

DENITRIFICATION IN RIPARIAN ZONES AND OTHER SATURATED SOILS OF A  
NORTHEASTERN AGRICULTURAL LANDSCAPE

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# DENITRIFICATION IN RIPARIAN ZONES AND OTHER SATURATED SOILS OF A NORTHEASTERN AGRICULTURAL LANDSCAPE

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Nitrogen (N), particularly nitrate ( $\text{NO}_3^-$ ), is a critical pollutant in many northeastern US watersheds that is especially detrimental to coastal marine ecosystems. Agricultural land, which receives fertilizers and/or animal manures, is a principal source of N loading to the environment. The most effective  $\text{NO}_3^-$  attenuation mechanism is probably microbial denitrification, i.e., the transformation of  $\text{NO}_3^-$  into N gases (e.g.,  $\text{NO}$ ,  $\text{N}_2\text{O}$ ,  $\text{N}_2$ ). Currently, our estimates of the magnitudes of denitrification rates at landscape scales are “tentative” at best, usually based on large-scale watershed budgets in which denitrification was estimated by difference. One reason that denitrification is hard to quantify is that a large amount of denitrification occurs in disproportionately small parts of the landscape (i.e., hotspots) and over relatively short periods (i.e., hot moments). Denitrification occurs primarily under anaerobic conditions by heterotrophic microbes and is expected to be vigorous in wet soils high in organic carbon. There is good evidence that these conditions correlate strongly with the likelihood of soil saturating, i.e., hydrological sensitivity, thus, by juxtaposing hydrology and biogeochemistry we can elucidate the distribution of denitrification hotspots across the landscape. We used nitrogen isotopes to quantify and characterize spatial patterns of denitrification in riparian zones and other saturated soils (i.e., the shallow saturated zone) of an agricultural landscape. *In situ* denitrification rates in shallow groundwater were determined monthly using the  $^{15}\text{N}\text{-NO}_3^-$  push-pull method. Annual rates of denitrification showed a strong positive relationship with topographic index, a well-

known wetness index and indicator of hydrologic similarity. The resulting relationship was used to distribute denitrification rates across the landscape and estimate denitrification N fluxes from the shallow saturated zone. Denitrification in the shallow saturated zone resulted in a N flux that was nearly half of the total denitrification from the landscape—in about a third of the area—as determined from an unusually well-constrained whole-farm N balance constructed from farm records and field measures. Denitrification N flux rates from saturated riparian soils were among the highest in the landscape, however the contribution of riparian areas to total landscape denitrification was less than 10 percent.

## BIOGRAPHICAL SKETCH

Todd Reif Anderson was born in Juneau, AK, the younger of two boys, to Norm Anderson and Linda Quesnell. He grew up in Juneau, and in 2002, obtained a B.S. in Environmental Science from University of Alaska Southeast. Shortly after graduating, Todd met his future wife, Denise, and holed up in a cabin near Haines, AK, with no running water and no electricity. While in Haines, Todd befriended the local brewer and was hired on as an apprentice. The small-town lifestyle and beautiful location nearly kept Todd and Denise rooted in Alaska. However, the prospects of a better career path led them to depart for Moscow, ID, where Todd obtained a M.S. in Environmental Science from University of Idaho in 2006. Immediately after graduating, Todd and Denise loaded up all their earthly possessions and a big black dog named Lou into their station wagon and headed east to Ithaca, NY, where Todd began to work on a Ph.D. in Biological and Environmental Engineering at Cornell University. Daughters Zoe and Ari were born in nearby Montour Falls during this period of Todd's academic endeavors.

## ACKNOWLEDGEMENTS

I want to thank my wife, Denise, who has supported me throughout this research with incredible patience and dedicated her time to our wonderful girls. I would also like to thank Todd Walter for providing me with this Ph.D. opportunity and guidance throughout, and for his eternal optimism and enthusiasm in all aspects of the project (and outside of it too). Thanks to my committee members and colleagues for their insight and constructive feedback regarding this work: Tim Fahey, Christy Goodale, Peter Groffman, and Sujay Kaushal. And lastly, thanks to Dan Fuka—fellow Soil and Water Lab member (and now doctor)—for moral support during the writing and defense process.

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## CHAPTER 1

### INTRODUCTION

Humans have dramatically altered the earth's nitrogen (N) cycle, largely through the industrial production of synthetic fertilizer to support agriculture (e.g., Vitousek et al., 1997; Galloway et al., 2008). Anthropogenic N applied by humans to the Earth's land surface now exceeds natural biological N<sub>2</sub> fixation in terrestrial ecosystems, resulting in substantial negative human and environment health effects (e.g., Galloway et al., 2004; Robertson and Vitousek, 2009). The main process that permanently removes reactive N from the environment is denitrification, the microbial production of N<sub>2</sub> from fixed N (Seitzinger et al., 2006). Denitrification is a facultative anaerobic process, with denitrifying bacteria requiring nitrate (reactive N to be reduced), an energy source (typically carbon), and sub-oxic or anoxic conditions (Boyer et al., 2006). Denitrification occurs in almost all terrestrial and aquatic ecosystems (see Seitzinger et al., 2006) and some human engineered systems (see Schipper et al., 2010). Despite many denitrification studies, there remains a need for more *in situ* denitrification measurements; few locations have measurements adequate to quantify rates or to understand factors controlling denitrification at the ecosystem scale (e.g., Boyer et al., 2006; Groffman et al., 2009b).

In a broad sense, hydrology has become key to understanding spatial and temporal rates and patterns of denitrification. Hydrology affects denitrification in two key ways: by transporting and mixing the necessary electron acceptors and donors (nitrate and carbon), and by changing the oxygen status of terrestrial soils or aquatic sediments (Boyer et al., 2006). These coupled eco-hydrological controls on oxygen, nitrate, and carbon availability give rise to hotspots and hot moments of denitrification activity in the landscape that are difficult to quantify and model (e.g.,

Groffman et al., 2009a). While riparian zones have received considerable attention as potential hotspots for denitrification (e.g., Hedin et al., 1998; McClain et al., 2003), similar conditions that may promote denitrification exist along a continuum throughout the landscape, and include areas prone to saturate permanently or periodically (e.g., Walter et al., 2000). Wet areas of the landscape are typically associated with sub-oxic or anoxic conditions; thus, partially to fully saturated soils and aquatic sediments are ideal sites for denitrification (e.g., Boyer et al., 2006; Groffman et al., 2009b). Despite this knowledge, spatiotemporal distributions of these denitrifying zones have not been quantifiably characterized and are often ignored in most denitrification studies.

The focus of the research presented in this dissertation is denitrification in riparian zones and other saturated soils of agricultural landscapes. It is presented as three separate papers, Chapters 2 – 4. The first paper (Chapter 2) explores denitrification in riparian zones of a typical northeastern US agricultural landscape, in which we quantify *in situ* rates of denitrification monthly over a one-year period and characterize physiochemical variables known to influence rates of denitrification. The second paper (Chapter 3) incorporates denitrification occurring in the shallow saturated zone from other parts of the landscape. We specifically develop and validate a model/method for scaling up point measurements of denitrification to larger scales (e.g., landscape or watershed). With a method for scaling our denitrification measurements to the entire agricultural landscape, the third paper (Chapter 4) constructs a whole farm-N balance as a means to investigate the contribution and relative importance of shallow saturated zone denitrification hotspots to the mitigation of nonpoint source N pollution. Unique to this body of work is the dataset of *in situ* denitrification measurements and associated physiochemical variables, presented in full at the end of this dissertation (Appendix).

The work herein supports the three fundamental areas for advancement targeted by the National Science Foundation Denitrification Research Coordination Network

(<http://www.denitrification.org>): (1) quantification of denitrification rates; (2) development of quantitative, process-based relationships between rates denitrification and controlling factors; and (3) production of spatially explicit, process-based models that can be used to scale-up site specific measurements to the ecosystem or larger scales.

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## CHAPTER 2

### DENITRIFICATION IN RIPARIAN ZONES OF A HEADWATER AGRICULTURAL LANDSCAPE\*

#### ABSTRACT

Riparian zones adjacent to cropped lands have been effective at reducing nitrate ( $\text{NO}_3^-$ ) loads to receiving water bodies primarily through plant uptake and denitrification. Denitrification represents both a permanent removal pathway and a greenhouse gas source, converting  $\text{NO}_3^-$  to inert  $\text{N}_2$  gas or nitrous oxide ( $\text{N}_2\text{O}$ ), and has been the subject of many studies in agricultural landscapes. Despite the prevailing notion that riparian zones can be areas of enhanced denitrification, there is a lack of *in situ* denitrification measurements from these areas that buffer streams and rivers from  $\text{NO}_3^-$  originating in upland cropped soils, especially over time scales that capture seasonal dynamics. We measured *in situ* denitrification rates in two riparian zones of an intensive dairy farm located in the headwaters of the Susquehanna River. Denitrification rates determined monthly over a one-year period with the  $^{15}\text{N}$ - $\text{NO}_3$  push-pull method ranged from 0 to  $4177 \mu\text{g N kg soil}^{-1} \text{ d}^{-1}$  with a mean of  $830 \pm 193 \mu\text{g N kg soil}^{-1} \text{ d}^{-1}$ . Denitrification showed a distinct seasonal pattern, with highest rates observed in the spring and summer, concomitant with warmer temperatures and decreasing dissolved oxygen. We estimate an annual N loss of  $470 \pm 116 \text{ kg yr}^{-1} \text{ ha}^{-1}$  of riparian zone via denitrification in the shallow saturated zone, with the potential for more than 20% of this amount occurring as  $\text{N}_2\text{O}$ . Total denitrification from shallow groundwater in the riparian zone was equivalent to 32% of manure N spread on the adjacent upland field, confirming the importance of riparian zones in agricultural landscapes at controlling N loads entering downstream waters.

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\* Anderson, T.R., P.M. Groffman, S.S. Kaushal, and M.T. Walter. Denitrification in riparian zones of a headwater agricultural landscape. *Journal of Environmental Quality*. <internal review>

## INTRODUCTION

Human alteration of the nitrogen (N) cycle has been well documented (e.g., Vitousek et al., 1997; Galloway et al., 2004; Galloway et al., 2008). There has been a doubling of the amount of available N in the terrestrial N cycle through increased production and use of industrial fertilizers, combustion of fossil fuels, cultivation of legumes, and other human activities (e.g., biomass burning). Agriculture is largely responsible for these changes. Additional N from fertilizers, manure, or N-fixation is largely presumed to be a crucial component of modern crop production, necessary to meet current and future food production demands. However, a substantial proportion of N added to cropping systems is lost to the environment in reactive forms. Major pathways include runoff and leaching of nitrate ( $\text{NO}_3^-$ ) to surface and groundwater, volatilization of ammonia ( $\text{NH}_3$ ) from soils, and fluxes of nitrous oxide ( $\text{N}_2\text{O}$ ) and other reactive N-containing gases ( $\text{NO}_x$ ) to the atmosphere (Robertson and Vitousek, 2009). Environmental consequences of these reactive N forms are well known and include eutrophication of coastal zones, compromised air and water quality, climate warming, and biodiversity changes in receiving ecosystems (e.g., Galloway et al. 2003).

Riparian areas have received considerable attention for their ability and potential to remove substantial amounts of  $\text{NO}_3^-$  from groundwater and shallow subsurface water before it is delivered to lakes and rivers (e.g., Gilliam, 1994; Hill, 1996). Denitrification and plant uptake are known to be the primary mechanisms of  $\text{NO}_3^-$  removal in riparian zones. Denitrification is of particular importance and interest because it provides a true N sink by converting  $\text{NO}_3^-$ , a biologically reactive form of N, into N gases (preferably inert  $\text{N}_2$ ) which return to the atmosphere; by contrast, plant uptake generally acts to temporarily immobilize reactive N, which may later be returned to hydrological flows to surface water bodies. Denitrification occurs

primarily under anaerobic conditions by heterotrophic microbes and is expected to be vigorous in wet soils (i.e., corresponding to low-oxygen conditions) high in organic carbon. Soils in riparian zones tend to satisfy these conditions and are often hot spots of denitrification and/or other biogeochemical transformations (e.g., Hedin et al., 1998; McClain et al., 2003; Vidon et al., 2010). Unfortunately, direct measurement of denitrification is problematic due to the high background concentration of  $N_2$  in the atmosphere; additional challenges arise from high spatial and temporal variation in the process itself, and scaling-up of measurements to ecosystem or watershed scales (e.g., Boyer et al., 2006; Groffman et al., 2006).

The majority of denitrification estimates from agricultural soils are based on laboratory studies of denitrification potential or unresolved N-balances, although in recent years there has been an increased effort to quantify *in situ* denitrification losses (Barton et al., 1999; Hofstra and Bouwman, 2005). The most common method for measuring denitrification is the acetylene ( $C_2H_2$ ) inhibition technique, generally applied to soil cores or soil slurries.  $C_2H_2$  inhibits the reduction of  $N_2O$  to  $N_2$ , so  $N_2O$  (which is much easier to measure) becomes the final product of denitrification. A widely recognized problem with the  $C_2H_2$  inhibition technique is that it also inhibits the production of  $NO_3^-$  via nitrification, which may lead to an underestimation of actual denitrification (Groffman et al., 2006). In agricultural landscapes which receive N additions (e.g., fertilizer, manure) and therefore have high  $NO_3^-$  concentrations, this is probably not much of a concern. However, the use of  $C_2H_2$  inhibition on soil cores or slurries may not yield representative measures of actual denitrification since soil samples are physically removed from their environment (biogeochemical disconnection) and often subjected to further alteration (e.g., changes in aeration status, soil structure, moisture content). A variety of  $^{15}N$  tracer methods have been used to quantify *in situ* denitrification in soils and sediments, surface waters, and groundwater. In most studies,  $^{15}N$ -labelled  $NO_3^-$  is directly added to soil or water, and measurement of the  $^{15}N$  gases generated represents actual denitrification. Depending on the

technique of  $^{15}\text{N}$  application and recovery, these methods require relatively little disturbance of *in situ* conditions.  $^{15}\text{N}$  tracer methods are best suited for agricultural soils or waters where the addition of N is less likely to show a fertilization effect and overestimate denitrification (Groffman et al., 2006). Direct measurements of denitrification in water-saturated soils are complicated by low gas diffusivity (Well and Myrold, 1999), but this has recently been overcome by combining  $^{15}\text{N}$  tracer techniques with a rapid, single-well “push-pull” method. The push-pull method assesses reaction rates of microbial activity by injecting a reactive solute plus tracer into groundwater and later extracting groundwater from the same location while measuring the reduction or accumulation of the various reactants and products (e.g., Trudell et al., 1986; Istok et al., 1997). The  $^{15}\text{N}\text{-NO}_3$  push pull method has been used to quantify denitrification in a variety of riparian settings, including fens (Well et al., 2001), freshwater and brackish riparian zones (Addy et al., 2002), pasture and grassland hydromorphic soils (Well et al., 2003), forested riparian sites (Kellogg et al., 2005; Watson et al., 2010), the riparian zone-stream interface of restored urban streams (Kaushal et al., 2008), seepage wetlands (Zaman et al., 2008), and urban wetlands (Harrison et al., 2011).

Measurements of denitrification from riparian zones in agricultural landscapes are important for estimating contributions to farm- and watershed-scale N budgets. Perhaps more importantly, these types of measurements can provide insight into the potential mitigation of agricultural nonpoint  $\text{NO}_3^-$  pollution by capitalizing on the natural ecosystem service of riparian denitrification. However, there is a lack of measurements of *in situ* denitrification rates in ephemeral headwaters and riparian zones in agricultural watersheds (Mayer et al., 2006). These areas are critical zones for denitrification. Ephemeral and low-order streams make up a large portion of cumulative stream length for high-order streams. Thus, the largest portion of annual stream nutrient loads enters watersheds from the headwaters (Alexander et al., 2007). Improved understanding of the factors that control denitrification in agricultural riparian zones is also an

important precursor to developing improved, targeted management practices to protect water quality. Part of the process to develop new strategies to control nonpoint source  $\text{NO}_3^-$  loads to surface waters typically relies on model simulations to compare outcomes of different possible scenarios (Boyer et al., 2006; Groffman et al., 2009). One critical short-coming of current agricultural water quality models is that they are calibrated against measurements of stream discharge and nutrient concentrations at a watershed's outlet, which provides little assurance that the internal processes are correctly represented or parameterized (e.g., Mehta et al., 2004; Garen and Moore, 2005). The ability to compare modeled predictions to measurements at different points within a watershed adds substantial confidence to overall model competence.

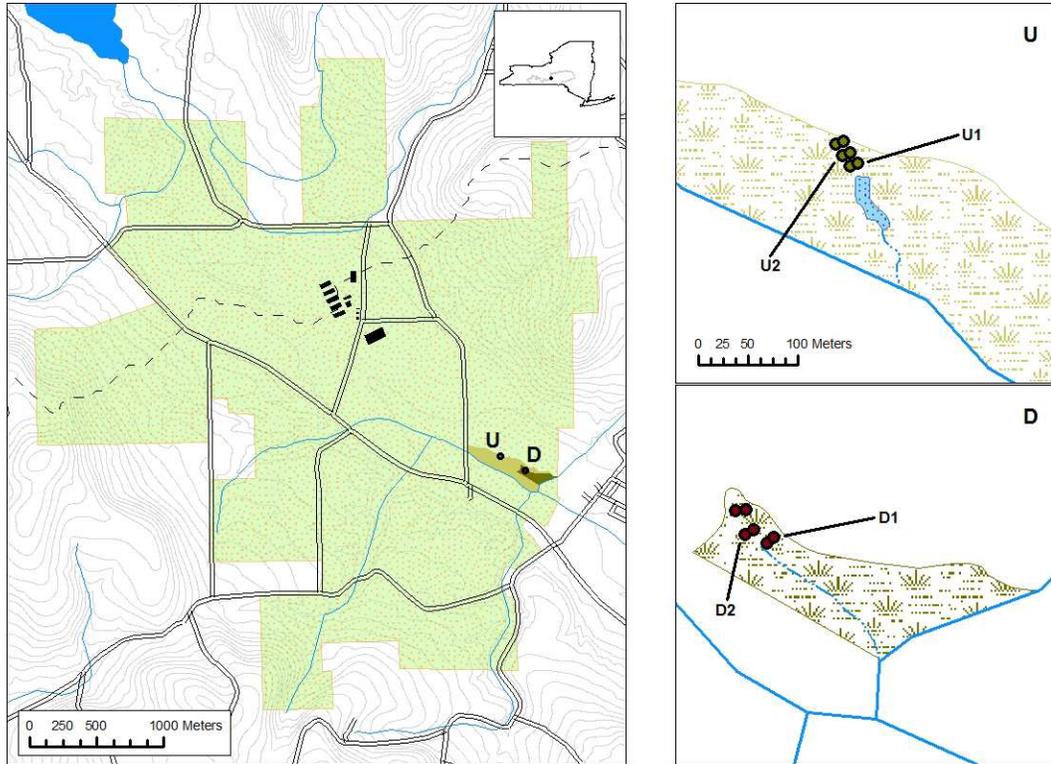
The objectives of this study were to: (1) quantify denitrification rates in two agricultural riparian zones of an active dairy farm in upstate New York; and (2) investigate potential seasonal factors influencing denitrification rates. We quantified *in situ* rates of  $\text{N}_2\text{O}$  and  $\text{N}_2$  production using the  $^{15}\text{N}\text{-NO}_3$  push-pull method at monthly intervals over a one-year period. We also characterized physiochemical variables known to influence rates of denitrification, particularly  $\text{NO}_3^-$ , carbon (C), and oxygen ( $\text{O}_2$ ). We hypothesized that riparian soils in agricultural landscapes have the potential to promote substantial denitrification year round and that the highest rates would be observed during the summer, concomitant with increasing temperatures and decreasing  $\text{O}_2$  (changes in environmental conditions known to promote denitrification). Our study site was located in the headwaters of the Susquehanna River watershed, the primary source to the Chesapeake Bay, which is highly impaired due to excess  $\text{NO}_3^-$  (e.g., Howarth et al., 2002; Kemp et al., 2005). The New York portion of the Susquehanna watershed has a current Total Maximum Daily Load (TMDL) N target of 8.77 million lbs  $\text{yr}^{-1}$  (3978 Mt  $\text{yr}^{-1}$ ) (USEPA 2010), which will require substantial reductions likely involving new and better management strategies (e.g., Boesch et al., 2001). This work is part of a larger, multidisciplinary study designed to

increase our knowledge of the sources and sinks of nutrients and sediments in the New York portion of the Susquehanna watershed (see Woodbury et al., 2008).

## **METHODS**

### **STUDY AREA DESCRIPTION**

We measured *in situ* denitrification rates in two riparian zones of a farm located at Cornell University's Animal Science Teaching and Research Center (T&R Center) near Harford, NY (Figure 2.1). The T&R Center occupies about 1050 ha (2600 acres) of land, of which 450 ha (1100 acres) are used for crop production (alfalfa and corn) and 160 ha (400 acres) are undeveloped pastureland used for grazing. The remaining acreage is utilized by the dairy, beef, and sheep facilities. The farm manages approximately 1000 dairy cows, 250 beef cattle, and 1100 sheep (T. Eddy, Director of Operations, personal communication, 2010). The T&R Center is situated in a broad valley floored with stratified drift, cut through bedrock ridges and drained by small headwater streams (Randall et al., 1988). A drainage divide runs thru the T&R Center with the area on the north side draining into the St. Lawrence River and the south draining into the Susquehanna River. The majority of the intensively farmed land is in the southern drainage system (Wang et al., 1999). The two riparian zones used in this study are adjacent to the easternmost cropped field within the T&R Center. At the time of this study, and two years prior, the field was under alfalfa rotation (i.e., continuously for three years). Farm records indicate that manure ( $130 \text{ kg N ha}^{-1} \text{ y}^{-1}$ ) was spread on the field (33 ha) during the winter and early spring months (Jan – Mar); no additional fertilizer was used (T. Eddy, personal communication, 2010). Soils in both sites are classified as Wayland (fine-silty, mixed, active, nonacid, mesic Fluvaquentic Endoaquepts), poorly drained silt loams belonging to the Howard association (Swader, 1972). Total riparian area delineated by this soil map unit (WbA) and confirmed using digital imagery plus soil survey data was 3 ha (NRCS, 2013). The streams that originate from



**Figure 2.1.** Location of study sites in upstream (U) and downstream (D) riparian zones of Cornell University's T&R Center near Harford, NY. The riparian sites are adjacent to the easternmost cropped field within the farm boundaries, and encompass intermittent streams that form headwaters for the Susquehanna River. A dashed line delineates the groundwater divide. The location of mini-piezometers reported on in this study are shown inset.

the riparian zones are intermittent, with higher flows occurring during periods of snowmelt or rain events and lower or no flow during periods of dry weather, primarily in the summer. The intermittent flow reflects the small watershed area, limited capacity for moisture storage in the upland soils, and heavy vegetative growth on the uplands (Swader, 1974).

## SITE INSTRUMENTATION

A series of mini-piezometers were installed in the two riparian sites to a depth of 50 cm below the soil surface in Fall 2007 (Figure 2.1). The mini-piezometers consisted of a stainless steel, mesh-screened well point (AMS, American Falls, ID) attached to 3/16-inch fluoropolymer tubing that extended above the ground surface. Mini-piezometers were installed in pairs at least 5 m apart and on opposite sides of observed surface water flow paths (i.e., surface water flows between the two mini-piezometers). The locations of individual mini-piezometers within pairs were selected based on accessibility and uniformity of vegetation and topography; locations of pairs within sites were selected based on observed differences in vegetation, topographic location, and/or wetness. The four mini-piezometers reported on in this study are a subset of locations used to consider denitrification patterns over the broader landscape and are considered to be representative of the general riparian zone (manuscript in preparation). Mini-piezometers are identified by riparian location, i.e., upstream (U1 and U2) or downstream (D1 and D2). Three PVC wells were installed at each site to a depth of approximately 35 cm below the soil surface and equipped with Odyssey Capacitance Water Level Probes (Odyssey House, Christchurch, New Zealand) to record water table fluctuations. Physiochemical characteristics for each site are shown in Table 2.1.

## SOIL SAMPLING AND ANALYSIS

Duplicate soil cores (7.2-cm diameter, 6.8-cm height) were taken by hand from 50 cm below the soil surface within 1 m of each mini-piezometer location over two consecutive months during late summer when water tables were low. Soil samples for bulk density were placed in weighing tins and oven dried at 105 °C for 24 hours. Particle density was determined using distilled water and a 100 mL volumetric flask (in place of a pycnometer). Soil C and N were determined using a NC 2100 soil analyzer (ThermoQuest Italia, Milan, Italy) and organic matter (OM) determined through loss on ignition (LOI) at the Cornell Nutrient Analysis Laboratory, Ithaca, NY.

## WATER SAMPLING AND ANALYSIS

Monthly groundwater and surface water samples were collected over the duration of this study. Shallow subsurface groundwater samples were collected from mini-piezometers using a Masterflex E/S Portable Sampler peristaltic pump (Cole Parmer, Vernon Hills, IL). Water samples from the intermittent streams within the riparian zones were collected when there was sufficient flow. Groundwater samples were collected from nearby shallow wells previously installed on the farm. All samples were collected in 125-mL low density polyethylene bottles (Fisher Scientific, Pittsburgh, PA) and stored on ice in the field until transport back to the laboratory. Samples were then refrigerated and filtered within 72 hours using Pall 0.45- $\mu$ m mixed cellulose ester filters (Pall Corporation, Ann Arbor, MI). Samples were analyzed for total dissolved nitrogen (TDN), nitrate-nitrogen ( $\text{NO}_3\text{-N}$ ), and dissolved organic carbon (DOC). TDN was determined using a Milton Roy Spectronic 501 spectrophotometer (Milton Roy Corporation, Rochester, NY) following persulfate digestion.  $\text{NO}_3\text{-N}$  was determined using a Dionex ICS-2000 ion chromatograph (Dionex, Sunnyvale, CA). DOC was determined using an O.I. Analytical 1010 total organic C analyzer (O.I. Corporation, College Station, TX). Dissolved oxygen (DO) content, temperature, and pH of the shallow subsurface groundwater were

measured in the field under continuous flow. DO and groundwater temperature were measured using a YSI 550A handheld DO instrument; pH was measured using a YSI 63 handheld pH and conductivity instrument (YSI, Yellow Springs, OH).

## DENITRIFICATION MEASUREMENT

*In situ* denitrification rates were measured monthly in each mini-piezometer from March 2009 to December 2009 using the  $^{15}\text{N}$ - $\text{NO}_3$  push-pull method (Istok et al., 1997; Addy et al., 2002; Zaman et al., 2008). Ambient groundwater was pumped from a mini-piezometer into a carboy the day before the push-pull and stored overnight in a cold room at 4°C. On the morning of the push-pull, a 10L enriched (32 mg N L<sup>-1</sup>) dosing solution was prepared consisting of ambient groundwater amended with isotopically enriched  $\text{NO}_3^-$  (20 atom percent  $^{15}\text{N}$ -enriched  $\text{KNO}_3$ ). Sulfur hexafluoride gas ( $\text{SF}_6$  100 mg L<sup>-1</sup>, He balance) (Airgas East, Salem, NH) was bubbled through the dosing solution prior to the push-pull to adjust DO concentrations to ambient groundwater conditions and serve as a conservative tracer; time of bubbling generally took 25-30 minutes. The carboy was capped, vents sealed, and headspace filled with  $\text{SF}_6$  for transport to the study site. The dosing solution was injected (pushed) into the mini-piezometer from which it came at a rate of 10 L hr<sup>-1</sup>; after an incubation period of 3.5 hours, groundwater was extracted (pulled) out of the mini-piezometer at a rate of 5 L hr<sup>-1</sup>. Water samples were collected at specific intervals during the push and pull phases as follows: two samples of the push phase at 1/3 and 2/3 of the total dosing volume (10L) and six samples during the pull phase at 0.5-L intervals of the first 3L extracted. An ambient groundwater sample was also collected prior to the push phase.

Water samples (20 mL) were taken from mini-piezometers through an air-tight sampling apparatus made of C-Flex tubing connected to the peristaltic pump and injected by syringe into evacuated serum bottles (125 mL). The remaining headspace was filled with ultra high purity grade He gas and the samples incubated for 24 hours at 4°C. Phase equilibration headspace extraction (Lemon and Lemon, 1981; Davidson and Firestone, 1988) was used to extract dissolved gases from the water samples. Gas samples (9 mL) were taken from the bottle headspace with a syringe and injected into evacuated gas vials (9 mL). Concentrations and isotopic composition of N<sub>2</sub>O and N<sub>2</sub> gases were determined on a ThermoFinnigan GasBench + PreCon trace gas concentration system interfaced to a ThermoScientific Delta V Plus isotope-ratio mass spectrometer (Thermo Fisher Scientific, Waltham, MA) at the Stable Isotope Facility, University of California at Davis, Davis, CA. Concentrations of SF<sub>6</sub> gas were determined on a Shimadzu GC 14 gas chromatograph (Shimadzu, Kyoto, Japan) at the Cary Institute of Ecosystem Studies, Millbrook, NY.

