areas can buffer negative impacts from different upslope land uses, however, forested riparian areas are considered to offer better protection than other types of vegetation (Haycock et al. 1997; Mayer et al. 2006). The importance of intact forested riparian zones as a BMP specifically for the protection of water quality has been proven time and again (Lowrance et al. 1984; Haycock et al. 1997; Hill 1996; Lowrance et al. 1997; Mayer et al. 2006; Secoges et al. 2013; Anderson et al. 2014). Riparian areas can slow down overland flow, allowing sediment- and contaminant-laden water to infiltrate into the soil prior to reaching the stream. Unsaturated lateral subsurface flow, or interflow, may pass through the rhizosphere where nutrient uptake and adsorption can occur (Fennessy and Cronk 1997). As shallow groundwater flow paths pass beneath the riparian zone and to the stream, biogeochemical processes, vegetative uptake, and adsorption to soil particles can attenuate nutrients and contaminants (Anderson, et al. 2014). Riparian buffer zones managed as SMZs may not be able to fully protect water quality of adjacent water bodies if contaminant inputs overload the ecosystem functions (Correll et al.1997) or if deep groundwater flow paths bypass this biologically-active zone (Groffman et al. 1998; Burt et al. 1999).

Width of SMZs for protection of water quality depend on landscape (land-use type, slope, presence and amount of impermeable area, soil characteristics) and hydrological factors (infiltration rates, hydraulic conductivity, depth to water table, dominant subsurface flow paths, stream size) of the watershed (Wenger 1999). In the US, each state recommends minimum SMZ widths in their respective BMP manual. The South Carolina Forestry Commission (1998) recommends a minimum of 12.3m (50 ft) riparian buffers.

Basics of the Nitrogen Cycle

Nitrogen is vital to the existence of life; it is present in amino acids which form proteins and DNA in all living organisms. It makes up 78% of our atmosphere as dinitrogen (N_2) and is abundant in the Earth's crust (Pepper et al. 2015). Even with these abundant nitrogen stocks, the reactive forms of nitrogen that can be readily used by organisms are considered a limiting factor for primary production (Galloway et al. 2004). This is due to the strong triple bond of N_2 which makes it a great inert gas for our atmosphere, but difficult for assimilation by plants and animals (Pepper et al. 2015). Microbial processes transform nitrogen into reactive forms of nitrogen that are easily assimilated by other organisms (Vitousek et al. 1997) making the nitrogen cycle fluid and complex (Figure 2.1).

The energy-intensive step of nitrogen fixation transforms inert dinitrogen from the atmosphere to reactive forms of nitrogen. Symbiotic relationships between microbes and plants are the dominant pathway of natural nitrogen fixation (Galloway et al. 2004). Ammonification, also known as mineralization, converts ammonia (NH_3) to ammonium (NH_4^+), the preferred form of nitrogen for most organisms including plants (Pepper et al. 2015). The oxygen-requiring microbes further transform ammonium to nitrate (NO_3^-) in the aerobic process of nitrification (Strauss et al. 2002). There are multiple pathways for nitrate. The inorganic anion (NO_3^-) can be assimilated by vegetative uptake, transformed back to ammonium through dissimilatory nitrate to ammonium (DNRA), or reduced to gaseous forms of nitrogen through denitrification (Seitzinger et al. 2006). The multi-step process of denitrification is dominated by anaerobic microbes that use nitrate for respiration in anoxic conditions and is the main pathway of nitrate reduction (Payne 1981; Boyer et al. 2006; Seitzinger et al. 2006; Rivett et al. 2008). Due to the high mobility of nitrate in water, nitrate may bypass transformation reactions and leach into groundwater systems, causing water quality issues (Vitousek et al. 1997). Nitrate leaching from soil into groundwater has increased due to the inputs of anthropogenic nitrogen to the environment (Korom 1992).

Anthropogenic Effects on Nitrogen Cycle

Anthropogenic activities have greatly altered the nitrogen cycle by introducing additional forms of reactive nitrogen into the environment (Fields 2004; Galloway et al. 2004; Gruber and Galloway 2008). Humans deliver more than 160 Tg of reactive nitrogen (N_r) into the environment annually with fossil fuel production and emissions contributing about 20 Tg of N_r, application of fertilizers contributing another 100 Tg of N_r, and nitrogen-fixing agriculture contributing the remaining 40 Tg of N_r (Fields 2004). These inputs affect all stages of the nitrogen cycle and cause detrimental effects to the ozone, climate change, water quality, and human health. The invention of the Haber-Bosch process to create inorganic forms of nitrogen that can be readily applied to crops allowed for the mass increase in agriculture, providing food to an increasing population (Gruber and Galloway 2008). This growth has led to over application of nitrogen-based fertilizers causing enormous amounts of nitrate leaching into water resources (Robertson and Vitousek 2009).

Nitrogen is a limiting nutrient in most ecosystems, and the presence of high concentrations of nitrate can lead to eutrophication causing algal blooms in freshwater and marine ecosystems (Howarth 2018). As algae die and are decomposed by other microorganisms, oxygen is depleted from the water creating areas of hypoxia, or dead zones, where organisms are unable to survive (Fields

2004; Robertson and Vitousek 2009). Nitrate contamination of drinking water sources (groundwater and surface water) can cause health issues for humans including methemoglobinemia ("blue baby disease") in infants and potential reproductive health issues in adults (Fields 2004). The U.S. Environmental Protection Agency (EPA) and World Health Organization developed a maximum limit of 10 mg-N L^{-1} for drinking water to protect human health (Fields 2004) which has been used as a level of comparison for non-point source nitrate pollution (Griffiths et al 2017).

Transport of Nitrate in Forested Watersheds

Nitrate is highly soluble in water (H. Wang et al. 2015). The fate of nitrate, how, when, and where it ends up in surface water bodies, is dependent upon hydrological processes beginning on the hillslope (Figure 2.2). These hydrological processes are controlled by climate (precipitation), terrain (slope), soil characteristics (soil texture, porosity, hydraulic conductivity), and vegetation (evapotranspiration) between uplands, hillslopes, and valleys (Jackson et al. 2004).

Overland flow in forested watersheds can be negligible due to high infiltration rates in the litter layer and topsoil (Hewlett and Hibbert 1967), however, in pine plantations managed for SRWC observed overland flow events may increase due to mechanical site prep and soil compaction from increased harvesting from short rotations (Griffiths et al. 2017). During precipitation events, saturation of the vadose zone from a rising water table can create areas on the landscape that may produce runoff to the stream called variable source areas (Hewlett and Hibbert

1967). Variable source areas shrink and swell based on degree of saturation and are commonly found in low lying areas with converging groundwater flow paths and water tables are near the surface (Hewlett and Hibbert 1967; Dunne et al. 1975).

Streamflow in humid, temperate forested watersheds can be dominated by two hydrological flow paths: groundwater flow and interflow (Klaus et al. 2015). Interflow occurs as lateral subsurface flow as water infiltrates into the vadose zone (Whipkey 1965; Weyman 1973). This lateral flow in the unsaturated zone can occur as preferential flow through soil media and macropores (Beven and Germann 1982), as indiscriminate flow or fingered flow (Selker et al. 1992), or lateral flow above a confining layer such as a perched water table (Whipkey 1965; Weyman 1973). Hydrologic connectivity between the hillslope and riparian zone is required for these vadose zone flow paths to be important in the transport of solutes to the stream (McGuire and McDonnell 2010; Ocampo et al. 2006). Hydrologic connectivity between uplands and riparian zones is not continuous (Stieglitz et al. 2003; Ocampo et al. 2006; McGuire and McDonnell 2010) and in some catchments, such as low-gradient watersheds in the Upper Coastal Plain, interflow along the hillslope may only be observed over short distances that are not adequate to transport solutes such as nitrate to the stream (Jackson et al. 2016; Du et al. 2016).

Water that infiltrates into the vadose zone can continue to percolate to the water table, even if interflow is observed, recharging the groundwater system (Jackson et al. 2014). Groundwater flows from high pressure to low pressure (Jackson et al. 2014), but not all groundwater flows the same. Groundwater flow paths differ spatially in all directions and temporally (residence time). In humid, temperate, forested watersheds water table topography may be a subdued imitation of the surface topography (Tóth 1963; Tóth 1972; Condon and Maxwell 2015). This is dependent on the isotropy or anistropy of the subsurface (Freeze and Witherspoon 1967). Heterogeneity within the subsurface can create a saturated zone that is vastly different from the overlying surface (Freeze and Witherspoon 1967; Jackson et al. 2016). As groundwater flows down pressure gradients, flowpaths converge, creating mixing and dilution effects of nitrate and other solutes (Altman and Parizek 1995).

Denitrification: A Complex Process (briefly) Explained

The fate of nitrate also depends on biogeochemical processes that are taking place along these hydrological flow paths. Denitrification is the only biogeochemical process that removes nitrate from the environment; it converts nitrate into gaseous forms of nitrogen (NO, N_2O, N_2) that will eventually be emitted to the atmosphere (Payne 1981). Other pathways, dissimilatory reduction of nitrate to ammonium (DNRA) and vegetative and microbial assimilation, transform nitrate to forms of nitrogen (ammonium and organic nitrogen, respectively) that remain in the environment until further processing (Simmons et al. 1992). The process of denitrification is a series of biochemical reactions carried out by facultative anaerobes, each step requiring a specific enzyme to reduce each intermediate nitrogen product (Pauleta et al. 2013).

The denitrification process, with corresponding enzymes for each step, is shown below:

$$NO_3^- \xrightarrow{1} NO_2^- \xrightarrow{2} NO \xrightarrow{3} N_2O \xrightarrow{4} N_2$$

[1] nitrate reductases— **nas**; **euk-nr**; **narG**; **napA**

[2] nitrite reductases—**nirK**; **nirS**

[3] nitric oxide reductases—**cnorB**; **qnorB**

[4] nitrous oxide reductase—**nosZ**

Bacteria have been identified as the primary denitrifiers with some species of fungi and Archaea contributing to the process (Wallenstein et al. 2006). The majority of microbes known to denitrify are heterotrophic bacteria that use organic carbon sources as an electron acceptor during reduction of nitrate (Payne 1981). Evidence of autotrophic denitrification has been recorded by microbes that use inorganic forms of carbon (CO_2), manganese (Mn^{2+}), or iron (Fe^{2+}) instead of organic carbon (Z. Wang et al. 2017). Autotrophic denitrifiers can even use reduced forms of sulfur as electron donors, and in turn create sulfate (SO_4^-) during the reaction (Rivett et al. 2008). Denitrification is a communal process; not all denitrifying microbes contain all the necessary enzymes required to fulfill the sequential reactions of denitrification (Wallenstein et al. 2006). Each step of the process has multiple enzymes that are capable of the reducing reaction except for the reduction of N_2O to N_2; nitrous oxide reductase (nosZ) is the only enzyme capable of this reaction (Pauleta et al. 2013).

Although denitrification provides a great ecosystem service of mitigating nitrate concentrations, the intermediate products of the process can have detrimental effects on Earth's environment. Nitric oxide (NO) is the main culprit in ozone depletion (Ravishankara 2009). Nitrous oxide (N_2O) in the atmosphere has a warming effect of over 300 times that of carbon dioxide (CO_2) making it a potent greenhouse gas that can remain in the atmosphere for over 100 years (Z. Wang et al. 2017). The only environmentally inert product of denitrification is the terminal product of N_2 (Payne 1981).

Certain environmental conditions are required for denitrification to occur and have been shown to control the process: (1) presence of nitrate or other electron acceptors (reduced forms of nitrate); (2) anoxic or hypoxic conditions; (3) presence of organic matter or other electron donors; (4) presence of microbial denitrifying communities (Korom 1992; Seitzinger et al. 2006; Rivett et al. 2008). Microbial respiration is energy-dependent; facultative microorganisms will use the most energy efficient electron acceptors present (Jackson et al. 2014). Table 2.1 shows redox reactions along a utilization preference gradient based on the amount of energy provided by the molecule serving as an electron acceptor. For microbial use of nitrate, oxygen must be depleted from the system (Payne 1981; Burgin et al. 2010). Evidence of aerobic denitrification has recently been observed in environments with fluctuating DO (Ji et al. 2015). Prolonged periods of soil saturation can create anoxic conditions allowing fluctuations in depth to groundwater and hydroperiods in wetlands to be important factors that can enhance or inhibit denitrification (Burgin et al. 2010). Fluctuation of these driving factors and process requirements may prevent the reduction of certain forms of nitrogen (Seitzinger et al. 2006; Rivett et al. 2008). Inhibition of certain steps may cause intermediate products to become terminal products of denitrification (Jurado et al. 2017). Other factors, such as low pH (< 5.0 pH), can inhibit complete denitrification as well, preventing the reduction of N_2O to N_2 (Nagele and Conrad 1990; Simek and Cooper 2002; Qu et al. 2014). An overabundance of nitrate in the system may provide microbial communities with an ample supply of energy efficient electron acceptors, stopping denitrification process at intermediate products, such as N_2O (Woodward et al. 2009; Jurado et al. 2017).

Presence of these driving factors can cause increased denitrification rates spatially, “hot spots”, or temporally, “hot moments”. These hot spots and hot moments can be largely controlled by hydrological flow paths (Senbayram et al. 2011; Musolff et al. 2016; Mcclain et al. 2018). Hydrological connectivity and increased groundwater residence times can increase the extent of denitrification (Welsh et al. 2017). The presence, or lack thereof, of these environmental reactants and ideal conditions make denitrification a spatially and temporally heterogenous biogeochemical reaction. However, some landscapes and ecosystems have been shown to be continuous hot spots of denitrification.

Denitrification in Riparian Groundwater

Numerous studies have focused on denitrification in groundwater (Groffman et al. 1998; Smith et al. 2004; Böhlke et al. 2002; Tesoriero et al. 2000; McAleer et al.

2017) especially along shallow groundwater flow paths in riparian zones (Weller et al. 1994; Hill et al. 2000; Jahangir et al. 2013; Anderson, Groffman, et al. 2014). Riparian zones are biogeochemical hotspots for several process, including denitrification due to hydrogeological and microbiological factors. Shallow groundwater can be a large conduit for nitrate attenuation through the denitrification process due to anoxic conditions established by saturation, relative proximity to organic-rich surface soils, and high mobility of nitrate in water (Simmons et al. 1992). A study of denitrification in the shallow groundwater of an Upper Coastal Plain riparian forest in southern GA showed little evidence of denitrification potential in the saturated zone unless the water table was within 60 cm of the surface, attributing vegetative uptake as the main mode of nitrate attenuation (Lowrance 1992). Recent studies, however, have provided ample evidence that shallow groundwater is a hot spot for denitrification depending on the level of environmental controlling factors of the process present along groundwater flow paths (Jahangir et al. 2017; Anderson et al. 2014; Anderson et al. 2014; McAleer et al. 2017). Studies of denitrification in the shallow groundwater system have been largely focused on agriculture-dominated watersheds and mitigation of nitrogen additions from fertilizer (Tesoriero et al. 2000; Rivett et al. 2008). Within the Chesapeake Bay watershed, an area widely affected by agriculture non-point source nitrate pollution, several studies have observed denitrification in groundwater as an important nitrate attenuation pathway (Fox et al. 2014).

A review of groundwater denitrification studies by Seitzinger et al. (2006) reported nitrate attenuation from 0-100% in groundwater, emphasizing that the microbial process varies depending on site-specific characteristics. Hydrological connectivity is a key component on the ability of riparian areas and groundwater systems to be hotspots of denitrification (Welsh et al. 2017). Rates of denitrification are dependent on the supply of NO_3^- and DOC (or other electron donors) along groundwater flow paths (Hill et al. 2000). The proximity of shallow groundwaters to the unsaturated-saturated zone interface allows for potentially high flux rates of these required factors and an anaerobic environment for denitrification to occur (Simmons et al. 1992). The presence of microbial communities is necessary and usually ubiquitous in the environment, but the abundance and diversity of the microbial community has been shown to not be as important as the site specific hydrological and biogeochemical factors (Boyer et al 2006; Wallenstein et al. 2006). Ye et al. (2017) concluded that hydrologic pulsing had a large influence on denitrification rates in a revegetated riparian zone rather than the structure of microbial communities, with the highest rates occurring in re-flooded soils underlying bald cypress and willow.

Denitrification in the Hyporheic Zone

Shallow groundwater pathways include the hyporheic zone of streams (Merill and Tonjes 2014). Defined as the saturated subsurface zone of water exchange (upwelling and downwelling) directly below and adjacent to surface waters, hyporheic zones have the potential for being hotspots for denitrification due increased DOC, lower DO concentrations, and slower flow rates compared to the stream system (Harvey et al. 2013; Merill and Tonjes 2014). The hyporheic zone is the interface of constant exchange; exchange of water, DO, organic matter, solutes (such as nitrate) that can occur vertically and laterally (Findlay 1995; Quick et al. 2016). This constant exchange allows for the hyporheic zone to be a biologically diverse environment (Merill and Tonjes 2014). The rate of exchange is dictated by stream geomorphology (streambed roughness, topography, slope), stream flow, hydraulic conductivity of streambed sediment, and hydraulic head of groundwater (Harvey et al. 2013; Quick et al. 2016; Reeder et al. 2018). These controls on hyporheic exchange change spatially and temporally (Quick et al. 2016) affecting the redox reactions such as denitrification occurring in the system (Merill and Tonjes 2014). The rate of hyporheic exchange is inversely related to residence time; faster hyporheic flow, shorter residence times and slower hyporheic flow, longer residence times (Harvey et al. 2013). Residence time is an important control on the denitrification process (Reeder et al. 2018). Flow paths that have shorter residence times in the hyporheic zone have observed higher rates of denitrification due to the constant supply of nitrate and DOC substrates (Harvey et al. 2013). More recent studies have shown that residence times also control the extent of denitrification with intermediate residence times predominantly emitting N_2O from denitrification (Quick et al. 2016; Reeder et al. 2018). Longer residence times were able to further reduce N_2O to N_2 (Reeder et al. 2018).

Methods of Measuring Denitrification

Researchers have been trying to understand the complex process of denitrification since the late 19th century (Payne 1981) with increasing interest in the past 20-30 years due to the increased anthropogenic inputs to the nitrogen cycle and the nitrate mitigating principles of the process (Groffman et al. 2006). As important of a process as denitrification is to the nitrogen cycle, it is one of the most difficult biological processes to measure because dinitrogen, the final end product of denitrification, comprises 78% of the Earth's atmosphere (Groffman et al. 2006). Several methods have been developed to estimate the extent of this microbial process by measuring the gaseous nitrogen products in different environments, all with advantages and disadvantages. An early and commonly used method that was developed to circumvent the atmospheric contamination of samples was introducing an acetylene solution (C_2H_2) to the media being sampled (Pinay, Roques, and Fabre 1993; Groffman et al. 2006). Acetylene solution inhibits the final step in the denitrification process allowing the intermediate product, N_2O, to be the final end product (Groffman et al. 2006). Although widely used (Groffman and Tiedje 1989), a disadvantage of this method is that it also inhibits nitrification, therefore stopping any further production of nitrate and underestimating denitrification in the process (Groffman et al. 2006).

Estimating denitrification through denitrification enzyme assays (DEA) can be coupled with the acetylene inhibition method. This method is really estimating the potential, or maximum amount, of denitrification that can occur at a given sampling point (Groffman et al. 2006). Several studies have used this method with laboratory soil slurries from riparian soils (Burt et al. 1999), sediments from streams (Bernard-Jannin et al. 2017), and lake and marine sediments (Seitzinger et al. 2006). During this laboratory experimental process, known amounts of nitrate are introduced to samples from a given environment while controlling environmental factors such as carbon content, pH, dissolved oxygen, and temperature. These lab-controlled conditions provide an ideal environment for microbes to thrive allowing the organisms to use as much nitrate for respiration as possible. The observations measured provide amounts of how much denitrification can potentially occur under optimal and desired conditions.