## RATE CALCULATIONS

Denitrification rates were calculated following the method developed by Addy et al. (2002) and used by subsequent researchers performing similar <sup>15</sup>N-NO<sub>3</sub> push-pull studies (e.g., Kaushal et al., 2008; Harrison et al., 2011). Rates were calculated using the average production of <sup>15</sup>N<sub>2</sub>O and <sup>15</sup>N<sub>2</sub> gases in the three ‘pull’ samples that had the highest SF<sub>6</sub> tracer recovery (C/C<sub>0</sub>), thus minimizing error from dilution and dispersion. Mass of N<sub>2</sub>O-N and N<sub>2</sub> were calculated for each headspace extraction sample using equations and constants from Tiedje (1982) and Mosier and Klemetsson (1994). The mass was transformed to mass of <sup>15</sup>N<sub>2</sub>O-N and <sup>15</sup>N<sub>2</sub> by multiplying by their respective <sup>15</sup>N sample enrichment proportion (the ratio of pulled atom percent to pushed atom percent, corrected for ambient atom percent). Sample <sup>15</sup>N<sub>2</sub>O-N and <sup>15</sup>N<sub>2</sub> gas production rates were expressed in conventional units of μg N kg soil<sup>-1</sup> d<sup>-1</sup> and calculated as [total mass of

$^{15}\text{N}_2\text{O-N}$  or  $^{15}\text{N}_2$  per volume of water pulled/(dry mass of soil per volume of water pulled x incubation time)] where each pulled sample represents 1L of groundwater pulled and the dry mass of soil occupied was calculated from soil bulk density and porosity. Sample denitrification rates were calculated as the sum of  $^{15}\text{N}_2\text{O-N}$  and  $^{15}\text{N}_2$  gas production rates (see Appendix A of Anderson 2013).

## STATISTICAL ANALYSES

Statistical analyses were performed using XLSTAT (Addinsoft, New York, NY). Significance was determined at  $\alpha=0.05$  (95% confidence). Unbalanced analysis of variance (ANOVA) was used to evaluate differences in mean values of ambient groundwater characteristics and denitrification rates for each mini-piezometer location. Pearson correlation analysis was used to examine relationships between denitrification rates and ambient groundwater characteristics including DOC,  $\text{NO}_3\text{-N}$ , DO, temperature, and pH.

## RESULTS

### AMBIENT GROUNDWATER CONDITIONS

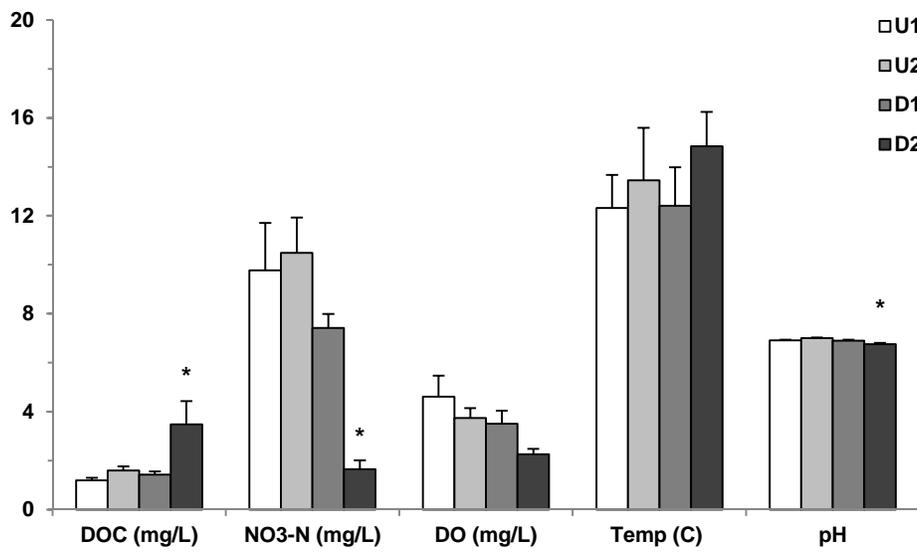
DOC concentrations ranged from 0.6 to 6.4  $\text{mg L}^{-1}$  across all mini-piezometers over the sampling period with a mean  $\pm$  SE of  $1.8\pm 0.2 \text{ mg L}^{-1}$ ;  $\text{NO}_3\text{-N}$  concentrations ranged from 1.0 to 15.9  $\text{mg L}^{-1}$  with a mean of  $7.6\pm 0.9 \text{ mg L}^{-1}$ ; DO concentrations ranged from 1.2 to 8.5  $\text{mg L}^{-1}$  with a mean of  $3.6\pm 0.3 \text{ mg L}^{-1}$ ; temperature ranged from 5.4 to 21.0  $^\circ\text{C}$  a mean of  $13.1\pm 0.8 \text{ }^\circ\text{C}$ ; and pH ranged from 6.5 to 7.1 with a mean of  $6.9\pm 0.0$ . Comparison of means showed that D2 had significantly higher DOC ( $3.5\pm 1.0 \text{ mg L}^{-1}$ ,  $F=6.2$ ,  $P<0.01$ ), lower  $\text{NO}_3\text{-N}$  ( $1.7\pm 0.4 \text{ mg L}^{-1}$ ,  $F=7.3$ ,  $P<0.01$ ), and lower pH ( $6.8\pm 0.1$ ,  $F=5.2$ ,  $P<0.01$ ) than all other mini-piezometers. Although not statistically significant, D2 also had lower DO ( $2.3\pm 0.2 \text{ mg L}^{-1}$ ,  $F=2.9$ ,  $P=0.11$ ) and higher

temperature ( $14.8 \pm 1.4$  °C,  $F=0.5$ ,  $P=0.69$ ) than all other mini-piezometers (Figure 2.2). As a whole, the downstream riparian site had higher DOC ( $2.2 \pm 0.5$  mg L<sup>-1</sup>,  $F=3.7$ ,  $P=0.07$ ), lower NO<sub>3</sub>-N ( $5.1 \pm 0.8$  mg L<sup>-1</sup>,  $F=10.6$ ,  $P<0.01$ ), lower DO ( $3.0 \pm 0.4$  mg L<sup>-1</sup>,  $F=3.8$ ,  $P=0.06$ ), higher temperature ( $13.4 \pm 1.1$  °C,  $F=0.1$ ,  $P=0.71$ ), and lower pH ( $6.8 \pm 0.0$ ,  $F=5.7$ ,  $P=0.03$ ) over the duration of the study (Table 2.1); however, only the NO<sub>3</sub>-N and pH differences were statistically significant.

## DENITRIFICATION RATES

Rates ranged from 0 to 4177 μg N kg soil<sup>-1</sup> d<sup>-1</sup> across all mini-piezometers (Figure 2.3) with a mean ± SE of  $830 \pm 193$  μg N kg soil<sup>-1</sup> d<sup>-1</sup>. The lowest rates were observed in U1 (0 μg N kg soil<sup>-1</sup> d<sup>-1</sup> measured in three consecutive spring months), while the highest rates were observed in D2 (>3000 μg N kg soil<sup>-1</sup> d<sup>-1</sup> measured in three consecutive summer months). A rate exceeding 1000 μg N kg soil<sup>-1</sup> d<sup>-1</sup> was measured at least once in each mini-piezometer. The mean denitrification rates for U1, U2, D1, and D2 were  $379 \pm 160$ ,  $593 \pm 121$ ,  $493 \pm 159$ , and  $2250 \pm 659$  μg N kg soil<sup>-1</sup> d<sup>-1</sup>, respectively (Figure 2.4). Comparison of means showed that D2 had a significantly higher denitrification rate than all other mini-piezometers ( $F=7.7$ ,  $P<0.001$ ); there was no significant difference in mean rate among the other mini-piezometers ( $F=0.4$ ,  $P=0.76$ ). As a whole, the downstream riparian site had a higher mean denitrification rate than the upstream site ( $1196 \pm 352$  and  $465 \pm 108$  μg N kg soil<sup>-1</sup> d<sup>-1</sup>, respectively), but this difference was not statistically significant ( $F=3.9$ ,  $P=0.06$ ).

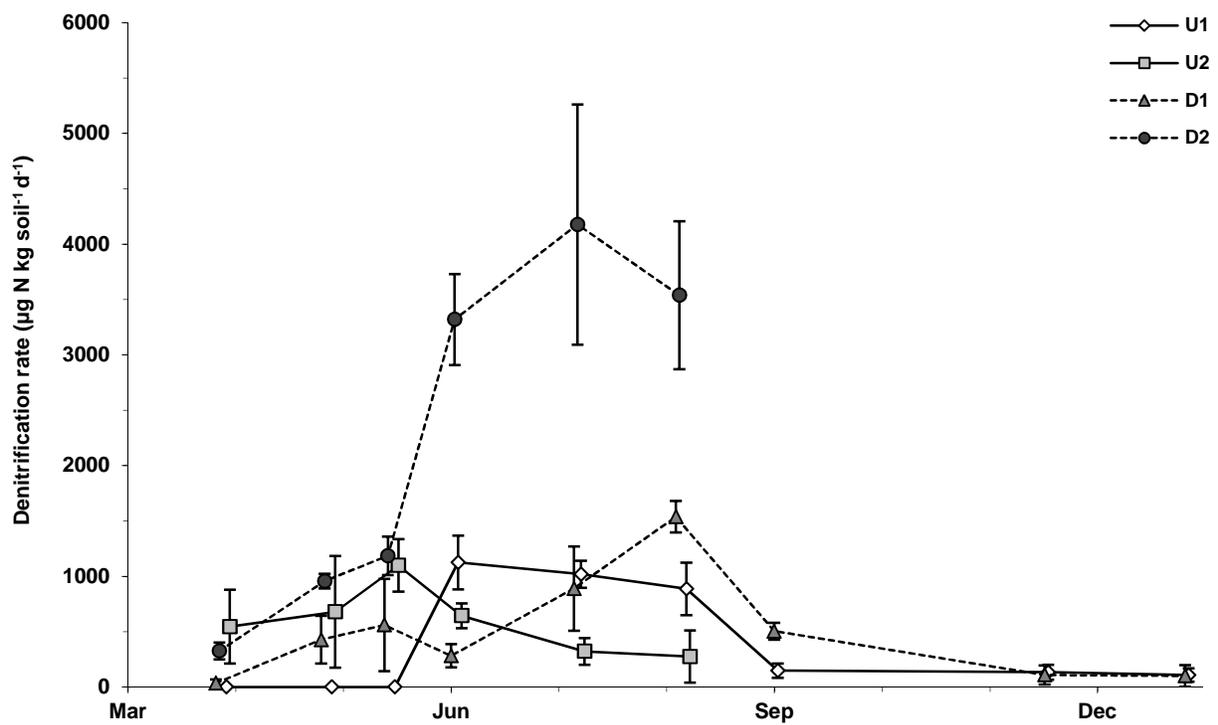
Strong seasonal patterns in denitrification coincided with notable seasonal variations in groundwater chemistry and temperature (Figure 2.5). Regression analyses showed significant relationships between denitrification rate and selected ambient groundwater characteristics when



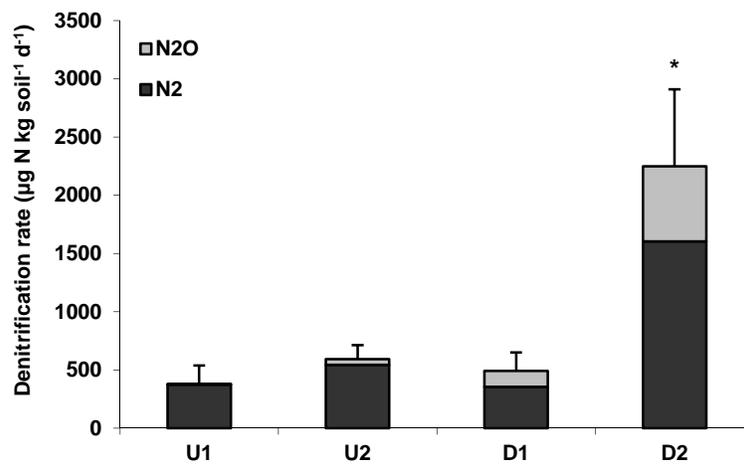
**Figure 2.2.** Mean  $\pm$  SE of ambient groundwater DOC, NO<sub>3</sub>-N, DO, temperature, and pH prior to *in situ* denitrification rate measurements at each mini-piezometer location. Asterisks (\*) indicate statistically significant means (P<0.05).

**Table 2.1.** Riparian site soil and groundwater characteristics

	<b>Upstream (U)</b>	<b>Downstream (D)</b>
Soil (n=4)		
OM (%)	10.3	16.0
C (%)	7.5	9.7
N (%)	0.7	0.8
pH	7.2	6.7
Bulk density (g cm <sup>-3</sup> )	0.63	0.47
Water (n=15)		
DOC (mg L <sup>-1</sup> )	1.4	2.2
TDN (mg L <sup>-1</sup> )	10.9	5.6
NO <sub>3</sub> -N (mg L <sup>-1</sup> )	10.1	5.1
DO (mg L <sup>-1</sup> )	4.3	3.0
Temperature (°C)	12.8	13.4
pH	7.0	6.8



**Figure 2.3.** *In situ* denitrification rates measured monthly at mini-piezometer location U1 (diamond), U2 (square), D1 (triangle), and D2 (circle). Values are mean  $\pm$  SE. Lines are provided to facilitate better visualization of each time series.



**Figure 2.4.** Mean  $\pm$  SE of *in situ* denitrification rates measured monthly for each mini-piezometer location. Rates are partitioned into mean N<sub>2</sub> and N<sub>2</sub>O production. Asterisks (\*) indicate statistically significant means (P<0.05).

**Figure 2.5.** *In situ* denitrification rates (solid circle) measured monthly at mini-piezometer location (a) U1; (b) U2; (c) D1; and (d) D2 plotted with selected ambient groundwater DOC (square), NO<sub>3</sub>-N (-x-), DO (triangle), and temperature (open circle). Lines are provided to facilitate better visualization of each time series. Note the difference in scale for denitrification rate in D2.

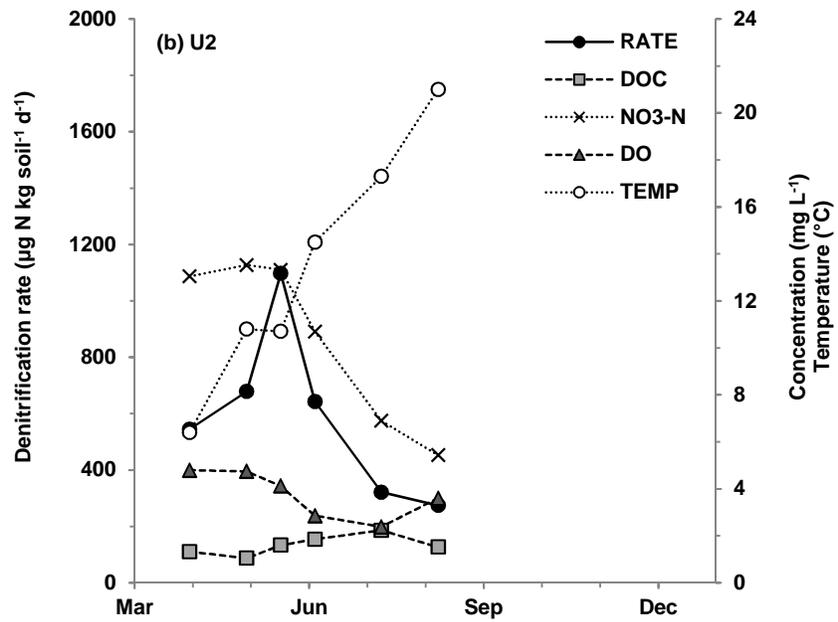
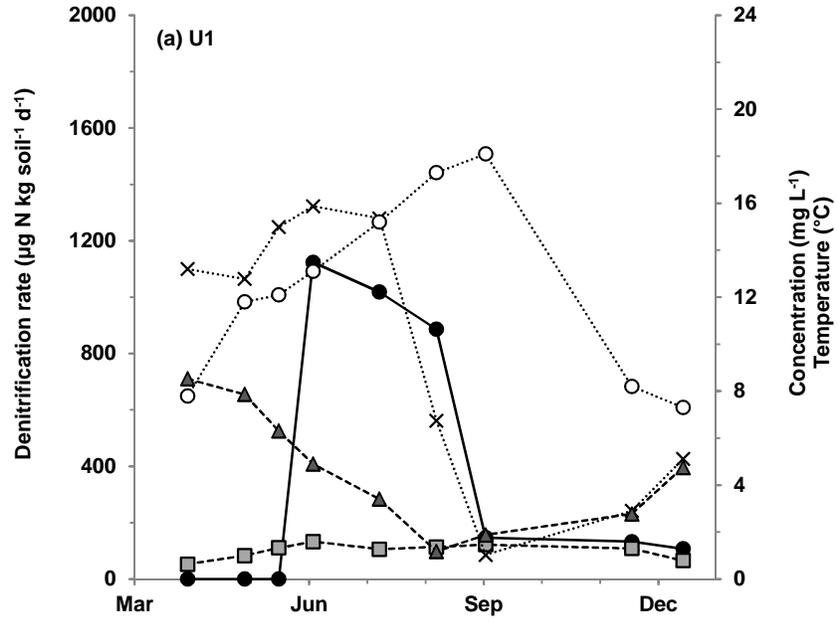
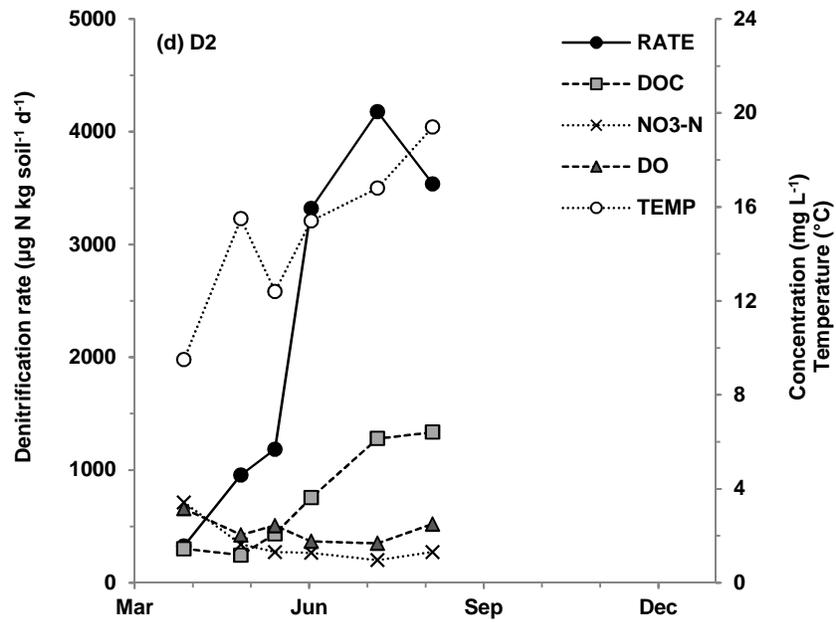
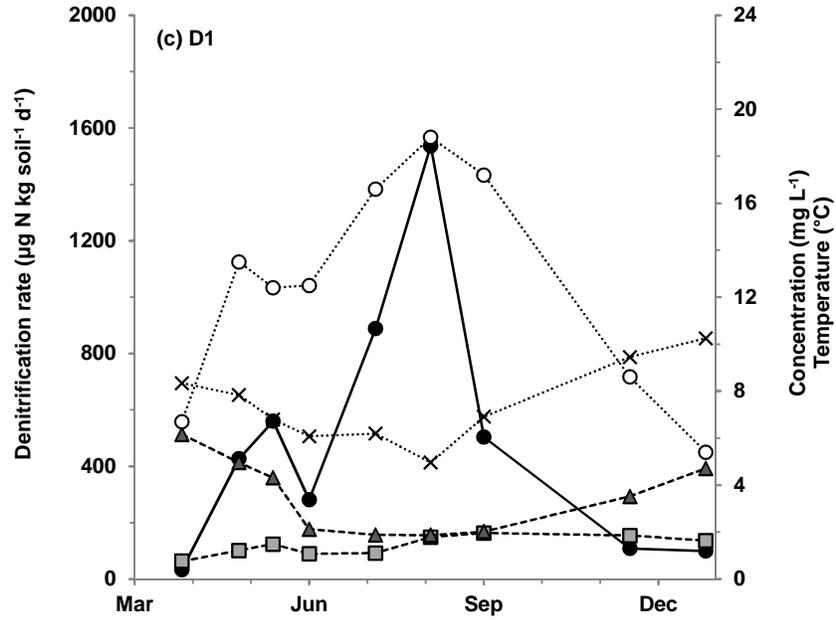


Figure 2.5 (Continued)



data were pooled across all mini-piezometers (Figure 2.6). Denitrification rates were positively correlated with DOC ( $r=0.89$ ) and temperature ( $r=0.46$ ), and negatively correlated with  $\text{NO}_3\text{-N}$  ( $r=-0.42$ ), DO ( $r=-0.46$ ), and pH ( $r=-0.30$ ). Only the pH relation was not statistically significant ( $r_{\text{crit}}=0.36$  at  $P=0.05$  with  $n=30$ ). A correlation matrix between denitrification rate and ambient groundwater characteristics is presented in Table 2.2.

$\text{N}_2$  was the dominant end product of denitrification over the study period (Figures 2.4 and 2.7).  $\text{N}_2\text{O}:\text{N}_2$  ratio ranged from 0 to 6.5 across all mini-piezometers during monthly measurements; the highest ratios were observed in the downstream riparian site (mean of 0.40).  $\text{N}_2\text{O}$  yield ( $\text{N}_2\text{O}/\text{N}_2\text{O}+\text{N}_2$ ) ranged from 0 to 0.87 across all measurements; the highest yields were also observed in the downstream riparian site (mean of 0.28). When combined, the riparian areas had a mean  $\text{N}_2\text{O}$  yield of 0.22 over the duration of the study.

## **DISCUSSION**

### **DENITRIFICATION RATE MAGNITUDES**

Comparison of denitrification rates across studies is complicated by differing methodologies and units of measurement, interpretation of results (i.e., representative of actual denitrification, denitrification capacity, or denitrification potential), and issues relating to both the spatial and temporal scale of the measurements taken (Groffman et al., 2006). To compare denitrification rates with previously published values, our *in situ* denitrification rates were converted to alternative units (from  $\text{mass N mass soil}^{-1} \text{ time}^{-1}$  to  $\text{mass N area}^{-1} \text{ time}^{-1}$ ; see Table 2.3) assuming that measured rates reflect conditions within the upper 0.5 m of the saturated zone of the riparian soil profile and that measured soil bulk density was constant/uniform in this portion of the soil (similar to Zaman et al., 2008). Denitrification rates from the downstream and upstream riparian

**Figure 2.6.** Relationships between *in situ* denitrification rate and ambient groundwater (a) DOC; (b) NO<sub>3</sub>-N; (c) DO; (d) temperature; and (e) pH over all study sites and sampling dates (n=30). Only the pH relation shown in (e) is not significant at P=0.05.

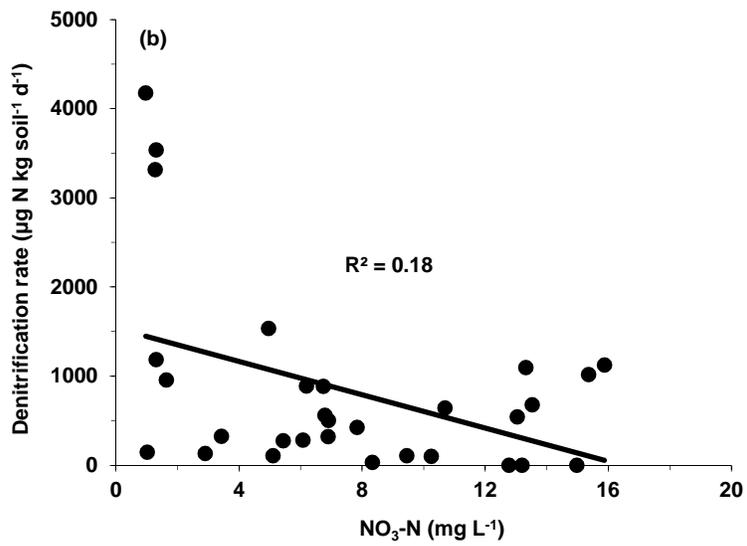
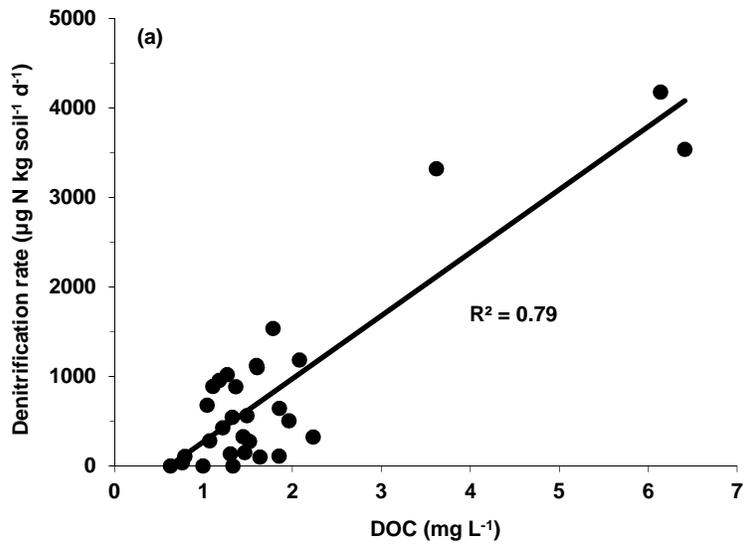


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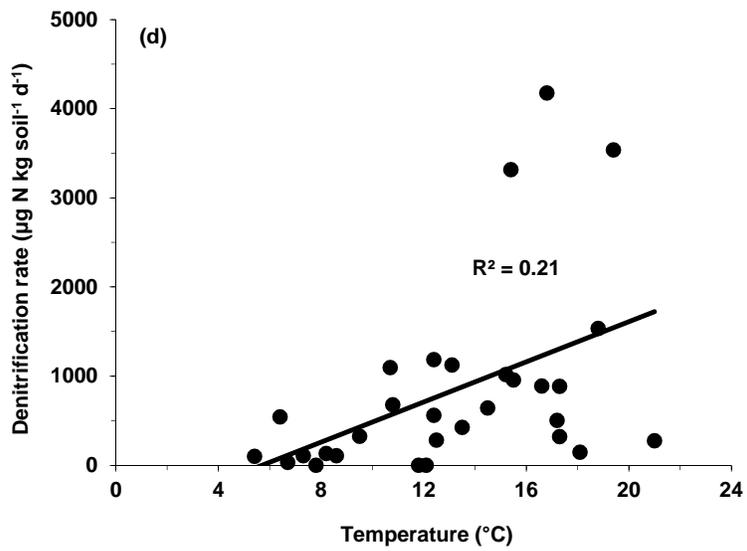
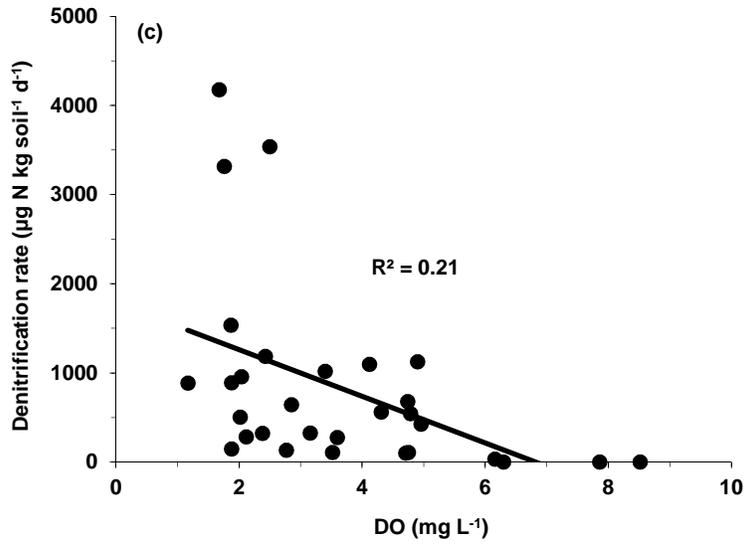
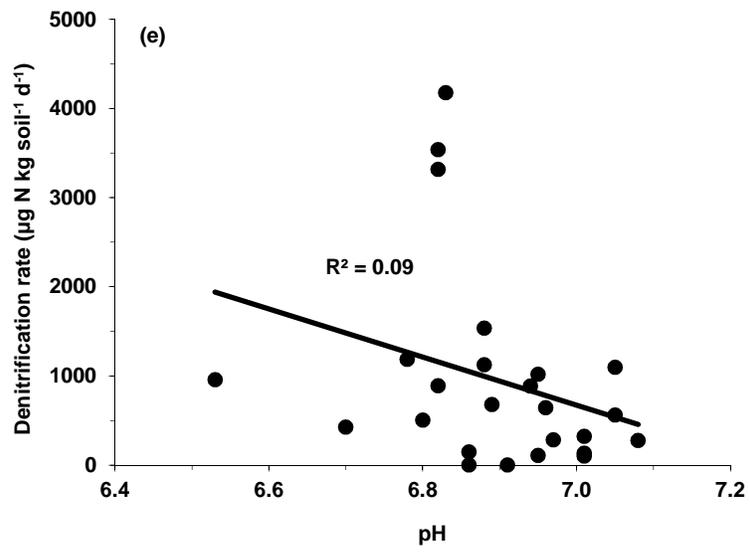


Figure 2.6 (Continued)



**Table 2.2.** Correlation matrix (r values) among *in situ* denitrification rate and ambient groundwater characteristics

	RATE	DOC	NO3	DO	TEMP	pH
RATE	<b>1</b>					
DOC	<b>0.892</b>	<b>1</b>				
NO3	<b>-0.423</b>	<b>-0.459</b>	<b>1</b>			
DO	<b>-0.460</b>	<b>-0.410</b>	<b>0.668</b>	<b>1</b>		
TEMP	<b>0.458</b>	<b>0.430</b>	<b>-0.393</b>	<b>-0.604</b>	<b>1</b>	
pH	-0.296	-0.157	0.344	0.185	-0.226	<b>1</b>

r values in **bold** indicate significance at P=0.05 (r=0.361)

**Figure 2.7.** Monthly *in situ* denitrification rates measured at mini-piezometer location (a) U1; (b) U2; (c) D1; and (d) D2. Rates are partitioned into mean N<sub>2</sub> and N<sub>2</sub>O production, and plotted with N<sub>2</sub>O yield [N<sub>2</sub>O/(N<sub>2</sub>+N<sub>2</sub>O)].

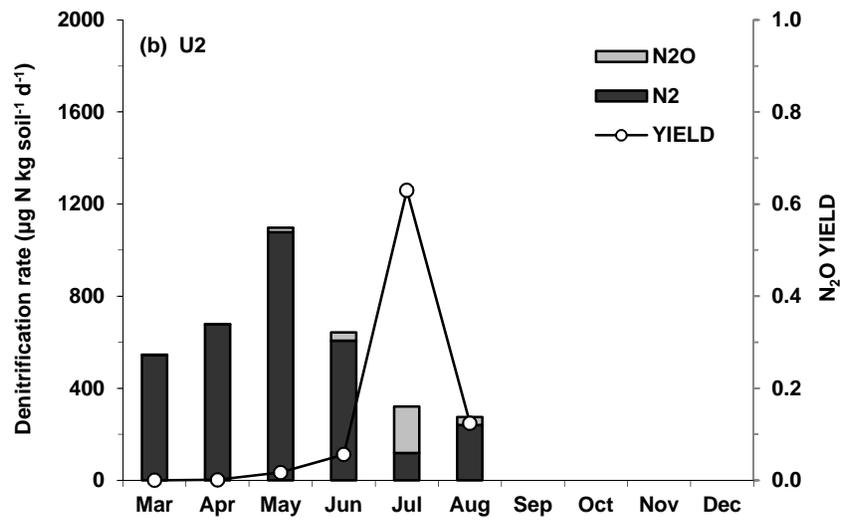
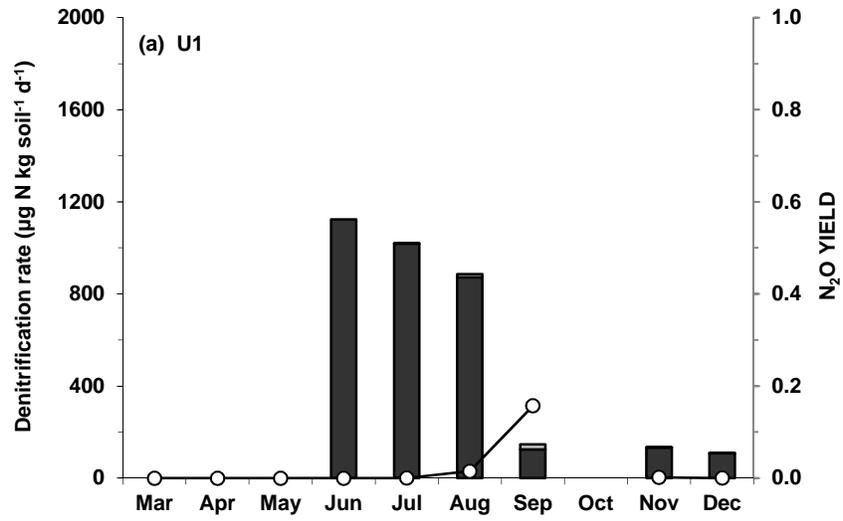
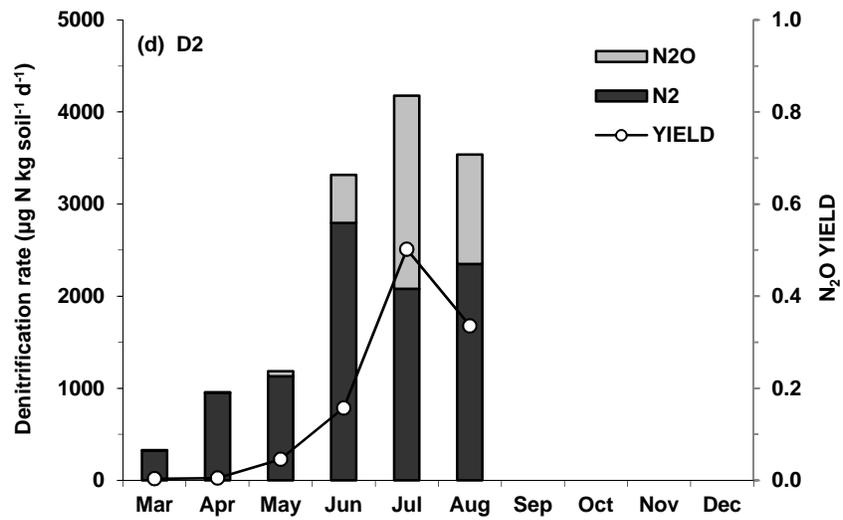
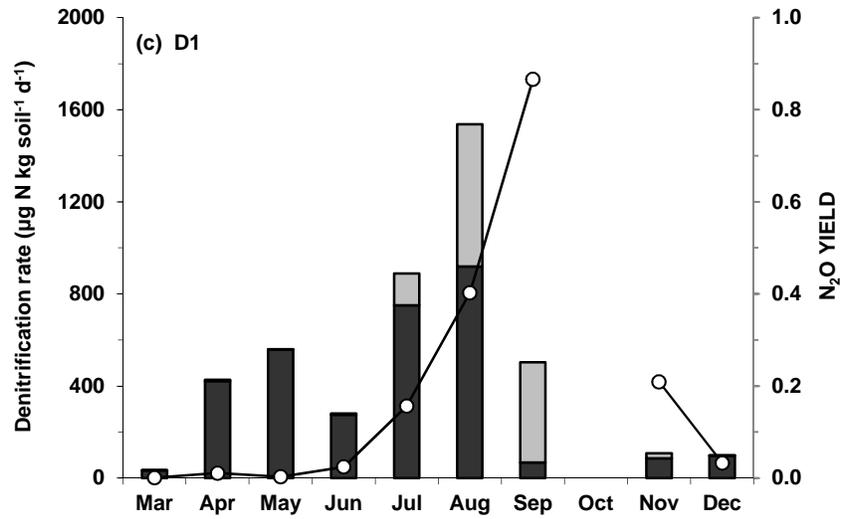


Figure 2.7 (Continued)



**Table 2.3.** Mean seasonal and annual denitrification rates for the riparian soils

	Denitrification rate			As removal rate
	$\mu\text{g N kg soil}^{-1}\text{d}^{-1}$	$\text{mg N m}^{-2}\text{d}^{-1}$	$\text{kg N ha}^{-1}\text{yr}^{-1}$	$\text{NO}_3\text{-N mg L}^{-1}\text{d}^{-1}$
Seasonal				
Spring	484	127	462	0.37
Summer	1501	367	1338	1.04
Fall	223	52	191	0.15
Winter	104	25	93	0.07
Mean	830	206	752	0.59
Annual				
Rate	543	129	470	0.37
Range (U – D)	291 – 747	85 – 172	310 – 629	0.25 – 0.48

areas were pooled together to calculate mean seasonal rates and an overall mean rate for the entire riparian area; an annual denitrification rate was estimated by assuming a value of zero for any month in which a measurement was not taken due to low water table or winter inaccessibility (Table 2.3). We estimated an annual denitrification flux from the shallow saturated zone in riparian soils of  $470 \pm 116 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  (mean  $\pm$  SE). Scaled up to the area of riparian zone (3 ha) amounts to a denitrification loss of 1353 kg N, equal to 32% of the manure N applied to the alfalfa field upland of the riparian zone.

Most studies that are of sufficient duration and sampling frequency to capture temporal variations (e.g., seasonally or monthly for one year) of *in situ* denitrification in riparian zones of agricultural landscapes use the  $\text{C}_2\text{H}_2$  inhibition technique on surface soil cores (Table 2.4). Our push-pull estimates of denitrification from the shallow groundwater in riparian zones are quite higher (Table 2.3), suggesting that denitrification fluxes from surface soils alone do not provide an accurate representation of this valuable ecosystem service. Unfortunately, there is a lack of analogous studies in this critical zone. Some studies have measured shallow groundwater denitrification rates in agricultural riparian zones—albeit over shorter periods—and these are comparable to the rates we measured (e.g., Bragan et al., 1997; Hill et al., 2000; Baker and Vervier, 2004; Vidon and Hill, 2004; Zaman et al., 2008). Zaman et al. (2008) is particularly relevant, as they measured denitrification rates in seepage wetland soils intercepting flow from a grazed dairy catchment using the  $^{15}\text{N}\text{-NO}_3$  push-pull method and report a groundwater denitrification rate of  $1.11 \text{ mg N L}^{-1} \text{ d}^{-1}$  (estimated areal rate of  $289 \text{ mg N m}^{-2} \text{ d}^{-1}$ ) in the late summer during low flow conditions. Our groundwater denitrification rates peaked in summer as well, with a mean of  $1.10 \text{ mg N L}^{-1} \text{ d}^{-1}$  across all four mini-piezometer sites prior to the water table dropping below two of four mini-piezometer installations. Baker and Vervier (2004) report a groundwater denitrification rate of  $2.67 \text{ mg N L}^{-1} \text{ d}^{-1}$  via  $\text{C}_2\text{H}_2$  push-pull during low flow conditions (summer) in a riparian forest bordering fields cropped wheat and corn. We measured

**Table 2.4.** Comparison of annual denitrification rates in riparian soils of agricultural landscapes

Riparian system, upland crop cover, and management	Denitrification Rate	Units	Method	Sampling frequency and study duration	No. sites (replicates per)	Reference
Georgia, USA Riparian forest Corn, peanut, soybean Fertilizer	31.5	kg N ha <sup>-1</sup> yr <sup>-1</sup>	C <sub>2</sub> H <sub>2</sub> inhibition Soil cores Field incubated	Monthly Two years	6 (0)	Lowrance et al. (1984)
France Riparian forest Corn, sunflower, orchard Fertilizer	55 – 115	kg N ha <sup>-1</sup> yr <sup>-1</sup>	C <sub>2</sub> H <sub>2</sub> inhibition Soil cores 0 – 10 cm Lab incubated, 10°C	Monthly One year	4 (3)	Pinay et al. (1993)
Georgia, USA Restored riparian forest wetland Bermudagrass pasture Fertilizer and manure (dairy)	68	kg N ha <sup>-1</sup> yr <sup>-1</sup>	C <sub>2</sub> H <sub>2</sub> inhibition Soil cores 0 – 24 cm Lab incubated, 25°C	Monthly Two years	24 (0)	Lowrance et al. (1995)
England Riparian buffer strip Intensively farmed arable lands upslope	26	kg N ha <sup>-1</sup> yr <sup>-1</sup>	C <sub>2</sub> H <sub>2</sub> inhibition Soil cores 0 – 10 cm Field incubated	Monthly One year	1 (6)	Burt et al. (1999)
France Riparian wetland Maize, wheat Fertilizer	565 – 819 175 – 689	kg N ha <sup>-1</sup> yr <sup>-1</sup> µg N kg <sup>-1</sup> d <sup>-1</sup>	C <sub>2</sub> H <sub>2</sub> inhibition Soil cores 0 – 50 cm Lab incubated, field temp	Seasonally One year	2 (27)	Clement et al. (2002) Clement et al. (2003)
France Riparian soils Maize, wheat Fertilizer and manure (dairy)	91	kg N ha <sup>-1</sup> yr <sup>-1</sup>	C <sub>2</sub> H <sub>2</sub> inhibition Soil cores 0 – 40 cm Field incubated	Monthly 16 months	7 (10)	Oehler et al. (2007)
Oregon, USA Noncultivated riparian area Ryegrass Fertilizer	20.2 – 59.0	kg N ha <sup>-1</sup> yr <sup>-1</sup>	C <sub>2</sub> H <sub>2</sub> inhibition Soil cores 0 – 15 cm Field incubated	Every 4 – 6 weeks Two years	2 (5)	Davis et al. (2008)
Oregon, USA Riparian forest Ryegrass Fertilizer	1.1 – 1.6	kg N ha <sup>-1</sup> yr <sup>-1</sup>	C <sub>2</sub> H <sub>2</sub> inhibition Soil cores 0 – 15 cm Field incubated	Every 4 – 6 weeks 18 months	2 (3)	Davis et al. (2011)

denitrification rates of similar magnitude in D2 only, with a peak of  $2.73 \text{ mg N L}^{-1} \text{ d}^{-1}$  in July. Denitrification rates in the other mini-piezometer locations ( $0.82$ ,  $0.30$ , and  $0.54 \text{ mg N L}^{-1} \text{ d}^{-1}$  in U1, U2, and D1, respectively) are closer to observed denitrification ( $0.54 \text{ mg N L}^{-1} \text{ d}^{-1}$ ) during late summer in a forest riparian zone below intensive potato production, as measured via  $\text{C}_2\text{H}_2$  push-pull (Hill et al., 2000). Vidon and Hill (2004) measured denitrification in a riparian forest downslope of fertilized corn fields when water tables were at their highest (April), and reported a groundwater denitrification rate of  $0.18 \text{ mg N L}^{-1} \text{ d}^{-1}$ . In our riparian sites, soils were saturated to the surface throughout March and most of April; shallow groundwater denitrification averaged  $0.28 \text{ mg N L}^{-1} \text{ d}^{-1}$  during this time.

#### DENITRIFICATION RATE PATTERNS

Denitrification showed a distinct seasonal pattern in each mini-piezometer (Figure 2.5). Rates were initially low coming out of the winter and increased during the spring, peaked in summer, then decreased in the fall before reaching their lowest in the middle of winter. This pattern of denitrification has been observed in other agricultural riparian areas (e.g., Watts and Seitzinger, 2000; Clement et al., 2003; Hefting et al., 2003; Oehler et al., 2007), but is opposite of what typically occurs in natural or unmanaged landscapes. In unmanaged landscapes, denitrification rates are highest in the late fall-winter-early spring months when demand for N is reduced; during the growing season, plant uptake of water and may N limit denitrification (Groffman and Tiedje, 1989). This seasonal pattern has also been observed in surface soils in agricultural riparian areas (e.g., Pinay et al., 1993; Pinay et al., 1998; Burt et al., 1999). Hefting et al. (2003) suggest that observed differences in seasonal dynamics can generally be attributed to  $\text{NO}_3^-$  availability, reflecting the competition between denitrifiers and vegetation, and ground water levels that provide optimal conditions for denitrification. In agricultural landscapes where N is abundant and available in excess beyond what is required for plant growth, primary controls on

denitrification shift to a dependence on C availability, O<sub>2</sub> status, and temperature (Barton et al., 1999). Depending on the cropping system (e.g., crop type, harvest method), tillage (till versus no-till), and manure disposal/spreading, there may be enough labile C present that denitrification rates are controlled primarily by O<sub>2</sub> status and temperature. In riparian areas of these systems, rates will be highest in the late spring-summer-early fall months when temperatures are warmer and O<sub>2</sub> lower, as long as the soils remain wet. While these patterns can be examined in Figure 2.5 for each mini-piezometer, they became more evident when data were pooled across all mini-piezometers. As denitrification rates increased through the spring and peaked in the summer, both temperature and DOC increased while both NO<sub>3</sub><sup>-</sup> and DO decreased. The patterns subsequently inverted after rates peaked, i.e., DOC and temperature decline while NO<sub>3</sub><sup>-</sup> and DO rise.

## DENITRIFICATION RATE RELATIONSHIPS

Factors influencing denitrification at the landscape, field, and plot scale have been well studied in a variety of ecosystems. At the organism level, activity of denitrifying bacteria is mainly regulated by the availability of O<sub>2</sub>, NO<sub>3</sub><sup>-</sup>, and C (Burgin and Hamilton 2007). Denitrifiers are ubiquitous in soil environments and, as such, many field denitrification studies measure O<sub>2</sub> (or soil moisture as a proxy), NO<sub>3</sub><sup>-</sup>, and/or C along with environmental conditions such as temperature and pH and omit specific microbial measures (Zumft 1997; Philippot et al., 2007). In the absence of limiting conditions, denitrification has been found to increase with increasing NO<sub>3</sub><sup>-</sup>, C, or temperature (positive correlation), and decrease with increasing O<sub>2</sub> (negative correlation) (e.g., Firestone, 1982; Knowles, 1982). The effect of pH on denitrification rate is not as pronounced, but researchers have found denitrification activity inhibited at low pH compared to the relatively neutral pH 6-8 range of soils (Firestone, 1982).

Our denitrification rate relationships (Figure 2.6) followed these idealized relations with a couple of exceptions, providing insight into the complex biogeochemical interactions that can drive denitrification in riparian zones found in agricultural landscapes. The strongest rate correlation we found was with DOC, followed by DO and temperature. As expected, both the DOC- and temperature-rate correlations were positive whereas the DO-rate correlation was negative. The strength of the DOC-rate correlation as compared to the nitrate-rate correlation can be explained if the system is not nitrate-limited, as often is the case in agricultural settings (e.g. Groffman et al., 1987). It is also important to note that  $\text{NO}_3^-$  is added in the push/pull incubations, making this method imperfect for assessing  $\text{NO}_3^-$  limitation of denitrification. If  $\text{O}_2$  status and temperature are suitable for denitrification in any given system, then either C or  $\text{NO}_3^-$  may be limiting. Also as expected, the temperature- and DO-rate correlations are nearly equal in magnitude (strength) but opposite in direction. DO in water is temperature dependent due to gas solubility constraints and biologic activity/kinetics. The negative nitrate-rate correlation does not fit the idealized relation, but was expected since the system was probably not nitrate-limited due to regular nearby manure spreading and legume cropping. Thus, we anticipate that  $\text{NO}_3^-$  does not drive denitrification, so  $\text{NO}_3^-$  levels are more indicative of how much denitrification is occurring (e.g., relatively low levels of  $\text{NO}_3^-$  indicate more denitrification). The relationships among DO, DOC, and denitrification are also not simple in that low DO is likely driven by high DOC that drives  $\text{O}_2$  consumption.

It is apparent that a few data points heavily influenced these correlations between denitrification rates and their potential controls, particularly the three highest rates we measured during the study period (Figure 2.6). We examined their influence by removing these three points to see how the relationships changed without them. The sign/direction of all correlations remained the same, but the correlation coefficients of the DOC- and nitrate-rate correlations (r values of 0.35 and -0.02, respectively) fell below the level of significance ( $r_{\text{crit}}=0.38$  at  $P=0.05$  with  $n=27$ ). The

strength of the DO-rate correlation ( $r=-0.49$ ) increased slightly, while the strength of the temperature-rate correlation ( $r=0.43$ ) decreased slightly; both remained statistically significant. These changes suggest that denitrification in agricultural riparian zones is largely controlled by  $O_2$  status and temperature, at least in landscapes which receive N and C additions from manure spreading.  $NO_3^-$  is not only abundant, but most likely in excess (non-limiting) compared to the C requirement; inputs of C appear capable of driving denitrification rate increases under suitable ambient DO and temperature conditions. DOC can also drive decreases in DO via  $O_2$  consumption, making conditions more favorable for denitrification. Results from a companion study support these findings (see Appendix B of Anderson 2013); specifically, we found increased denitrification rates in some mini-piezometers amended with sodium acetate as an additional DOC source in the dosing solution; rates were more enhanced (as compared to the control) in the summer/fall months when temperatures were warmer and DO lower.

## NITROUS OXIDE PRODUCTION

There is great interest in  $N_2O$  production from agricultural landscapes because of the importance of  $N_2O$  as a greenhouse gas (GHG) (e.g., Mosier et al., 1998; Davidson 2009). Denitrification is one potential source of  $N_2O$ , but it is not clear how much this contributes to total  $N_2O$  production from the landscape. The denitrification pathway at its most basic level is enzymatic reduction of  $NO_3^-$  to  $N_2$  thru a series of intermediates, the last of which is  $N_2O$ . Research has shown  $N_2$  to be the dominant end product of denitrification in a range of ecosystems, but it is possible to see incomplete denitrification whereby a large portion of the  $N_2O$  produced does not undergo further reduction (Schlesinger 2009; Beaulieu et al., 2011). Schlesinger (2009) compiled  $N_2O$  yields (as a ratio,  $N_2O/N_2O+N_2$ ) from various laboratory and field studies of denitrification, and found that mean yields in freshwater wetland/flooded soils were generally low ( $0.082\pm 0.024$ ) while yields in agricultural soils were considerably higher ( $0.375\pm 0.035$ ). To develop a better understanding

of N<sub>2</sub>O production in these soils, N<sub>2</sub>O yields should be discussed in the context of overall denitrification rates. For example, upland agricultural soils may exhibit larger N<sub>2</sub>O yields than wetland soils, but corresponding denitrification rates tend to be much higher in wetland soils.

When pooled across all mini-piezometers, the mean N<sub>2</sub>O yield (0.22) fell near the midpoint between values expected for wetland soils and agricultural soils given in Schlesinger (2009) (0.082 – 0.375). When looked at individually, the downstream riparian mini-piezometers exhibited mean yields more commonly found in agricultural soils, whereas the upstream riparian mini-piezometers exhibited mean yields typically found in wetland soils. We found considerable range in N<sub>2</sub>O yields within mini-piezometers, from nearly pure N<sub>2</sub>O (values close to 1) to nearly pure N<sub>2</sub> (values close to 0) across all measurements. However, much like overall denitrification rate, a distinct seasonal pattern is apparent in each individual mini-piezometer (Figure 2.7). N<sub>2</sub>O yield was very small (<0.01) in the spring, increased into the summer, peaked mid- to late-summer, then decreased through the fall and remained low in the winter. The peak N<sub>2</sub>O yield exceeded 0.50 in all mini-piezometers except U1, indicating that more N<sub>2</sub>O was being produced than N<sub>2</sub> during denitrification. Fortunately, from a GHG flux standpoint, the seasonal pattern of N<sub>2</sub>O yield lagged that of denitrification by a month, so these peaks generally coincided with decreasing denitrification rates (the post-peak recession). This was not always the case though, as peak N<sub>2</sub>O yield in D2 occurred at the same time as peak denitrification rate, which also happened to be the largest rate we measured over the course of the study.

Our results suggest that N<sub>2</sub>O emissions may have been a significant portion (>20%) of total denitrification from the riparian zones in our agricultural landscape over the duration of the entire study. It is important to note that because the push-pull method involves addition of NO<sub>3</sub><sup>-</sup>, it inherently overestimates N<sub>2</sub>O yield during denitrification, which is strongly influenced by

$\text{NO}_3^-$  concentrations (Firestone et al. 1980). It is also possible that our relatively short incubation period lead to an additional overestimation of  $\text{N}_2\text{O}$  yields, since  $\text{N}_2\text{O}$  is produced as an intermediate and can be further reduced to  $\text{N}_2$  in denitrifying environments (Well et al., 2003). Li et al. (2011) measured a mean  $\text{N}_2\text{O}$  flux of  $105 \mu\text{g N m}^{-2} \text{hr}^{-1}$  (equivalent to  $9.2 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ) within the same downstream riparian area during late spring and early summer (Mar – May) using static chambers arranged near D1. To put our measurements in context of the total  $\text{N}_2\text{O}$  flux, we estimate the  $\text{N}_2\text{O}$  produced via denitrification during the same months to be  $19 \mu\text{g N m}^{-2} \text{hr}^{-1}$  ( $1.7 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ), or about 18% of the total  $\text{N}_2\text{O}$  flux from the riparian zone.

## CONCLUSIONS

The riparian zones we studied can remove substantial  $\text{NO}_3^-$  via denitrification year round under *in situ* conditions, with higher rates during the summer occurring with increasing temperature and decreasing  $\text{O}_2$ . Total denitrification from shallow groundwater in the riparian zone was equal to 32% of manure N spread on the field above directly above the buffer. Our results are in agreement with the prevalent management practice of establishing and/or maintaining riparian zones in agricultural landscapes as a way to reduce N loads to aquatic systems. However, we also found that  $\text{N}_2\text{O}$  produced through denitrification may account for a significant portion (22%) of the total N flux, with larger  $\text{N}_2\text{O}$  yields associated with larger denitrification rates. The  $^{15}\text{N}$ - $\text{NO}_3$  push-pull method for measuring denitrification in saturated soils inherently increases the  $\text{N}_2\text{O}:\text{N}_2$  ratio of  $^{15}\text{N}$  gases generated, but the magnitude of this increase is unknown.

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## CHAPTER 3

### USING A SOIL TOPOGRAPHIC INDEX TO DISTRIBUTE DENITRIFICATION FLUXES ACROSS A NORTHEASTERN HEADWATER CATCHMENT<sup>2</sup>

#### ABSTRACT

Conceptually, riparian zones are considered potential hotspots of denitrification because they allow for the confluence of necessary electron acceptors (nitrate) and donors (carbon) via hydrologic flowpaths in low oxygen (reducing) conditions. While these areas have received considerable attention, other parts of the landscape exhibit similar qualities, namely those areas that are prone to saturate. We quantified *in situ* denitrification rates in the shallow saturated zone, a dynamic portion of the landscape, across a range of hydroperiodicities, i.e., frequencies and durations of saturated conditions, as characterized by a topographic index in a small mixed land-use headwater catchment in central New York State. We found a strong positive relationship between topographic index and denitrification, indicating that the highest rates of denitrification occur in the relatively small portion of the landscape prone to saturation. We used the resulting relationship to distribute denitrification rates across the catchment and estimate denitrification fluxes from the shallow saturated zone. While the highest rates of denitrification were observed in wetter portions of the landscape, including riparian zones, we found that the shallow saturated zone beneath drier upland soils contributed to a larger portion of whole-catchment denitrification due to a larger areal extent. A topographic index-denitrification model is a promising and simple tool that allows for scaling of *in situ* denitrification rates across the landscape and provides insight into the spatial organization of denitrification at the catchment scale.

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<sup>2</sup> Anderson TR, Groffman PM, Walter MT. Using a soil topographic index to distribute denitrification fluxes across a northeastern headwater catchment. Biogeochemistry. <internal review>

## INTRODUCTION

Denitrification is an important part of the nitrogen (N) biogeochemical cycle and a valuable ecosystem service for protecting aquatic habitats from nonpoint source pollution. Unfortunately, it is also extremely difficult to quantifiably measure in the environment. It is commonly estimated at landscape or watershed scales as the difference between all the measurable N-inputs and –outputs (e.g., van Breemen et al. 2002; Gentry et al. 2009). While this type of large-scale mass balance approach is powerful, it is difficult to meaningfully attribute denitrification rates across a heterogeneous landscape. In fact, it is widely recognized that there are biogeochemical hotspots, which account for small fractions of the landscape but, by definition, show disproportionately high reaction rates relative to the surrounding area (McClain et al. 2003). Conceptually, research has shown that denitrification rates are especially high in carbon (C)-rich parts of the landscape with low oxygen ( $O_2$ ) levels and sufficiently high nitrate ( $NO_3^-$ ) concentrations (e.g., Hill et al. 2000; Burgin et al. 2010); these conditions are commonly associated with riparian areas, wetlands, and other parts of the landscape prone to wet or saturated soil conditions. Because these types of areas are recognized as likely denitrification hotspots, many studies have focused on measuring denitrification from these spots. However, these areas are, for the most part, only qualitatively characterized with respect to denitrification, which makes it difficult to translate these point measurements to landscape or watershed scales. Finding ways to quantifiably synthesize our understanding of denitrification across scales may help environmental planners develop better strategies for targeting management practices for reducing nonpoint source N pollution in streams, lakes, and coastal ecosystems (Walter et al. 2007).