One method that more accurately assesses denitrification rates in the groundwater system is known as the push-pull method (Groffman et al. 2006). In this method, a prepared nitrate-enriched solution with a distinct isotopic signature, usually ^{15}N, is injected into a borehole (pushed) and then samples are collected at different time intervals (pull) (Groffman et al. 2006; Anderson et al. 2014). Dissolved gases concentrations produced in the collected groundwater with the same isotopic signature ($^{15}N_2$ and $^{15}N_2O$) are used to calculate denitrification rates (Addy et al. 2002). The *in situ* factor and known interval of the push-pull method have allowed it to gain much support and use in recent years (Addy et al. 2002; Woodward et al. 2009; Jahangir et al. 2017; Anderson, Groffman, et al. 2014). This method, however, is still assessing denitrification potential rates due to the created nitrate-enriched solution being introduced to the natural system.

Due to advances in sampling equipment and analysis instrumentation, a method for measuring *in situ* end products of denitrification in closed systems such as groundwater, has become more prominent in recent years (Groffman et al. 2006). One such method uses the known atmospheric ratio of dinitrogen and argon to directly measure excess N_2 in groundwater. The concept of excess air in groundwater was first proposed by Vogel et al. (1981) and attributed higher N_2 concentrations in groundwater than expected concentrations in water equilibrated with air to the biogenic process of denitrification. The development of membrane inlet mass spectrometry (MIMS) which measures gas ratios to obtain dissolved gas concentrations at an increased analysis accuracy and precision has allowed this method to be more advantageous (Kana et al 1994). Dissolved gas analysis using MIMS has become more frequently used (Singleton et al. 2007; Heiss, Fields, and Fulweiler 2012; Jahangir et al. 2013; Fox et al. 2014) because it requires only a small amount of sample water (<10 mL), short processing times (~30 samples/hour), and precision is (±0.03%) (Kana et al. 1994). Temperature dictates gas solubility (Weiss 1970) so for any dissolved gas analysis the temperature of the water must be known. In groundwater systems, temperature at the interface of the vadose zone and water table can be used as the recharge temperature (Jahangir et al 2013).

Differences within similar methodologies exist making comparisons between studies somewhat difficult. For instance, studies using the N_2:Ar method have used measured Ar concentrations to determine recharge temperature of sampled groundwater (Fox et al. 2014), the temperature at the unsaturated-saturated zone interface (Jahangir et al. 2013), or mean groundwater temperature (Weymann et al. 2008). Different groundwater sampling equipment and protocols can vary between studies, creating subtle differences in measured and calculated denitrification parameters.

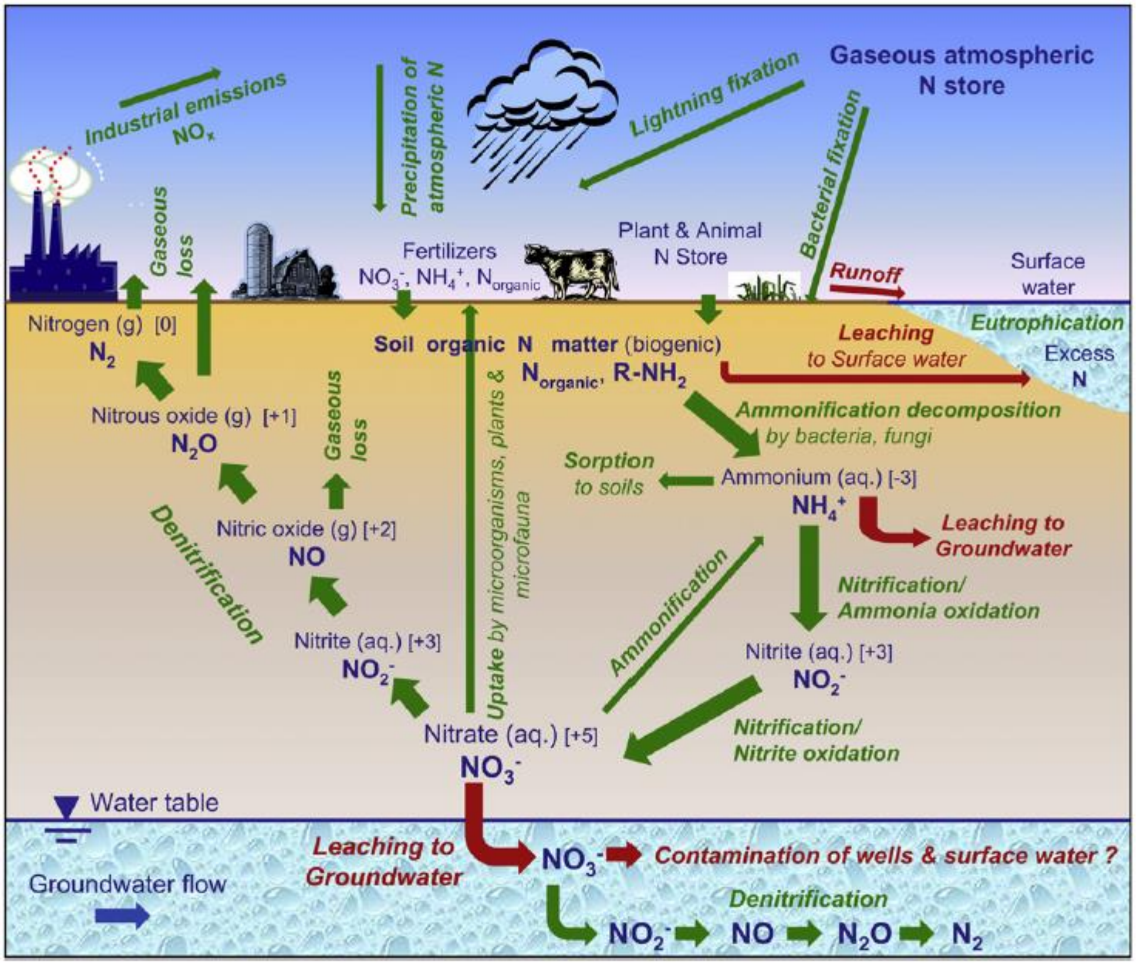

Figure 2.1: Overview of the nitrogen cycle and connections to surface and groundwater systems (Rivett et al. 2008)

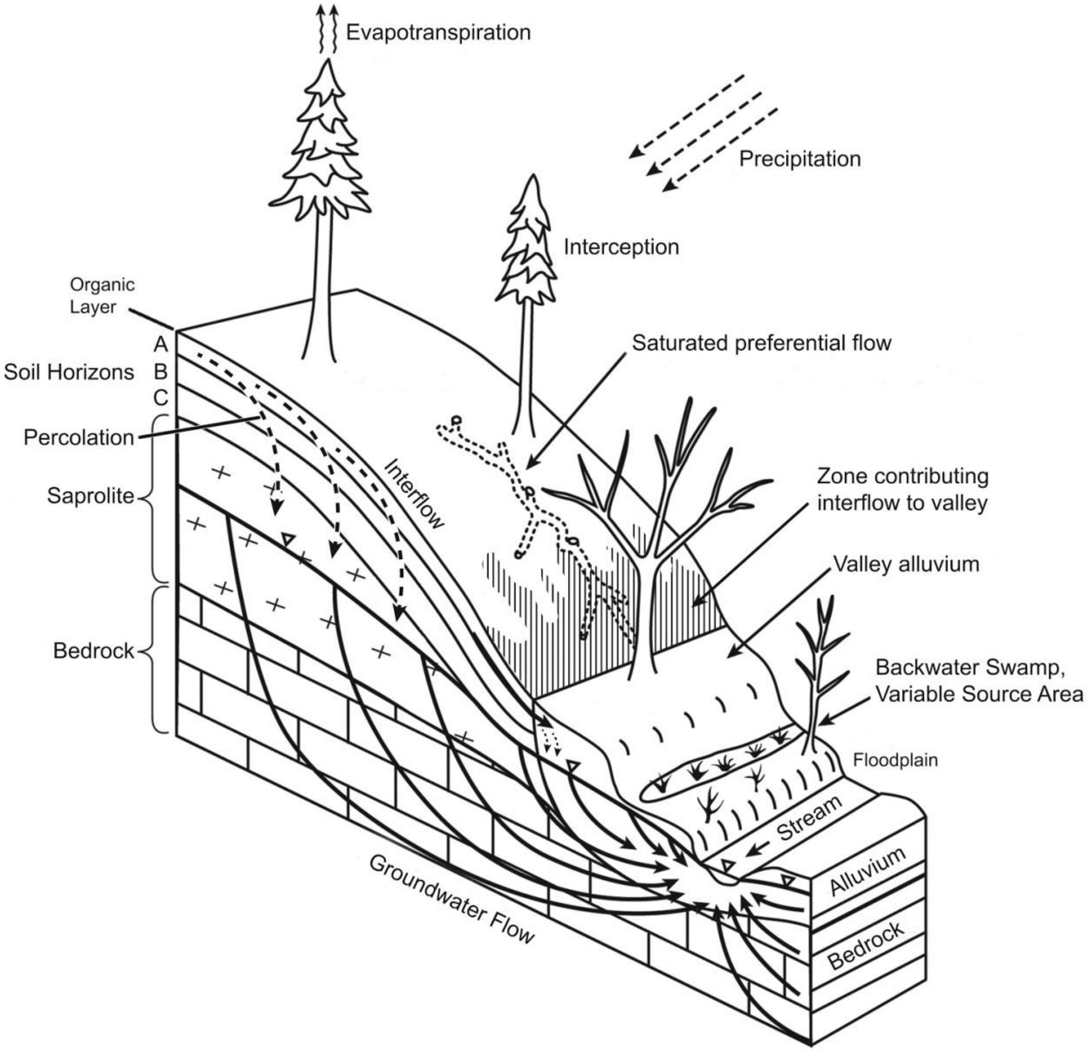

Figure 2.2: Hydrological processes and flow paths that determine fate of solutes, such as nitrate, in forested watersheds (Jackson et al. 2014).

Table 2.1: Sequential order of microbial use of a variety of electron acceptors and measured ORP (v) of reactions. Energy obtained drops as you move down the list (from Jackson et al. 2014; adopted from Bohn et al. 1985).

Reaction	Electrode Potential, pH 7 (v)	Measured Redox Potential in Soils (v)
$\frac{1}{2} O_2 + 2e^- + 2H^+ \leftrightarrow H_2O$	0.82	0.6 to 0.4
$NO_3^- + 2e^- + 2H^+ \leftrightarrow NO_2^- + H_2O$	0.54	0.5 to 0.2
$MnO_2 + 2e^- + 4H^+ \leftrightarrow Mn^{2+} + 2H_2O$	0.4	0.4 to 0.2
$FeOOH + e^- + 3H^+ \leftrightarrow Fe^{2+} + 2H_2O$	0.17	0.3 to 0.1
$SO_4^{2-} + 6e^- + 9H^+ \leftrightarrow HS^- + 4H_2O$	–0.16	0 to –0.15
$H^+ + e^- \leftrightarrow \frac{1}{2} H_2$	–0.41	–0.15 to –0.22
$(CH_2O)_n \leftrightarrow {}^n/_2\, CO_2 + {}^n/_2\, CH_4$	—	–0.15 to –0.22

CHAPTER 3

METHODS

Site Description

The Savannah River Site (SRS) is an 80,267-ha Department of Energy (DOE) Site located in the Upper Atlantic Coastal Plain southeast of Aiken, South Carolina (Figure 3.1). Its namesake, the Savannah River, borders the site on the western edge. Row-crop agriculture was the primary land use prior to the site's construction in 1951 as a nuclear materials development and management site that focused on producing tritium at its onset (Wyatt and Harris 2004; Kilgo and Blake 2005). In the 1970's the SRS was established as the first National Environmental Research Park by the DOE (Wyatt and Harris 2004). After decades of reforestation and environmental restoration, SRS is now approximately 90% forested with several species of pine covering the upland regions (loblolly, slash, and longleaf pine) and mixed hardwoods (sweetgum, ash, American holly) dominating the lowland and riparian areas. Well-drained, sandy soils overlaying an impeding argillic layer dominate the soil profiles of much of the SRS upland landscape. Riparian soils are organic-rich sandy soils (Soil Conservation Service 1990; Kilgo and Blake 2005) . Average temperatures (18^{o}C) and precipitation (1225mm) reflect the humid, subtropical climate (Kilgo and Blake 2005). These unique soils, vegetation, and climate of the Upper Coastal Plain create dynamic hydrological connections within the forested watersheds. Headwater streams are tannic and intermittently flowing with low gradient, sandy channels in broad riparian valleys.

Hydrologic Dynamics and Nitrogen Transport in the Fourmile Watershed

A continuous eight-year study investigating the environmental effects of short-rotation pine management for the production of bioenergy within the Fourmile Watershed at the SRS has monitored nutrient concentrations in deep and shallow groundwater, interflow, riparian groundwater, and stream flows (Griffiths et al. 2017). Two experimental watersheds (B and C) were clear cut and planted loblolly pine (*Pinus taeda*) seedlings were managed as SRWC. A reference watershed (R) was left unmanaged (Figure 3.2). Detailed experimental design is described in Griffiths et al. (2017), but fertilizer applications are displayed in Table 3.1. Forestry BMPs were followed throughout the silvicultural process in accordance with South Carolina Forestry Commission, including SMZ width of at least 12.3m (50 ft) (South Carolina Forestry Commission 1998).

A spike in nitrate concentrations ($\Delta NO_3^- \sim 0.5$ mg L^{-1}) was observed in shallow groundwater in watersheds B and C after the initial harvest and site prep (2012) and a larger increase ($\Delta NO_3^- \sim 1$ mg L^{-1}) occurring after planting and initial fertilizer application (2013) (Griffiths et al. 2017). This nitrate concentration signal stabilized in shallow groundwater but was not seen in riparian groundwater or stream flows. Evidence of nitrate leaching into the subsurface has been observed from 2013-2016, declining each year with only 10% of applied nitrogen leaching in to the subsurface in 2016 (Rau, unpublished data). Nitrate is mobile in soil and can leach into groundwater or be transported to surface waters by lateral subsurface flow (interflow) from hillslopes. In some forested watersheds, interflow is the main hydrologic component contributing to streamflow. Previous research within the Fourmile Watershed on hillslope hydrological processes has shown that groundwater flow in the surficial aquifer is the main hydrologic pathway contributing to stream flow (Klaus et al. 2015; Du et al. 2016; Griffiths et al. 2016) . A conservative tracer-based model showed that increased nitrate concentration signal should have already reached the stream during the monitoring period with upland locations having an estimated groundwater travel times between 8-13 years and near-stream locations having travel times of 1-3 years (Vache, unpublished data). Nitrogen and oxygen isotopic analysis has confirmed these findings and established that riparian denitrification may be the sink for the missing N in the watershed (Klaus et al. 2015; Griffiths et al. 2016).

Study Site

The study site is located within a sub-catchment of watershed C (Unit 4) where clearcutting and planting of loblolly pine to be managed as a SRWC occurred (Figure 3.3). SMZs were left unmanaged similar to the other experimental units with SMZ averaging 25 meters from an intermittent, low-gradient stream with an undefined channel, typical of streams found throughout the SRS and the Upper Atlantic Coastal Plain. The main soil series found in the study area are the Fuquay sand, Vauclase – Ailey complex, and Pickney series. Both the Fuquay sand and the Vauclase – Ailey complex are well-drained sandy soils that include an impeding yet permeable argillic (clay) layer, with a fragipan horizon (Btx) in the Vauclase – Ailey complex between 50 and 140 cm from the surface. The Pickney series are poorly-drained, extremely permeable sands mainly found along the floodplains of streams (Soil Conservation Service 1990). Slopes in the sub-catchment from the uplands to the stream can be characterized as low-grade, convex slopes (Figure 3.4). A swale drains the uplands into the riparian zone of an intermittent stream of the Fourmile Branch system.

Piezometer Network

A network of 21 piezometers was established within Unit 4, a sub-catchment of watershed C in the Fourmile Experimental Watershed at the Savannah River Site. Seventeen piezometer locations were identified and installed along transects of potential groundwater flow paths. Transect locations included a hillslope within a 3.5-year old short-rotation pine stand, a drainage swale, the boundary between the pine stand and SMZ, a forested hillslope within the SMZ, and the hyporheic zone of an intermittent stream (Figure 3.5). Due to an argillic soil horizon commonly found within the Fourmile Branch Watershed, pairs of nested wells were installed at four hillslope locations to identify a potential perched water table above the clay layer. Nested piezometers were located at W03 & W02 along a hillslope within the pine plantation and W11 & W12 along the forested hillslope within the SMZ (Figure 3.6). Piezometers were installed based on modeled depth to groundwater (Figure 3.7). All piezometers were made of 5.08 cm diameter polyvinyl chloride (PVC). Deep piezometers (4 m) included a 1.5 m slotted screen attached to two 1.5 m risers.

Shallow piezometers (1.5 m) consisted of a 0.6 m screen attached to a 1.5 m riser. All piezometer risers were trimmed and varied in height above the soil surface. Removable caps above ground and conical pointed caps below ground sealed the piezometers. Construction-grade filtered sand was used to secure each piezometer into each hand-augured hole and bentonite was used in the top 20 cm to seal the hole from surface flow and contamination. Piezometers were installed in October 2016 and sampling began in March 2017.

Sampling Protocols

Piezometers were sampled for a year at monthly intervals. Prior to each sampling event (24-48 hours), piezometers were purged with a Teflon bailer completely or until recharge rate was equal to purging rate to allow for fresh groundwater recharge in the piezometer and ensure a representative groundwater sample was collected during sampling. Depth to groundwater was recorded for each piezometer prior to purging.

For each sampling event, depth to groundwater was recorded and a series of three different groundwater samples were collected at each piezometer. Groundwater samples to be analyzed for dissolved gases (N_2, Ar, and N_2O) were collected using a peristaltic pump (GeoTech Geopump) and 6 mm silicone tubing (Geotech) at a pumping rate of 90 ml/minute to minimize any ebullition (degassing). Groundwater samples to be analyzed for N_2 and Ar concentrations were collected in 12 mL exetainers by overflowing 12 mL of groundwater, adding 0.2 ml of a 50% $ZnCl_2$ solution (as a preservative), and capping with no headspace.

The sample was inverted in a 50-mL centrifuge tube and stored on ice. A second groundwater sample was collected for nitrous oxide concentrations using a 160-mL glass serum bottle. The silicone tubing was placed at the bottom of the serum bottle and 160-mL of groundwater was overflowed. The glass serum bottle was capped with no headspace using a butyl rubber septa and aluminum crimp caps. Each sample was placed on ice immediately and stored until sample processing. Once both dissolved gas samples were collected, the peristaltic pump was put on the highest speed to collect a groundwater sample for nutrient concentrations in a 500-mL high-density polyethylene (HDPE) bottle (Nalgene). Each sample was immediately placed and stored on ice until sample filtering and processing. Water chemistry parameters including temperature, pH, specific conductivity, dissolved oxygen (DO), and oxidation-reduction potential (ORP) were measured using an *in situ* handheld probe (YSI Quadrocapble ProPlus).