Because denitrification hotspots are commonly associated with wet soils, several researchers have suggested that there is good potential for capturing hotspot activity by coupling

hydrological and denitrification processes (e.g., Vidon and Hill 2004; Burt and Pinay 2005; Tague 2009). Parallel to the biogeochemical hotspot concept, the variable source area (VSA) concept in hydrology describes how storm flow in many humid areas is largely generated on the small parts of the watershed that are especially prone to becoming saturated (e.g., Hewlett and Hibbert 1967; Dunne and Black 1970a,b) or at least wet enough to allow rapid lateral flows (e.g., Anderson and Burt 1978; Lyon et al. 2006b). In the northeastern US, regional hydrology is largely characterized by VSA processes (e.g., Walter et al. 2000). The soils are generally very permeable relative to typical rainfall rates (Walter et al. 2003). Thus, most rainfall infiltrates and is redistributed by gravity-driven subsurface flow. While the resulting patterns of soil moisture are highly heterogeneous in space and time, they are relatively predictable with VSA concept hydrological models (e.g., Holko and Lepisto 1997; Mehta et al. 2004). These hydrological models may thus be a useful tool for accurately modeling the occurrence of biogeochemical hotspots (Richardson et al. 2007).

The soil topographic index (STI) (e.g., Walter et al. 2002; Lyon et al. 2004; Agnew et al. 2006), a slight variation of the topographic index (TI) developed by Beven and Kirkby (1979), incorporates many of the landscape-scale features indicative of primary denitrification controls, specifically (1) upland drainage-area size; (2) depth and permeability of saturated sediments; and (3) topographic slope (Vidon and Hill 2004):

$$STI = \ln\left(\frac{a}{\tan(\beta)K_{sat}D}\right) \quad (1)$$

where  $a$  is upslope contributing area per unit contour length (m),  $\tan(\beta)$  is the local surface topographic slope,  $K_{sat}$  is the mean saturated hydraulic conductivity of the soil ( $\text{m d}^{-1}$ ), and  $D$  is

the soil depth (m). According to the TI/STI concept, soil moisture and groundwater level of a location are a result of the upslope contributing area and drainage (expressed as a slope). The TI (also referred as ‘topographic wetness index’) is considered an index of hydrological similarity: the higher the index value, the wetter the point and the more frequently a point will be saturated to a given level, relative to other points in the same landscape (Ambroise et al. 1996). TI has been shown to be a good predictor of soil moisture content or shallow groundwater level (e.g., Burt and Butcher 1985; Moore et al 1993; Western et al. 1999; Lyon et al. 2006a,b; Schneiderman et al. 2007; Easton et al. 2008) and has been used to quantify hydroperiodicity, i.e., frequencies and durations of saturated conditions (e.g., Agnew et al. 2006). Furthermore, several studies have indicated additional relations between TI and other physiochemical properties of soil (or its porewater) that are known to influence denitrification, including soil organic matter (OM) and C, soil N, dissolved organic carbon (DOC),  $\text{NO}_3^-$ , pH, soil texture, and bulk density (e.g., Moore et al. 1993; Florinsky et al. 2004; Ogawa et al. 2006; Seibert et al. 2007).

Despite the connection between TI and soil moisture, and research indicating that topographic control of soil moisture may promote denitrification (e.g., Pennock et al. 1992; Van Kessel et al. 1993), we found only one study that has evaluated the TI-denitrification relation (Florinsky et al. 2004). This is likely because it is only with the maturation of geographic information systems (GIS) and readily available, high resolution digital elevation model (DEM) data that we have had the easy ability to generate TI/STI maps at resolutions of interest. Florinsky et al. (2004) studied the effect of topography on the activity of denitrifiers under different soil moisture conditions in an agroecosystem and found that denitrification rates generally increase with increasing TI under wet conditions, but not drier conditions. Their physical interpretation was that some threshold amount of water was required to maintain topographic control on the spatial distribution of denitrifier activity. In other words, when it is wet (above some threshold), gravity-driven

transport of nutrients coupled with anaerobic conditions promote denitrification in higher TI areas; conversely, when it is dry, one or both of those components are likely missing and denitrification is low in most parts of the landscape. However, their study only looked at one wet period and one dry period, both lasting a single month. Agnew et al. (2006) demonstrated a clear, regionally consistent relationship between STI and probability of saturation with strong correlation throughout the year. Combined, these two studies suggest that TI/STI and denitrification should show a positive correlation on an annual basis, and the resulting relation may be a useful tool to model or predict the spatial distribution of denitrification at larger scales (landscape or watershed).

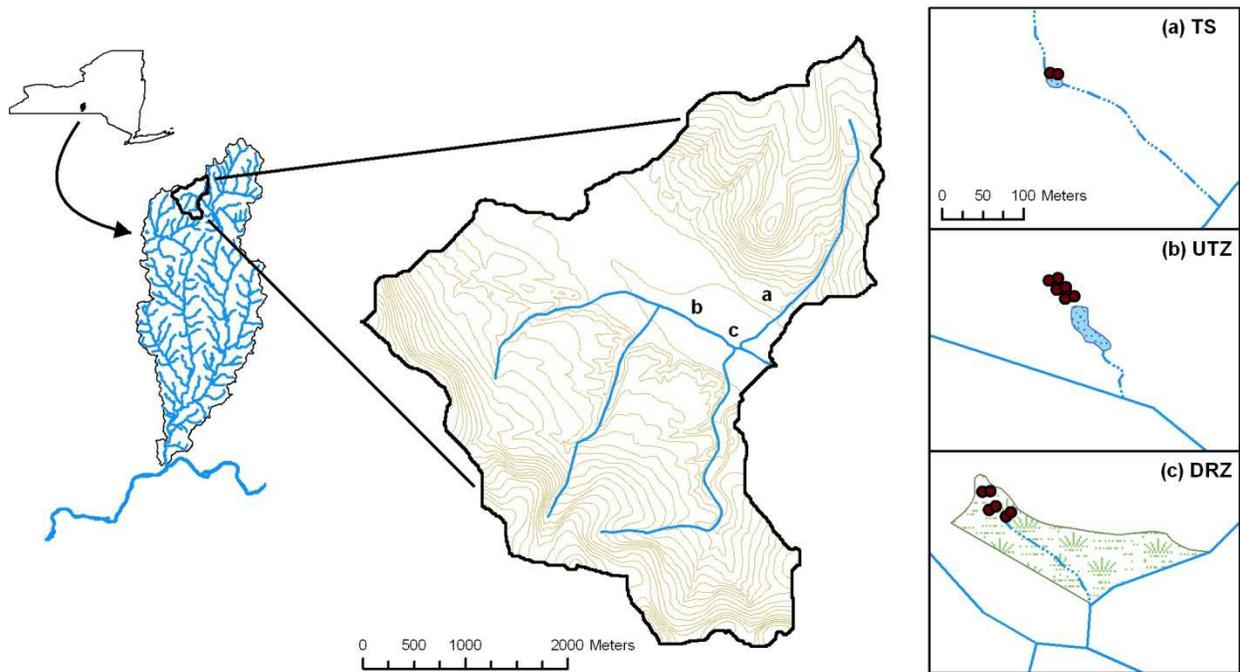
The objective of this study was to quantify *in situ* denitrification across a range of hydroperiodicities as characterized by the STI in a small mixed land-use headwater catchment in central New York State. We measured denitrification rates using the  $^{15}\text{N}\text{-NO}_3$  push-pull method at monthly intervals across a range of STI over a one-year period. We also characterized physiochemical variables known to influence rates of denitrification and related those back to STI. The goal of the study was to determine if annual denitrification rates correlated with STI within the catchment, and if so, use that relation to distribute rates (as fluxes) across a watershed to estimate whole-catchment denitrification occurring in the shallow saturated zone. This is a critical area for denitrification, but there has been no analysis of its variation in this zone and its link to surface topography.

## METHODS

### STUDY AREA DESCRIPTION

We measured *in situ* denitrification rates across gradients of hydroperiodicity, as defined by STI, in a small headwater catchment near Harford, NY, USA (Figure 3.1). The catchment boundary lies along a major drainage divide between the St. Lawrence and Susquehanna River Basins, within the latter system. More locally, the catchment is a sub-basin of the Owego-Wappasening Watershed which drains into the Susquehanna River near the NY-PA state border. The catchment is 1561 ha (15.6 km<sup>2</sup>) in size, with elevation ranging from 360 m at the outlet to 614 m along the valley ridgetops. Records (2005-2011) from a recently deployed NOAA U.S. Climate Reference Network monitoring station on site (NY Ithaca 13 E, 42.44 °N, 76.25 °W, 374 m elevation) indicate an annual mean temperature of 7.4 °C with monthly mean temperatures ranging from -5.3 °C in February to 19.4 °C in July (NCDC 2012; <http://www.ncdc.noaa.gov/>). Mean annual precipitation is 1076 mm yr<sup>-1</sup> with more precipitation on average in summer (114 mm mo<sup>-1</sup>) than winter (75 mm mo<sup>-1</sup>). Snowfall averages 1583 mm yr<sup>-1</sup>. Land cover in the catchment is predominantly agricultural (44%) and forested (41%) with the rest in shrub/grassland (8%), wetlands (4%), or developed (3%) according to the 2006 NLCD (Fry et al. 2011; MRLC 2012; <http://www.mrlc.gov/>).

The catchment encompasses a broad valley floored with stratified drift cut through bedrock ridges along the basin divide and drained by a small headwater stream. This geomorphologic feature, termed a through valley, is uncommon in that a drainage divide typically follows the crest of ridges, with headwater streams descending steep slopes on each side of the divide. However, through valleys are recurrent along the northern boundary of the Susquehanna River Basin as a result of continental glaciation and associated glaciofluvial processes. Groundwater and surface water interactions in the valley have been studied and reported by the USGS



**Figure 3.1.** Location of the Harford valley headwater catchment along the northern drainage divide of the Owego-Wappasening watershed in New York, USA. Three sites within the catchment were instrumented with mini-piezometers (red circles) to explore the relation between *in situ* denitrification and the propensity of saturation characterized by a topographic index. These sites include: (a) a toeslope (TS) above the valley floor; (b) an upland transition zone (UTZ) between the cropped portion of the valley floor and riparian wetlands; and (c) a downstream riparian zone (DRZ).

(Randall et al. 1988). Briefly, small streams originating in the uplands are intermittent and lose water by seepage as they flow across the valley-bottom stratified drift, leaving lower reaches dry for much of the year. Groundwater is derived from these losses plus precipitation on the stratified drift, and drains downvalley as underflow. Where the water table intersects the land surface, groundwater is discharged to streams. The water table subsides during the summer as groundwater continues to drain downvalley but is not recharged from precipitation due to high rates of evapotranspiration. As the water table subsides, the point at which it intersects the land surface migrates downvalley, leaving a dry channel upvalley (Swader 1974; Randall et al. 1988).

Soils in the catchment are largely Inceptisols or Alfisols developed in till and outwash deposited over bedrock shale during the period of the Wisconsin glaciation. The uplands that border the valley consist of bedrock mantled with till that was deposited directly from the last ice sheet when it covered the region; soils here are shallow, gently sloping, and somewhat poorly drained Dystrudepts, Fragiaquepts, and Fragiudepts of the Lordstown-Volusia-Mardin soil association. The sides of the valley consist of till-mantled bedrock slopes, with the lower slopes flanked by terraces and knolls that formed when the uplands were ice-free; soils here are steeply sloping and moderately well drained Dystrudepts, Hapludalfs, and Fragiudepts of the Valois-Howard-Langford soil association. The valley floor consists of sand and gravel outwash deposited by meltwater; soils here are deep, nearly level or gently sloping, and well drained Hapludalfs of the Howard soil series (Swader 1972; Randall et al. 1988). The Howard soil series is most favorable for agriculture, as erosion and drainage are not major problems; not surprisingly, most of the cultivated land is accordingly situated upon the valley floor. Conversely, the upland soils—which often contain a fragipan—are considered extremely difficult to manage for cropland and kept in permanent pasture or forest (Swader 1972).

## SITE CHARACTERIZATION AND INSTRUMENTATION

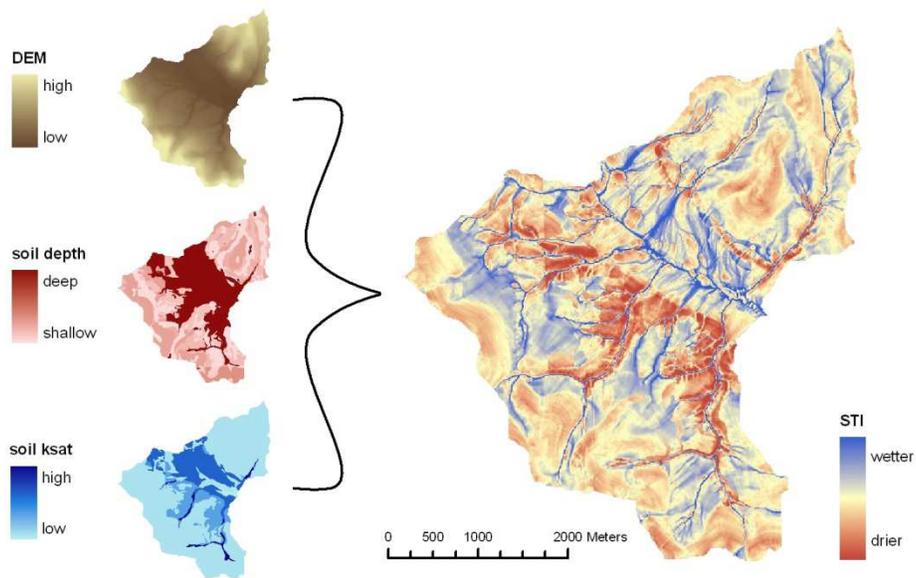
Three sites in the catchment were chosen to investigate the relation between *in situ* denitrification and the propensity of saturation. In order of decreasing elevation, these sites in the landscape include: (a) a toeslope (TS) above the valley floor; (b) an upland transition zone (UTZ) between the cropped portion of the valley floor and riparian wetlands; and (c) a downstream riparian zone (DRZ) (Figure 3.1). The TS site is located on a glacial terrace covered by northern hardwood forest; an ephemeral stream forms at the base of the steep bounding hillslope following snowmelt or precipitation events and flow accumulates temporarily in a vernal pool. The UTZ is located on the valley floor, in an herbaceous grassland adjacent to both the cropping and riparian system; the area is excluded from cultivation due to wetness and occasional overflow of a kettle pond nearby. The DRZ is a mixed riparian forest/shrub wetland with perennial herbaceous vegetation, located in a topographic depression near the confluence of headwater tributaries in the catchment; a headwater stream originates in the upper portion of the riparian zone during the winter and spring, but migrates downzone during the summer and fall. Land cover above TS is predominantly forest (80%) and shrub/grassland (12%). The hillslope immediately above TS is pastureland grazed by cattle and receives no fertilizer input. Conversely, land cover above UTZ and DRZ is mixed between forest (59%) and agriculture (33%). The field immediately above UTZ was cropped alfalfa. Farm records indicate that 90 kg N ha<sup>-1</sup> yr<sup>-1</sup> was spread as manure on the field during winter months of the study (Jan – Mar).

Mini-piezometers consisting of small stainless steel mesh-screened well points (Dedicated Gas Vapor Tip; AMS, American Falls, ID, USA) attached to fluoropolymer tubing were installed to a depth of 50 cm below the soil surface in the fall of 2007 at each site—two in TS, six in UTZ, and six in DRZ. Mini-piezometers were installed as pairs separated by a distance of at least 5 m to avoid potential complications during simultaneous denitrification measurements (e.g., change in

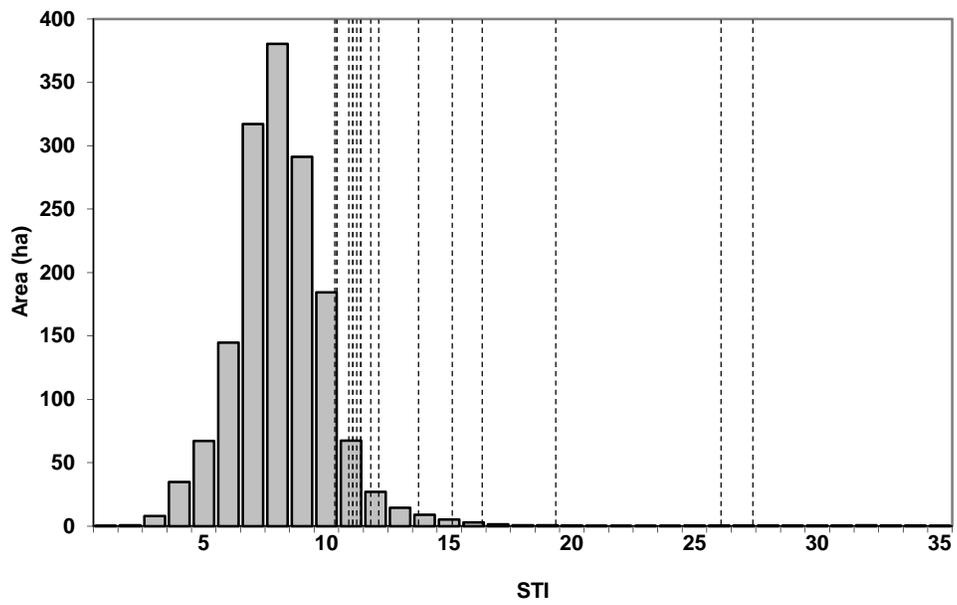
local flow conditions, dilution, mixing) (Figure 3.1). The location and placement of mini-piezometer pairs in UTZ and DRZ was based on differences in vegetation, topography, and/or wetness (i.e., typically along or near observed surface flow paths, representing a range in frequency and duration of saturated conditions); separation distance between consecutive pairs within site was at least 10 m, but generally 15-20 m. Mini-piezometer locations were recorded using a Garmin GPSmap 60CSx handheld GPS unit (Garmin International, Olathe, KS, USA). Three PVC wells were installed at each site to a depth of approximately 35 cm below the soil surface and equipped with Odyssey Capacitance Water Level Probes (Odyssey House, Christchurch, New Zealand) to record water table fluctuations. Monthly shallow groundwater samples were collected from mini-piezometers when there was sufficient flow using a Masterflex E/S Portable Sampler peristaltic pump (Cole Parmer, Vernon Hills, IL, USA). Duplicate soil cores (7.2-cm diameter, 6.8-cm height) were taken by hand from 50 cm below the soil surface within 1 m of each mini-piezometer location over two consecutive months during late summer when water tables were low.

## SOIL TOPOGRAPHIC INDEX

Hydroperiodicity was characterized across the catchment using a STI (Eq. 1). STI was determined for every point in the landscape using a GIS with DEM and soil data inputs (Figure 3.2). STI was calculated in ArcGIS 9.3 (ESRI, Redlands, CA, USA) using Eq. 1 following the multi-direction flow algorithm of Tarboton (1997). Upslope contributing area and slope were derived from 10-m resolution USGS DEMs (USGS 2012; <http://seamless.usgs.gov/>); soil depth and saturated hydraulic conductivity were obtained from USDA-NRCS SSURGO soil data (NRCS 2012; <http://soildatamart.nrcs.usda.gov/>). STI across the catchment ranged from 0.5 to 34.7 with a mean of 7.7 and a nearly normal distribution about the mean with positive skew (Figure 3.3). Less than 1% (15.3 ha) of the landscape was located in an area of STI greater than



**Figure 3.2.** Soil topographic index (STI) of the Harford valley catchment created in GIS using readily available digital elevation model (DEM) and soil data. Soil depth indicates the depth to bedrock or some other restrictive layer (e.g., fragipan) and ksat is the saturated hydraulic conductivity of that upper soil layer. High values of STI are in blue and indicate generally wetter areas. Conversely, drier portions of the landscape corresponding to low values of STI appear in red.



**Figure 3.3.** Histogram showing the range and distribution of STI values in the Harford valley catchment (1561 ha total). Dashed lines indicate STI values of mini-piezometer locations from which *in situ* denitrification measurements were made.

14. STI for individual mini-piezometer locations was calculated on an areal weighted-average basis using the horizontal accuracy limits reported by the GPS unit (generally  $\pm 2-4$  m) and STI values that fell within this radius. The sites where we measured *in situ* denitrification rates represented STI values that ranged from 9.9 to 26.9 (9.2% of the landscape).

### *IN SITU* DENITRIFICATION

We measured rates of *in situ* denitrification using a  $^{15}\text{N-NO}_3$  push-pull method (Istok et al. 1997; Well et al. 2001; Addy et al. 2002). Detailed descriptions of the method can be found in Addy et al. (2002) and Harrison et al. (2011). Briefly, we introduced a plume of previously collected groundwater amended with  $^{15}\text{N-NO}_3$  and a gaseous conservative tracer ( $\text{SF}_6$ ) into a single mini-piezometer and then extracted the plume from the same mini-piezometer after an incubation period; introduced groundwater was adjusted to ambient dissolved oxygen (DO) conditions prior to injection. Analysis of ambient, introduced, and recovered groundwater samples for  $^{15}\text{N}_2$ ,  $^{15}\text{N}_2\text{O}$ , and  $\text{SF}_6$  provide information on the recovery of the introduced plume and allow for calculation of *in situ* denitrification rates. Push-pull tests were attempted monthly over the course of one year, beginning in the spring of 2008. Push-pull measurements are limited to saturated soils, so it was not possible to get a measurement from each mini-piezometer in some months due to the nature of water table dynamics across the catchment (e.g., lower water tables in summer). However, this was anticipated and acceptable since the goal of this study was to relate measured *in situ* denitrification rates to a range of hydroperiodicities, including areas which only experience saturated conditions for brief durations (e.g., following snowmelt or storm events).

Ambient groundwater was collected the day before the push-pull and stored at 4°C. A 10-L dosing solution consisting of ambient groundwater enriched with nitrate (32 mg NO<sub>3</sub>-N mg L<sup>-1</sup> as KNO<sub>3</sub>, 20 atom % <sup>15</sup>N) (Cambridge Isotope Laboratories, Andover, MA, USA) was prepared the day of the push-pull. Sulfur hexafluoride gas (100 mg L<sup>-1</sup> SF<sub>6</sub>, He balance) (Airgas East, Salem, NH, USA) was bubbled through the dosing solution to adjust DO concentrations to ambient groundwater conditions and saturate the solution with a conservative tracer. The dosing solution was reintroduced (pushed) into the mini-piezometer from which it came and groundwater was recovered (pulled) from the mini-piezometer after a 4-hr incubation period. Low flow rates were controlled during the push and pull phases (10 and 5 L hr<sup>-1</sup>, respectively) via peristaltic pump to avoid generating air bubbles and minimize changes in water table elevation. Groundwater samples were collected at specific intervals throughout the push-pull for both water chemistry and dissolved gas analysis. The latter were taken through an airtight sampling apparatus made of gas-impermeable tubing connected to the peristaltic pump and injected by syringe into evacuated serum bottles. The remaining headspace was filled with ultra high purity grade He. Serum bottles were stored on ice in the field and at 4 °C upon return to the laboratory. Following a 24-hr equilibration period, gas samples were taken from the bottle headspace with a syringe and injected into evacuated gas vials.

## DENITRIFICATION RATE CALCULATION

Denitrification rates were calculated from six gas sample replicates taken from the first 3 L of the recovered groundwater plume (i.e., the portion that consistently contained the highest tracer recovery) to minimize the effects of dispersion and dilution (Addy et al. 2002). Measured concentrations of N<sub>2</sub>O-N and N<sub>2</sub> gases from headspace extraction were converted to concentrations of the dissolved gases in collected water samples as described by Tiedje (1982) and Mosier and Klemetsson (1994) taking into account the volume of the gas vial, the volume

of water sample collected in the serum bottle, the headspace volume of the serum bottle, and Bunsen solubility coefficients for  $\text{N}_2\text{O}$  and  $\text{N}_2$ . These concentrations, representing  $\text{N}_2\text{O}$  and  $\text{N}_2$  gas production in the groundwater, were transformed to  $^{15}\text{N}_2\text{O-N}$  and  $^{15}\text{N}_2$  generation rates over the incubation using their respective  $^{15}\text{N}$  sample enrichment proportion (the ratio of pulled atom % to pushed atom %, corrected for ambient atom %). Denitrification rates were calculated as the sum of  $^{15}\text{N}_2\text{O-N}$  and  $^{15}\text{N}_2$  generation rates and expressed as  $\mu\text{g N kg soil}^{-1} \text{d}^{-1}$  using soil bulk density and porosity (i.e., converted from  $\text{mg N L}^{-1} \text{d}^{-1}$ ; see Appendices A and C of Anderson 2013).

## STREAM DISCHARGE AND NITROGEN

A streamgauge was established at the catchment outlet in July 2009. Stream stage (height of the water surface) was recorded at 15-minute intervals using a Odyssey Capacitance Water Level Probe (Odyssey House, Christchurch, New Zealand) housed in a slotted and screened PVC pipe mounted to a piece of iron rebar pounded into the streambed in a channelized portion of the stream (along cement support walls, under a bridge). Periodic measurements of discharge were made immediately upstream of the streamgauge within the channelized portion of the stream as follows. The channel was divided into 10 to 15 subsections depending on flow conditions, and cross-sectional areas were measured using a measuring tape (width) and wading rod (depth). Water velocity in each subsection was measured using a Marsh-McBirney Flo-Mate 2000 electromagnetic velocity flow meter (Hach Company, Loveland, CO, USA). Discharge in each subsection was calculated by multiplying cross-sectional area by the measured velocity, and total discharge was determined by summing the discharge of each subsection. A stage-discharge relation was developed using 18 discharge measurements made over a 6-month period to capture a sufficient range of stages and streamflows (e.g., low-baseflow in fall, high-snowmelt in spring,

also pre- and post-storm events). The stage-discharge relation was applied to the stage record to obtain a continuous record of discharge.

Stream water at the catchment outlet was collected approximately monthly for one year after establishing the streamgage (August 2009 to July 2010). Samples were analyzed for  $\text{NO}_3\text{-N}$  and compared to concomitant discharge values. Trends in  $\text{NO}_3\text{-N}$  concentration relative to discharge were characterized using a hyperbolic dilution model (Johnson et al. 1969; Salmon et al. 2001). The nitrate-discharge relation was developed using 14 concentration-discharge measurements over the 1-year period to capture variable conditions (e.g., seasonal weather, growing and dormant seasons, agricultural practices in the catchment). The nitrate-discharge relation was applied to the discharge record to estimate  $\text{NO}_3\text{-N}$  concentrations in the stream as a function of flow. Annual  $\text{NO}_3\text{-N}$  export was estimated by multiplying measured daily discharge by estimated daily  $\text{NO}_3\text{-N}$  concentration and summing over the 1-yr period Aug 1 – Jul 31 (see Appendix D of Anderson 2013).

## ANALYTICAL METHODS

Soil samples for bulk density were placed in weighing tins and oven dried at  $105\text{ }^\circ\text{C}$  for 24 hours. Particle density was determined using distilled water and a 100 mL volumetric flask (in place of a pycnometer). Soil C and N were determined using a NC 2100 soil analyzer (ThermoQuest Italia, Milan, Italy) and OM determined through loss on ignition (LOI) at the Cornell Nutrient Analysis Laboratory, Ithaca, NY. Groundwater and stream samples for water chemistry analysis were stored on ice in the field and then refrigerated at  $4\text{ }^\circ\text{C}$  upon return to the laboratory and filtered ( $0.45\text{-}\mu\text{m}$ ) within 72 hours. Samples were analyzed for total dissolved nitrogen (TDN),  $\text{NO}_3\text{-N}$ , and DOC. TDN was determined using a Milton Roy Spectronic 501

spectrophotometer (Milton Roy Corporation, Rochester, NY, USA) following persulfate digestion.  $\text{NO}_3\text{-N}$  was determined using a Dionex ICS-2000 ion chromatograph (Dionex, Sunnyvale, CA, USA). DOC was determined using an O.I. Analytical 1010 total organic C analyzer (O.I. Corporation, College Station, TX, USA). DO content and temperature were measured in the field under continuous flow using a YSI 550A handheld DO instrument (YSI, Yellow Springs, OH, USA). Concentrations and isotopic composition of  $\text{N}_2\text{O}$  and  $\text{N}_2$  gases were determined on a ThermoFinnigan GasBench + PreCon trace gas concentration system interfaced to a ThermoScientific Delta V Plus isotope-ratio mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) at the Stable Isotope Facility, University of California at Davis, Davis, CA, USA. Concentrations of  $\text{SF}_6$  gas were determined on a Shimadzu GC 14 gas chromatograph (Shimadzu, Kyoto, Japan) at the Cary Institute of Ecosystem Studies, Millbrook, NY, USA.

## STATISTICAL ANALYSES

Statistical analyses were performed using XLSTAT (Addinsoft, New York, NY, USA). Significance was determined at  $\alpha = 0.05$  (95% confidence). Pearson linear correlation ( $r$ ) was used to examine the relationship between STI and ambient groundwater characteristics including TDN, DOC,  $\text{NO}_3\text{-N}$ , DO, and temperature, and soil characteristics including bulk density, pH, %OM, %C, and %N. Linear regression with multiple cross validation was used to develop the relationship between STI and denitrification rate.

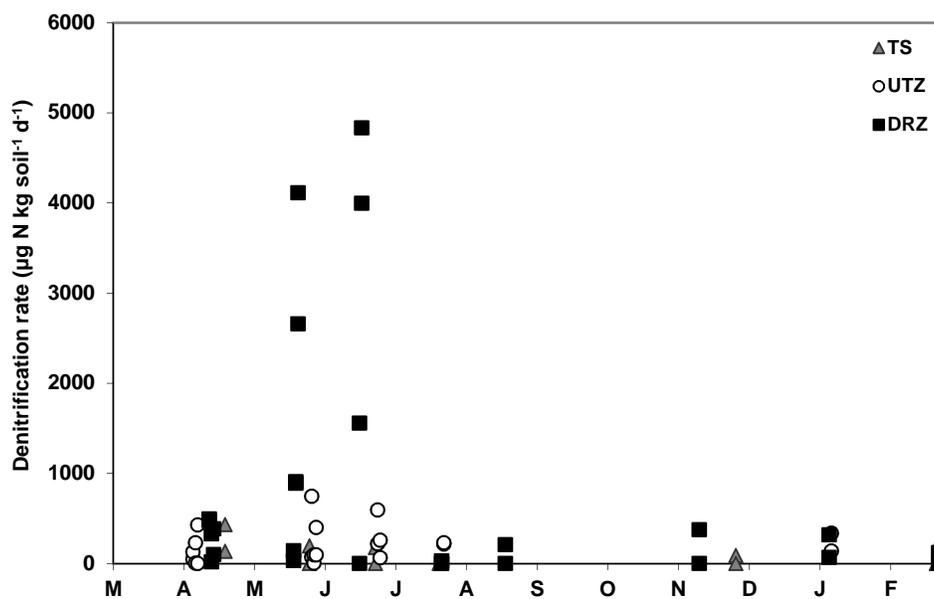
## RESULTS AND DISCUSSION

### DENITRIFICATION RATES

Denitrification showed a seasonal pattern in the catchment, with rates increasing through the spring, peaking in the summer, then decreasing through the fall and remaining low during winter

(Figure 3.4). Measured *in situ* denitrification rates ranged from 0 to 4831  $\mu\text{g N kg soil}^{-1} \text{d}^{-1}$  across all mini-piezometers, with a mean value  $\pm$  standard error of  $568 \pm 156 \mu\text{g N kg soil}^{-1} \text{d}^{-1}$ . Annual denitrification rates at each mini-piezometer estimated by assuming a value of zero for any month in which a measurement was not taken (due to low water table or winter inaccessibility) ranged from 19 to 484  $\mu\text{g N kg soil}^{-1} \text{d}^{-1}$  with a mean of  $162 \pm 44 \mu\text{g N kg soil}^{-1} \text{d}^{-1}$ . A similar seasonal pattern and range of denitrification rates were observed the following year in a subset of locations used to consider denitrification in riparian areas (manuscript in preparation). However, annual rate estimates in this study are lower, presumably because (1) we are including non-riparian areas and (2) the duration of saturated conditions was shorter across all sites.

The observed seasonal pattern and denitrification rate magnitudes reflect agriculture within the catchment. In natural (i.e., unmanaged) systems, plant uptake is thought to be a primary N removal mechanism and demand for N is often greatest during the growing seasons; denitrification rates are subsequently highest during the late fall-winter-early spring months (Groffman and Tiedje 1989a). In agricultural systems, the pattern is likely to be reversed because N is often abundant and available in excess of what is required for plant growth; denitrification rates will be highest during late spring-summer-early fall when temperatures are warmer (e.g., Hefting et al. 2003). Greater denitrification rates in agricultural landscapes are generally attributed to the larger N inputs, and can be orders of magnitude higher on an annual basis compared to unmanaged landscapes (Barton et al. 1999).

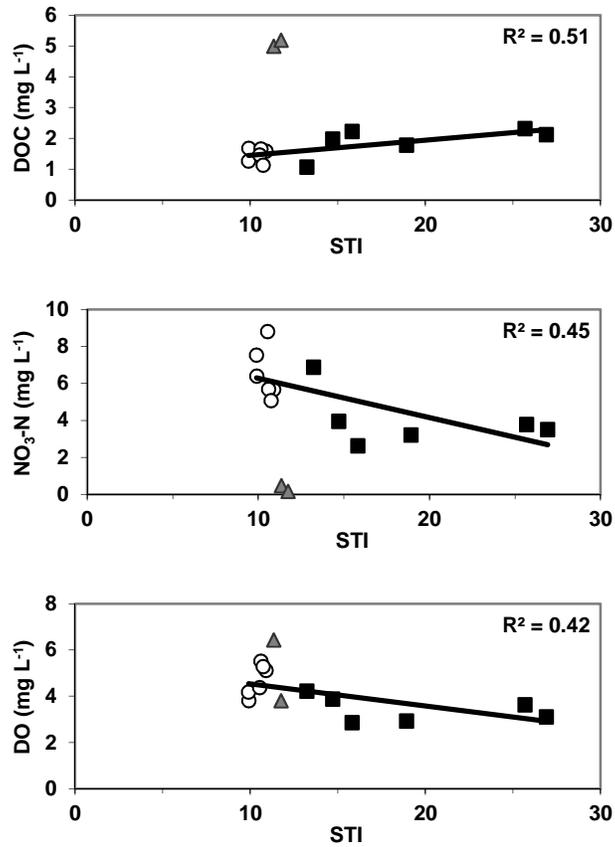


**Figure 3.4.** *In situ* denitrification rates measured monthly from mini-piezometers within the saturated zone of sites TS (triangle), UTZ (circle), and DRZ (square). Values are the mean of six replicate gas samples taken from a recovered groundwater plume that had been amended with <sup>15</sup>N-nitrate. Standard error bars are omitted to facilitate better visualization, but average  $\pm 43\%$  of the mean (generally a higher percentage for the lower rates, and *vice versa*).

## STI AND GROUNDWATER CHARACTERISTICS

There were significant relationships between STI and select ambient groundwater characteristics in UTZ and DRZ sites, but not in the TS site (Figure 3.5). STI was positively correlated with DOC ( $r=0.72$ ), and negatively correlated with TDN ( $r=-0.82$ ),  $\text{NO}_3\text{-N}$  ( $r=-0.67$ ), DO ( $r=-0.65$ ), and temperature ( $r=-0.49$ ). Only the temperature relationship was not statistically significant ( $r_{\text{crit}}=0.58$  at  $P=0.05$  with  $n=12$ ). Groundwater characteristics—particularly DOC and  $\text{NO}_3\text{-N}$ —do not follow the same trends in TS, probably due to location within the catchment. TS is positioned at a higher elevation and has a relatively small upland contributing area, more typical of conditions near a watershed boundary; therefore, we expect groundwater characteristics to reflect mainly local conditions. High DOC (mean  $5.1 \text{ mg L}^{-1}$ ) was likely a result of slow and consistent decomposition of leaf litter (plus other woody debris) on the forest floor. Low  $\text{NO}_3\text{-N}$  (mean  $0.3 \text{ mg L}^{-1}$ ) was likely a result of lower N inputs (since the hillslope above receives no fertilizer or manure spreading) and inferred N flushing (Creed et al. 1996; Burns 2005). DO and temperature in TS were similar to UTZ and DRZ. Including groundwater characteristics from TS in the correlation analyses weakened the correlations between variables to the point of negating statistical significance, except for the STI-DO relation ( $r=-0.58$  with new  $r_{\text{crit}}=0.50$  at  $P=0.05$  when  $n=14$ ). A full correlation matrix is presented in Table 3.1.

The observed STI-chemistry relationships agree with previous studies investigating the influence of topography on hydrology and soil (discussed below), and demonstrate why the STI itself is likely to be a good predictor of denitrification activity. Denitrification is the reduction of  $\text{NO}_3^-$  to N-gases through a chain of microbial oxidation-reduction reactions, requiring an electron donor as an energy source (typically C) and  $\text{NO}_3^-$  as an electron acceptor in the absence of  $\text{O}_2$ . Accordingly, factors that control  $\text{O}_2$ , labile C, and  $\text{NO}_3^-$  availability are thought to be the main



**Figure 3.5.** Significant ( $p < 0.05$ ) relationships between STI and average ambient groundwater DOC ( $r = 0.72$ ),  $\text{NO}_3\text{-N}$  ( $r = -0.67$ ), and DO ( $r = -0.65$ ) measured over the study period in UTZ (circle) and DRZ (square) mini-piezometers. Values from TS (triangle) are shown, but only follow the DO relation ( $r = -0.58$ ).

**Table 3.1.** Correlation matrix (r values) among STI, denitrification rate, and select groundwater and soil physiochemical characteristics

	Groundwater							Soil				
	STI	RATE	TDN	DOC	NO3	DO	TEMP	BD	PH	OM	N%	C%
STI	<b>1</b>											
RATE	<b>0.929</b>	<b>1</b>										
TDN	<b>-0.636</b>	<b>-0.535</b>	<b>1</b>									
DOC	<b>0.717</b>	<b>0.837</b>	<b>-0.682</b>	<b>1</b>								
NO3	<b>-0.673</b>	<b>-0.730</b>	<b>0.767</b>	<b>-0.730</b>	<b>1</b>							
DO	<b>-0.581</b>	<b>-0.713</b>	0.309	<b>-0.625</b>	0.546	<b>1</b>						
TEMP	-0.366	-0.280	0.311	-0.475	<b>0.656</b>	-0.099	<b>1</b>					
BD	<b>-0.615</b>	<b>-0.576</b>	0.296	<b>-0.594</b>	0.407	<b>0.582</b>	0.222	<b>1</b>				
PH	-0.026	0.102	<b>0.533</b>	-0.134	0.249	-0.177	0.229	-0.096	<b>1</b>			
OM	<b>0.894</b>	<b>0.870</b>	-0.253	<b>0.609</b>	-0.538	<b>-0.559</b>	-0.344	<b>-0.639</b>	0.233	<b>1</b>		
N%	<b>0.692</b>	<b>0.777</b>	-0.060	0.540	-0.479	<b>-0.690</b>	-0.189	<b>-0.600</b>	0.487	<b>0.878</b>	<b>1</b>	
C%	<b>0.660</b>	<b>0.768</b>	-0.083	0.538	-0.546	<b>-0.749</b>	-0.205	<b>-0.597</b>	0.463	<b>0.823</b>	<b>0.983</b>	<b>1</b>

r values in **bold** indicate significance at p=0.05  
shaded cells omit samples from TS location

regulators of denitrification at the field scale, with other environmental variables exerting a secondary influence (Korom 1992; Barton et al. 1999; Rivett et al. 2008).

O<sub>2</sub> is considered the principal regulator or critical limiting factor in denitrification, as the process requires anaerobic or low O<sub>2</sub> conditions. Increasing soil moisture often restricts soil aeration and results in lower O<sub>2</sub> conditions promoting denitrification (e.g., Groffman and Tiedje 1986b; Burgin and Groffman 2012). Similarly, water table fluctuations have been shown to greatly influence denitrification rates (e.g., Simmons et al. 1992; Hefting et al. 2004). We found that DO in the shallow groundwater decreased with increasing STI. To our knowledge, no study has found this relation explicitly; however, it is well known that soil moisture and probability of saturation increase with various topographic indices (e.g., Gunter et al. 2004; Sorenson et al. 2006; Agnew et al. 2006; Lyon et al. 2006a,b). Also, O<sub>2</sub> depletion along topographic and rainfall gradients have been measured in the field (Silver et al. 1999; Burgin et al. 2010; Burgin and Groffman 2012).

Most denitrifying bacteria are heterotrophic, i.e., they use organic C for energy. Quantity and quality of OM are known to influence denitrification (e.g., Burford and Bremner 1975; Hill and Cardaci 2000), with the amount of DOC in groundwater or porewater most often related to denitrification rate (e.g., Hedin et al. 1998; Hill et al. 2000). Increasing soil moisture often increases OM within the soil by promoting plant growth and slowing rates of decomposition, and DOC is readily leached from this material (e.g., Parton et al. 1987; Neff and Asner 2001). Again omitting the TS location, we found that DOC in the shallow groundwater increased with increasing STI, consistent with the DOC flushing hypothesis (Hornberger et al. 1994) and other studies that have explored the relation between DOC and topographic indices (e.g., Welsch et al.

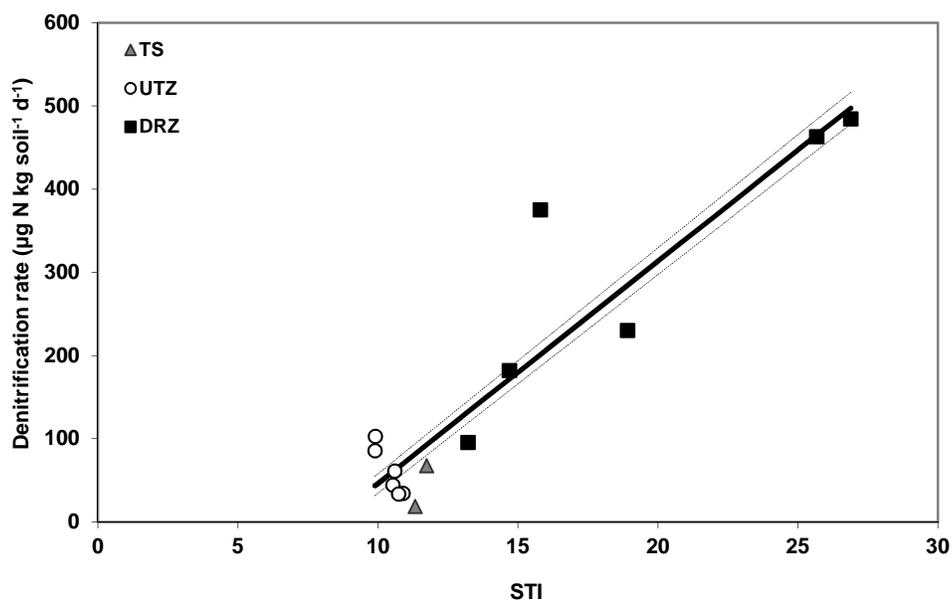
2001; Ogawa et al. 2006). A positive relation between soil OM and TI has also been observed in previous studies (e.g., Moore et al. 1993; Pei et al. 2010) and at our site (Table 3.1).

Low  $\text{NO}_3^-$  concentrations can limit denitrification, with  $1 \text{ mg NO}_3\text{-N L}^{-1}$  often reported as the value above which reaction kinetics become zero order, i.e., independent of concentration and non-limiting (e.g., Barton et al. 1999; Rivett et al. 2008).  $\text{NO}_3^-$  in the landscape is largely controlled by land use and total N inputs (e.g., Jordan et al. 1997; Boyer et al. 2002) and is subject to multiple removal pathways in addition to plant uptake or denitrification (Burgin and Hamilton 2007).  $\text{NO}_3^-$  is very mobile in water and, in the absence of plant uptake or biologic transformation, hydrologic flows transport  $\text{NO}_3^-$  in soil from higher elevations to lower elevations where it concentrates (e.g., Hairston and Grigal 1994; Hook and Burke 2000).

Topography and development of saturated areas is also thought to regulate the  $\text{NO}_3\text{-N}$  flushing mechanism that has been observed in field studies (e.g., Creed and Band 1998). A positive correlation between TI and  $\text{NO}_3\text{-N}$  has been reported for storm events (Welsch et al. 2001) and other studies have found a positive relation between TI and soil N (e.g., Johnson et al. 2000; Seibert et al. 2007). We found the opposite—a negative correlation between STI and  $\text{NO}_3\text{-N}$  (and TDN) in shallow groundwater, although our analysis was limited to the high-end of the watershed's STI range and omits the TS location. Ogawa et al. (2006) also found a negative relationship between TI and  $\text{NO}_3\text{-N}$ , and suggested that decreasing  $\text{NO}_3\text{-N}$  was partly the result of denitrification within riparian wetlands of their forested catchment.

## STI AND DENITRIFICATION RATE

There was a significant positive relationship between STI and annual denitrification rate (Figure 3.6). Multiple cross validation was conducted to assess the stability of the regression model



**Figure 3.6.** STI-denitrification rate relation determined by linear regression ( $r=0.93$ ,  $p<0.01$ ) and subsequently used to extrapolate denitrification fluxes according to STI value across the catchment. Denitrification rates are annual estimates in units of  $\mu\text{g N kg soil}^{-1} \text{d}^{-1}$  from mini-piezometer locations in sites TS (triangle), UTZ (circle), and DRZ (square). Dashed lines indicate one standard deviation of the linear regression coefficients determined via multiple cross validation and represent model performance/error.

(Mosteller and Tukey 1977). A single STI-rate pair was removed from the data set and the linear regression analysis repeated to determine new regression coefficients. This process was repeated sequentially—removing only one data pair at a time—for the whole data set, resulting in 14 sets of regression coefficients. The mean values of the regression coefficients were used to develop the overall regression model with uncertainty taken as one standard deviation of the regression coefficients. This method of multiple cross validation is more appropriate for small datasets than excluding a portion of the data for validation, since the model will have more predictive power if a larger dataset is used.

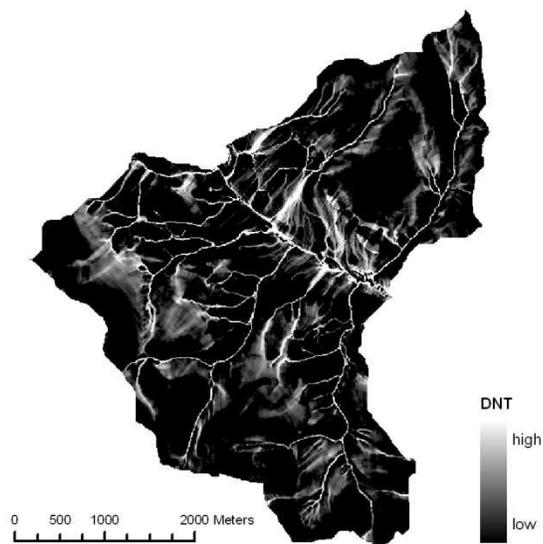
Our findings are in agreement with Florinsky et al. (2004) who studied the effect of topography on denitrifier activity under contrasting hydrologic conditions. They found a positive correlation between TI and denitrification rate under wet soil conditions, but the relation was not significant during dry soil conditions. Based on other measures like microbial biomass C, denitrifier enzyme activity, and most probable number of denitrifiers, the authors concluded that the decrease in denitrification rate was in response to more oxic conditions brought on by a decrease in soil moisture. It is not appropriate to compare our study to theirs in this regard, since we are using the push-pull method to measure denitrification and inherently under saturated conditions. However, since we are focusing on an annual representation of denitrification based on STI, drier conditions are accounted for in both the annual denitrification rate and the STI value, i.e., low STI areas are generally drier over the course of a year than high STI areas and a denitrification rate of zero was assigned to any month in which mini-piezometer locations were dry.

A positive correlation between STI and denitrification rate indicates that denitrification is likely to be greater in high STI areas; those areas likely to be wettest in the landscape. While much denitrification research has been focused on riparian or near-stream areas, other parts of the

landscape are prone to saturating. These include areas where topography converges, where restrictive layers or water tables are shallow, and/or at toeslopes (e.g., Beven 1986, Walter et al. 2000). Indeed, other studies exploring topographic influence on denitrification have found increased denitrification in these very areas. For example, Pennock et al. (1992) and Van Kessel et al. (1993) examined denitrification rates in distinct landform elements classified on the basis of slope gradient and curvature, and found that topographic controls of soil aeration via hydrologic and pedologic processes had a significant effect on landscape-scale patterns of denitrification. Specifically, footslopes (toeslopes) showed greater denitrification activity than shoulder elements (upper hillslope), and concave landforms (e.g., depressions) showed greater denitrification than convex landforms. Also, denitrification rates measured in spring/seep areas (indicative of shallow water table) are generally higher than the surrounding landscape (e.g., Ashby et al. 1998; Pinay et al. 2003).

## DISTRIBUTED DENITRIFICATION

To estimate denitrification fluxes across the catchment, denitrification rates in the regression model were first converted from units of  $\mu\text{g N kg soil}^{-1} \text{d}^{-1}$  to  $\text{kg N ha}^{-1} \text{yr}^{-1}$  using measured soil bulk density values and assuming that (1) the rates reflect conditions within some upper 0.5 m portion of the saturated zone and (2) soil bulk density was constant/uniform in this portion of the soil. Denitrification rates (now annual fluxes on an areal basis) were distributed across the catchment in GIS according to the regression model equation and spatial distribution of STI (Figure 3.7). A flux value of zero was assigned to any location with a STI corresponding to a denitrification rate below the x-intercept of the STI-rate relation, i.e.,  $\text{STI} = 8.3$ .



**Figure 3.7.** Distributed denitrification (DNT) fluxes across the catchment according to STI. A flux value of zero (black) was assigned to any location with a STI value below the x-intercept of the STI-DNT rate relation. Light areas delineate hotspots of denitrification activity in the landscape attributed to frequency and duration of saturated conditions.

Ignoring for a moment the widespread use of the TI in hydrologic modeling (e.g., TOPMODEL – Beven and Kirkby 1979; Ambroise et al. 1996, VSLF – Schneiderman et al. 2007; Easton et al. 2008a, SWAT-VSA – Easton et al. 2008b, 2011) few studies have used a TI to predict or distribute denitrification-related fluxes across a landscape. Vilain et al. (2012) adapted the TI into a “concentration flux position index” by incorporating landform element information, i.e., footslope, slope, or shoulder, and subsequently upscaled measured N<sub>2</sub>O emissions from closed chambers on two plots to the Orgeval basin (area = 106 km<sup>2</sup>) in France according to this new index plus land use. A handful of denitrification studies—often the modeling variety—have used TOPMODEL combined with a denitrification model (or a suite of biogeochemical models which include a denitrification routine) to produce catchment-scale estimates of denitrification (e.g., Beaujouan et al. 2002; Whelan and Gandolfi 2002). RHESSys, a model that couples C and N cycling with hydrologic processes based on the topographic approach of TOPMODEL, has been used in a variety of watershed-scale N simulations that include denitrification (e.g., Band et al. 2001). Whelan and Gandolfi (2002) assumed the spatial distribution of soil C was imperfectly correlated with TI in an attempt to model landscape-scale denitrification in a catchment similar to the one used in our study. TOPMODEL was used to model the spatial distribution of water and denitrification modeled as a function of soil organic C availability, i.e., nitrate-non-limited. Beaujouan et al. (2002) developed the topography-based nitrogen transfer and transformation (TNT2) model, where the hydrologic model was a water balance with transfer calculations based on TOPMODEL and N transformations were based on a generic plant-soil model. In these instances, TI is controlling soil moisture and the delivery of C and/or NO<sub>3</sub><sup>-</sup> needed for denitrification; thus, the TI-denitrification rate relation exists, but is not readily apparent.

## CATCHMENT N FLUXES

Denitrification in the shallow groundwater of the catchment over one year was estimated to be 20541 kg N. If taken over the entire area of the catchment (1561 ha), this represents an annual denitrification N flux of  $13.2 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ; for the area (523 ha) where  $\text{STI} > 8.3$ , the denitrification N flux was  $39.2 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ . As mentioned previously, the push-pull method for measuring denitrification is limited to saturated areas, so using these values likely underestimates total denitrification across the catchment because denitrification undoubtedly occurs in other parts of the landscape not accounted for in the STI relation: even low STI areas will be wet and anaerobic for brief periods following rain events, snowmelt, etc.

Our watershed-scale estimates of shallow groundwater denitrification are comparable to previous studies. Mehnert et al. (2007) found that  $5.7 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  of N was denitrified in the shallow groundwater of a tile-drained agricultural watershed using isotopic ratios of N and O in coupled with push-pull tests to determine  $\text{NO}_3\text{-N}$  reduction rates. Lowrance et al. (1984) measured denitrification in riparian zones of an agricultural watershed of  $31.5 \text{ kg N yr}^{-1} \text{ ha}^{-1}$  of riparian forest; this was equal to  $9.5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  over the entire 1568 ha watershed, 30% of which was riparian. Whelan and Gandolfi (2002) predicted average whole-catchment denitrification losses of  $33.1\text{-}49.3 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ , with more than half occurring in the saturated zone. In an application of the TNT2 model validated with denitrification measurements from soils in a farming catchment area, Oehler et al. (2009) simulated a catchment-scale denitrification N flux of  $47.0 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ . The aforementioned studies were of catchments similar in size and land use to ours. At much larger scales and using a N mass balance approach, Van Breemen et al. (2002) estimated denitrification losses of  $12.5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  in soils from 16 mixed land use (72% forest, 19% agriculture) large watersheds in the northeastern US. Broken down by land use, they estimated  $55.3$  and  $2.0 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  was denitrified in agricultural and forest soils, respectively.

Ashby et al. (1998) also found a relatively small annual N flux ( $1.2 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ) from denitrification in a forested headwater catchment; denitrification rates were measured in soil cores and rates were distributed across the catchment according to soil type. It is important to note that the studies of Lowrance et al. (1984), Oehler et al. (2009), and Ashby et al. (1998) all use the acetylene inhibition method to measure denitrification in surface soil cores from riparian zones, so there are distinct differences between methodologies and interpretations of their results as compared to our push-pull measurements from shallow groundwater. They are relevant studies nonetheless, for all measured denitrification rates at depth (generally down to 40 cm) from particularly wet soils, and either modeled or distributed those rates according to soil properties across entire watersheds to estimate whole-catchment denitrification fluxes.

Stream  $\text{NO}_3\text{-N}$  export over one year was estimated to be 12180 kg N, or  $7.8 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  over the whole catchment area (see Appendix D of Anderson 2013). The estimated annual N flux via denitrification from our catchment was thus 69% greater than the annual stream  $\text{NO}_3\text{-N}$  export. The ratio of annual denitrification flux to stream  $\text{NO}_3\text{-N}$  export (1.7) is similar to that reported for other mixed agricultural use watersheds (e.g., 2.4, Lowrance et al. 1984; 1.1, Oehler et al. 2009). In contrast, Ashby et al. (1998) found that N export from denitrification in a forested catchment was only 0.7 of stream N export, which they attributed to lower N loading and fewer organic C pools. Similar ratios can be found in Van Breemen et al. (2002) for forest (0.9) and agricultural (2.9) lands. From that same study, a denitrification to stream N ratio of 1.7 was obtained when N losses were compiled across all watersheds and land use ( $12.5$  and  $7.2 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  for denitrification N and riverine N export, respectively).

## LANDSCAPE PATTERNS OF DENITRIFICATION

The landscape pattern of denitrification that emerges from this study is consistent with past studies or modeling efforts at the catchment-scale: denitrification rates are generally highest in riparian or near-stream areas (wet), and lower in drier upland soils. For this reason, riparian systems are often regarded as hotspots of denitrification (McClain et al. 2003; Groffman et al. 2009; Vidon et al. 2010). In a broader sense, denitrification hotspots are likely to occur at the interface between terrestrial and aquatic systems for the same reasons we see them in riparian zones: appropriate redox conditions brought on by restricted O<sub>2</sub> diffusion and delivery of necessary electron donors and/or acceptors via hydrologic flowpaths (e.g., Hedin et al. 1998; Vidon et al. 2010). In this study we find that denitrification rates correlate with STI, and use that relation to model the spatial distribution of denitrification hotspots within the shallow saturated zone, which suggests that upland connectivity, soil depth, and drainage capacity (i.e., saturated hydraulic conductivity and hydraulic gradient) are also potentially important watershed-scale controllers of denitrification.

We directly measured denitrification in a relatively small portion of the watershed, i.e., STI values of 9.9-26.9 made up only 9.2% of the catchment. The STI-denitrification rate relation we found allows for some extrapolation, covering STI values of 8.3-34.7 or 34% of the catchment, with most of the additional area coming from the low end. Clearly, if no other denitrification were occurring in the catchment, denitrification in saturated soils or shallow groundwater would represent a hotspot of activity in the landscape. As discussed in the previous section (CATCHMENT N FLUXES), there are likely additional denitrification fluxes. However, our estimated denitrification flux of 13.2 kg N ha<sup>-1</sup> yr<sup>-1</sup> and denitrification:stream N ratio of 1.7 are similar to other studies that measured, modeled, or estimated (through mass balance) whole-catchment denitrification in the region or in catchments of similar size, climate, and land use.

This suggests that we are capturing a significant portion of denitrification in the catchment, although interpretation and comparison of denitrification fluxes and N flux ratios from different studies really depend on both having comparable methods for measuring/estimating denitrification and having similar N input rates. A detailed N balance for the catchment could provide additional context and bounds for whole-catchment denitrification, but is complicated by the presence of an intensive dairy farm straddling the watershed boundary within the valley, and unequal distribution of N inputs, i.e., records are available only at the whole-farm level, not for individual watersheds within the farm boundary. Even so, the hotspot behavior of denitrification is apparent over the range of STI in our analysis. For instance, 1.0 % of the catchment is in an area of STI greater than 14, yet this area is responsible for 17.8% of the denitrification we measured. Choosing an STI value of 12 as an indicator of riparian area (falling between the highest STI in the UTZ of 10.9 and the lowest STI in the DRZ of 13.2), we find that 29.6% of the measured denitrification was occurring in only 2.5% of the catchment on an areal basis. Conversely, this indicates that shallow groundwater beneath drier upland soils (STI between 8 and 12) was responsible for the bulk of the observed groundwater denitrification (~70%).