Sampling Processing

Samples that required pre-analysis processing included N_2O samples collected in serum bottles and nutrient samples collected in 500-mL HDPE bottles. Groundwater samples to be analyzed for N_2 and Ar did not require additional processing and were refrigerated between 4-7^{o}C until shipped to a laboratory for analysis (Cary Institute for Ecosystem Studies, Millbrook, NY). Samples collected to be analyzed for N_2O and nutrients were stored on ice and processed the following day after each sampling event prior to analysis. A headspace equilibration technique was used to create an inert headspace (3:1, He:water) and extract a representative headspace sample from the serum bottles to be analyzed for N_2O (Jahangir et al. 2012). Two-inch hypodermic needles were inserted into the serum bottle through the rubber septa. Stopcocks and silicone tubing were attached to each needle. Helium (He) was injected into the serum bottle at 15-20 pounds per square inch (PSI) through one needle-and-tubing apparatus. Sample water from the serum bottle flowed though the other needle-and-tubing apparatus into a graduated cylinder. Once a headspace was created, serum bottles were placed on a horizontal shaker (140 oscillations per minute) for 13 minutes. After shaking, each bottle was removed from the shaker and left to stand at room temperature for 63 minutes. A 15-mL sample of headspace was extracted using a 20-mL syringe and injected into a 12-mL evacuated exetainer. Headspace samples were stored at room temperature until shipment to an analytical lab for analysis (Cary Research Institute for Ecosystem Studies, Millbrook, NY).

Processing for nutrient samples included vacuum filtration (0.7 μm filter pore size; 45 mm GF/F GE Healthcare Whatman) and sample preservation (method dependent on type of sample). Three different water samples were taken from the 500-mL groundwater chemistry sample collected from each piezometer during each sampling event. A 60-mL unfiltered sample was collected in a HDPE bottle (Nalgene) to be analyzed for total nitrogen (TN). Filtered samples included a 60-mL sample for nitrate and ammonium (HDPE bottle; Nalgene) and a 40-mL sample for dissolved organic carbon (DOC) in an amber glass vial that was preserved with 0.1 mL (2 drops) of 6N hydrochloric acid (HCl). Samples for nitrogen concentrations (NO_3^-,

NH_4^+, and TN) were frozen (-20°C) until analysis while dissolved organic carbon samples were refrigerated (4°C) until analysis. All samples were transported to Oak Ridge National Laboratory (Oak Ridge, TN) for analysis.

Sample Analysis

Groundwater samples to be analyzed for dissolved gas concentrations (N_2, Ar, O_2, and N_2O) were sent, in bulk (per 3 sampling events), to the Groffman Lab at the Cary Institute of Ecosystem Studies in Millbrook, NY. Membrane inlet mass spectrometer (MIMS) was used to analyze samples for N_2, Ar, and O_2. Water baths were used to hold atmosphere-equilibrated water (AEW) standards at temperatures similar to groundwater temperature at time of sampling (+/- 2°C). AEW standards were run prior to any sample analysis and between every 3-4 samples. Triplicate sub-samples were analyzed from each groundwater sample collected.

Headspace samples were analyzed using gas chromatography. A series of six standards of varying concentration (0.25 – 75 ppm) were analyzed before and after all sample analysis to correct for instrument drift and calculate concentrations of samples. Triplicate subsamples from each headspace sample were analyzed. Trip standards were used to calculate any leakage during sample storage and shipping and backup samples were collected from each headspace (analyzed at UGA Marine Science Lab) in case of any damage during shipping.

All nutrient samples were analyzed at Oak Ridge National Laboratory in Oak Ridge, Tennessee. The unfiltered TN samples were analyzed using a combustion oxidation and chemiluminescence detection method (Shimadzu TOC-L CHS/CSN analyzer). Cadmium reduction method (SEAL Analytical AA3 autoanalyzer) was used to analyze filtered samples for nitrate concentrations. Ammonium concentrations from filtered samples were analyzed using the phenol hypochlorite method (SEAL Analytical AA3 autoanalyzer). All nitrogen concentrations results were reported in μg-N L^{-1} and converted to mg-N L^{-1}. Preserved filtered samples were analyzed for DOC concentrations using the high-temperature combustion catalytic oxidation method on a Shimadzu TOC-L CSH/CSN analyzer. Results were reported in mg L^{-1} of DOC.

Calculations

Mass signals for N_2 (28 m/z), Ar (40 m/z), and O_2 (32 m/z) were compared to provide gas ratios of N_2:Ar and O_2:Ar. Using gas solubility coefficients (Weiss 1970) and AEW standard temperatures, expected concentrations of N_2 and Ar along with N_2:Ar ratios were calculated. Linear regression between AEW standards of same temperatures was used to correct for the instrument drift. Drift-corrected ratios of the standards were compared to expected N_2:Ar ratio at AEW standard temperatures to calculate a calibration factor for Ar concentrations (Fox et al. 2014). Dissolved dinitrogen in excess of AEW at the sampling temperature was assumed to be from the biological process of denitrification occurring in the groundwater.

Excess N_2 was calculated using the below equation originally derived by Weymann et al. (2008) and used in previous *in situ* denitrification studies (Jahangir et al. 2013; McAleer et al. 2017):

$$X_{\text{EXCESS } N_2} = X_{\text{TOTAL } N_2} - X_{\text{EA } N_2} - X_{\text{EQ } N_2} \qquad \text{(Eq. 3.1)}$$

where X is the molar concentration of different dissolved N_2 components. $X_{TOTAL\ N_2}$ is the total amount of N_2 measured in the sample. $X_{EA\ N_2}$ is N2 from dissolution of excess air (explained below). $X_{EQ\ N_2}$ represents the amount of N_2 in equilibrium with the atmosphere.

Excess nitrogen ($X_{EA\ N_2}$) and argon can be dissolved into infiltrating water through entrapment and complete or partial dissolution of gas bubbles in the unsaturated zone (Heaton and Vogel 1981). This can be estimated by the known N2:Ar ratio in the atmosphere, total measured Ar concentrations in the water sample ($X_{TOTAL\ Ar}$), and the measured Ar concentrations of the water equilibrated with the atmosphere at the sampling temperature ($X_{EQ\ Ar}$) (Weymann, Well, Flessa, von der Heide, et al. 2008).

$$X_{EA\ N_2} = (X_{TOTAL\ Ar} - X_{EQ\ Ar}) * (X_{ATM\ N2} / X_{ATM\ Ar}) \qquad \text{(Eq. 3.2)}$$

Uncertainty within the calculation of the excess-nitrogen was determined by the calculation below:

$$U_{EA\ N_2} = (X_{TOTAL\ Ar} - X_{EQ\ Ar}) * (X_{ATM\ N_2} / X_{ATM\ Ar} - X_{EQ\ N_2} / X_{EQ\ Ar}) \qquad \text{(Eq. 3.3)}$$

Previous *in situ* denitrification studies have used measured end products of denitrification (N_2 and N_2O) and other forms of nitrogen in the groundwater to estimate initial nitrogen concentrations in the groundwater:

$$N_{INTIAL} = NO_3^-\text{-N} + NH_4^+\text{-N} + \text{EXCESS } N_2\text{-N} + N_2O\text{-N} \qquad \text{(Eq. 3.4)}$$

Due to different nitrogen analysis methods, either TN or (NO_3^--N + NH_4^+-N) was used depending on which value was greater in order to overestimate residual nitrogen ($N_{INITIAL}$) at each sampling point. This overestimation leads to an underestimation of the progress of the denitrification reaction, known as reaction progress (RP). This can be estimated by comparing the intermediate and final end products of denitrification to the estimated initial nitrogen concentrations (Weymann, Well, Flessa, von der Heide, et al. 2008; Jahangir et al. 2013; McAleer et al. 2017):

$$RP = (\text{EXCESS } N_2\text{-N} + N_2O\text{-N}) / N_{\text{INITIAL}} \qquad \text{(Eq. 3.5)}$$

The amount of incomplete denitrification has been estimated using a ratio of the intermediate product N_2O compared to the sum of all end products of denitrification. In this study we will use the sum of N_2O and N_2 as the total end products of denitrification.

$$N_2O\text{-N} / N_2O\text{-N} + \text{Excess } N_2\text{-N} \qquad \text{(Eq. 3.6)}$$

Some studies have referred to this as the molar ratio of N_2O or N_2O yield and has been used to calculate indirect emissions of N_2O in groundwater (Weymann et al. 2008; Jahangir et al. 2013; McAleer et al. 2017).

Statistical Analysis

Differences in nutrient, dissolved gas, and denitrification data were analyzed by landscape locations and by sampling event (month). The Shapiro-Wilks test was used to test for violation of normality. All data violated the assumption of normality, so a non-parametric statistical analysis was used. Kruskal-Wallis H test was used to compare ranked sums of data between landscape locations. Kruskal-Wallis H test was also used to test for seasonality of dissolved gas, nutrient, and denitrification parameters. Statistically significant differences were further analyzed using the

Dunn test. A Spearman correlation analysis was used to analyze relationships between water chemistry parameters, nutrient concentrations, dissolved gas concentrations, denitrification measurements, landscape characteristics (elevation), and hydrological characteristics (depth to groundwater and groundwater fluctuations). A principal component analysis (PCA) was performed to further investigate variations and correlations of driving factors of denitrification and denitrification parameters between landscape locations. A Spearman correlation was conducted between the principal components from the PCA of driving factors of denitrification and denitrification parameters. All statistical analysis was conducted in R statistical software.

Table 3.1: Fertilizer annual applications and application rates expressed as applied nitrogen for the C watershed in the Fourmile Watershed at the Savannah River Site. Total rate of application and total applied fertilizer values are cumulative.

Year	Type of Fertilizer	Rate of Application (kg N/ha)	Total Rate of Application (kg N/ha)	Total Applied Fertilizer (kg N)
2013	diammonium phospate	50.6	--	3,170
2014	urea	111	162	10,120
2015	blended urea; diammonium phosphate	107	269	16,800
2016	urea	196	465	29,100

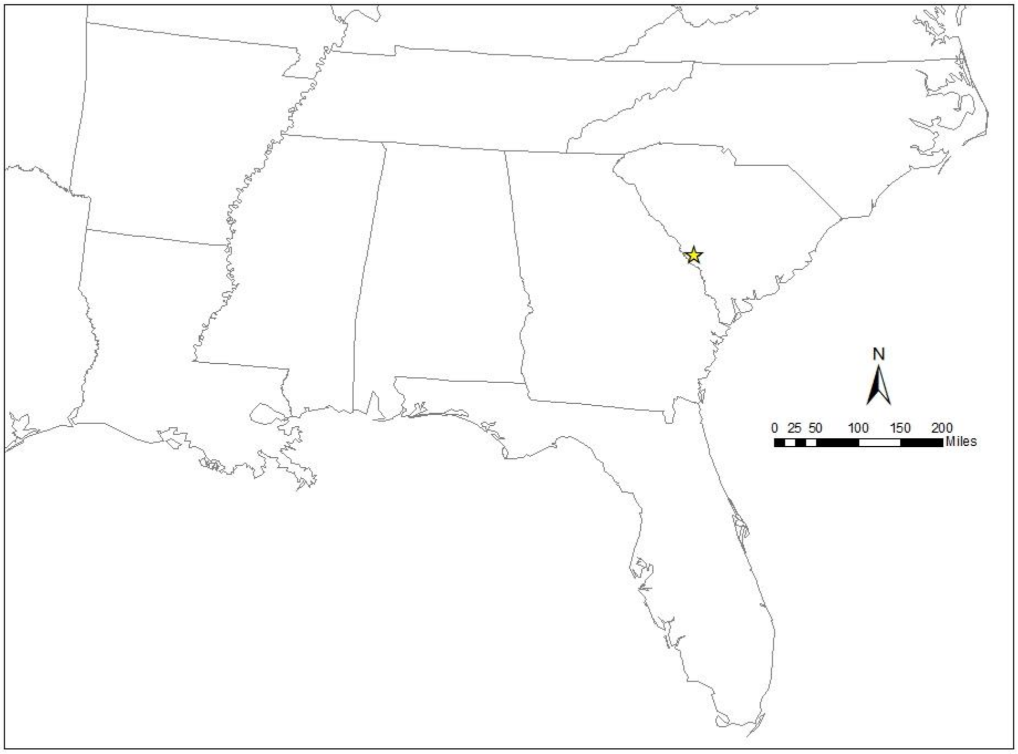

Figure 3.1: Location of the Savannah River Site (SRS), marked by the yellow star, in the Upper Coastal Plain of South Carolina.

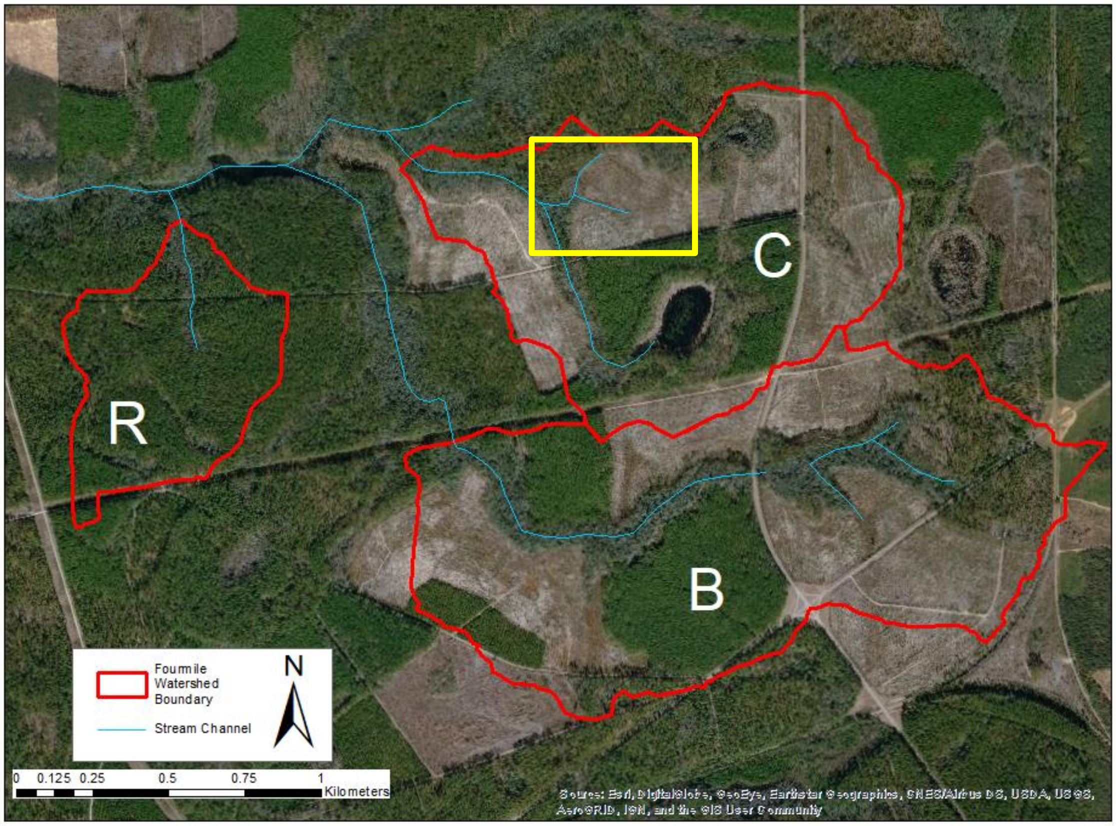

Figure 3.2: Location of the Fourmile Watershed at SRS and the three experimental watersheds (R, B, C) from Griffiths et al. (2017) used to investigate the environmental effects of silviculture practices of short-rotation pine management. The denitrification study site is located in C watershed denoted by the yellow box.

Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community

Figure 3.3: Piezometer network within sub-catchment Unit 4

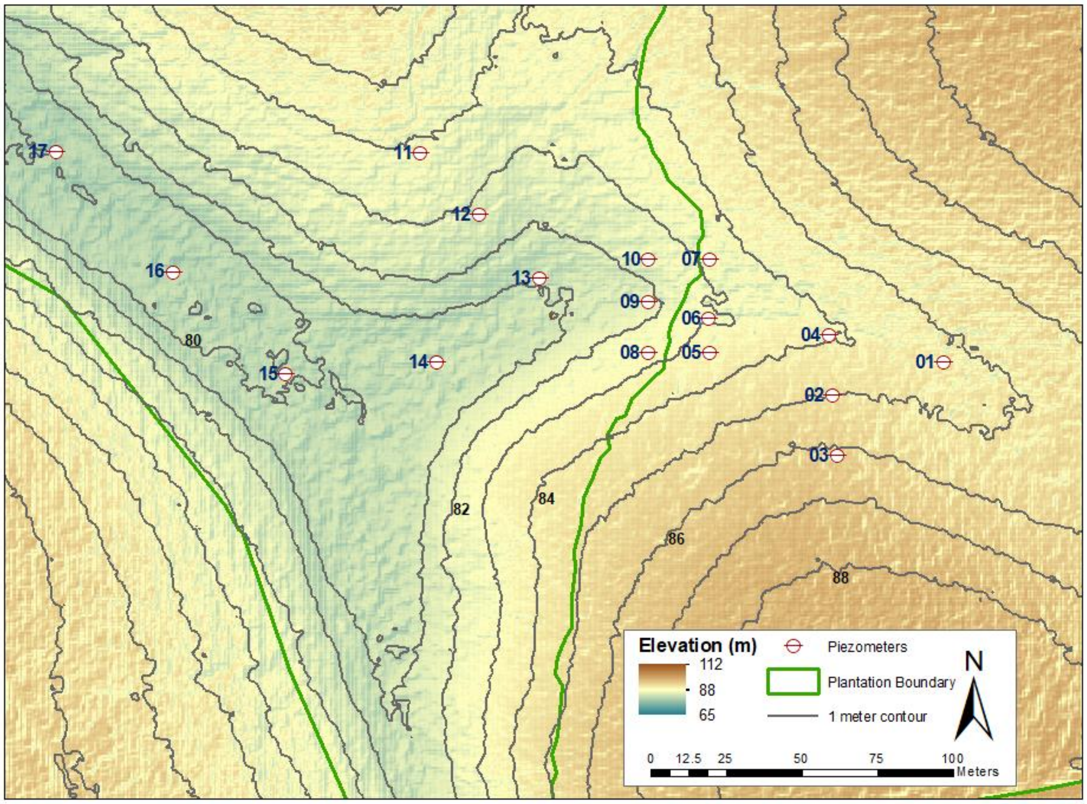

Figure 3.4: Elevation of study site and piezometers within sub-catchment Unit 4 of C watershed.

Figure 3.5: Examples of piezometers located throughout the study site: (a) W06 located in the swale at the edge of the SMZ (a deeper well was installed for the second year of sampling), (b) W09 located in the middle of the swale entering the SMZ, (c) W13 located in the valley of the SMZ, (d) W16 located in the hyporheic zone of an intermittent stream in the SMZ.

Figure 3.6: Examples of nested wells installed to monitor the establishment of a perched water table above an argillic layer commonly found in the Fourmile Watershed: (a) W03 & W03D located on the hillslope of the short rotation pine plantation, and (b) W11 & W11D located on the forested hillslope within the SMZ.