The landscape pattern of denitrification depicted in Figure 3.7 is nearly identical to that of Whelan and Gandolfi (2002), with higher rates occurring near streams and in hillslope depressions extending to the streams, although they did not provide details on relative rates or amount denitrified. Ashby et al. (1998) found annual rates of denitrification in wet, spring-fed soils and near-stream riparian areas up to five times greater than in upland surface soils; however, because these areas were such a small part of the landscape (<3%), riparian denitrification amounted to only 1.8% of whole-catchment activity. Dry upland soils that covered a large portion of the landscape (71%) accounted for 91% of the denitrification. Oehler et al. (2009) also found higher rates of denitrification in the riparian zone of their study catchment compared to the hillslopes, and simulations indicated 40% of the total catchment

denitrification was occurring in only 20% of the area. Results from these studies and ours confirm the importance of denitrification in riparian areas, but also demonstrate that upland areas contribute significantly to catchment-scale denitrification, i.e., the amount of N denitrified in a watershed is generally larger in upland portions of the terrestrial-freshwater continuum (Seitzinger et al. 2006).

## UNCERTAINTIES AND FUTURE DIRECTIONS

*Nitrate supply.* The STI-based approach to distributing denitrification fluxes implies that STI is the sole factor controlling denitrification, but fundamentally this is not true. No matter how high the STI at a given location, if there is no  $\text{NO}_3^-$  available to denitrify, then denitrification rates should be low. The STI-based approach we used does not explicitly take  $\text{NO}_3^-$  supply into consideration: low STI areas in cropped fields receiving manure N are assigned the same denitrification flux as equivalent low STI areas in upland forests distant from large N sources. This is evident in our landscape, at the TS position. We cannot discern whether the low denitrification rates are due to low STI or low  $\text{NO}_3^-$ , or if the rates measured in TS were actually enhanced through the addition of  $\text{NO}_3^-$  necessary to perform the push-pulls. If the push-pull method has overestimated denitrification rates from these locations, then we would be overestimating denitrification fluxes distributed across the watershed. To highlight the importance of considering  $\text{NO}_3^-$  supply and its potential limitation on denitrification, we lowered measured rates from TS by an order of magnitude and repeated above methods and analyses. The denitrification N flux from the shallow saturated zone decreased by 21%, down to 16231 kg N over the study period with a slight decrease (less than 2%) in areal extent.

*Denitrification rates.* Uncertainty in our denitrification rates develop through the conversion of units from mass-based to area-based, which was necessary to distribute fluxes across the landscape. Individual rates were determined using measurements of gaseous isotopic ratios, concentrations, and quantities scaled up to the physical sample volume. This is a rather straight forward process whereby the mean denitrification rate and uncertainty (e.g., standard error) are easily calculated and expressed in units of  $\text{mg N L}^{-1} \text{d}^{-1}$ , or  $\mu\text{g N kg soil}^{-1} \text{d}^{-1}$  using soil bulk density and porosity. Thus, the STI-denitrification rate relation shown in Figure 3.6 with uncertainty limits taken as one standard deviation of the regression analysis should be an accurate representation of expected denitrification rates as a function of landscape STI value. However, distribution of these rates across the landscape requires flux units on an areal basis. Rates were first converted to a flux on a volume basis (using soil bulk density), and then multiplied by an “effective depth” to obtain flux on an areal basis, which introduces uncertainty in the magnitude of our denitrification estimates. For this analysis, we chose 50 cm, consistent with previous push-pull studies (e.g., Zaman et al. 2008) and corresponding to the minimum depth above which soil organic C was greater than 0.5% (Burford and Bremner 1975; Beauchamp et al. 1980) when push-pulls were performed (see Appendix C of Anderson 2013). Had we chosen a larger effective depth, denitrification fluxes on an areal basis would be larger (e.g., 100 cm would double the fluxes we found). Moreover, we did no detailed analysis of variation in activity within our estimate of effective depth.

*Denitrification rate relation shape and extent.* Annual denitrification rates clearly show a positive correlation with STI (Figure 3.6), however, the expected shape of this relationship is not known. While we chose linear regression to characterize the rate relation (Figure 3.6), other nonlinear functions (e.g., exponential or power) fit the data well within the range of STI from which we measured denitrification without introducing substantial bias in the residuals. Over this range, the shape of the relation is not without consequence; curvature of exponential or

power functions result in slightly higher rates at the low STI values and considerably higher rates at high STI values. The net effect is a 7-12% increase in the amount of denitrification over the range of STI from which we performed push-pulls, depending on model shape, and even greater if we extrapolate beyond either STI endpoint. There is appeal in using an exponential or power model that can be extrapolated to the minimum STI within the catchment, but we feel we did not have enough measurements to justify a non-linear model for this study. We did extrapolate the linear model back to the x-intercept of the STI-denitrification rate relation (STI value of 8.3) to produce a first approximation of whole-catchment denitrification. We feel this was reasonable given the duration of saturated conditions observed in the lowest STI mini-piezometers, i.e., areas with STI values of 9.9-10.3 were saturated for at least two months and displayed low denitrification rates, so we can assume that areas of relatively lower STI will be saturated for a shorter duration of time and have even lower rates of denitrification. This assumption adds considerably to the whole-catchment estimate of denitrification; per-area denitrification rates in the low STI areas were small, but the extrapolated area occupies 24% of the landscape and represents 40% of our estimated denitrification N flux.

*Limitations of the push-pull method.* The push-pull method for measuring denitrification rates has two main benefits: (1) rates are determined under *in situ* conditions and (2) the volume of groundwater and soil encompassed is relatively large compared to other methods. The latter is particularly important for capturing micro-scale hotspots ('patchiness') of denitrification and scaling up *in situ* observations to the landscape or whole-catchment. Despite these benefits, the push-pull method has its limitations (as do all methods, see Groffman et al. 2006). First, push-pull tests are time consuming and expensive. The labor and expense allow fewer measurements of denitrification than other techniques, which are greatly needed at increasingly larger spatial and temporal scales. Second—and more importantly—the push-pull method is limited to saturated areas, necessarily limiting the scale or scope of the study. While this can be good

given the first limitation, it means the push-pull technique may not be the best method to estimate total whole-catchment denitrification unless most of the denitrification is occurring in the shallow saturated zone. The magnitude of estimated N fluxes suggest a good portion of denitrification is occurring in these areas, but to better ascertain the relative magnitude requires a combination of additional measurements of denitrification from other portions of the landscape and modeling or a detailed N balance for the catchment.

*Future work.* The annual denitrification flux amounts reported in this study can be improved upon in subsequent landscape or catchment denitrification studies that utilize the push-pull method. As discussed previously, transforming denitrification rates from a per volume (water) or per weight (soil) basis to a rate or flux per area requires an assumption of “effective depth” that introduces uncertainty. Given that the push-pull method for measuring denitrification can only be used in saturated areas and rates are initially calculated as  $\text{mg N L}^{-1} \text{d}^{-1}$  requiring no assumptions about soil properties or extent, it stands to reason that incorporating a hydrologic model and estimating water fluxes coupled with the STI-distributed denitrification rates would result in more spatially and temporally precise estimates of denitrification fluxes, although accuracy may not be improved. Alternatively, the “effective depth” parameter used to transform rates could be determined in greater detail, depending on available data. For example, measured or modeled water table elevations could be used in conjunction with minimum threshold values of C and/or  $\text{NO}_3^-$  in the saturated soil to indicate the upper and lower levels of the effective depth for each measurement. Or denitrification rate versus depth relationships could be determined.

## CONCLUSIONS

The strong positive relationship between (soil) topographic index and *in situ* denitrification rate confirm that the highest rates of denitrification occur over a relatively small portion of the landscape and correlate with those areas prone to saturate. We used this relationship to estimate distributed denitrification fluxes from the shallow saturated zone across a study catchment. Our results suggest that a large portion of whole-catchment denitrification occurs in the shallow saturated zone and that these areas should be conceptualized as hotspots of potential denitrification activity in the landscape. Although our study included riparian zones, we also found hotspot behavior in portions of the upland landscape where water tables are shallow and dynamic, experiencing event- or seasonal-scale fluctuations that lead to periodically saturated surface soils, consistent with the variable source area concept, and simultaneous increases in denitrification.

The techniques used in and findings of this study support the three fundamental areas for advancement targeted by the National Science Foundation Denitrification Research Coordination Network (<http://www.denitrification.org>): (1) quantification of denitrification rates; (2) development of quantitative, process-based relationships between rates denitrification and controlling factors; and (3) production of spatially explicit, process-based models that can be used to scale-up site specific measurements to the ecosystem or larger scales. A TI-denitrification model is a promising and simple tool that allows for scaling of *in situ* denitrification rates across the landscape and provides insight into the spatial organization of denitrification at the catchment scale. Improved understanding of the distribution and magnitudes of denitrification—particularly in landscapes impacted by agriculture—has good potential to facilitate new, novel, and better management practices for controlling N loading to streams and rivers. Indeed, the very areas that appear to have a propensity to harbor

denitrification, i.e., areas prone to be wet, are often artificially drained as part of standard agricultural practices, which effectively increase N loading to rivers and contributes to downstream eutrophication. This practice not only reduces the frequency that these areas are likely to be anaerobic, but it constitutes a rapid N transport pathway between the landscape and streams.

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## CHAPTER 4

### ASSESSING DENITRIFICATION FROM SEASONALLY SATURATED SOILS IN AN AGRICULTURAL LANDSCAPE: A FARM-SCALE MASS-BALANCE APPROACH<sup>3</sup>

#### ABSTRACT

Riparian zones have received considerable attention as potential hotspots of denitrification at the landscape- or watershed-scale. Conceptually, the conditions that promote denitrification in these zones are also found in other parts of the landscape, namely those areas that are prone to saturate. However, spatiotemporal characterization and quantification of these potential denitrification hotspots are lacking, despite their importance to land managers tasked with mitigation of nitrogen (N) pollution, particularly in human-dominated landscapes. We quantified denitrification fluxes from the shallow saturated zone of an agricultural landscape using a topographic index-denitrification model, which facilitates scaling of *in situ* denitrification rates across the landscape based on frequency and duration of saturated conditions. Denitrification in the shallow saturated zone resulted in a N flux that was nearly half of the total denitrification from the landscape—in about a third of the area—as determined from a well-constrained whole-farm N balance constructed from farm records and field measures. Denitrification flux rates from saturated riparian soils were among the highest in the landscape, however the contribution of riparian areas to total landscape denitrification was less than 10 percent.

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<sup>3</sup> Anderson, T.R., Groffman, P.M., Walter, M.T. Assessing denitrification from seasonally saturated soils in an agricultural landscape: a farm-scale mass-balance approach. *Agriculture, Ecosystems & Environment*. <internal review>

## INTRODUCTION

The issue of nitrogen (N) pollution and the dominant role of agriculture as a major nonpoint source of N pollution is well recognized (e.g., Galloway et al., 2003; Robertson and Vitousek, 2009; Smil, 1999). Agricultural production results in unavoidable losses of reactive N to the environment via multiple pathways, such as leaching of nitrate ( $\text{NO}_3^-$ ) to surface and groundwater, volatilization of ammonia ( $\text{NH}_3$ ) from soils, and fluxes of nitrous oxide ( $\text{N}_2\text{O}$ ) and other reactive N-containing gases ( $\text{NO}_x$ ) to the atmosphere. Environmental consequences of these reactive N forms include eutrophication of coastal zones, compromised air and water quality, climate warming, and biodiversity changes in receiving ecosystems (Davidson et al., 2012). Successful management of N in agroecosystems attempts to maximize crop and/or animal production while minimizing environmental loss of N (referred to as nitrogen use efficiency, or NUE). This task is complicated by multiple transformation processes within the N cycle (e.g., mineralization, immobilization, volatilization, fixation, nitrification, denitrification) (Galloway et al., 2003). Denitrification, the microbial transformation of  $\text{NO}_3^-$  to N gases, is of particular interest and importance in agricultural landscapes because it is capable of reducing a reactive form of N ( $\text{NO}_3\text{-N}$ ) to a non-reactive form (inert  $\text{N}_2$ ) (Seitzinger et al., 2006).

Denitrification is a facultative anaerobic process utilized by specific groups of heterotrophic microbes that are ubiquitous in terrestrial soils; oxygen ( $\text{O}_2$ ) and available carbon (C) and nitrate ( $\text{NO}_3^-$ ) are widely regarded as the main factors controlling denitrification activity at the organism scale (Firestone, 1982; Knowles, 1982). However, estimating denitrification fluxes at larger scales (e.g., landscape or watershed) is problematic due to high spatial and temporal variability of the environmental regulators of  $\text{O}_2$ , C, and  $\text{NO}_3^-$ , giving rise to hotspots and hot moments of denitrification (Groffman et al., 2009; McClain et al., 2003). Denitrification hotspots can be biogeochemically process-driven and/or transport-driven; the former due to locally anoxic

conditions and the presence of labile C, the latter due to solute fluxes in water (Vidon et al., 2010).

Riparian zones have received considerable attention as potential hotspots of denitrification because they allow for the confluence of necessary electron acceptors ( $\text{NO}_3^-$ ) and donors (C) via hydrologic flowpaths in low  $\text{O}_2$  (reducing) conditions (e.g., Hedin et al., 1998; Vidon and Hill, 2004a). However, these conditions exist along a continuum throughout the landscape, and include areas prone to saturate both permanently and periodically (e.g., Hill 2000; Walter et al., 2000). Indeed, groundwater fluctuations and their control on anaerobic conditions and nutrient fluxes have been shown to promote denitrification in riparian buffers, wetlands, and other areas experiencing saturation (e.g., Hefting et al. 2004; Reddy and Patrick, 1975; Woli et al., 2010). Similarly, increased hydrological connectivity via shallow groundwater is thought to enhance  $\text{NO}_3^-$  removal through denitrification (e.g., Kaushal et al., 2008; Roley et al., 2012; Vidon and Hill, 2004b,c).

Despite the emerging importance of denitrification hotspots, there remains a critical knowledge gap: how much of the denitrification in a landscape or watershed can be attributed to these hotspots? Spatiotemporal distribution of these denitrifying zones have not been quantifiably characterized. This is partially due to the fact that, until recently, techniques for measuring denitrification *in situ* at specific points in the landscape were not well developed (Groffman et al., 2006). And only recently—with the maturation of geographic information systems (GIS)—have we had the ability to model hydrologic processes in a fully distributed manner. Scaling-up site specific measurements to ecosystem or larger scales has been identified as a critical need for denitrification research (Boyer et al., 2006; NSF Denitrification RCN, 2012).

In a companion study, we quantified *in situ* denitrification rates across a range of hydroperiodicities, i.e., frequencies and durations of saturated conditions, as characterized by a topographic index (TI) in a small, agricultural headwater catchment (manuscript in preparation). TI (also referred as ‘topographic wetness index’) is considered an index of hydrological similarity: the higher the index value, the wetter the point in the landscape and the more frequently the point will be saturated relative to other points in the same landscape (Ambrose et al. 1996). We found a strong positive relationship between TI and denitrification, indicating that the highest rates of denitrification occur over the relatively small portion of the landscape prone to saturation. We used the resulting relationship to distribute denitrification rates across the catchment and estimate denitrification fluxes from the shallow saturated zone. We compared rates/fluxes to other published values in similar settings, and concluded that a large portion of whole-catchment denitrification was occurring in the shallow saturated zone, and that these areas should be conceptualized as hotspots of potential denitrification activity. However, our analysis did not evaluate denitrification fluxes in context of other major components of the N cycle or the total N budget for the watershed.

N budgets or balances are useful tools for expanding our understanding of the N cycle at any scale of interest. In agroecosystems, N budgets are typically used to document the major N flow paths, sources, and sinks as a way to develop estimates of N use efficiencies, evaluate N management strategies, and/or identify areas of environmental N loss (Meisinger et al., 2008; Meisinger and Randall, 1991; Mosier et al., 2003). Each N balance is unique: tailored to a specific set of goals and requiring a clear definition of spatial and temporal boundaries which ultimately determines the N flow paths and sources/sinks to be considered. Conservation of mass is the principle on which N balances are based:

$$N_{inputs} = N_{outputs} + \Delta N_{storage} \quad (1)$$

where the mass of N entering is equal to the mass of N leaving plus the change of N stored in the system, over a given time period. Major N inputs into agroecosystems may include atmospheric deposition, inorganic fertilizers, animal manure, biological fixation, and feed imports; major N outputs may include harvested crops, animal products, volatilization losses from manure or fertilizer, denitrification, leaching losses, and surface runoff. A quasi steady-state condition is often assumed to simplify agricultural N balances, so the  $\Delta N$  term is taken to be zero. This condition implies that soil N mineralized from soil on an annual basis is balanced by N immobilized (e.g., in residues and roots or as new soil microbial biomass). Consistent application of the same N management practices over many years is key to attaining a quasi steady-state condition; other factors include climate, soil properties, tillage, N additions, and cropping system (Meisinger et al., 2008). N balances, as described above, have been used to make estimates of landscape- or watershed-scale denitrification. In this method, all the N inputs and N outputs—other than denitrification—are estimated or measured, and the resulting N surplus is assumed to be balanced by denitrification (e.g., Gentry et al., 2009; Puckett et al., 1999; van Breemen et al., 2002).

Our goal in this study was to quantify denitrification occurring in shallow saturated zone hotspots of an agricultural landscape, and compare it to total denitrification and other N fluxes in the same landscape to investigate the contribution and relative importance of these hotspots to the mitigation of nonpoint source N pollution. We used the methodology developed in and results from our companion study (manuscript in preparation) to estimate the denitrification flux

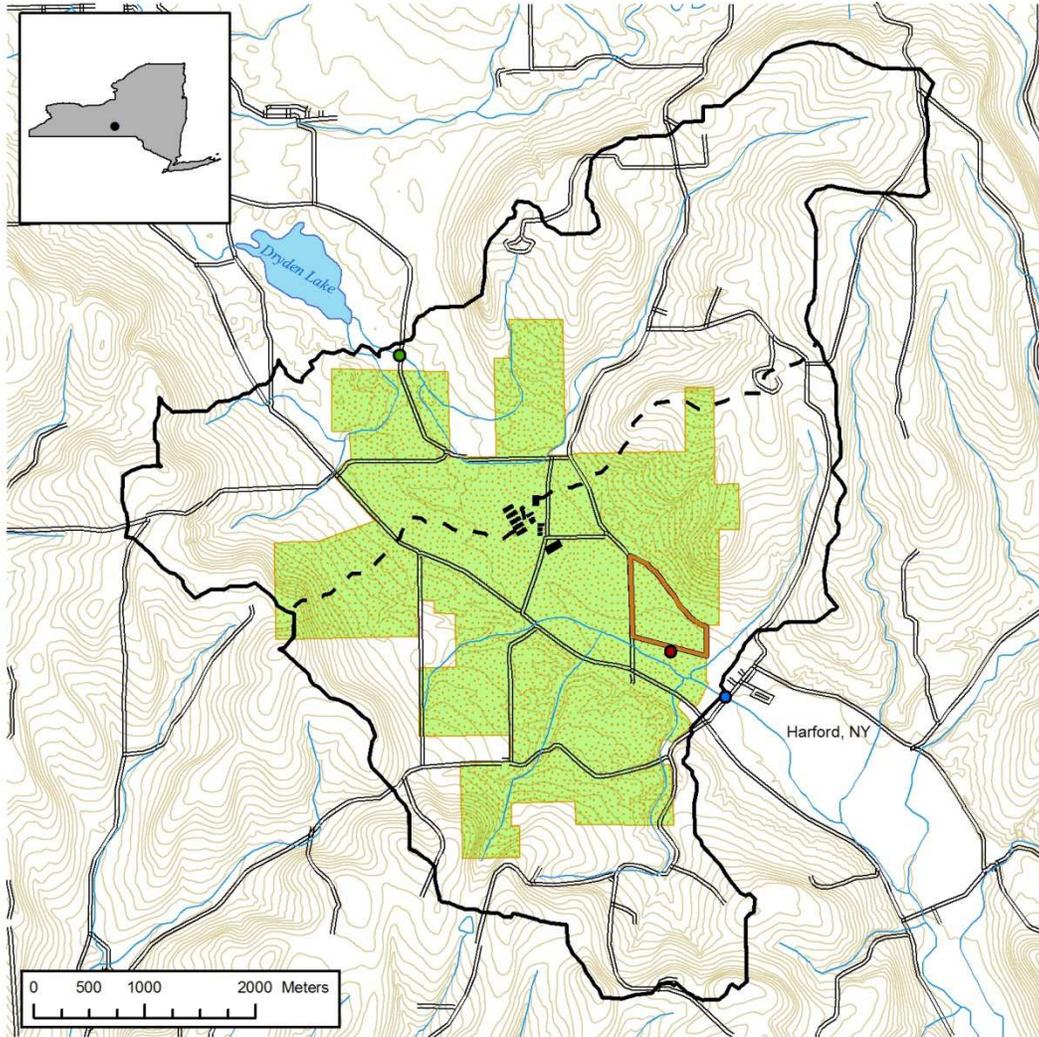
from the shallow saturated zone of an intensive dairy farm under a corn/alfalfa production system. A whole-farm N balance was constructed from detailed farm records and direct measurements to estimate the remaining N sources and sinks, including total denitrification (via the difference method).

## **MATERIALS AND METHODS**

### **DESCRIPTION OF STUDY AREA**

The study was carried out at the Cornell University Animal Science Teaching and Research Center (T&R Center) located near Harford, NY, USA (Figure 4.1). The T&R Center occupies 1052 ha of land, of which 159 ha was in pasture and 456 ha was cropped corn or alfalfa in rotation to support intensive dairy production. The remaining acreage is utilized by dairy, beef, and sheep unit facilities. Daily average herd size during the study was 1050 dairy cattle, 195 beef cattle, and 1100 sheep. All manure is stored on site and surface spread without incorporation into the soil, often on a daily basis. Animal products sold include milk and wool, plus live animals to adjust herd size and composition. NPK fertilizers are used during the planting of corn as a starter and later as a sidedress depending on rotation (e.g., corn not following alfalfa) and/or results of pre-sidedress nitrogen tests. Harvested crops, primarily corn silage and alfalfa hay silage, stay on the farm and are used to supplement imported feed. Alfalfa is harvested in three cuts during the summer and fall; corn is harvested once in the fall. Reduced tillage practices follow harvest and precede most seeding operations (T. Eddy, T&R Center Director of Operations, personal communication).

The T&R Center is situated in a broad valley floored with stratified drift cut through bedrock ridges—one of which is a groundwater divide between Fall Creek to the north and Owego Creek



**Figure 4.1.** Location of the T&R Center (green shade) and drainage divide (black dashed line) between Fall Creek and Owego Creek near Harford, New York, USA. 14 sites in or around field #10 (solid brown line) were instrumented with mini-piezometers to measure *in situ* denitrification and related to the propensity of saturation as characterized by a topographic index. Nitrate ( $\text{NO}_3^-$ ) concentrations were monitored in a groundwater well (red circle) and in northern and southern streams (green and blue circle, respectively) leaving the farm. Solid black lines delineate the catchment encompassing the farm area.

to the south (Figure 4.1)—and drained by small headwater streams (Randall et al., 1988). The valley floor is nearly level with deep and well-drained soils surrounded by hillsides of shallow and intermediate to poorly-drained soils (Randall et al., 1988; Swader, 1972). The valley soils are most favorable for agriculture, as erosion and drainage are not major problems. Accordingly, most crop production and manure spreading occur along the bottom of the valley with hillsides left in grassland pasture (used for grazing) or woodlands (Wang et al., 1999). Groundwater and surface water interactions in the valley have been described elsewhere (Randall et al., 1988; Swader, 1974). Briefly, intermittent streams originating in the uplands lose water by seepage as they flow across the valley floor. Groundwater is derived from these losses plus precipitation on the valley floor, and drains downvalley as underflow. Where the water table intersects the land surface, groundwater is discharged to form a stream. The water table subsides during the summer as groundwater continues to drain downvalley but is not recharged from precipitation due to high rates of evapotranspiration. As the water table subsides, the point at which it intersects the land surface migrates downvalley, leaving a dry channel upvalley.

Records (2005-2011) from a recently deployed NOAA USCRN monitoring station on site (NY Ithaca 13 E, 42.44 °N, 76.25 °W, 374 m elevation) indicate an annual mean temperature of 7.4 °C with monthly mean temperatures ranging from -5.3 °C in February to 19.4 °C in July (NCDC, 2012; <http://www.ncdc.noaa.gov/>). Mean annual precipitation is 1076 mm yr<sup>-1</sup> with more precipitation on average in summer (114 mm mo<sup>-1</sup>) than winter (75 mm mo<sup>-1</sup>). Snowfall averages 1583 mm yr<sup>-1</sup>.

## NITROGEN INPUTS

### *Atmospheric deposition*

Rate of background atmospheric N deposition was obtained from a nearby US EPA Clean Air Status and Trends Network (CASTNet) site located on Connecticut Hill, about 45 km to the west of the T&R Center (CTH110, 42.40 °N, 76.65 °W, 501 m elevation). CASTNet sites estimate dry N deposition by combining measurements of ambient dry N species ( $\text{NO}_3^-$  and  $\text{NH}_4^+$ ) with deposition velocities determined using a multilayer model (CASTNet, 2012; <http://www.epa.gov/castnet>). For estimates of wet N deposition, CASTNet uses reported values from the closest National Atmospheric Deposition Program/National Trends Network (NADP/NTN) site, which is located at the Aurora Research Farm, about 60 km to the northwest of the T&R Center (NY08, 42.73 °N, 76.66 °W, 249 m elevation). NADP/NTN sites estimate wet N deposition based on measurements of wet N species in precipitation (NADP, 2012; <http://nadp.sws.uiuc.edu/>). Total N deposition over the one-year study period was estimated to be equal to the 2008 value of 6.06 kg N ha<sup>-1</sup> (1.34 and 4.72 kg N ha<sup>-1</sup>, dry and wet N, respectively). *Uncertainty/error.* We used rates of total N deposition in years bracketing the study period to estimate the range of variability that might be expected locally. These rates, as reported by CASTNet (2012), were 7.24 and 5.58 kg N ha<sup>-1</sup>, in 2007 and 2009, respectively.

### *Fertilizer*

Fertilizer N was estimated from fertilizer application records combined with target NPK composition of the fertilizer blend used. Most of the fertilizer used during the study period was applied as urea and ammonium nitrate for corn production on 225 ha of cropland. Fertilizer applications were split to increase N use efficiency. N starter was applied in bands next to seed rows when planting corn in the spring, followed by an optional early summer sidedressing of N

prior to the time of maximum crop uptake. The average fertilizer N rate for corn was 63 kg ha<sup>-1</sup>. A lesser amount of N (about 1% of total fertilizer N) was applied as ammonium sulfate and monoammonium phosphate in an alfalfa seeding blend. *Uncertainty/error.* Synthetic fertilizer use on farms is typically one of the more constrained inputs, with less than 5% uncertainty (Oenema et al., 2003). As such, we assume ±5% fertilizer N for this study.

### *Nitrogen fixation*

N fixation was estimated from crop yield in conjunction with nutrient analysis of crop samples in NY State over the study period. Hay silage was harvested in three cuts from alfalfa production on 231 ha of cropland. The average yield on a dry matter (DM) basis was 8.42 Mg ha<sup>-1</sup> (3.26 tons per acre). Crude protein (CP) content was estimated to be 20.5% of DM using values for MML (mixed mostly legume) silage reported by the Dairy One Forage Laboratory feed composition library (Dairy One, 2012; <http://www.dairyone.com/Forage/FeedComp>). N content is calculated as CP content divided by 6.25 (NRC, 2001,2003). Only a portion of total N is due to N fixation. We estimated the amount of fixed N based on Heichel et al. (1994), who found that fixed N comprised 59% of total N harvested over a 4-yr alfalfa stand, covering multiple cuttings and stand ages. *Uncertainty/error.* We used the standard deviation of DM and CP content reported by Dairy One (2012) as the normal range of values expected in NY state for MML silage.

### *Feed*

N imported in feed and bedding materials was estimated from purchase records together with producer-reported feed composition or regional feed composition data over the study period. Major N containing feed imports include proprietary mineral and grain mixes, corn, and

distiller's grain for ration formulization. Other significant imports include grass hay and sawdust for bedding. For each input, total weight was converted to DM weight, and N content was calculated as the CP content (on a DM basis) divided by 6.25. For the proprietary mineral and grain mixes, CP was reported by the producer and a 90% DM was assumed. For the remaining feed and bedding imports, values of DM and CP were taken from the Dairy One Forage Laboratory feed composition library (Dairy One, 2012) or NRC feed composition tables (NRC, 2001). *Uncertainty/error.* We used the standard deviation of DM and CP content reported by Dairy One (2012) as the normal range of values expected in NY state for feed and bedding import composition. We also analyzed the five most used proprietary mineral and grain mixes (largest contributors on a dry weight basis) for total N using a vario EL III elemental analyzer (Elementar Analysensysteme, Hanau, Germany). Samples were oven dried prior to N analysis to determine DM content of stored mixes. We used the mean  $\pm$  one standard deviation of the differences between expected N content (from producer-reported CP content) and observed N content as the normal range of variability in all mixes (Table 4.1).