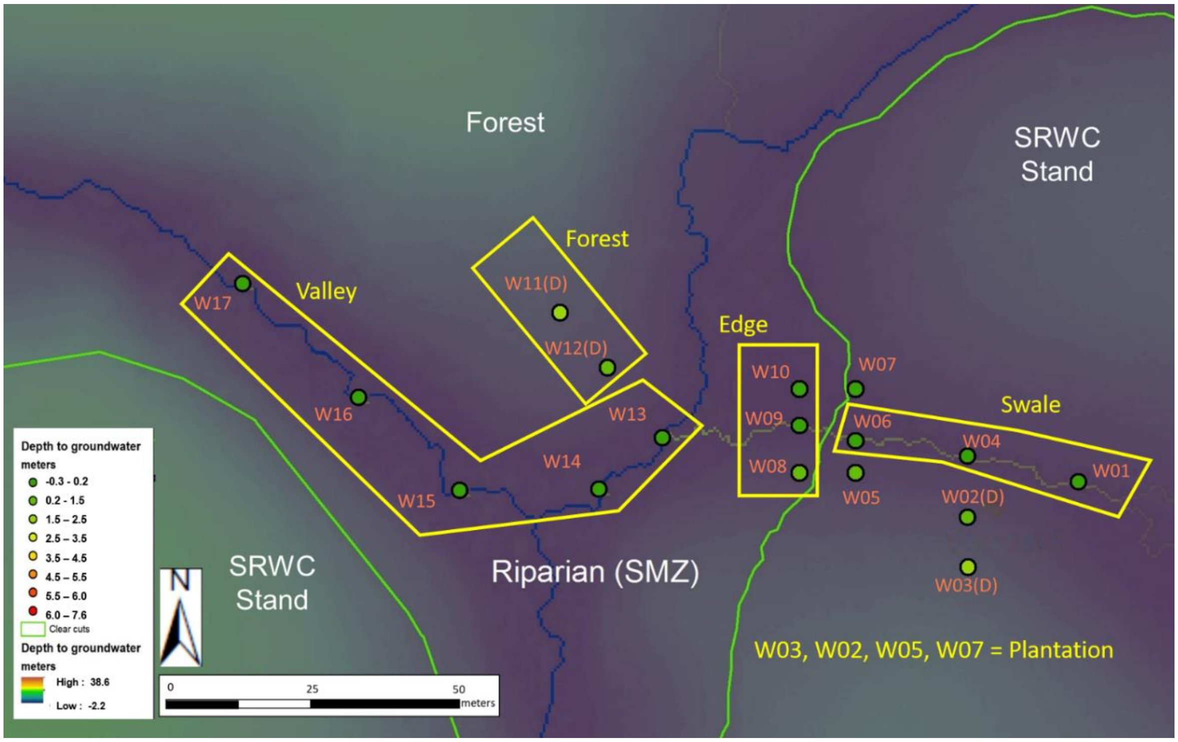

Figure 3.7: Map of modeled depth to groundwater (DTG) with piezometers grouped based on landscape position

CHAPTER 4

RESULTS

Depth to groundwater

Seasonal drops in groundwater levels resulted in fewer collected samples at some piezometers (Table 4.1). Sampling was temporarily suspended during a period of low water tables (August 2017) but resumed the following month (September 2017). All the wells in the valley of the SMZ contained water throughout the study period. DTG was deepest in the pine plantation (mean depth=2.66 m) but had similar mean fluctuations as the SMZ edge and forested hillslope (Figure 4.1). Valley fluctuations were not as drastic (0.02 m). The edge and swale had similar mean DTG at just over 1 m.

Nutrient Data

Nitrate concentrations varied significantly across landscape positions (χ^2 = 65.617, $p<0.001$, df=4). Landscape locations within the SMZ (valley and forest) had significantly different levels of NO_3^- than all other landscape positions. Median concentrations were lowest in the valley (0.00427 mg-N L^{-1}) compared to all other landscape positions (Figure 4.2). Highest median NO_3^- concentrations were observed in the pine plantation (8.56 mg-N L^{-1}) with groundwater along the swale and edge positions being relatively similar (3.53 mg-N L^{-1} and 3.48 mg-N L^{-1}, respectively). Observations over the US EPA standard of 10 mg-N L^{-1} were observed in piezometers in the pine plantation (10.5 mg-N L^{-1}) and the edge of the SMZ (16.7 mg-N L^{-1}). Over the course of the year, shallow groundwater NO_3^- varied monthly (χ^2=26.25, p=0.003, df=10). Concentrations in March and April (Spring) were significantly higher than Fall (September, October, November) and Winter (December, January, February) months. March and April did not differ significantly than the summer months (June and July).

Concentrations of ammonium varied significantly (χ^2=35.647, p<0.001, df=4) between the SMZ valley and all other landscape positions (Figure 4.2). Concentrations were higher along the SMZ valley than any other part of the sub-catchment. Unlike nitrate, there was no significant effect of seasonality on NH_4^+ concentrations during the sampling period across the sub-catchment (χ^2=15.746, p=0.12, df=10).

Total nitrogen concentrations in sampled groundwater followed a similar pattern to nitrate concentrations; there was a significant difference across landscape positions (χ^2=61.618, p<0.001, df=4) and there was a seasonality effect (χ^2=22.917, p=0.01, df=10). TN in groundwater samples from the upslope region of the catchment (pine plantation, swale, and edge) were higher than TN concentrations in the valley and forest (Figure 4.2). The valley and forest TN median concentrations, 0.17 mg L^{-1} and 0.11 mg L^{-1}, did not differ. The months of March and April had higher TN concentrations than other months during the sampling period.

Groundwater DOC varied between the valley (median = 3.31 mg L^{-1}) and the rest of the sub-catchment (χ^2=33.83, p<0.001, df=4). The pine plantation, swale, and edge of the SMZ all had similar median DOC concentrations, 1.40 mg L^{-1}, 1.40 mg L^{-1}, and 1.16 mg L^{-1} respectively, but varying ranges (Figure 4.2). The pine plantation (0.99 mg L^{-1} to 12.7 mg L^{-1}) and forested hillslope within the SMZ (0.78 mg L^{-1} to 18.59 mg L^{-1}) had a large range of DOC concentrations while the swale (0.74 mg L^{-1} to 2.3 mg L^{-1}) and edge (0.89 mg L^{-1} to 2.21 mg L^{-1}) positions had a more dampened range. There was no seasonality effect on DOC concentrations across the site (χ^2=5.13, p=0.88, df=10).

Excess N_2 and N_2O in shallow groundwater

The terminal end product of complete denitrification (excess-N_2) ranged from -1.32 mg-N L^{-1} to 3.40 mg-N L^{-1} across the entire study site. Negative values indicate estimated dissolved excess N_2 concentrations that are less than the expected N_2 concentrations in water equilibrated with the atmosphere at the recharge temperature. Several reasons for potential degassing are discussed later. In order to analyze results, only observations with positive excess N_2 concentrations were used in the analysis. Total number of observations at each landscape position along with median, minimum, and maximum values of dissolved N_2, N_2O, and denitrification parameters are provided in Table 4.2.

Excess-N_2 produced by denitrification varied significantly across landscape locations (χ^2=15.38, p=0.004, df=4). The swale had significantly more excess N_2 levels than the plantation and the SMZ valley. Compared to the valley, the edge of the SMZ had significantly higher excess N_2 groundwater concentrations. A maximum of 3.40 mg-N L^{-1} was observed in the swale with a site minimum of 0.03 mg-N L^{-1} measured in the pine plantation. Concentrations of excess-N_2 varied significantly month to month over the entire study site (χ^2=19.08, p=0.02, df=9).

Nitrous oxide concentrations were high across the site (Table 4.2). Levels of the intermediate product of denitrification varied across landscape positions (χ^2=50.27, $p<0.001$, df=4) with the valley and forested hillslope having significantly lower levels than the other landscape locations. Seasonality had an effect on nitrous oxide production in shallow groundwater across the study site (χ^2=25.93, p=0.002, df=10).

Denitrification Parameters

The sum of N_2O-N and N_2-N produced in shallow groundwater provides the total amount of reduced nitrogen from denitrification. Calculated denitrification end products followed similar patterns as of N_2O-N concentrations, significantly varying between the upslope locations (plantation, swale, and edge) and the SMZ valley (χ^2=44.24, $p<0.001$, df=4). The forested hillslope was significantly different from the plantation, swale, and edge, but not significantly different compared to the valley. Monthly concentrations were significantly higher in March and April compared to all other monthly sampling events. Nitrogen reduced by denitrification in the month of May was significantly more than September and November. The percentage of intermediate denitrification is represented by the ratio N_2O-N/(N_2O-N + N_2-N). The denitrification ratio followed the same gradient as total denitrification end products varying between the plantation, swale, and edge locations and the valley and forest (χ^2=42.87, df=4, p<0.001). Median denitrification end products and denitrification ratios based on landscape position are shown in Figure 4.3. The percentage of nitrogen reduced to N2O-N varied during the months of March and April compared to January and November (χ^2=17.50, p=0.04, df=9).

The extent of denitrification occurring within the sub-catchment was estimated using the reaction progress of denitrification (RP). There was no significant variation in RP over the course of the sampling period (χ^2=10.36, p=0.32, df=9). Median RP (Table 4.2) varied between landscape locations similar to other denitrification parameters (N_2O-N, end products, and ratio) with pine plantation locations differing from the valley and forest locations (χ^2=41.03, p<0.001, df=4).

Environmental factors

Observations of water chemistry parameters (temperature, pH, DO, specific conductivity, and ORP) were highly controlled by the availability of water present in the piezometers. When the depth to groundwater was high (low water table) there was not sufficient volume of water within the piezometers to measure the desired parameters. Even if water samples were collected for nutrients and dissolved gases, groundwater was depleted to a level below a depth that was adequate to submerge the YSI probe, and recharge rates were too slow to return to the piezometer to record same-day measurements. These conditions led to incomplete measurements during the sampling period for the plantation, swale, and edge landscape locations.

Measurements were able to be obtained for each monthly sampling in the valley and forest locations.

Temperatures of shallow groundwater ranged from 11.6°C to 22.3°C with higher temperatures in the summer months (June and July) and lower temperatures in the winter months (December, January, February). The mean groundwater temperatures below the forested hillslope within the SMZ continued to rise until September (Figure 4.4).

Groundwater across the entire sub-catchment was acidic with pH ranging from 5.7 to 4.1 (Figure 4.5). There was a malfunction with the pH probe on the YSI probe during the month of April and no values were used for this sampling event. The probe was replaced, and pH monitoring resumed in May and continued for the duration of the sampling period.

During the first sampling event (March) dissolved oxygen (DO) concentrations in groundwater were similar between landscape locations, but throughout the year these concentrations varied significantly (Figure 4.6). The valley groundwater DO remained near or below 1 mg L^{-1} for much of the year until November. Groundwater at the forested hillslope had two peak DO concentrations above 5 mg L^{-1}. The pine plantation observed DO concentrations above 2 mg $L^{-1}$for the majority of the measurements obtained.

Specific conductivity remained fairly constant at each landscape position during the sampling period (Figure 4.7). The valley had the lowest, corresponding to low TN concentrations. The pine plantation had the highest mean specific conductance with the edge and swale observing similar values of specific conductivity.

Mean oxidation-reduction potential (ORP) across the site ranged from 74.6 (valley in March) and 396.4 (pine plantation in May) during the sampling period (Figure 4.8). Mean values of ORP decreased from May to September/October for most of the sub-catchment (pine plantation, swale, edge, forest). Valley groundwater trends upward over the course of the entire sampling period.

Relationships between measured parameters

Only complete observations were used for Spearman correlations to examine relationships between parameters. Correlations between nutrient and dissolved gas concentrations, denitrification parameters, depth to groundwater measurements, and water chemistry parameters are displayed in Figure 4.9. Those that were not significant were marked by an "X" over the correlation value. Reaction progress is negatively correlated with temperature (-0.43, $p<0.05$) and fluctuation in depth to groundwater (delta.DTG; -0.24) but positively correlated with excess-N2, the terminal end product of denitrification (0.64). Nitrate (NO3.N) is highly correlated with denitrification end product, N_2O, DO, and DTG (0.66, 0.60, 0.45, and 0.80 respectively). DOC is negatively correlated with most parameters including denitrification end products and nitrate but positively correlated with ammonium (0.58). Ammonium is negatively correlated to both excess-N2 and N2O (-0.56 and -0.55, respectively) along with nitrate (-0.61). Investigating scatter plots of some of these relationships suggested that some distinct groupings exist based on landscape location (Figures 4.10-4.13).

Principal component analysis (PCA) was performed using all measured and calculated parameters (Figure 4.14). The two largest principal components explained 50.9% of the variation between all variables. Since several of the measured and calculated parameters were dependent of each other, several PCAs were performed excluding such dependent parameters (Figure 4.15-4.17). A PCA was conducted including reaction progress, environmental factors (water chemistry parameters, nutrients, and water table measurements), and dissolved gas measurements (Figure 4.15). PC1 and PC2 from this PCA only accounted for 49% of the variation between parameters at the landscape locations. PCA with the environmental factors and RP (Figure 4.16) accounted for 49.2% of all variation with specific conductivity, nitrate concentrations, and DTG contributing significantly to PC1 (27.9% of all variation) and ORP and ammonium significantly contributing to PC2 (21.3 % of all variation).

A fourth PCA was performed using only the potential driving factors of denitrification (environmental factors), only including independent variables. Approximately 53.1% of all variation between landscape locations was explained by PC1 and PC2 (Figure 4.17). Again, DTG, NO_3^--N, and specific conductivity contributed to PC1 (30.9% of all variation). Ammonium, ORP, and DOC significantly contributed to PC2 which explained 22.2% of the variation between landscape locations. A third principal component (explaining 10.9% of the variation) was plotted with PC1 (Figure 14.8). The three principal components were correlated with denitrification parameters (Table 4.3). Total denitrification end products (Excess-N_2 + N_2O-N) had a strong negative correlation with PC1 (-0.84) and a weak positive correlation with PC3 (0.25). Both excess-N_2 and N_2O-N had moderate negative correlations with PC1 (-0.51 and -0.65, respectively). Excess-N_2 had a moderate positive correlation with PC3 (0.51) along with denitrification reaction progress (0.56). No denitrification parameter had strong or moderate correlations with PC2.

Table 4.1: Total number of complete observations within each landscape position and median, minimum, and maximum depth to groundwater measurements, in meters, and median monthly fluctuations of vadose zone (ΔDTG, in meters)

Landscape Position	**Total # of observations**	**Depth to groundwater (m)**			**Mean ΔDTG (m)**
		Mean	*Min*	*Max*	*Mean*
Pine Plantation	14	2.66	0.78	3.98	0.57
Swale	10	1.12	0.29	1.49	0.28
Edge	12	1.01	0.15	1.38	0.58
Valley	55	0.23	-0.29	0.85	0.02
Forest	21	2.20	0.96	3.93	0.52

Table 4.2: Mean, median, minimum, and maximum measured values for denitrification reaction end products (excess-N_2 and N_2O), denitrification end product ratio (N_2O / excess-N_2+N_2O), and denitrification reaction progress.

Landscape Position	**# of observations**	**Excess N_2-N (mg L^{-1})**			**N_2O-N (mg L^{-1})**			**Ratio**			**RP**		
		Median	*Min*	*Max*	*Median*	*Min*	*Max*	*Median*	*Min*	*Max*	*Median*	*Min*	*Max*
Plantation	16	0.67	0.03	1.66	5.01	1.16	24.34	0.95	0.74	0.99	0.61	0.28	0.90
Swale	10	1.41	0.81	2.05	4.11	1.16	6.54	0.71	0.69	0.94	0.60	0.38	0.79
Edge	11	1.10	0.23	2.33	3.38	0.44	33.80	0.51	0.41	0.98	0.47	0.34	0.84
Valley	37	0.32	0.05	1.69	0.29	0.08	26.54	0.51	0.28	0.99	0.87	0.41	0.99
Forest	16	0.73	0.18	1.78	0.41	0.11	1.13	0.36	0.17	0.90	0.94	0.24	0.98

Table 4.3: Spearman correlation coefficients between denitrification parameters and principal component scores from PCA of environmental factors shown in Figure 4.17 and 4.18.

	PC1	PC2	PC3
Excess N_2-N	-0.51	-0.05	0.51
N_2O-N	-0.65	-0.15	-0.05
Total Denitrification End Product	-0.84	-0.05	0.25
Ratio	-0.22	0.03	-0.51
RP	-0.01	-0.27	0.56

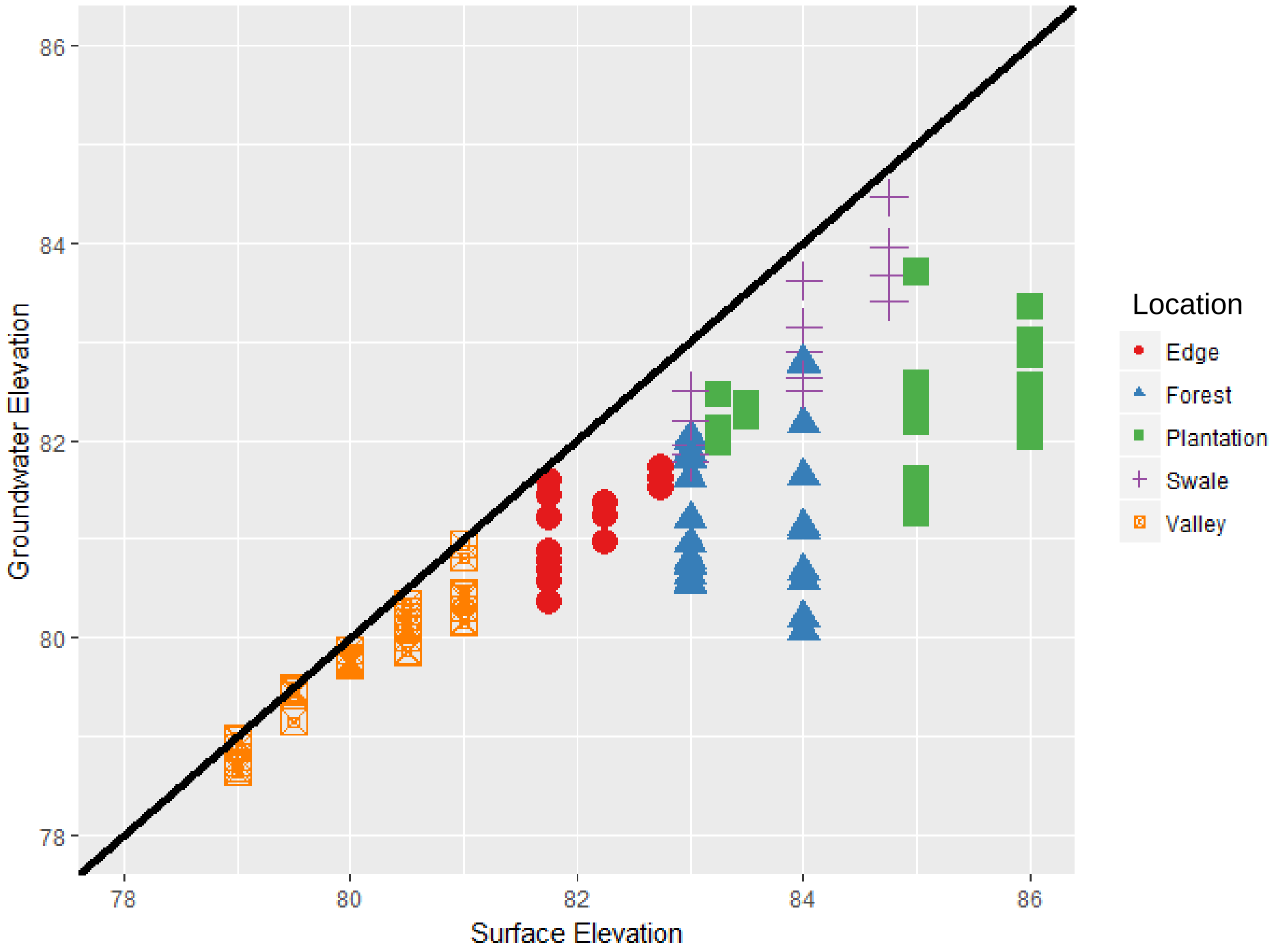

Figure 4.1: Groundwater elevation of piezometers with sufficient water level to obtain water samples during the sampling period. The 1:1 line represents the surface elevation.