## NITROGEN OUTPUTS

### *Animal products*

Major N containing animal products that are exported from the T&R Center include live animals (sold), milk, and wool. N exported in animal sales was estimated from live weights reported in sales records and the typical N content of live, growing cattle and sheep, 2.6 and 2.3 percent, respectively (NRC, 2003). N exported in milk was estimated from total milk production and the average daily milk CP content reported by the Dairy One Milk Laboratory; N content of milk is calculated as CP content divided by 6.38 (NRC 2001,2003). N exported in wool was estimated from the dry weight of sheared, clean wool assuming a CP content of 100 percent; N content is then 100 divided by 6.25, or 16 percent. *Uncertainty/error.* We used the range of typical N

**Table 4.1**

Nitrogen (N) determination of five major rations—grain and mineral mixes—utilized at the T&R Center

Ration	DM (kg)	%N		Diff
		CP/6.25	N analyzer	
Herd #1	593462	4.5	6.3	1.8
Herd #5	89049	3.7	4.0	0.3
Herd HS	336901	3.2	4.3	1.1
Heifer #7	31543	5.0	5.5	0.5
Heifer #8	78771	7.7	7.5	-0.2

contents reported for live cattle (2.0-2.9%) and sheep (2.0-2.5%) in all stages of growth (NRC, 2003) to estimate the minimum and maximum amount of N exported via livestock sales. One standard deviation of the reported daily milk CP content was used to represent the normal range and variability of daily values over the study period.

### *Ammonia volatilization*

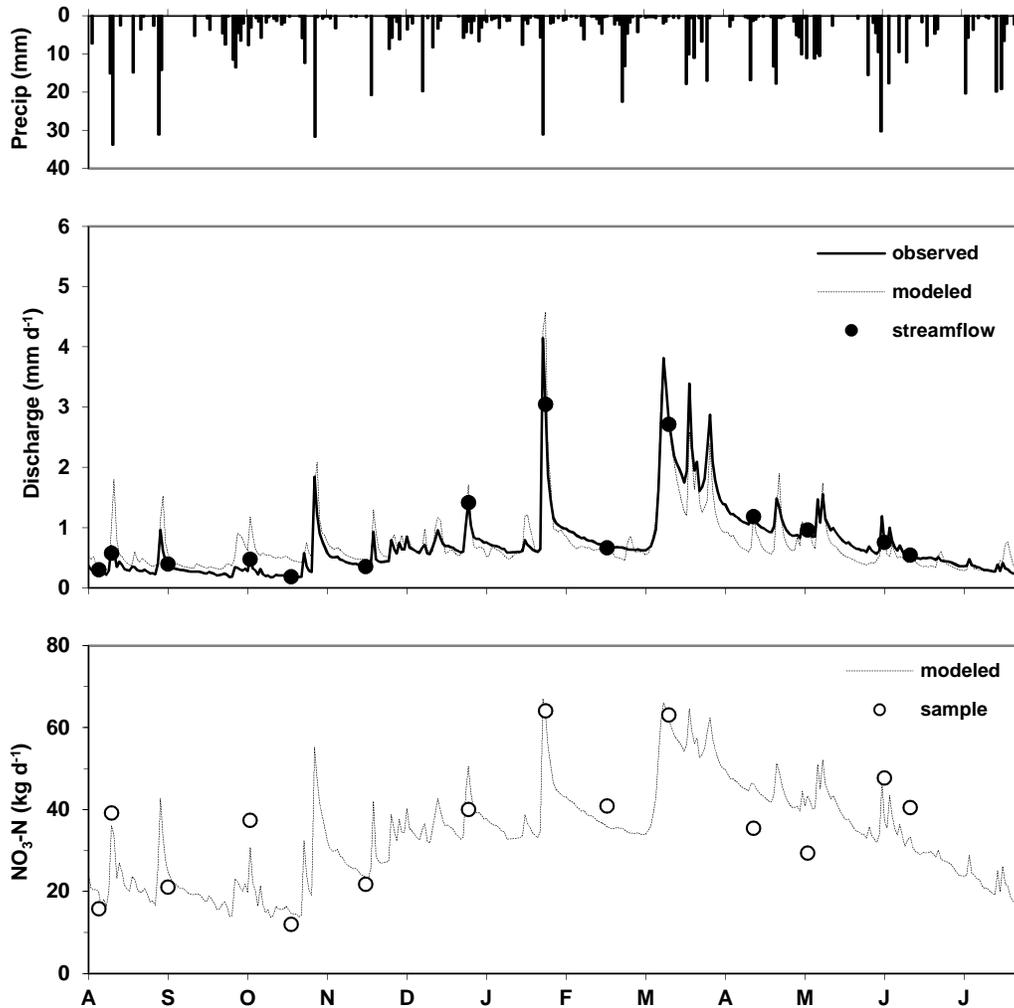
Ammonia N ( $\text{NH}_3\text{-N}$ ) lost during volatilization was estimated from manure application records coupled with manure analysis reports. A variety of manure collection and storage systems are in place at the T&R Center, including slatted floor systems with both aboveground and underground containment tanks, solid floor with scrape systems, and bedded pack systems. Containment tanks are emptied two times per year. Scraped systems have no long-term storage (daily haul and spread). Bedded packs are removed 3-5 times per year. There is also an open feedlot for the beef unit, with a storage tank that collects surface runoff to be applied during the winter. Manure samples are analyzed each year for total Kjeldahl N (TKN),  $\text{NH}_3\text{-N}$ , and organic N by the Dairy One Agronomy Laboratory. All manure is surface spread and not incorporated into the soil. Manure application records track the cumulative TKN and  $\text{NH}_3\text{-N}$  amounts applied using averaged values of recent manure analyses. We estimated the loss of  $\text{NH}_3\text{-N}$  by volatilization based on Lauer et al. (1976), who found that 85% of  $\text{NH}_3\text{-N}$  is lost after surface application of dairy manure, across all seasons, with a half-life of 3.44 days. The portion of  $\text{NH}_3\text{-N}$  that is redeposited locally was estimated to be 25%, based on a 2-4 km transport distance (Asman and van Jaarsveld, 1992; Fahey et al., 1999). *Uncertainty/error.* We used the standard deviation of  $\text{NH}_3\text{-N}$  content reported for each manure type in the master manure analysis report to represent the normal range and variability of manure  $\text{NH}_3\text{-N}$  over the study period.

### *Nitrate export*

Nitrate N (NO<sub>3</sub>-N) lost in stream water was modeled from continuous discharge monitoring and routine stream water sampling conducted the following year. Details of monitoring and sampling methods—plus analyses and results—can be found in Anderson et al. (YEAR). Briefly, we installed a streamgage on the southern stream draining the T&R Center and developed a rating curve to establish the stage-discharge relation. NO<sub>3</sub>-N concentrations in stream water collected at the streamgage were compared to simultaneous discharge values. Trends in NO<sub>3</sub>-N concentration relative to discharge were characterized using a hyperbolic dilution model (Barco et al., 2012; Johnson et al., 1969). The resulting concentration-discharge relation was applied to the discharge record to estimate NO<sub>3</sub>-N concentrations in stream as a function of flow. To back estimate over this study period (Mar 1 2008 – Feb 28 2009), we applied the same hyperbolic dilution model to a modeled discharge record based on the drainage-area ratio method (Hirsch, 1979). Discharge was modeled after Fall Creek (USGS 04234000, 42.453 °N, 76.473 °W, 242 m elevation, 326 km<sup>2</sup> drainage area) and further calibrated using linear regression based on the closest one-year long discharge record at the T&R Center (Aug 1 2009 – Jul 31 2010) (Figure 4.2).

NO<sub>3</sub>-N lost in groundwater was estimated from concentrations measured in a shallow groundwater well at the T&R Center and a water balance to determine the amount of water entering the groundwater system over the study period:

$$P = ET + Q + GW \tag{2}$$



**Figure 4.2.** Model results for discharge and nitrate-N ( $\text{NO}_3\text{-N}$ ) loading in the southern stream leaving the T&R Center from Aug 2009 – July 2010. (a) Precipitation measured at a weather station on site. (b) Observed discharge (solid line) developed from streamflow measurements (solid circle); discharge was modeled using a watershed area-adjusted approach from the nearby Fall Creek discharge record. (c) Measured  $\text{NO}_3\text{-N}$  loading (open circle); loading was modeled using a hyperbolic dilution discharge-concentration relation. Model results are shown as a dashed line in (b) and (c).

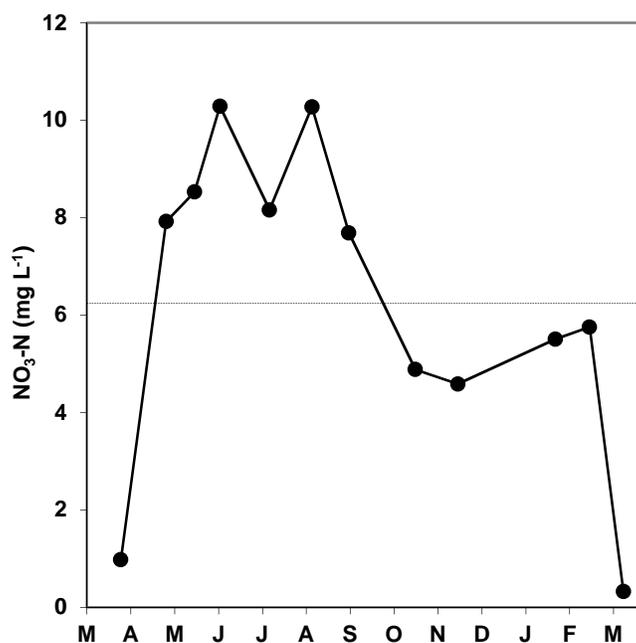
where  $P$  is precipitation,  $ET$  is evapotranspiration,  $Q$  is stream discharge, and  $GW$  is the flux to groundwater. To estimate  $ET$ , a daily Thornthwaite-Mather soil water budget was developed which accounts for inputs and outputs of water (Steenhuis and Van der Molen, 1986; Thornthwaite and Mather, 1955). Precipitation was obtained from the weather station on site. Potential evapotranspiration (PET) was calculated using the Penman-Monteith equation (Allen et al., 1998; Monteith, 1965; Penman, 1948). Available water capacity (AWC) was determined from USDA-NRCS SSURGO soil data (NRCS, 2012; <http://soildatamart.nrcs.usda.gov/>).  $P$  and PET were used in calculations on a daily time-step to determine available water (AW) and actual evapotranspiration (AET); each calculation was dependent on whether the soil was drying, wetting and below AWC, or wetting and above AWC. AET was summed to estimate  $ET$  over the study period. Rearranging the water balance (Eqn. 2), the contribution to groundwater was the difference between inputs (precipitation) and outputs (actual evapotranspiration and stream discharge) (Table 4.2). The mean concentration of  $NO_3-N$  in drainage water was estimated from monthly sampling of a shallow well (#64) which skims the groundwater beneath the valley (Figure 4.3). Samples were analyzed for  $NO_3-N$  using a Dionex ICS-2000 ion chromatograph (Dionex, Sunnyvale, CA, USA).

To estimate  $NO_3-N$  export in the northern drainage system encompassing the T&R Center, we adjusted values from the southern drainage (described above for both stream water N and groundwater N) according to the proportion of agricultural land in each area, i.e., the ratio of farmland in the north (306 ha) to farmland in the south (746 ha), or 0.41. *Uncertainty/error*. We used root-mean-square-error (RMSE) as an indicator of stream  $NO_3-N$  load model uncertainty. We calculated RMSE between the southern stream ‘observed’ and Fall Creek ‘modeled’ daily  $NO_3-N$  load for the one-year period that the model was calibrated on, and applied that value to back-modeled estimates of stream  $NO_3-N$  load for this study. A similar approach was used to estimate uncertainty in the loss to groundwater  $NO_3-N$ . We calculated RMSE between the

**Table 4.2**

Monthly water balance for the T&R Center over the study period Mar 2008 – Feb 2009

	P (mm)	PET (mm)	AET (mm)	Q (mm)
Mar	154.4	41.3	40.9	62.7
Apr	55.0	95.4	76.0	39.1
May	50.8	104.2	64.1	19.1
Jun	97.3	123.1	68.6	14.2
Jul	142.4	127.8	67.7	19.4
Aug	73.3	113.7	56.0	14.9
Sep	81.1	83.0	25.9	9.5
Oct	96.2	54.3	22.7	13.7
Nov	86.4	27.9	16.2	22.8
Dec	84.1	27.2	24.7	41.1
Jan	60.6	20.9	20.7	22.8
Feb	49.5	31.5	30.8	25.5
Total	1031.1	850.1	514.2	304.8



**Figure 4.3.** Time series of nitrate-N ( $\text{NO}_3\text{-N}$ ) concentration in shallow groundwater well #64 at the T&R Center from Mar 2008 – Mar 2009. The dashed line represents the mean  $\text{NO}_3\text{-N}$  concentration across all sample dates ( $6.2 \text{ mg L}^{-1}$ ,  $n=12$ ) and is the value used to estimate the amount of N leached in groundwater.

‘observed’ and ‘modeled’ daily stream discharge, and applied that value to back-modeled estimates of stream discharge for this study to estimate upper and lower limits of the contribution to groundwater (through Eqn. 2).

### *Denitrification*

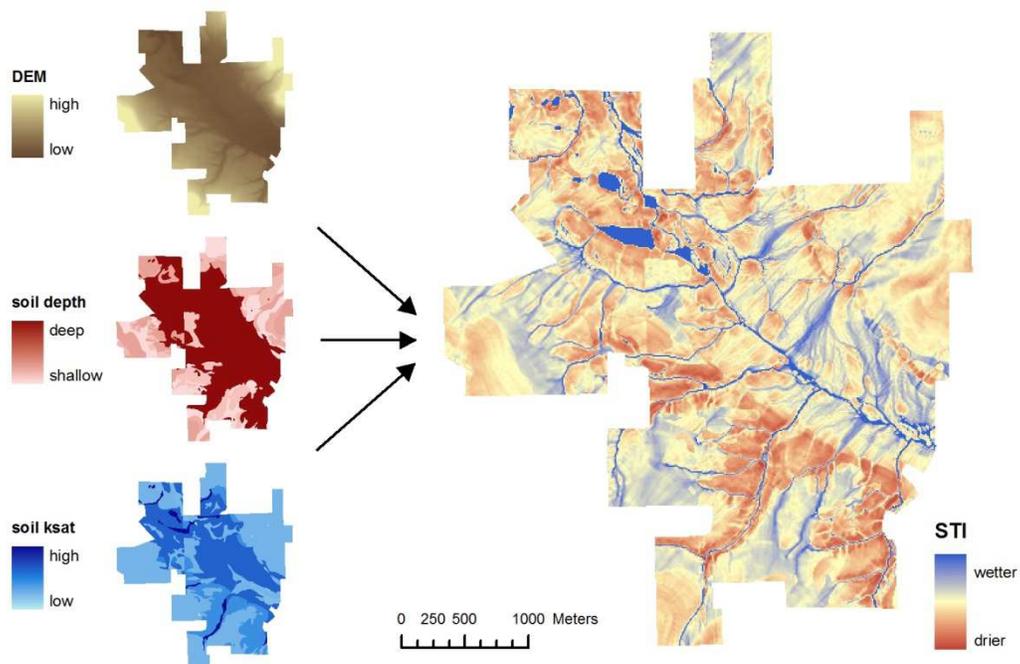
Total denitrification across the landscape was estimated as the difference between N inputs and N outputs. Of this total, the amount occurring in the shallow saturated zone was estimated using a topographic index-denitrification model. Details of the methodology and model development can be found in Anderson et al. (YEAR). Briefly, we quantified *in situ* denitrification rates using the  $^{15}\text{N}\text{-NO}_3$  push-pull method (e.g., Addy et al., 2002) across a range of hydroperiodicities, i.e., frequencies and durations of saturated conditions, as characterized by a soil topographic index (e.g., Agnew et al., 2006) in the southern drainage system of the T&R Center (Figure 4.4). We found a strong positive relationship between soil topographic index and *in situ* denitrification rate on an annual basis (Figure 4.5), and the relation was used to distribute denitrification fluxes from the shallow saturated zone over the entire agricultural landscape (Figure 4.6).

*Uncertainty/error.* Total denitrification estimated through a mass balance approach such as this accumulates the uncertainties and errors of each input and output term. Multiple cross validation was used to determine uncertainty in the topographic index-denitrification model and bound estimates of denitrification in the shallow saturated zone (see Anderson et al., YEAR).

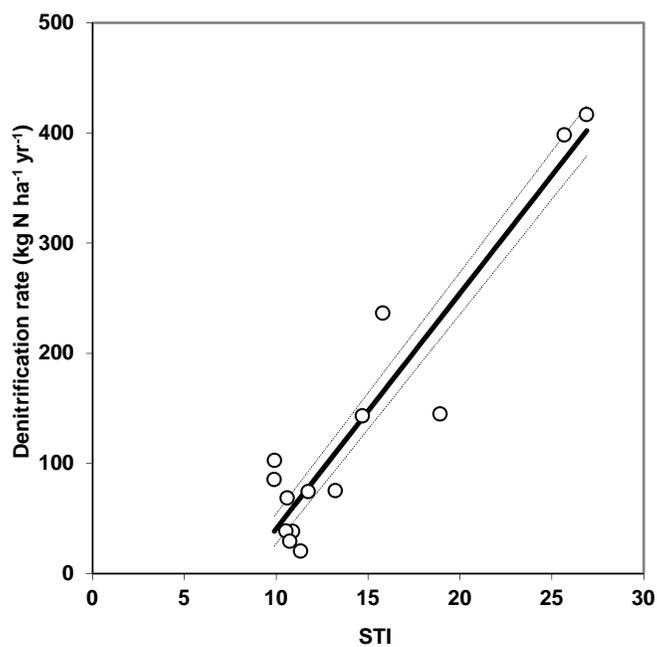
## **RESULTS**

### **NITROGEN BALANCE**

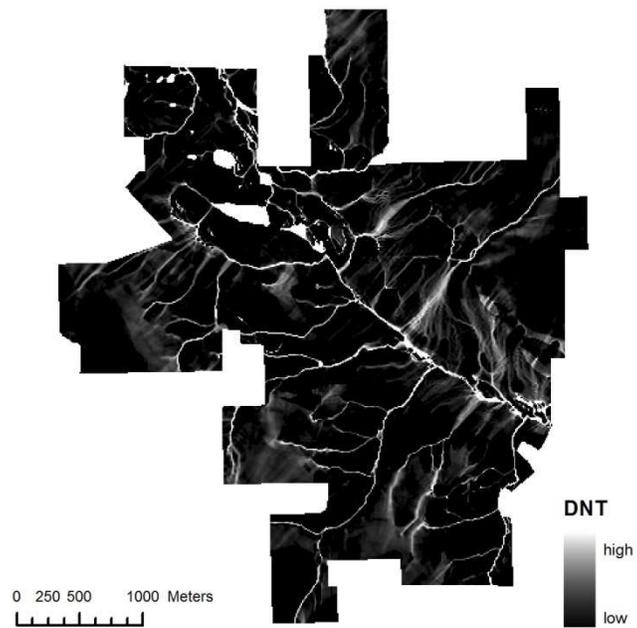
N inputs exceeded N outputs at the T&R Center (Table 4.3; Figure 4.7). The largest inputs were in feed products, specifically purchased feed (63% of the total N input) and a lesser amount from



**Figure 4.4.** Soil topographic index (STI) of the T&R Center created in GIS using digital elevation model (DEM) and soil data. Soil depth indicates the depth to bedrock or some other restrictive layer (e.g., fragipan) and ksat is the saturated hydraulic conductivity of that upper soil layer. High values of STI are in blue and indicate generally wetter areas. Conversely, drier portions of the landscape corresponding to low values of STI appear in red.



**Figure 4.5.** STI-denitrification rate relation determined by linear regression and used to extrapolate denitrification fluxes from the shallow saturated zone according to STI value across the T&R Center. Denitrification rates are shown as annual estimates in units of  $\text{kg N ha}^{-1} \text{ yr}^{-1}$ . Dashed lines indicate one standard deviation of linear regression coefficients determined via multiple cross validation and represent model uncertainty. Modified from Anderson et al. (YEAR).



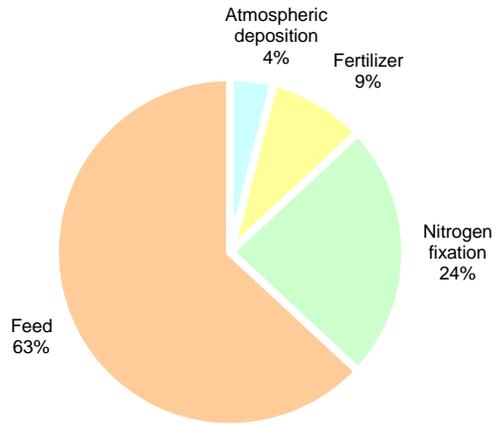
**Figure 4.6.** Distributed denitrification (DNT) fluxes across the T&R Center according to STI. Light areas indicate hotspots of denitrification activity in the landscape attributed to frequency and duration of saturated conditions.

**Table 4.3**

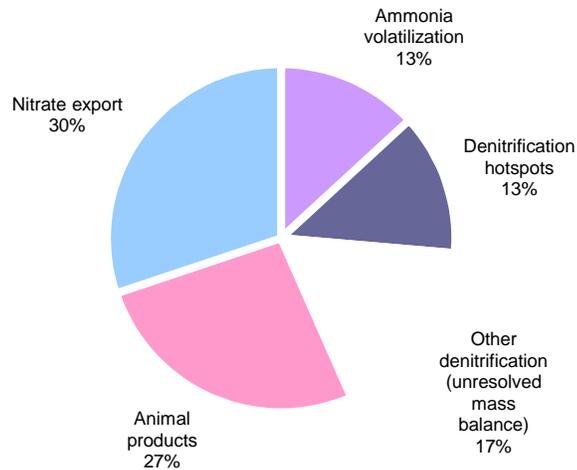
Nitrogen (N) balance for the T&amp;R Center over the study period Mar 2008 – Feb 2009

	Mass (kg)	Min	Max
<b>N Inputs</b>			
Atmospheric deposition	6376	5871	7618
Fertilizer	14197	13487	14906
N fixation	37610	24361	53374
Feed	99430	91066	125408
Total	157613		
<b>N Outputs</b>			
Animal products	41723	39778	42887
NH <sub>3</sub> volatilization	20719	12936	28503
NO <sub>3</sub> export	47485	31080	63890
Denitrification hotspots	20833	16550	26787
Total	130760		
Balance	26853		

**(a) N inputs**



**(b) N outputs**



**Figure 4.7.** Pie charts depicting the overall nitrogen (N) balance of (a) inputs and (b) outputs for the T&R Center over the study period Mar 2008 – Feb 2009. The ‘missing piece’ of the N balance was attributed to denitrification occurring in other parts of the landscape not measured during this study. See Table 4.3 for mass balance values.

N fixation in alfalfa (24%). Fertilizer use (9%) and atmospheric N deposition (4%) were considerably smaller sources of N to the farm. The largest output from the T&R Center was  $\text{NO}_3\text{-N}$  exported in stream and groundwater (30%), followed by N sold off the farm in animal products (26%). Volatilization loss of  $\text{NH}_3\text{-N}$  from manure (13%) was the smallest N output. Considering  $\text{NO}_3\text{-N}$  loss in stream and groundwater separately, more N was lost to groundwater (29128 kg, or 18%) than stream water (18356 kg, or 12%). Excluding denitrification, 30% of total N inputs are not accounted for.

## DENITRIFICATION

Total denitrification is assumed, here, to balance total N inputs and outputs—the missing sink—at 47686 kg N (Table 4.3; Figure 4.7). It becomes the single largest N output, slightly exceeding total  $\text{NO}_3\text{-N}$  export in water. Denitrification in the shallow saturated zone makes up a significant portion of total denitrification at 20833 kg N (44% of the total), and occurs in a relatively small portion of the landscape (366 ha, or about 35% of the total area). The aggregate denitrification flux from these hotspots is  $57 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ . The remaining piece of the N balance—assumed to be denitrification not measured—amounts to 26853 kg N. Taken over the entire agricultural landscape (1052 ha) results in an average denitrification flux of  $26 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ .

## DISCUSSION

### NITROGEN BALANCE

Interpretation of our results (as they pertain to denitrification) depend largely on the accuracy of our estimates of the various N input and output terms. For each N input or output, we have estimated a range of variability or uncertainty and report these values in Table 4.3. However, additional discussion may be helpful for establishing context and confidence.

Our results are consistent with past whole-farm N balances, in that the largest input for dairy farms with supplementary crop production tends to be feed imports followed by N fixation, and the major outputs are animal products (e.g., Spears et al., 2003; Wang et al., 1999). The smallest N input in our study area was background atmospheric N deposition, which is not unusual given the large N inputs required for crop production (fertilizer and/or manure) or animal production (feed) in agricultural areas (e.g., Barry et al., 1993; Puckett et al., 1999). Precision N management of corn is practiced at the T&R Center, with fertilizer N applied according to crop guidelines for NY State after taking into account all N sources (Ketterings et al., 2003). Overall, corn was fertilized at an average rate of  $63 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ , which is a typical economic rate for corn 1-3 years following alfalfa, grown on productive soils with manure amendments (Klausner, 1997). The amount of N fixation based on crop yield of alfalfa equaled  $163 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ , also in agreement with reported literature values (e.g.,  $150\text{-}250 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ , Brady and Weil, 2002;  $114\text{-}224 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ , Heichel et al., 1984;  $82\text{-}254 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ , Heichel et al., 1991;  $43\text{-}471 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ , Russelle and Birr, 2004).

While the outputs of N in animal products are well constrained, there is less certainty in the remaining output terms (Table 4.3). Nevertheless, there are multiple reasons to believe those estimates are relatively accurate. Similar values of  $\text{NH}_3\text{-N}$  loss to volatilization are obtained using two different calculation methods. The first uses EPA emission factors suggested in Battye et al. (1994)— $18.83 \text{ kg N cattle}^{-1}$  and  $2.77 \text{ kg N sheep}^{-1}$ —yielding a total N loss of 17241 kg (83% of the value estimated from manure records). The second approach is to assign N loss fractions based on the type of manure management facility. According to Bouldin et al. (1984), we would estimate a 40% N loss for having free stalls with daily haul and cleaning, plus slotted

floor systems with underlying pit storage and infrequent cleaning. This approach results in a loss of 23039 kg N (111% of the value estimated from manure records).

Observed stream water  $\text{NO}_3\text{-N}$  patterns in the southern stream at the T&R Center are consistent with groundwater-surface water interactions in the valley reported by the USGS (Randall et al., 1988). Stream flow is primarily groundwater discharge of a relatively constant source of  $\text{NO}_3\text{-N}$ , which experiences seasonal or event dilution. A similar pattern has been documented in other headwater areas locally (e.g., Goodale et al., 2009) as well as in the state (e.g., Burns et al., 1998). Uncertainty in stream  $\text{NO}_3\text{-N}$  export comes from using measurements taken the following year to estimate stream flow and  $\text{NO}_3\text{-N}$  concentrations during the actual study period. Qualitatively, the two years are comparable in terms of the factors that contribute to both stream flow and N loading, namely precipitation and N management. Total precipitation and the distribution thereof, i.e., seasonal totals and patterns, were similar in both years. In terms of N management, there were minimal changes in herd sizes and crop acreage between the two years, with no change in specific management practices. Thus, model performance is critical in establishing confidence in our stream  $\text{NO}_3\text{-N}$  estimates. Modeled discharge tended to slightly overestimate flow during the fall and underestimate flow during the spring (Figure 4.2b); over the one-year calibration period, the predicted discharge totaled 270 mm as compared to 268 mm observed (+ 0.5%). Modeled  $\text{NO}_3\text{-N}$  load over 14 sampling events spanning all seasons and flow conditions captured trends and peaks (Figure 4.2c); summing these days, the predicted  $\text{NO}_3\text{-N}$  export totaled 521 kg N as compared to 508 kg N measured (+ 2.5%).

A network of streams also converge and discharge to the north, although most of the farm lies within the southern drainage system (Figure 4.1). The drainage systems are nearly equal in size (1482 and 1561 ha for the northern and southern, respectively). To account for  $\text{NO}_3\text{-N}$  export in

the northern landscape, we assumed that the amount of N was proportional to the area of agricultural land in the southern landscape (306 to 746 ha, or 0.41). To validate this assumption, we measured flow and NO<sub>3</sub>-N concentration in the northern and southern streams on seven independent days in late fall, winter, and early spring covering a range of low and high flow events. The ratio of daily NO<sub>3</sub>-N load (N/S) was  $0.45 \pm 0.23$  (mean  $\pm$  standard deviation).