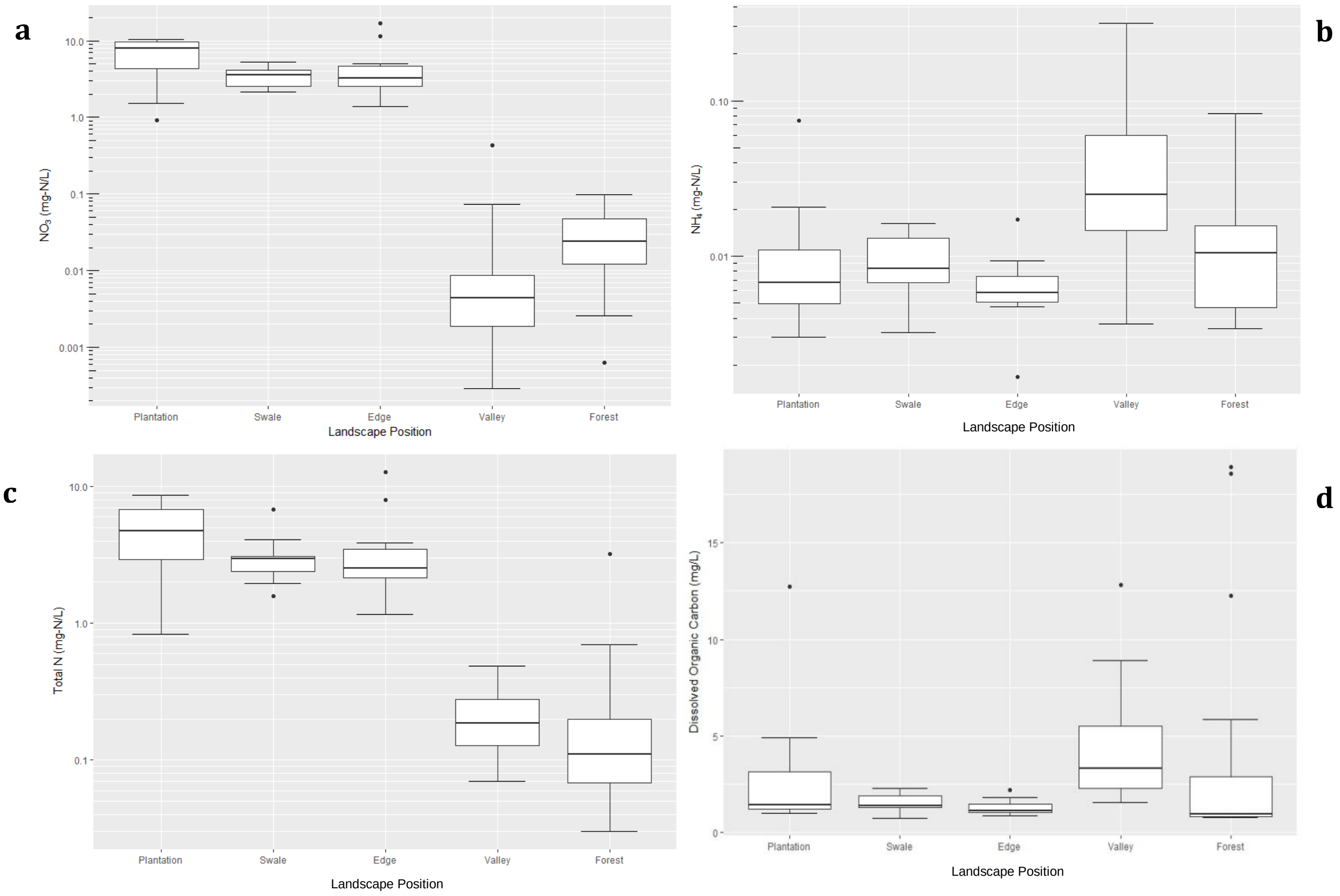

Figure 4.2: Boxplots of (a) NO_3^-, (b) NH_4^+, (c) TN, and (d) DOC based on landscape position for the full duration of the sampling period. The y-axis for NO_3^-, NH_4^+, and TN are on a log scale. The y-axis for DOC is on a linear scale.

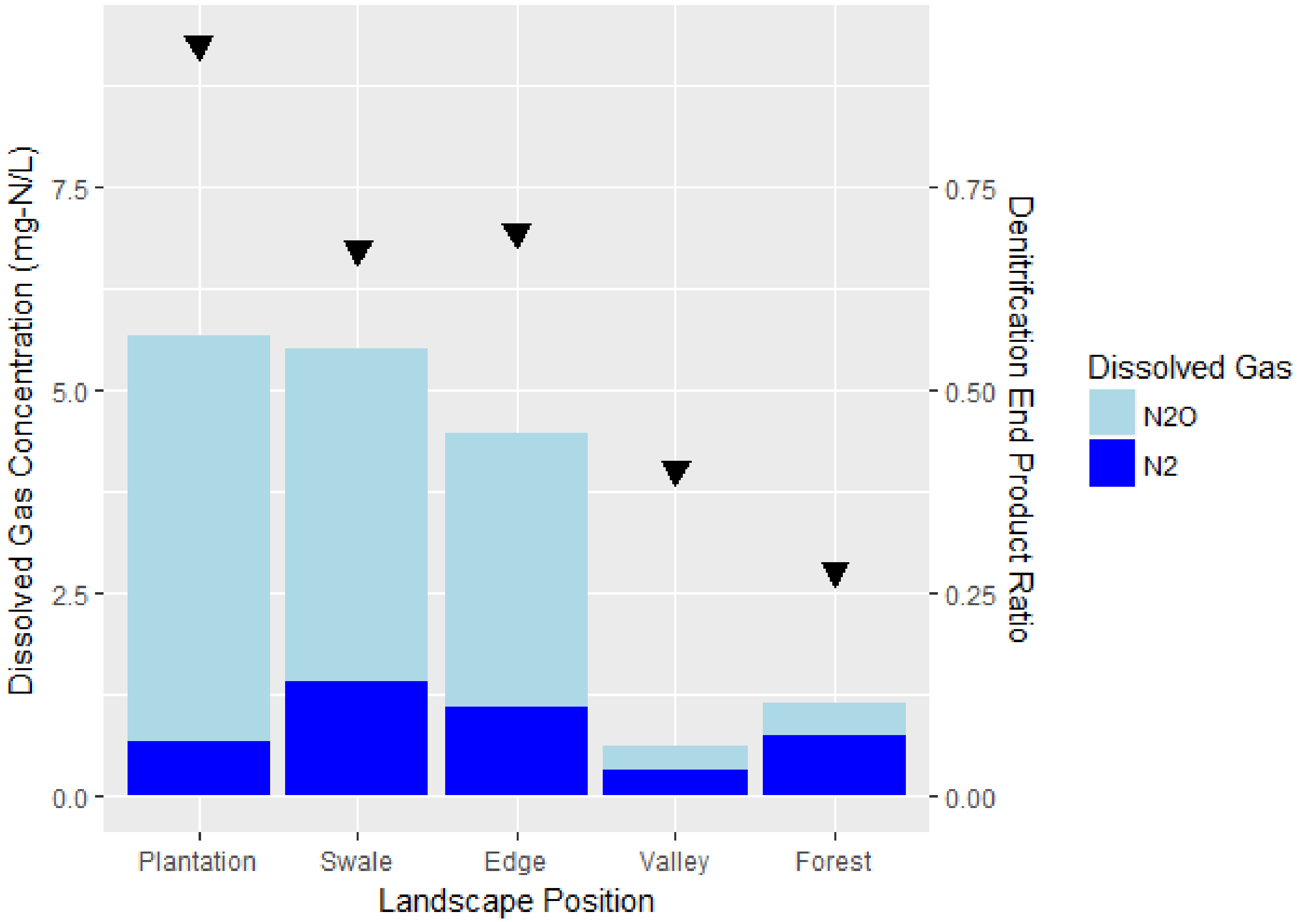

Figure 4.3: Median denitrification end products (N_2 and N_2O) represented in the bars with ratio of end products (N_2O-N/N_2O-N +N_2-N) marked with ▼ for corresponding landscape position.

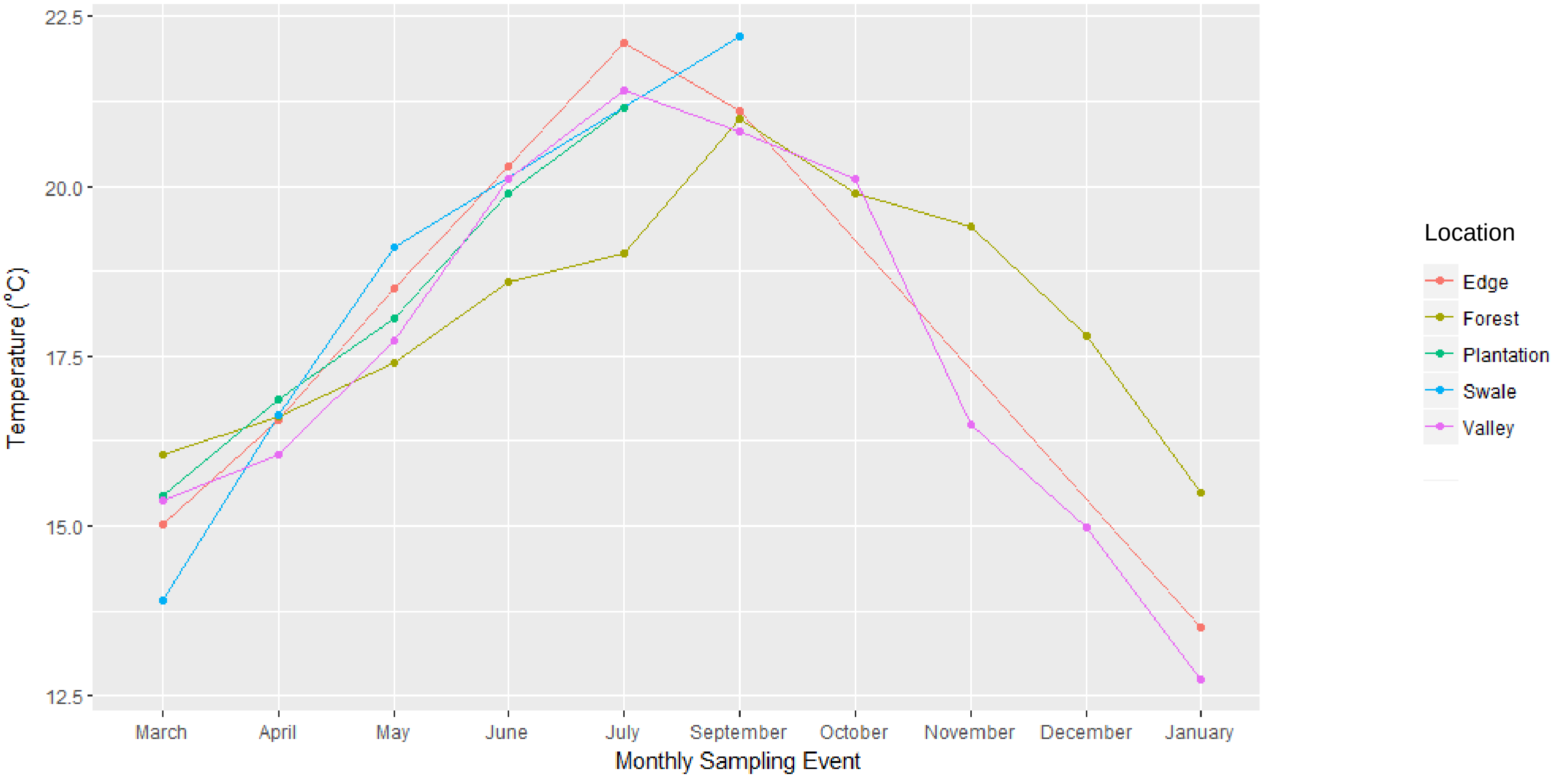

Figure 4.4: Mean groundwater temperatures (°C) for each landscape position over the course of the sampling period.

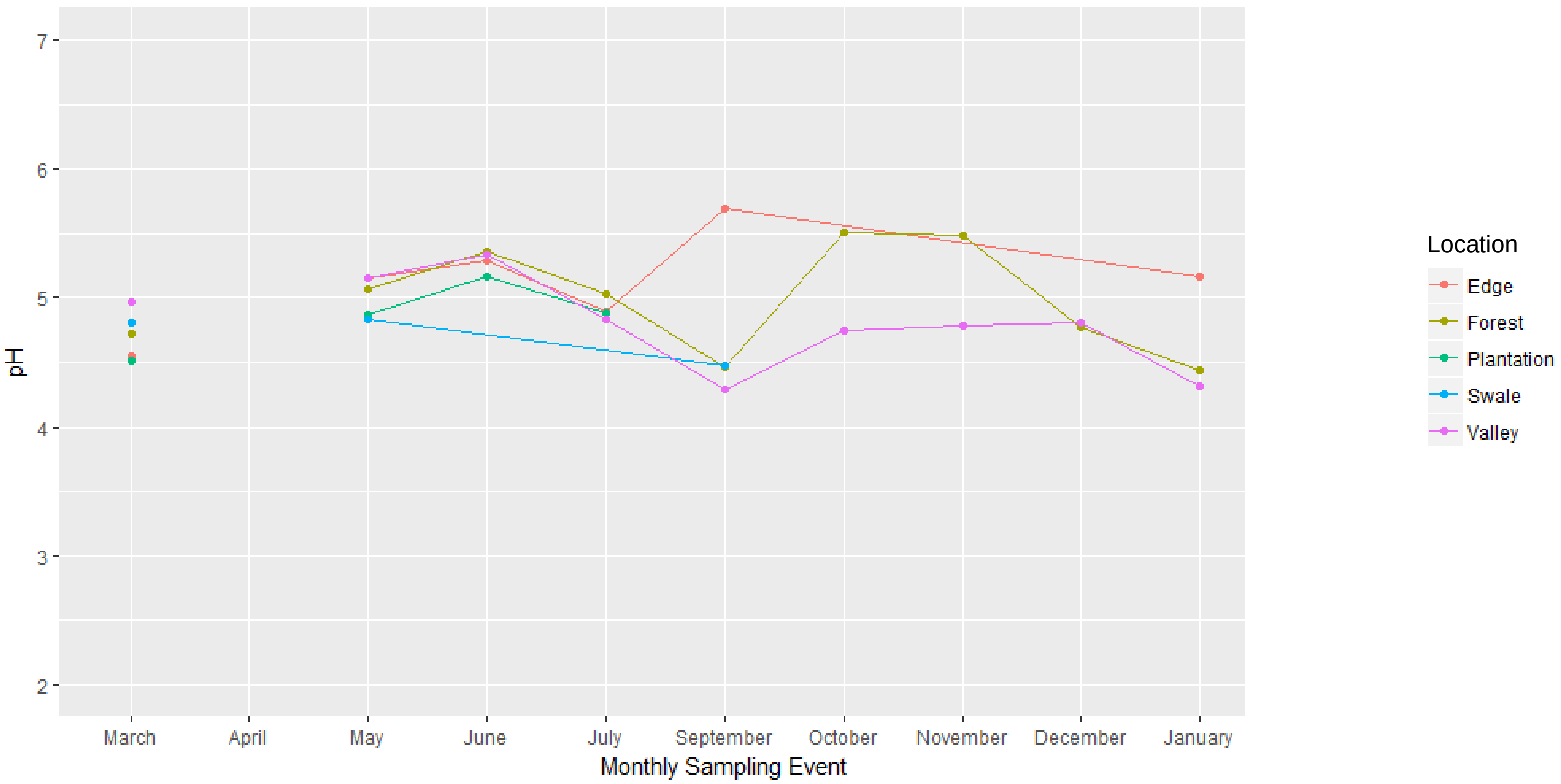

Figure 4.5: Mean groundwater pH for each landscape position over the course of the sampling period.

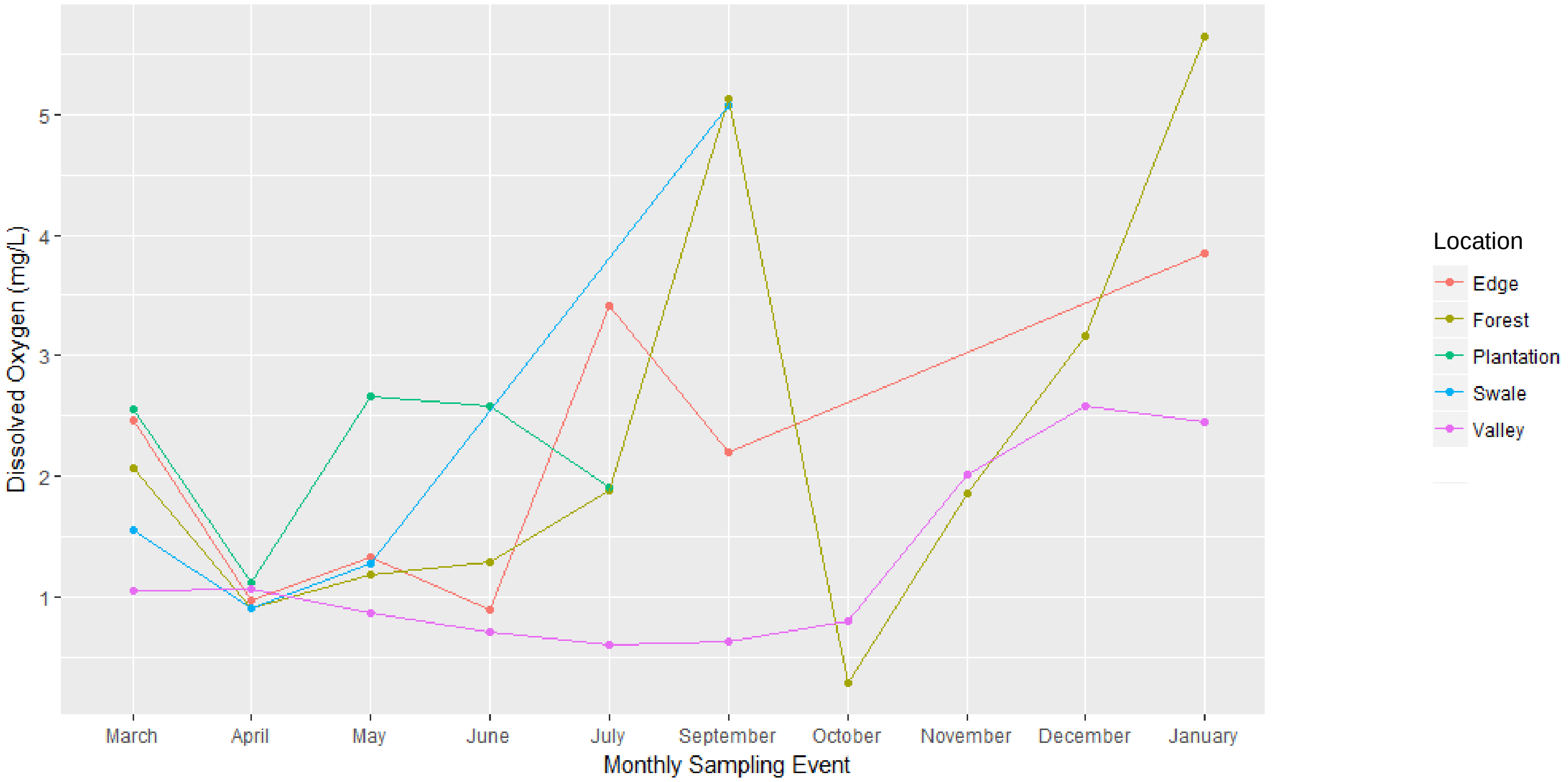

Figure 4.6: Mean groundwater dissolved oxygen (DO) concentrations for each landscape position over the course of the sampling period.

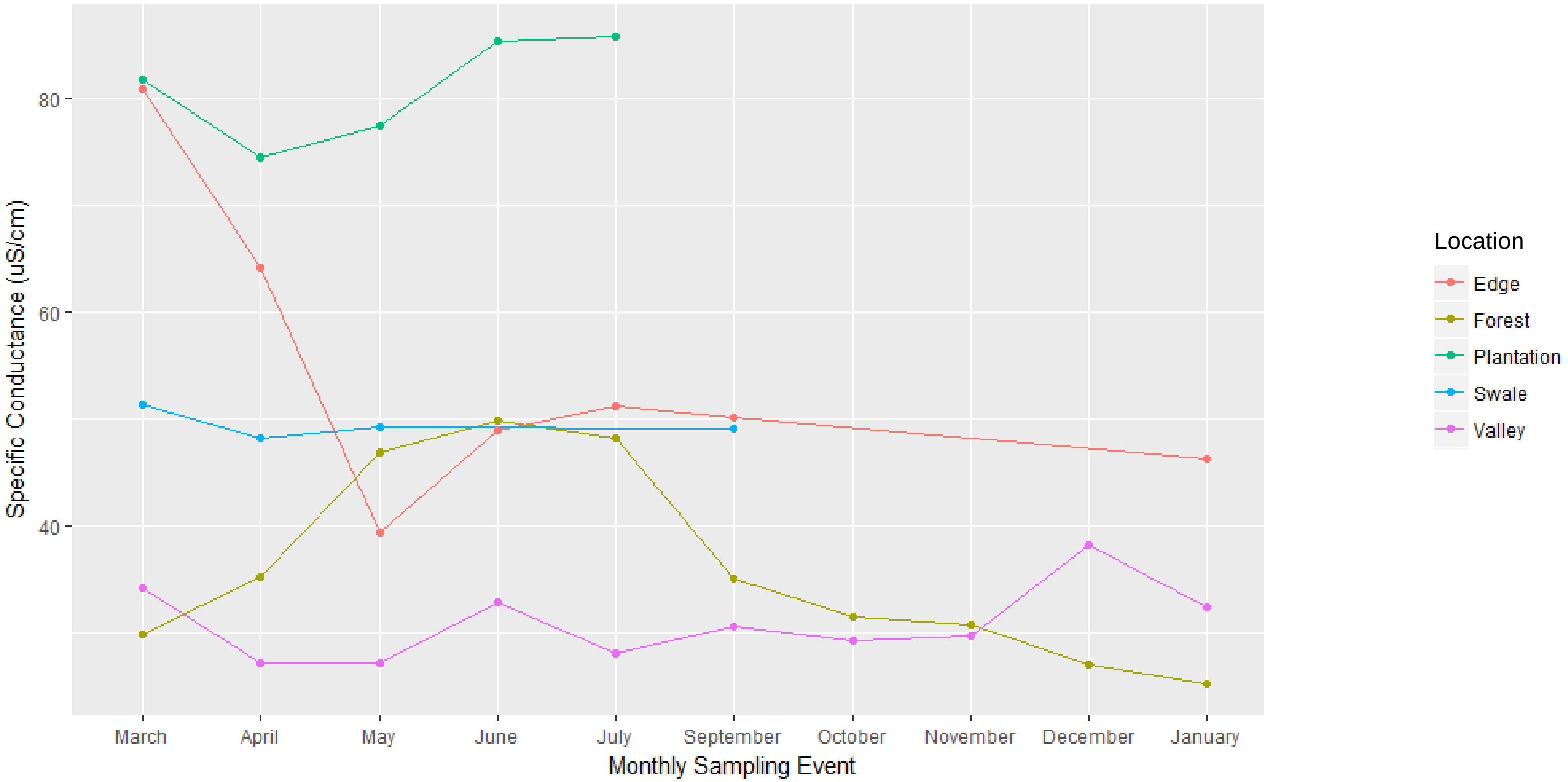

Figure 4.7: Mean groundwater specific conductance (µS/cm) for each landscape position over the course of the sampling period.

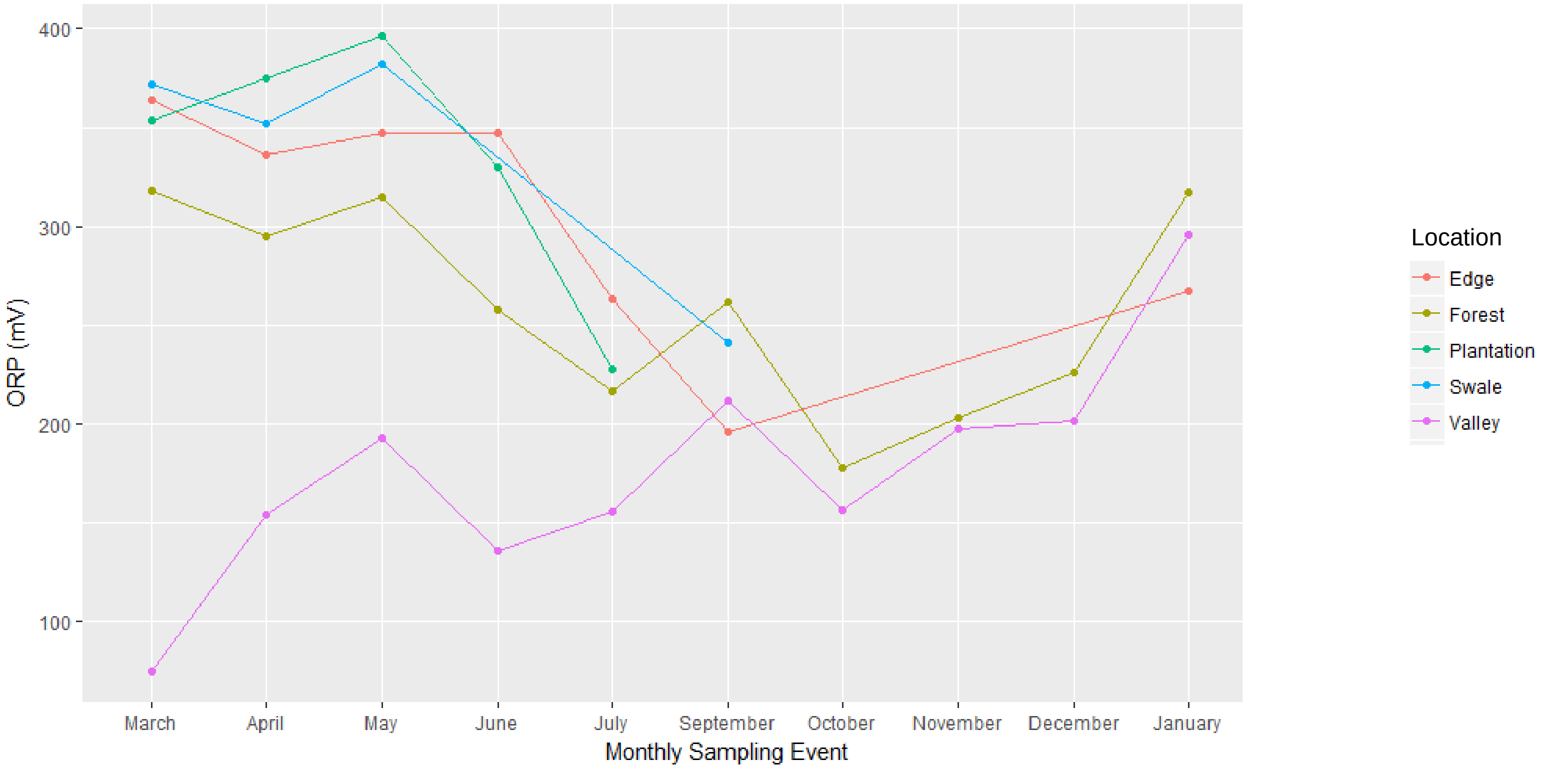

Figure 4.8: Mean oxidation-reduction potentials (ORP) for each landscape position over the course of the sampling period.

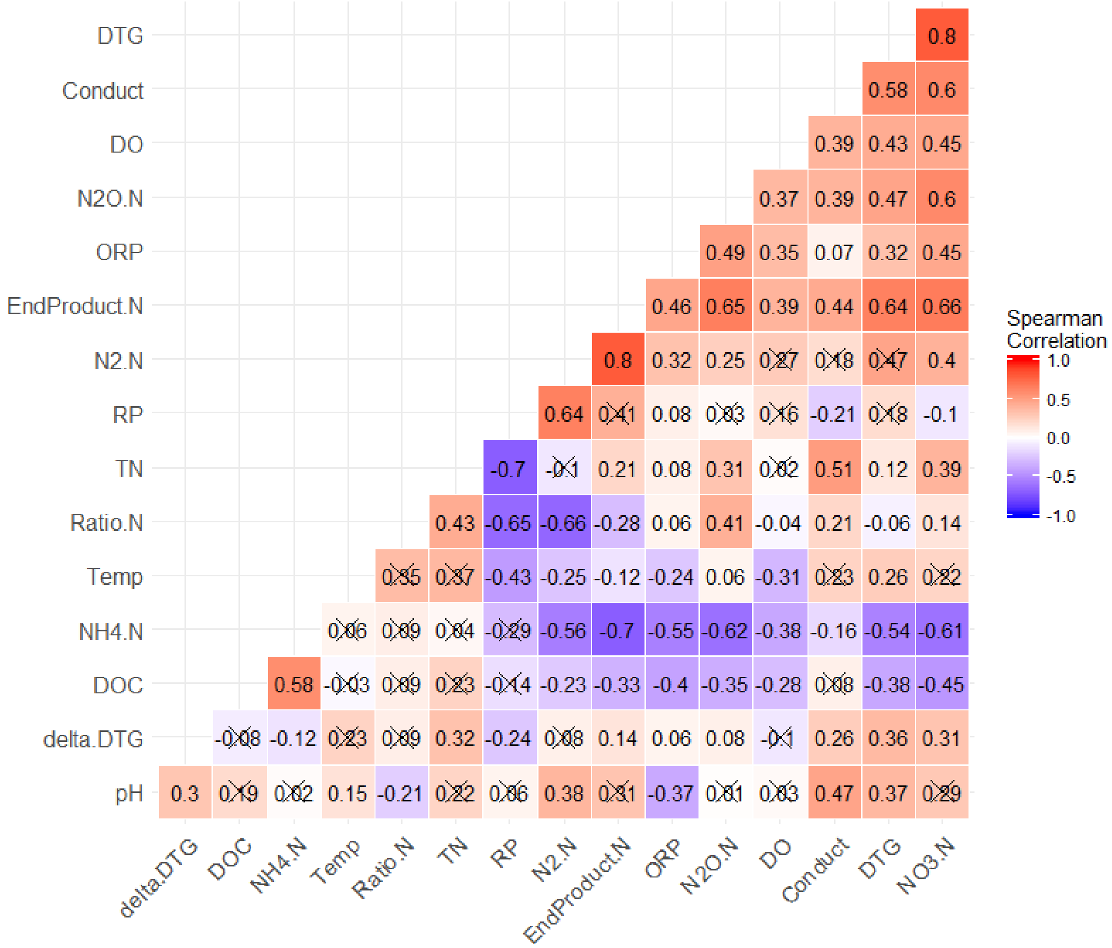

Figure 4.9: Spearman correlation map showing significant correlation coefficients ($p<0.05$) and non-significant correlations ($p\geq0.05$; marked with an X).

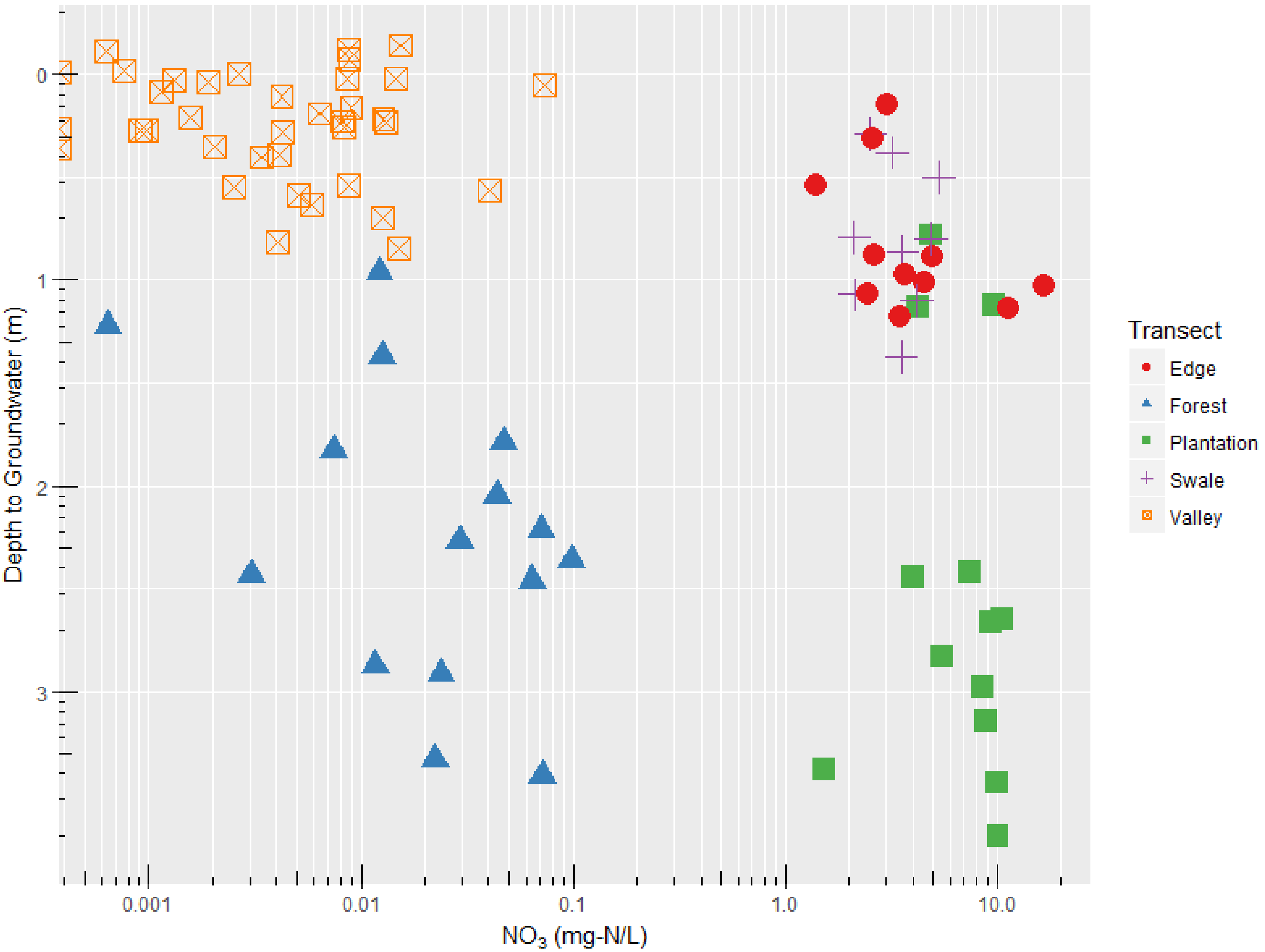

Figure 4.10: Scatter plot of nitrate concentrations and depth to groundwater from the surface (m). The x-axis is on a log scale.

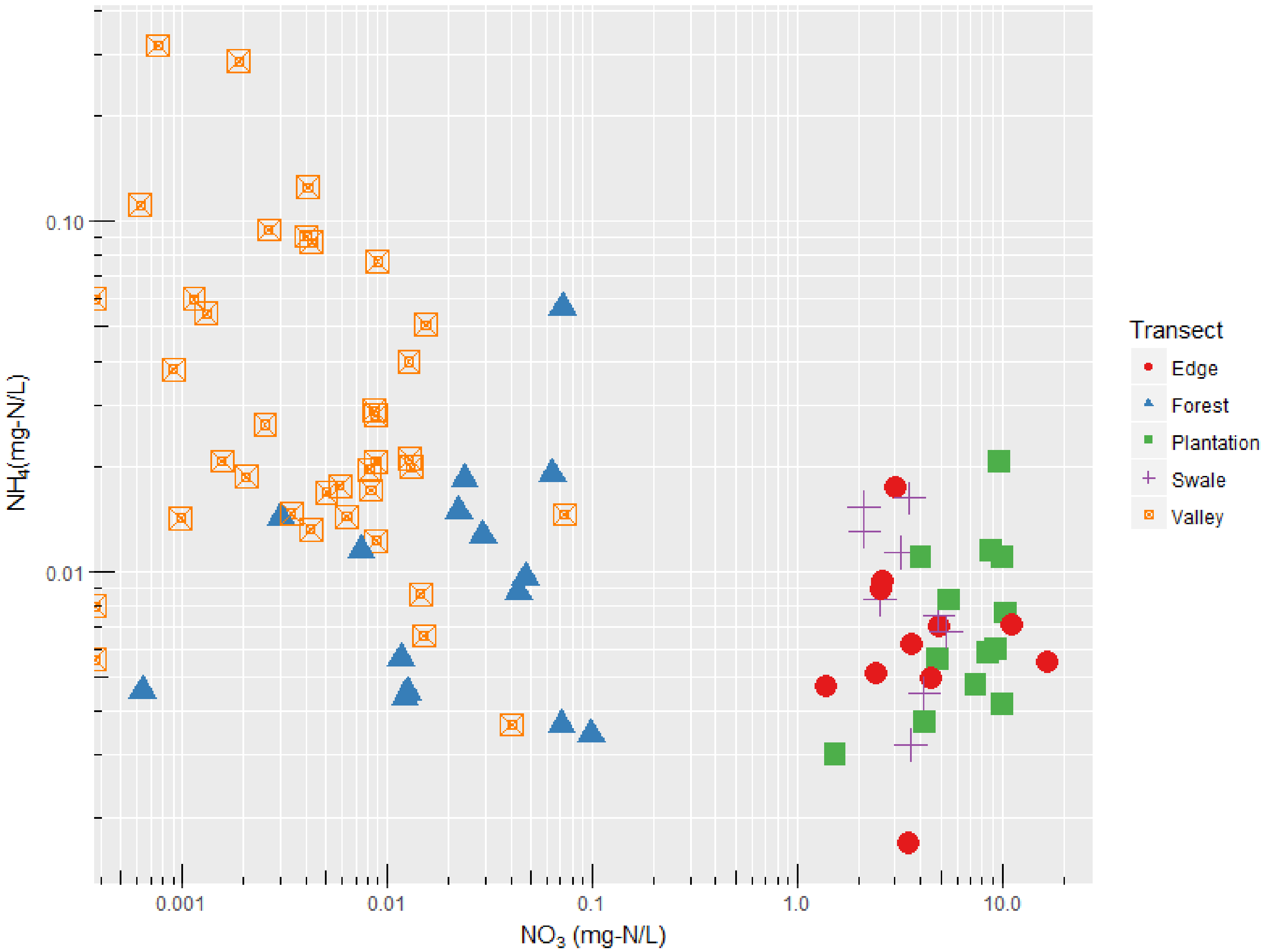

Figure 4.11: Scatter plot of ammonium concentrations and nitrate concentrations. Both y-axis and x-axis are on a log scale.

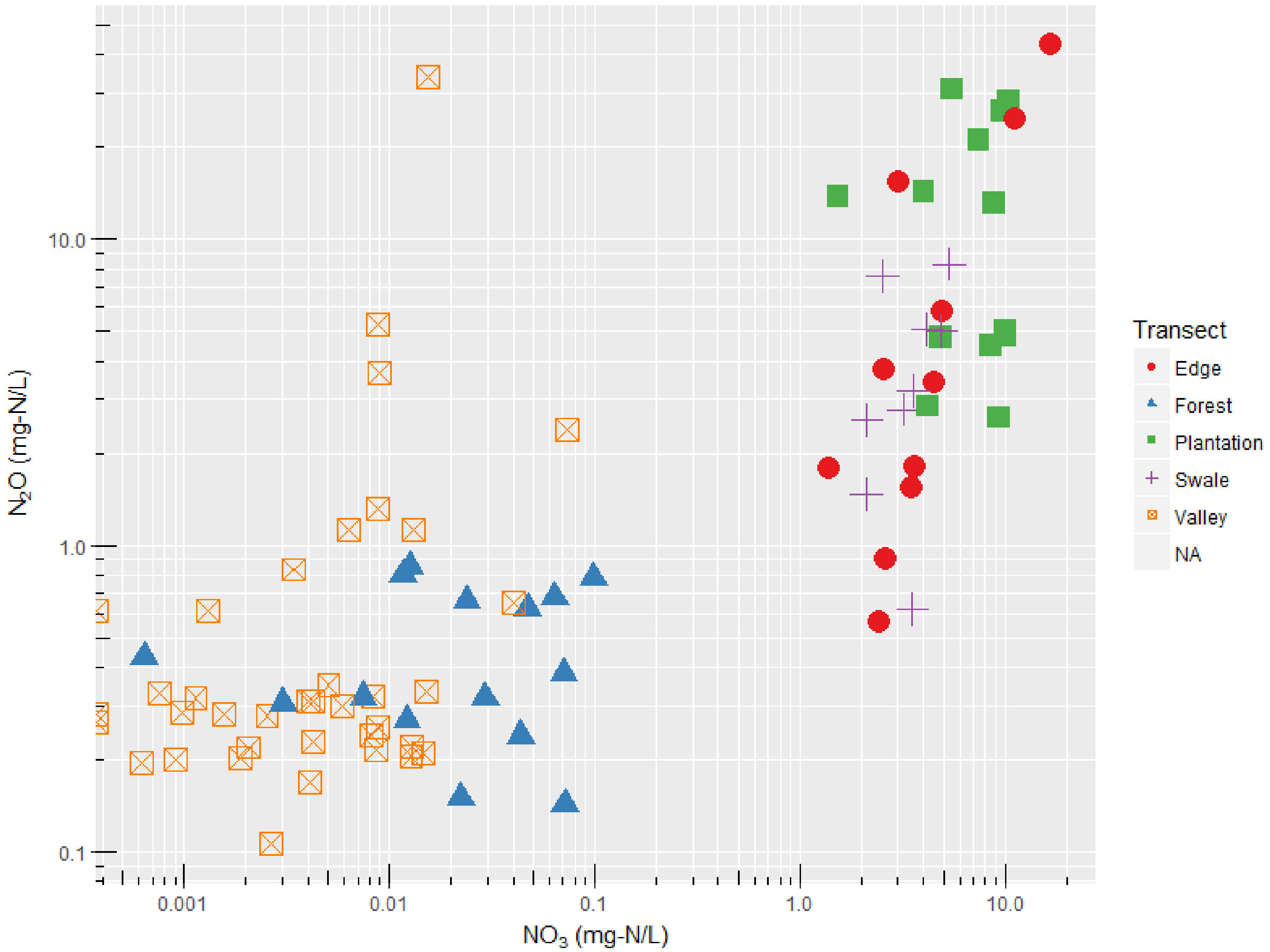

Figure 4.12: Scatter plot of nitrous oxide and nitrate concentrations. Both y-axis and x-axis are on a log scale.

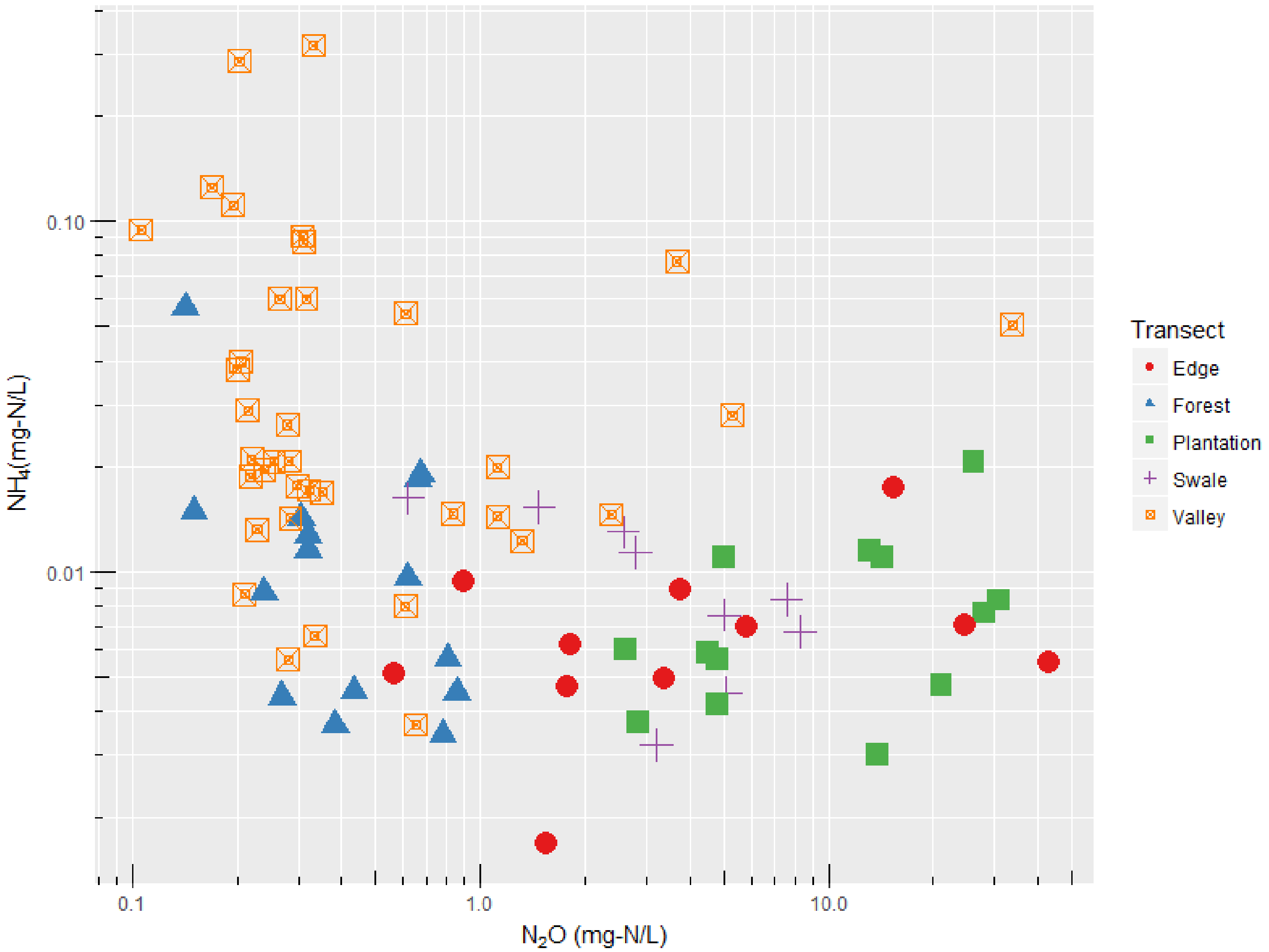

Figure 4.13: Scatter plot of ammonium and nitrous oxide concentrations. Both y-axis and x-axis are on a log scale.

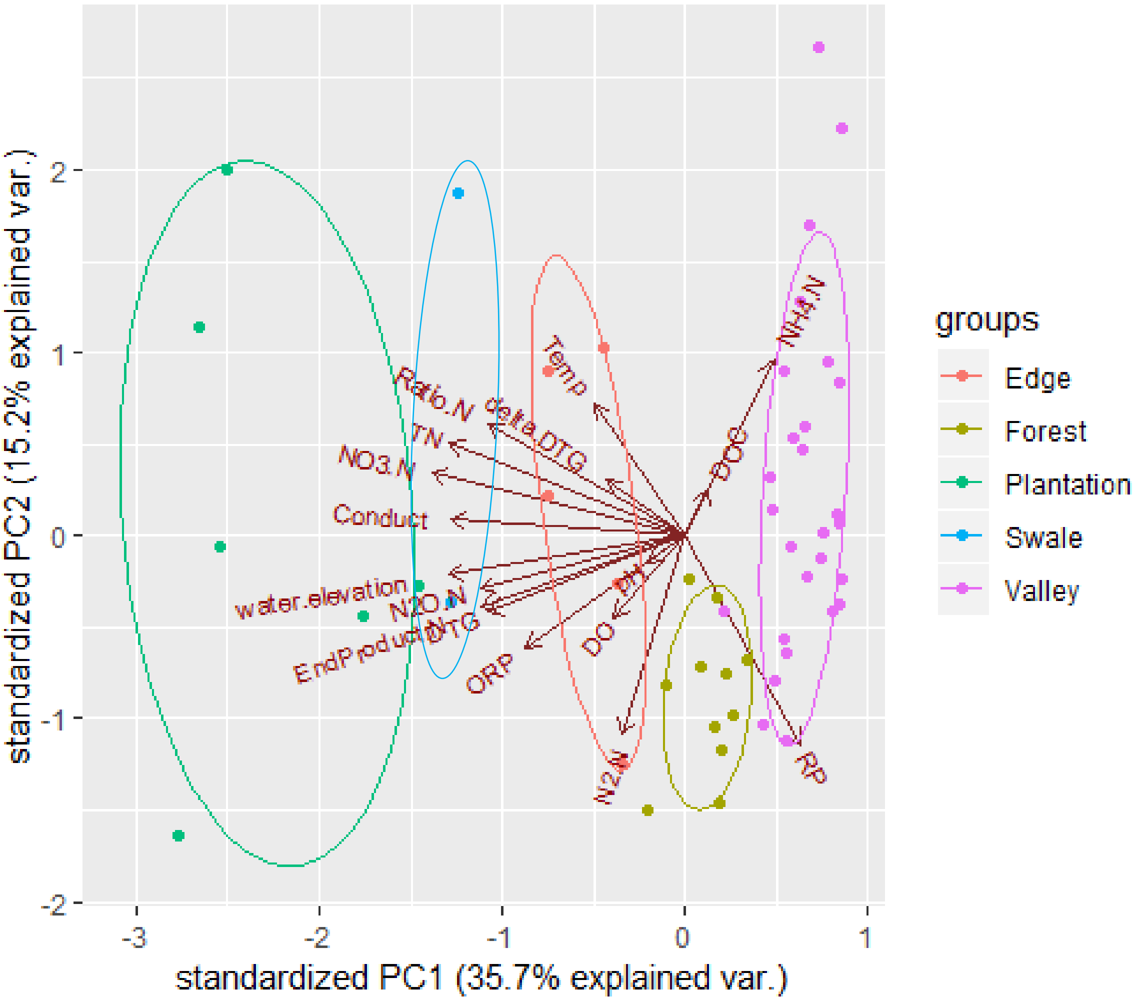

Figure 4.14: Principal component analysis of all measured and calculated parameters (site-specific environmental factors, dissolved gas concentrations, and denitrification parameters) at different landscape locations.

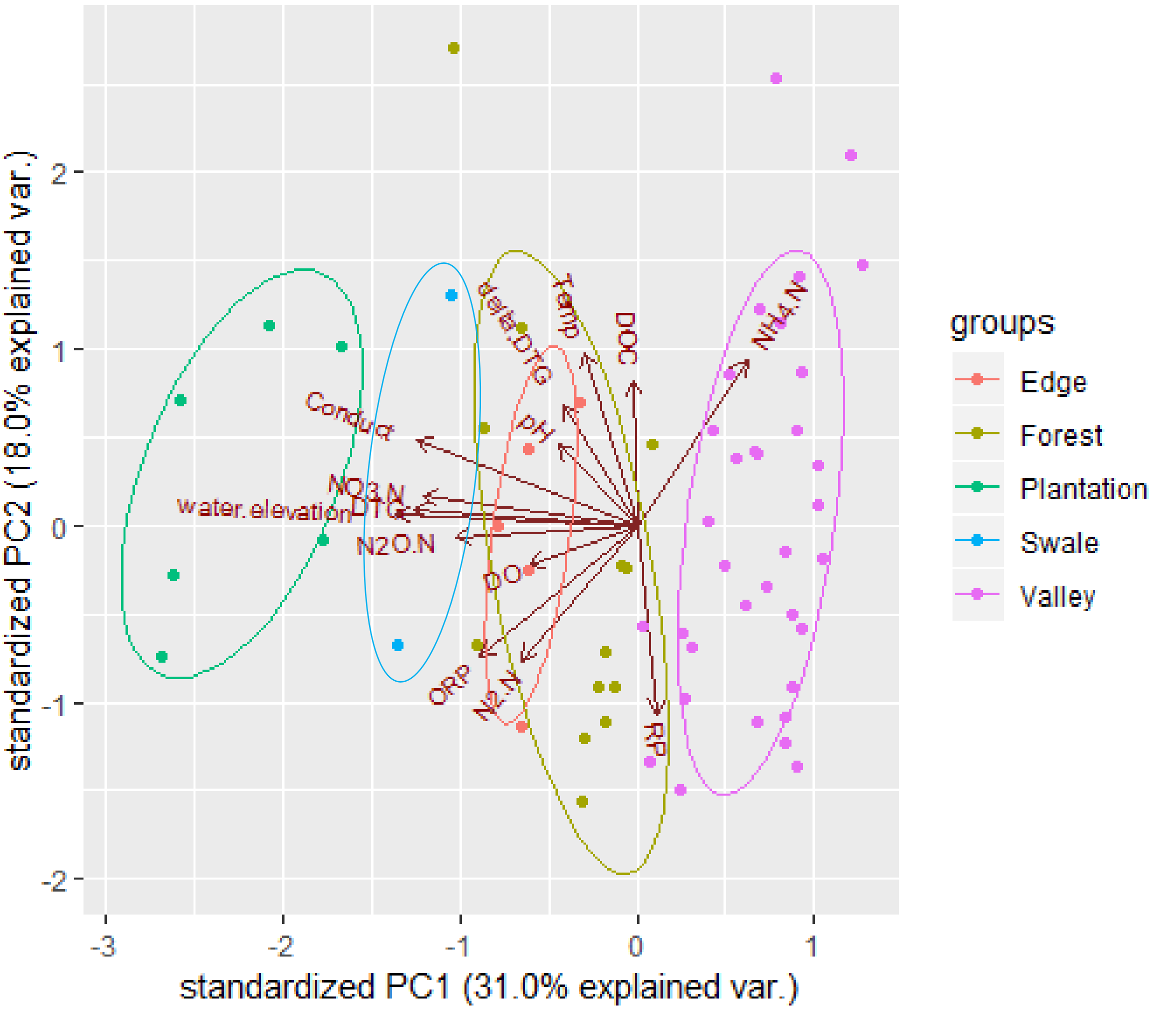

Figure 4.15: Principal component analysis of site-specific environmental factors, reaction progress (RP), and dissolved gas concentrations at different landscape locations.

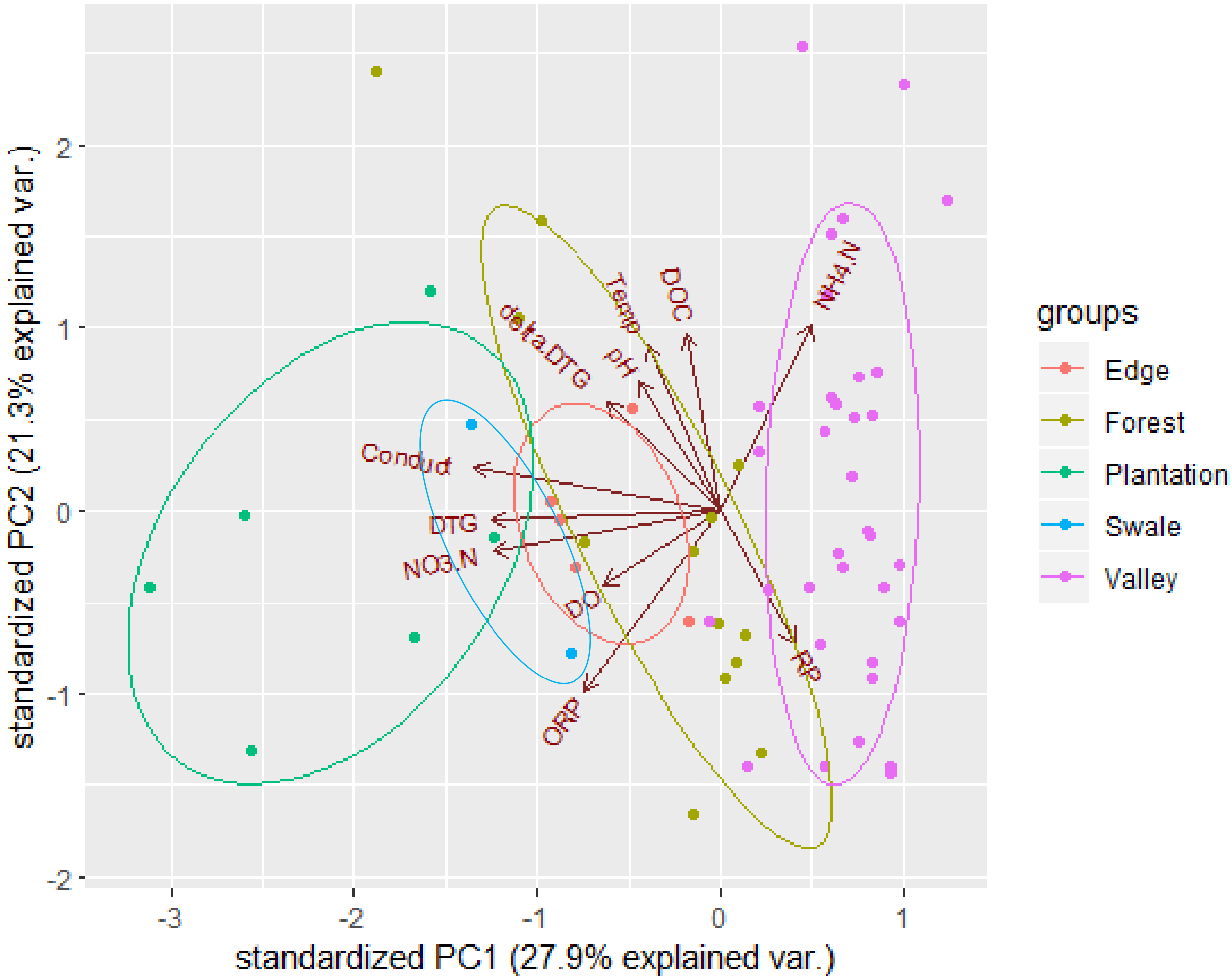

Figure 4.16: Principal component analysis of site-specific environmental factors and reaction progress (RP) at different landscape locations.

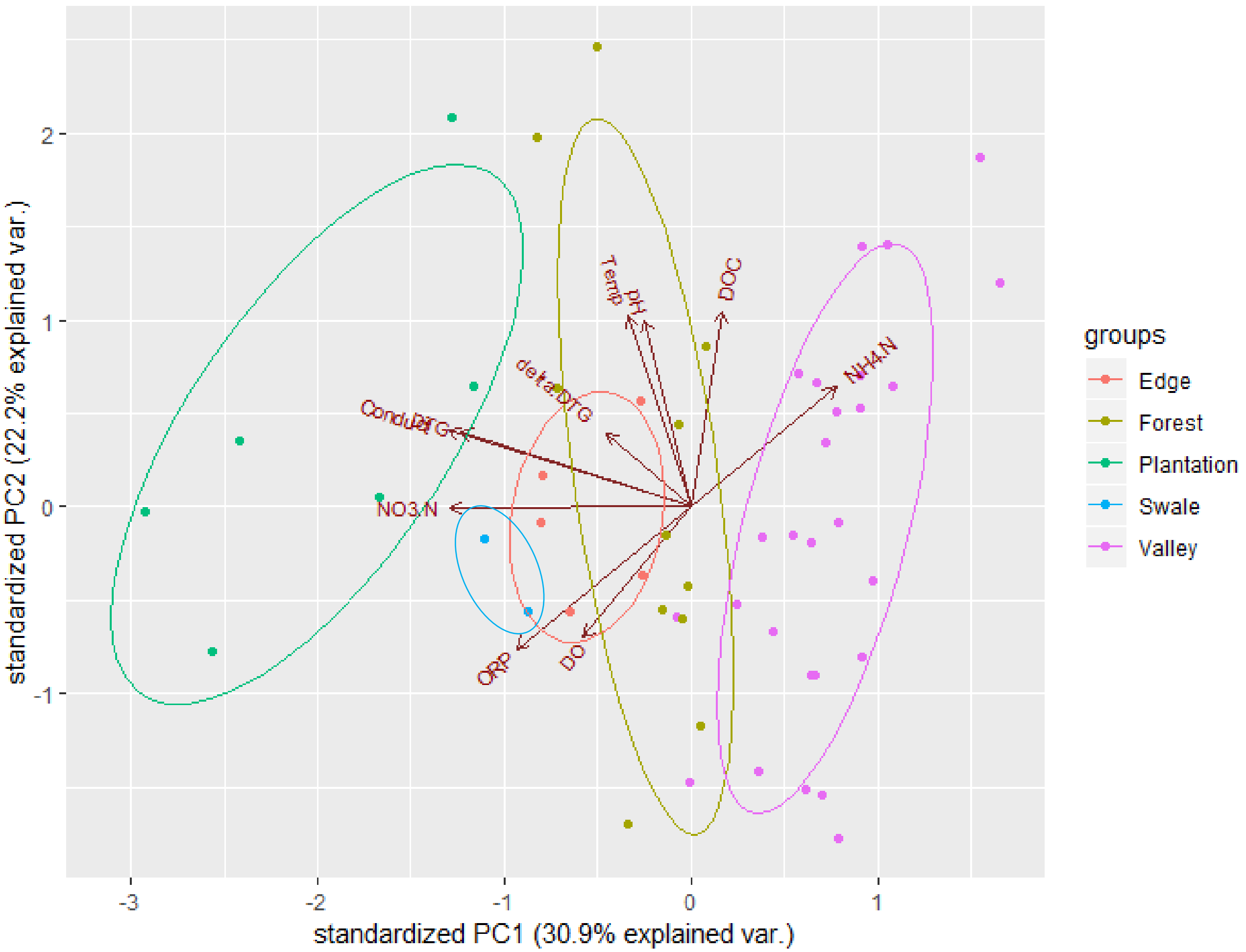

Figure 4.17: Principal component analysis of site-specific environmental factors at different landscape locations.

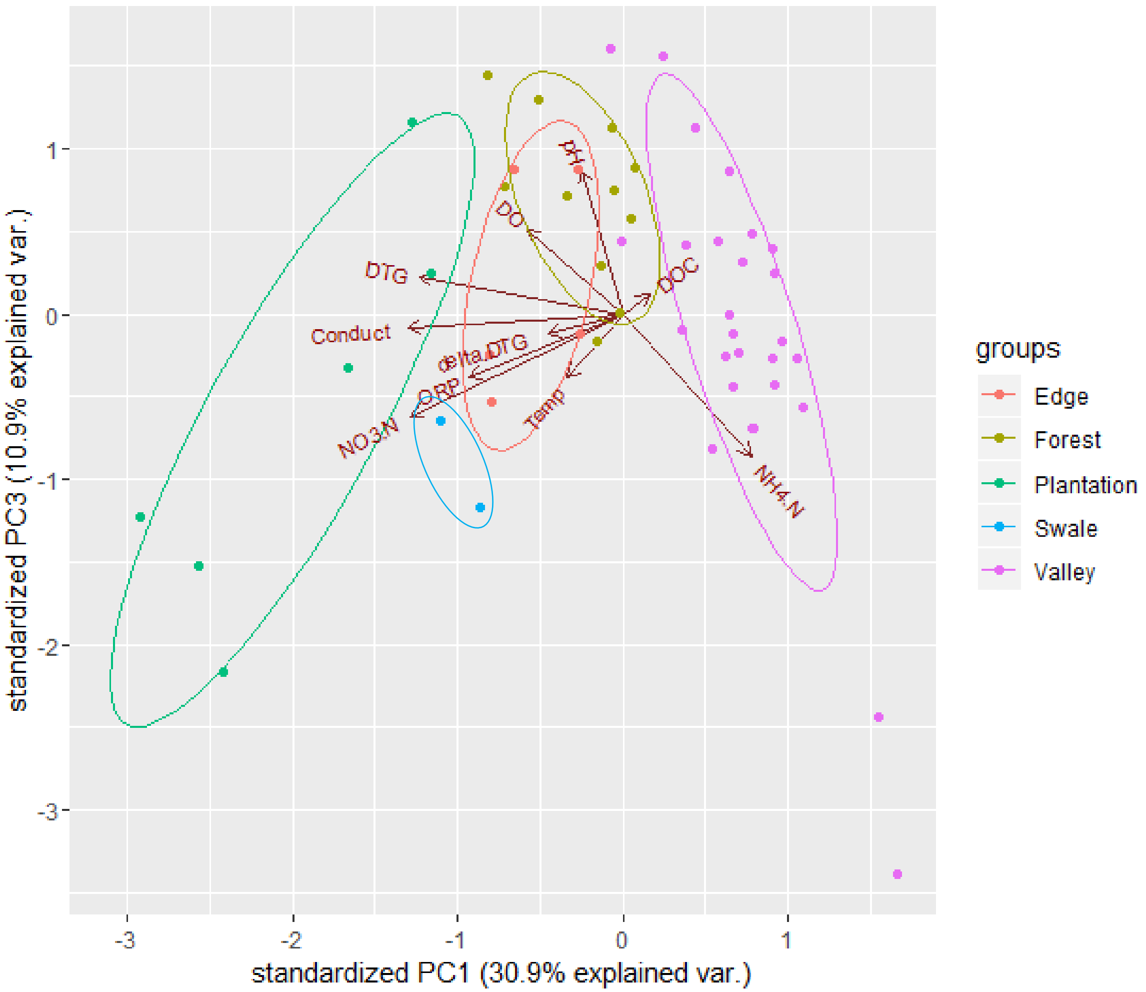

Figure 4.18: Principal component analysis of site-specific environmental factors at different landscape locations.

CHAPTER 5

DISCUSSION

In-well degassing

Observations of excess N_2 below expected concentrations of water equilibrated with the atmosphere occurred in the deep piezometers of the nested pairs (W02D, W03D, W11D, W12D) and valley piezometers (W14-W17) at least once, and multiple times at some of the piezometers in the valley (W14, W15, W17). Most of the observations of negative N_2 were measured during sampling events in September and October.

Negative concentrations of excess N_2 can be caused by degassing within the system or during sampling. Ebullition during sampling has been a concern using peristaltic pumps but using a slow pumping rate (below 100 mL minute^{-1}; (Blicher-Mathiesen et al. 1998; Weymann et al. 2008; Jahangir et al. 2013; Fox et al. 2014) reduces the risk of degassing during sampling.

Degassing can also occur due to oversaturation of dissolved gases from in gas producing processes such as denitrification. Few studies (Blicher-Mathiesen et al. 1998; Mookherji et al. 2003) have investigated degassing phenomenon in shallow groundwater. Blicher-Mathiesen et al. (1998) provided a calculation based on differences in Ar concentrations between piezometers in a transect. This equation was not appropriate for our experimental setup because our piezometer transects in this study ranged from 20-40 meters apart with differing temperatures.

Temperature controls the amount of dissolved gases in water (Weiss 1970). In contrast, the piezometer transect in the study by Blicher-Mathiesen (1998) study was only 5 meters long with temperatures differing by 0.16°C. In our study, changes in monthly groundwater temperature may play an important factor in the decrease in excess N_2 concentrations since negative concentrations and decreases in excess N_2 were measured primarily in months with warm groundwater temperatures.

Degassing of N_2 can occur due to stripping from other gas production such as methane (Blicher-Mathiesen et al. 1998) and if the sum of partial pressures of all dissolved gases in solution exceeds hydrostatic pressure (Weyman et al. 2008). Hydrostatic pressure in piezometers can be calculated from the height of water above the screened portion of the piezometer (Blicher-Mathiesen et al. 1998). This can be easily calculated for the piezometers in this study, but not knowing the total number of gases dissolved in the groundwater (carbon dioxide across the system and possible hydrogen sulfide in the riparian zone) prevents our ability to calculate the potential of degassing due to partial pressures of dissolved gases exceeding the hydrostatic pressure in each piezometer.

Residence times and gas production in shallow groundwater

The estimates from the conservative tracer model suggests that groundwater ages in the uplands and hillslopes of the pine plantation in unit 4 are around 11-13 years (Vache, unpublished data). The near stream estimates are around 1-3 years. Studies investigating the effect of residence times on denitrification have shown that longer residence times facilitate complete denitrification with more N_2 production while intermediate residence times produce N_2O (Jurado et al. 2017; Reeder et al. 2018). Based on current data, residence time is not a governing factor of N_2 or N_2O production on our site, as we observed higher N_2O in the uplands. However, longer residence time may enable more denitrification to occur producing higher concentrations of dissolved gases.

Complete Denitrification Inhibited

This begs the question, why are N_2O concentrations much higher in the locations where there are longer residence times? There are several possible answers. Overall, N_2O production was high across the study site. This may be due to the low pH of the groundwater (4-5). Acidic soil and groundwater have been shown to inhibit complete denitrification and enhance the production of N_2O (Nagele and Conrad 1990; Simek and Cooper 2002; Jurado, Borges, and Brouyère 2017). The correlation between pH and N_2O production was not significant, but this may be due to the low range of pH values across the study site during the sampling period.

Abundance of nitrate has also been linked to inhibition of complete denitrification (Weyman et al 2008). With plenty of nitrate present in the groundwater system in the pine plantation, microbes are able to continue to use nitrate for respiration rather than further reducing N_2O to N_2 (Rivett et al. 2008; Jurado et al. 2017). Weyman et al. (2008) cited that the inhibiting characteristic of low pH on N_2O reduction to N_2 is amplified by high levels of NO_3^-.

Although oxygen levels were higher in the pine plantation when compared to the other four landscape locations, for the majority of sampling events the concentrations of DO were within the range of conditions for denitrification (< 2 mg L^{-1}; Jahangir et al. 2013). The vadose zone is much thicker in the pine plantation allowing more availability of atmospheric air within the pore space of the unsaturated zone. Fluctuations of this unsaturated zone measured by changes in depth to groundwater could incorporate this air into the groundwater system, introducing DO to the groundwater. DTG fluctuations (ΔDTG) in this study were negatively correlated with reaction progress (-0.24 Spearman correlation rank, $p<0.05$).

Net denitrification: a combination of nitrogen reducing processes

The measure of dissolved gases for denitrification is an estimate of net denitrification. Without separating the processes that produce N_2O, it is hard to decipher which process is dominating one part of the landscape versus the other. Comparing driving (or inhibiting) factors of denitrification can elucidate between these different processes.

Dissimilatory nitrate reduction (DNRA) also produces N_2O as it transforms nitrate to ammonium. This process is mainly observed when nitrate levels are low (Rivett et al. 2008). There is an observed increase in ammonium between the SMZ edge and the valley of the intermittent stream where nitrate levels were observed at the lowest concentrations across the site. The valley has higher DOC concentrations and lower ORP values which favor DNRA (Jurado et al. 2017) so this may be another nitrogen reducing process taking place within the SMZ valley. Based on the scatterplot of ammonium concentrations plotted against nitrous oxide concentrations suggests that perhaps instead of DNRA occurring in the valley, that nitrification may be occurring as ammonium concentrations decline as nitrous oxide concentrations increase (Figure 4.13).

Other sources of N_2O

Nitrification could be happening in the unsaturated soil above the groundwater, reducing NH_4^+ levels reaching the groundwater system and providing NO_3^- through leaching from the unsaturated zone. Nitrification produces N_2O when NH_3 or NH_4^+ is oxidized to NO_3^-. This process predominantly occurs in aerobic conditions and has long been considered a minor source of N_2O; however, in environments with high levels of available organic matter, low pH, and high oxygen, the potential for N_2O production increases (Wrage et al. 2001; Strauss, Mitchell, and Lamberti 2002). High availability of ammonium, DO, and DOC are a limiting factor for nitrification to take place (Strauss et al. 2002). In the shallow groundwater system in sub-catchment 4 of watershed C, ammonium levels are extremely low.

Coupled nitrification-denitrification occurs at the interface of oxic-anoxic environments, where fluctuations in DO persist, or at intermediate levels of DOC, nitrogen content, and DO (Wrage et al. 2001; Marchant et al. 2016). These conditions are present in the upslope landscape locations, especially within the pine plantation. Another nitrogen reduction pathway, nitrifier denitrification, has been hypothesized to produce substantial levels of N_2O (Wrage et al. 2001). This nitrogen cycling process is conducted by microorganisms that can both nitrify and denitrify (autotrophic ammonia-oxidizers), and can occur intensively in conditions of high nitrogen content (NH_3), low DOC, low DO, and (possibly) low pH environments (Wrage et al. 2001). These conditions are present at the study site, but concentrations of NH_4^+ are low in the groundwater.

Nitrogen gradients and a tale of two nitrogen cycling systems

There are clear distinctions in nitrogen cycling between upslope locations (pine plantation, swale, and edge) and SMZ locations (valley and forest). The non-parametric statistical analysis noted significant differences between these groups in several of the denitrification parameters and nutrient concentrations, especially between the valley and the three upslope locations. Observed differences between these two systems are accentuated by plotting correlations between some of the measured and calculated parameters. Although there is an overall trend of nitrate decreasing with increasing depth to groundwater (Figure 4.10) the obvious grouping between landscape locations suggests that the location is more important than the depth to groundwater. This pattern of similar trends, but obvious grouping, is seen in graphing other relationships (Figure 4.11 and Figure 4.12) and in the multiple PCAs performed with different parameters. Each PCA shows a clear grouping based on landscape location with different parameters controlling the variation between landscape locations. The correlations between the denitrification parameters and the first principal component of environmental factors (Table 4.3), driven by nitrate concentrations (Figure 4.17), further emphasize that the abundance of nitrate in the pine plantation is an important component to incomplete denitrification.

These upland sites where fertilizer has been applied are also characterized by higher DO concentrations, lower DOC concentrations, and higher ORP values than the valley. This emphasizes the observed gradient of nitrogen species and net denitrification processing across the site and suggests it is dominated by the environmental factors of the sub-catchment.

Comparison of reaction progress with other studies

Difficulty comparing between studies due to difference in methods used to measure denitrification. Even studies that have used the same method (N_2:Ar) different intricacies in methodology (such as differences in measuring recharge temperature, sampling equipment, calculations) may not warrant accurate comparisons. With this in mind, comparisons between sites can be beneficial to…

Similar studies that have used N_2:Ar method to directly measure excess N2 to calculate reaction progress as an estimation of denitrification are the most pertinent studies to compare findings. Jahinger et al (2013) quantified denitrification in shallow groundwater at 4 different agricultural sites using a similar reaction progress calculation and observed mean RP in sites with low permeability between 0.46 and 0.77 and sites with high permeability between 0.04 and 0.08. McAleer et al (2017) investigate denitrification in varying depths of groundwater (shallow and deep) in two catchments with different underlying bedrock (slate and sandstone) and found denitrification reaction progress ranging from 0-32% in in the slate catchment and 4-94% in the sandstone catchment. The dominant land use in both catchments was agriculture. Our calculated ranges of reaction progress fall within these ranges for all locations. Jahinger et al (2013) noted a maximum of reaction progress in some low permeability sites were between 0.97 and 0.99 which is similar to our maximum observed RP.

CHAPTER 6

CONCLUSIONS

Streamside management zones currently used as BMPs for forestry management in the Fourmile Experimental Watershed at the Savannah River Site are sufficient for mitigating nitrogen inputs from short rotation pine management. There is a gradient of nitrate reduction occurring between the pine plantation and other upland locations (swale and edge) and the SMZ valley. Based on the reaction progress calculated from dissolved gas and initial nitrogen concentrations from data collected, net denitrification is reducing more than 80% of the nitrogen in the shallow groundwater system within the valley of the SMZ with upland locations reducing between 47-60%. Due nitrate abundance from fertilization and site-specific environmental factors, complete denitrification is inhibited in the upslope regions of this sub-catchment making N_2O the dominant end product of net denitrification.

Recommendations for Future Research

This study summarizes findings from one annual cycle of a two-year study. After the second year of data collection, all observations will be reanalyzed. More observations will strengthen results and conclusions from the study and help identify any outlying values. Deeper piezometers along the edge of the SMZ boundary were added which will fill gaps in data collected from this landscape location. Analysis of water samples for isotopic nitrate signatures will help elucidate the source(s) of nitrate within the sub-catchment and will provide further information to the importance of denitrification along the groundwater flow path. With N_2O as the dominant end product of denitrification, further research about indirect emissions of this potent greenhouse gas from short-rotation pine management should be investigated further.

Denitrification is a complex process to monitor in the environment. Factors such as atmospheric contamination, degassing, and multiple biological processes that produce gaseous forms of nitrogen, make it difficult to estimate the extent of denitrification in groundwater. Within similar methods of measuring denitrification there are subtle differences that could potentially create hinderances of comparing groundwater denitrification estimates across landscapes. Environmental factors and scientific objectives will dictate methodology but there should be more studies on the differences of sampling equipment and techniques for measurement of denitrification in groundwater systems that result in a set of standards or best practices. Even with all the intricacies and potential complications, the need to estimate the extent of denitrification, especially in groundwater systems where leached NO_3^- can be transported to surface waters, is vital to balancing watershed budgets of nitrogen inputs and outputs, estimating the effect of fertilization on the production of potent greenhouse gases (NO and N_2O), and studying nitrate attenuation processes.

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