As explained above, a daily Thornthwaite-Mather procedure for calculating AET and ultimately recharge (GW in Eqn. 2) from the soil moisture balance was used estimate the amount of N leached to groundwater. The commonly used FAO dual crop coefficient method (Allen et al., 1986) yields a nearly identical value of AET—515.1 mm—as compared to the soil moisture balance—514.2 mm. Both methods utilize the Penman-Monteith estimate of PET, widely recognized as the most physically correct PET model (e.g., Shaw and Riha, 2011; Sumner and Jacobs, 2005). Rearranging Eqn. 2, we estimate recharge as the difference between inputs (precipitation) and outputs (stream discharge and AET) over the one-year study period (see Table 4.2). The average NO<sub>3</sub>-N concentration of leached groundwater was estimated to be 6.2 mg L<sup>-1</sup> based on monthly sampling from a shallow groundwater well (Figures 4.1 and 4.3). This value is consistent with recent sampling of groundwater monitoring wells at the T&R Center (e.g., wells 50 & 98 from 2002-2004, range 2.5-13.4 mg L<sup>-1</sup>, mean 6.4 mg L<sup>-1</sup>, n=24, Geohring, unpublished data; well 98 from 1992-1994, mean 6.9 mg L<sup>-1</sup>, n=64, Wang et al., 1999). The NO<sub>3</sub>-N load to groundwater (leached) was estimated as the product of recharge (Q<sub>gw</sub>) and NO<sub>3</sub>-N concentration (C<sub>gw</sub>), and totaled 29128 kg. Taken over the entire agricultural landscape, this amounts to a loss of 28 kg N ha<sup>-1</sup> yr<sup>-1</sup>, within the range of NO<sub>3</sub>-N leaching losses (14.5-34.9 kg N ha<sup>-1</sup> yr<sup>-1</sup>) measured in field plots of loamy sand soils seeded to corn following alfalfa plowdown and fertilized at a rate of 22-134 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Sogbedji et al., 2000).

## DENITRIFICATION

Our results are in agreement with previous studies that have quantified denitrification at the agroecosystem landscape- or watershed-scale. The average landscape denitrification rate ( $45 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ), as estimated using the whole-farm N balance, is similar to the value estimated by van Breemen et al. (2002) also using a N mass balance approach for agricultural lands in the NE US ( $55 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ). Their analysis looked at 16 watersheds of varying size and mixed agriculture-forest-urban landuse, with N budget terms estimated from (coarser) county-level data, possibly leading to an overestimation of denitrification; across all watersheds, the percent of N inputs unaccounted for in agricultural land (which they attributed to denitrification) ranged from 34-63% and averaged 49%. Other N mass balance studies supplemented with field measures of denitrification have found results comparable to ours. For example, Gentry et al. (2009) used measurements of in-stream and shallow groundwater denitrification in conjunction with a N mass balance to estimate 'field denitrification' in a midwest US agricultural watershed under corn and soybean production. They estimated field and total denitrification to be 27 and  $34 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ , respectively; shallow groundwater denitrification was estimated to be  $6 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ . The authors note that their estimate of shallow groundwater denitrification represents a minimum value, and thus both it and total denitrification are likely underestimated. Also, they speculate that extensive tile drainage decreases the potential for shallow groundwater denitrification. Indeed, our estimate of field denitrification (i.e., total minus shallow saturated zone) is similar at  $26 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ , while shallow saturated zone denitrification is higher at  $19 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  on a total landscape area basis.

Oehler et al. (2009) simulated a watershed-scale denitrification flux of  $47 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  from a small dairy farming catchment in NW France under maize and winter wheat production using the agro-hydrologic TNT2 model, calibrated with field data including *in situ* measurements of

denitrification. Soils in the catchment were organized in two domains: well-drained hillslopes and poorly-drained hydromorphic soils at the bottom of slopes where the water table is often near the soil surface. Denitrification flux from the 'riparian' domain (mean of  $93 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ) was greater than from the 'hillslope' (mean of  $35 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ); however, the areal extent of the hillslope domain was much larger, resulting in a greater contribution to whole-catchment denitrification. On the whole, denitrification in the riparian domain accounted for 40% of the total denitrification in only 20% of the watershed area. In another modeling study, Whelan and Gandolfi (2002) used a formulation the TOPMODEL hydrologic model coupled with a simple process-based denitrification model to estimate spatial patterns of denitrification from a small mixed landuse catchment in the United Kingdom under 'nitrate non-limiting' conditions (similar to what might be expected in an agriculturally dominated landscape). They predicted average whole-catchment denitrification fluxes of  $33\text{-}49 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  for differing soil organic carbon scenarios, with more than half of this occurring in the saturated zone. More importantly, the landscape pattern of denitrification that emerged from their study was nearly identical to that shown in Figure 4.6, with higher fluxes occurring near streams and in hillslope depressions extending to the streams.

Our results indicate that at the landscape- or watershed-scale, the traditional classification of riparian zones as hotspots for denitrification can be expanded to include outlying areas which are prone to saturate for any length of time or where water tables remain shallow for extended periods. Current state recommendations for minimum riparian buffer widths range from 15.5 to 24.2 m (Mayer et al., 2005). We created a 25 m riparian buffer in ArcGIS around stream and wetland areas within or along the farm boundary, and estimated the denitrification flux from the shallow saturated zone using the method described above (Anderson et al., YEAR) The estimated denitrification flux from our 'riparian buffer' is  $63 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ , but this occurs in such a small portion of the landscape—60 ha, or about 6%—that the mass flux is only 3749 kg N

yr<sup>-1</sup>, or just 18% of the shallow saturated zone ‘hotspot’ denitrification (and only 8% of total landscape denitrification), suggesting that a STI-based approach may allow for better depiction of landscape hotspots than a simple delineation of riparian zones.

## CONCLUSION

Spatiotemporal characterization and quantification of potential denitrification hotspots are essential to land managers and policymakers tasked with developing and implementing better strategies for reducing nonpoint source N pollution to receiving water bodies. The STI-based approach is a simple and rapid tool to improve our depiction of hotspots in the landscape, and can be readily incorporated into geospatial approaches for assessing denitrification sinks at the landscape- and watershed-scale (e.g., Kellogg et al., 2010). We used a STI-denitrification model to distribute denitrification fluxes from the shallow saturated zone across an agricultural landscape and assess its contribution to whole-farm N sources and sinks. We found that denitrification in the shallow saturated zone accounted for 44% of the unresolved whole-farm N balance, confirming that a large portion of landscape-denitrification occurs in upland hotspots, relative to riparian zones, where water tables are shallow and dynamic, experiencing event- or seasonal-scale fluctuations that lead to periodically saturated surface soils. Spatiotemporal characterization of these hotspots is among the first steps necessary to develop targeted management practices, particularly for agricultural landscapes. Future intra-hotspot research is needed to see if untapped potential exists, e.g., ‘hyperfunctioning’ via natural accumulation of carbon (e.g., through no till practices) or via engineered approaches (e.g., bioreactors, denitrification walls, induced flooding).

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## APPENDIX A

### DENITRIFICATION RATE CALCULATION

#### <sup>15</sup>N<sub>2</sub> CALCULATIONS

sample N<sub>2</sub> (μmol) = μmol N<sub>2</sub> (from analysis results; UC Davis Stable Isotope Facility)

$$\text{concentration N}_2 (\mu\text{mol L}^{-1}) = \frac{\text{sample N}_2 (\mu\text{mol})}{\text{gas vial volume (mL)}} \times 1000$$

$$\text{sample atom \%} = \frac{R_{\text{sample}}}{R_{\text{sample}+1}} \times 100$$

$$R = \frac{^{15}\text{N}}{^{14}\text{N}}$$

$$R_{\text{sample}} = R_{\text{standard}} + \frac{R_{\text{standard}} \times \text{delta air}}{1000}$$

$$R_{\text{standard}} = 0.0036765$$

$$\text{delta air} = \text{delta } ^{15}\text{N} (\text{from analysis results; UC Davis Stable Isotope Facility}) = 1000 \times \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}}$$

$$\text{mass of N}_2 \text{ in vial } (\mu\text{mol}) = \text{concentration N}_2 (\mu\text{mol L}^{-1}) \times \frac{\text{gas vial volume (mL)}}{1000}$$

dosing sample atom % = average atom % of dosing samples (i.e., DOS1 and DOS2 vials)

dosing mass (μmol) = average mass of N<sub>2</sub> in vials (i.e., DOS1 and DOS2 vials)

mass of N<sub>2</sub> attributed to denitrification (μmol) =

$$\text{mass of N}_2 \text{ in vial} \times (\text{sample atom \%} - \text{standard atom \%}) - \frac{\text{dosing mass} \times (\text{dosing atom \%} - \text{standard atom \%})}{(\text{EN} - \text{standard atom \%})}$$

$$\text{standard atom \%} = 0.366303286$$

$$\text{EN} = \text{enrichment \%} = \frac{^{15}\text{N added as NO}_3\text{-N}}{^{14}\text{N} + ^{15}\text{N added as NO}_3\text{-N} + \text{ambient N as NO}_3\text{-N}}$$

concentration of N<sub>2</sub> attributed to denitrification (μmol L<sup>-1</sup>) =

$$\frac{\text{mass of N}_2 \text{ attributed to denitrification } (\mu\text{mol})}{\text{gas vial volume (mL)}} \times 1000$$

total mass of N<sub>2</sub> from denitrification (μmol) =

$$\text{concentration of N}_2 \text{ attributed to denitrification } (\mu\text{mol L}^{-1}) \times [\text{volume of headspace (L)} + \alpha \times \text{volume of liquid sample (L)}]$$

$$\alpha = \text{Bunsen coefficient for at } 5 \text{ }^\circ\text{C} (0.0211 \text{ for N}_2)$$

## <sup>15</sup>N<sub>2</sub>O CALCULATIONS

sample N<sub>2</sub>O (μL L<sup>-1</sup>) = ppm N<sub>2</sub>O (from analysis results; Cary Institute of Ecosystem Studies)

concentration N<sub>2</sub>O (μg L<sup>-1</sup>) = sample N<sub>2</sub>O (μL L<sup>-1</sup>) × ρN<sub>2</sub>O (g L<sup>-1</sup>)

$$\rho_{\text{N}_2\text{O}} = 1.948 \text{ g L}^{-1} \text{ at } 4 \text{ }^\circ\text{C}$$

atom % = atom % (from analysis results; UC Davis Stable Isotope Facility) =  $\frac{R_{\text{sample}}}{R_{\text{sample}+1}} \times 100$

$$R = \frac{^{15}\text{N}}{^{14}\text{N}}$$

$$R_{\text{sample}} = R_{\text{standard}} + \frac{R_{\text{standard}} \times \text{delta air}}{1000}$$

$$R_{\text{standard}} = 0.0036765$$

$$\text{delta air} = \text{delta } ^{15}\text{N} \text{ (from analysis results; UC Davis Stable Isotope Facility)} = 1000 \times \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}}$$

mass of N<sub>2</sub>O in vial (μg) = concentration N<sub>2</sub>O (μg L<sup>-1</sup>) ×  $\frac{\text{gas vial volume (mL)}}{1000}$

mass of N<sub>2</sub>O-N in vial (μmol) =  $\frac{\text{mass of N}_2\text{O in vial (}\mu\text{g)}}{44 \text{ g mol}^{-1}}$

dosing atom % = average atom % of dosing samples (i.e., DOS1 and DOS2 vials)

dosing mass (μmol) = average mass of N<sub>2</sub>O-N in vials (i.e., DOS1 and DOS2 vials)

mass of N<sub>2</sub>O-N attributed to denitrification (μmol) =

$$\frac{(\text{mass of N}_2\text{O-N in vial} \times \text{atom \%}) - (\text{dosing mass} \times \text{dosing atom \%})}{(\text{EN} - \text{standard atom \%})}$$

$$\text{standard atom \%} = 0.366303286$$

$$\text{EN} = \text{enrichment \%} = \frac{^{15}\text{N added as NO}_3\text{-N}}{^{14}\text{N} + ^{15}\text{N added as NO}_3\text{-N} + \text{ambient N as NO}_3\text{-N}}$$

concentration of N<sub>2</sub>O-N attributed to denitrification (μmol L<sup>-1</sup>) =

$$\frac{\text{mass of N}_2\text{O-N attributed to denitrification (}\mu\text{mol)}}{\text{gas vial volume (mL)}} \times 1000$$

total mass of N<sub>2</sub>O-N from denitrification (μmol) =

concentration of N<sub>2</sub>O-N attributed to denitrification (μmol L<sup>-1</sup>) × [volume of headspace (L) + α × volume of liquid sample (L)]

α = Bunsen coefficient for at 5 °C (1.06 for N<sub>2</sub>O)

## DENITRIFICATION RATES BASED ON SF<sub>6</sub> RECOVERY

mass N<sub>2</sub> (μmol) = average of total mass of N<sub>2</sub> from denitrification (μmol) from three samples with highest SF<sub>6</sub> C/C<sub>0</sub>

mass N<sub>2</sub>O-N (μmol) = average of total mass of N<sub>2</sub>O-N from denitrification (μmol) from three samples with highest SF<sub>6</sub> C/ C<sub>0</sub>

SF<sub>6</sub> C = concentration of SF<sub>6</sub> in sample

SF<sub>6</sub> C<sub>0</sub> = average concentration of SF<sub>6</sub> in dosing samples (i.e., DOS1 and DOS2 vials)

mass N<sub>2</sub> (μg) = mass N<sub>2</sub> (μmol) × [28(1-EN) + 30(EN)]

mass N<sub>2</sub>O-N (μg) = mass N<sub>2</sub>O-N (μmol) × [28(1-EN) + 30(EN)]

$$\text{EN} = \text{enrichment} = \frac{15N \text{ added as } NO_3-N}{14N + 15N \text{ added as } NO_3-N + \text{ambient } N \text{ as } NO_3-N}$$

Denitrification rate (μg N kg soil<sup>-1</sup> d<sup>-1</sup>) =

$$\frac{\text{mass } N_2 (\mu\text{g}) + \text{mass } N_2O-N (\mu\text{g})}{\text{volume of sample (L)}} \times \frac{1\text{L of water}}{\text{mass of soil holding 1L of water (kg)}} \times \text{incubation time (hrs)} \times 24$$

$$\text{mass of soil holding 1L of water (kg)} = \frac{\text{bulk density (g cm}^{-3}\text{)}}{\text{porosity}}$$

$$\text{porosity} = 1 - \frac{\text{bulk density (g cm}^{-3}\text{)}}{\text{particle density (g cm}^{-3}\text{)}}$$

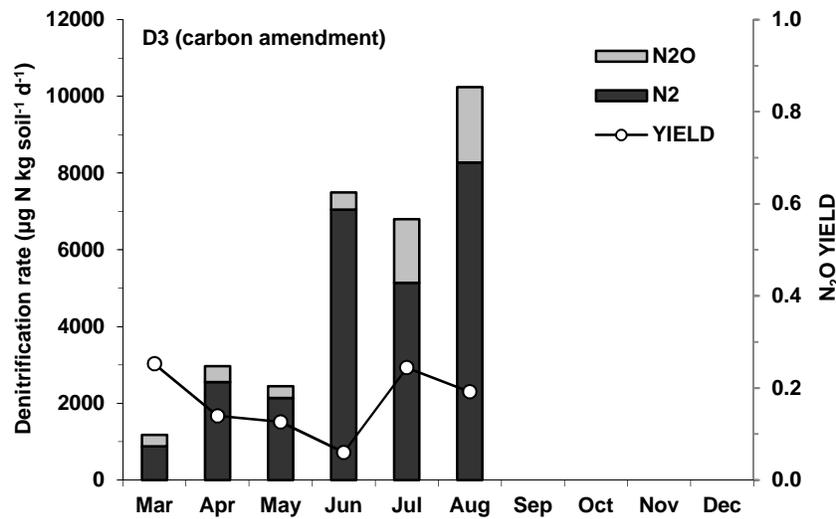
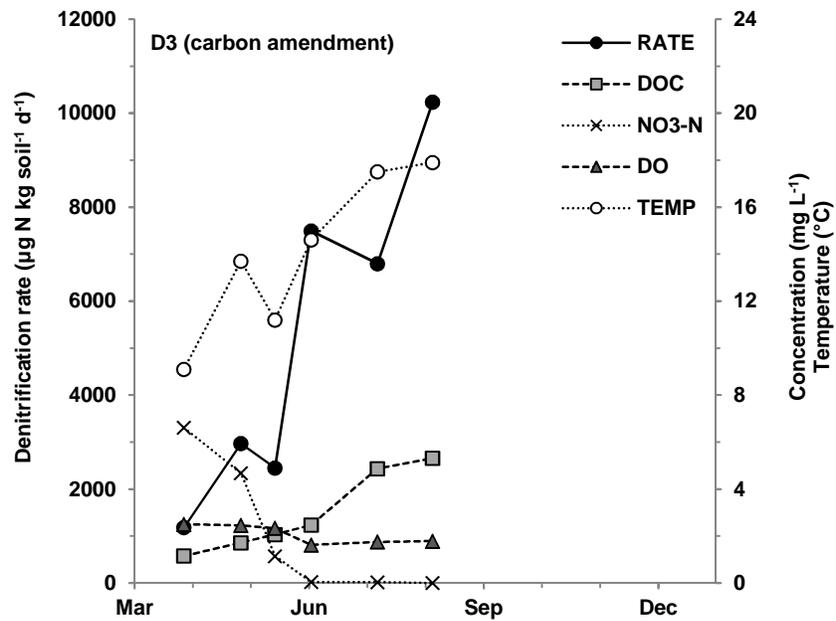
## APPENDIX B

### CARBON AMENDED PUSH-PULLS

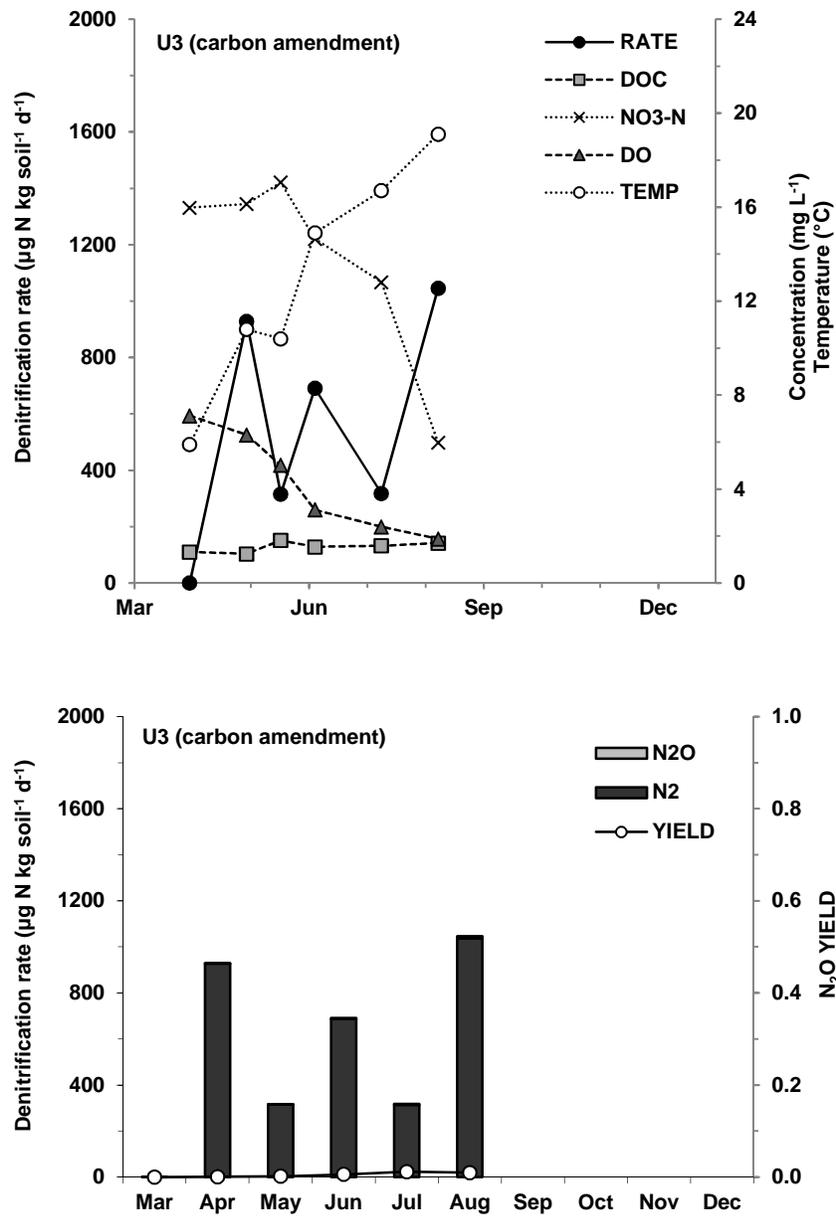
Carbon (C) amended push-pulls were performed in both the upstream and downstream riparian zones, mini-piezometer locations U3 and D3, respectively. 128 mg C L<sup>-1</sup> as sodium acetate was added to the dosing solution as a source of labile C to raise the C:N ratio to approximately 4:1, the optimal ratio for denitrification (Payne, 1981). All other push-pull protocols (i.e., methodology and calculations) followed the description given in Chapter 2. Monthly denitrification rates are plotted with ambient groundwater conditions, and further broken down into N<sub>2</sub> and N<sub>2</sub>O components for each C-amended push-pull (Figures B.1 and B.2).

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**Figure B.1.** Carbon amended push-pulls in D3. *In situ* denitrification rates (solid circle) measured monthly and plotted with selected ambient groundwater DOC (square), NO<sub>3</sub>-N (-x-), DO (triangle), and temperature (open circle). Rates are partitioned into mean N<sub>2</sub> and N<sub>2</sub>O production, and plotted with N<sub>2</sub>O yield [N<sub>2</sub>O/(N<sub>2</sub>+N<sub>2</sub>O)].



**Figure B.2.** Carbon amended push-pulls in U3. *In situ* denitrification rates (solid circle) measured monthly and plotted with selected ambient groundwater DOC (square), NO<sub>3</sub>-N (-x-), DO (triangle), and temperature (open circle). Rates are partitioned into mean N<sub>2</sub> and N<sub>2</sub>O production, and plotted with N<sub>2</sub>O yield [ $\text{N}_2\text{O}/(\text{N}_2+\text{N}_2\text{O})$ ].

## APPENDIX C

### SOIL PROPERTIES AND CARBON PROFILES

#### **SOIL PROPERTIES**

Two soil samples were taken from each mini-piezometer location for bulk density (BD) and particle density (PD) determination, as well as analysis for organic matter (OM), nitrogen (N), and carbon (C) content (Table C.1). Soil samples were taken in mid- to late-summer when water tables were below 50 cm. Soil pits were dug down to 50 cm, and intact cores extracted by hand using a 7.2-cm diameter and 6.8-cm height soil ring/cylinder. Soil samples for BD and PD determination were oven dried at 105 °C for 24 hours. Soil samples for OM, N, and C analysis were sent to the Cornell Nutrient Analysis Lab (CNAL).

Bulk density was calculated as the dry mass of soil divided by volume of soil extracted (i.e., the volume of the cylinder). Particle density was determined using a modified pycnometer method. Approximately 10 g of dry soil was weighed out and poured into an empty, pre-weighed 100-mL volumetric flask. After recording the initial weight, approximately 50 mL of distilled water was added to the flask, and the soil-water mixture brought to a gentle boil for 10 minutes. The flask was removed from the heat source and allowed to cool, capped, and let sit for 24 hours. The cap was removed and distilled water added to bring the total volume to 100 mL. At this point the total soil-water mixture plus flask was weighed (and total volume of water added known). Particle density was calculated as dry mass of soil divided by volume of that soil, where the volume is determined by displacement, i.e., the volume of water required to bring the entire soil-water mixture up to 100 mL.

A complete description of CNAL analyses for OM, N, and C can be found at <http://cna1.cals.cornell.edu/analyses/index.html>. Briefly, N and C were determined using a NC 2100 soil analyzer (ThermoQuest Italia, Milan, Italy) and OM determined through loss on ignition (LOI) at 500 °C for two hours.

**Table C.1.** Soil properties at each mini-piezometer location (n=2 per)

	BD (g cm <sup>-3</sup> )	PD (g cm <sup>-3</sup> )	%OM	%N	%C
TS1	0.61	1.91	7.9	0.5	5.2
TS2	0.59	1.83	8.1	0.5	5.8
DRZ1	0.46	1.57	17.8	0.9	11.4
DRZ2	0.38	1.61	16.6	0.9	10.6
DRZ3	0.50	1.75	13.5	0.7	7.8
DRZ4	0.38	1.60	14.1	0.7	8.5
DRZ5	0.34	1.57	25.1	1.0	10.9
DRZ6	0.36	1.64	21.5	1.0	10.2
UTZ1	0.39	1.71	11.2	0.8	9.1
UTZ2	0.57	1.84	12.5	0.7	9.1
UTZ3	0.60	1.88	10.6	0.6	7.1
UTZ4	0.68	1.92	11.2	0.7	8.1
UTZ5	0.49	1.79	16.2	0.8	8.6
UTZ6	0.56	1.68	14.6	0.8	8.8

## SOIL CARBON PROFILES

Soil carbon (C) profiles were created for each site (Figure C.1). Soil pits were dug down to the water table (a depth of approximately one meter) in mid-summer, and soil samples taken every 10 cm using a hand trowel. Samples were oven dried at 105 °C for 24 hours. Organic matter (OM) content was determined through loss on ignition (LOI) at 550 °C for five hours. A conversion factor of 1.724 was used to convert OM to organic C (Nelson and Sommers, 1996):

$$\%OM = (\%LOI * 0.7) - 0.23$$

$$\%C = \%OM/1.724$$

An exponential decay function was used to extrapolate soil organic C at depth (e.g., Jobbagy and Jackson, 2000):

$$C(z) = C_0 e^{-kz}$$

where  $C(z)$  is soil organic C (%) at depth  $z$  (cm),  $C_0$  is the soil organic C content at the surface (%), and  $k$  a scaling constant ( $\text{cm}^{-1}$ ). The parameters  $C_0$  and  $k$  were determined via linear fit of log transformed values of  $C$  with depth  $z$ :

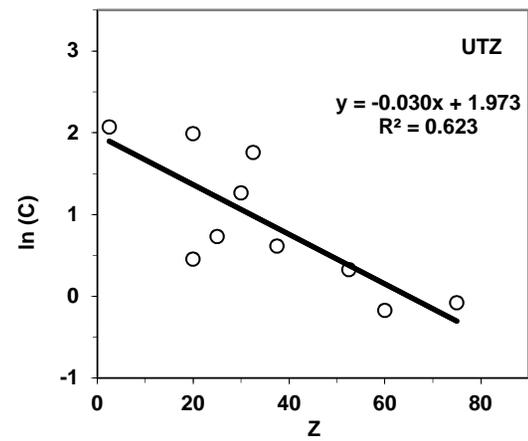
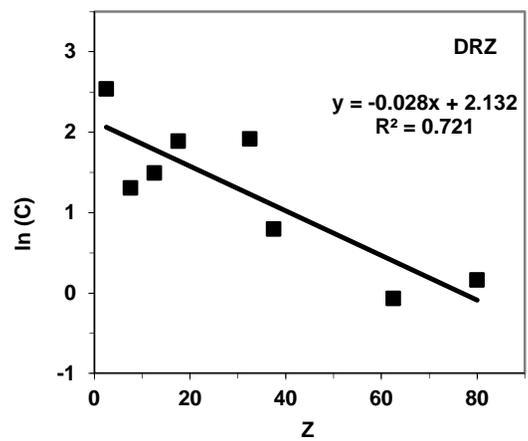
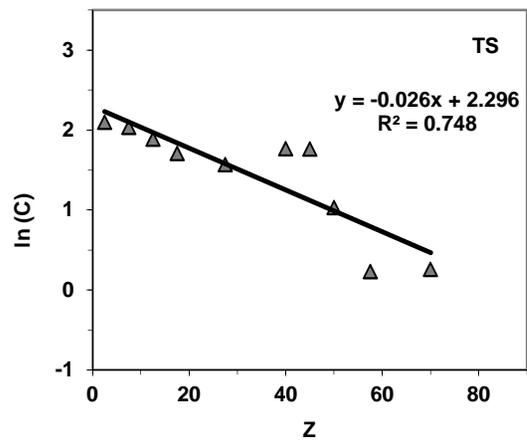
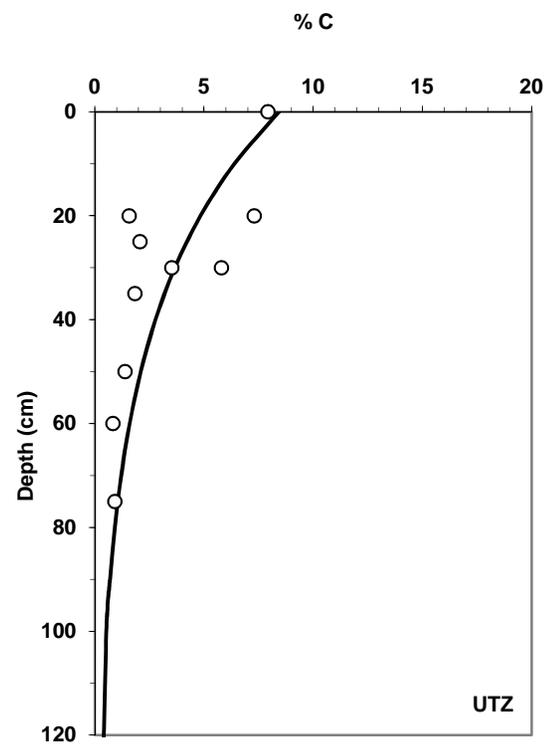
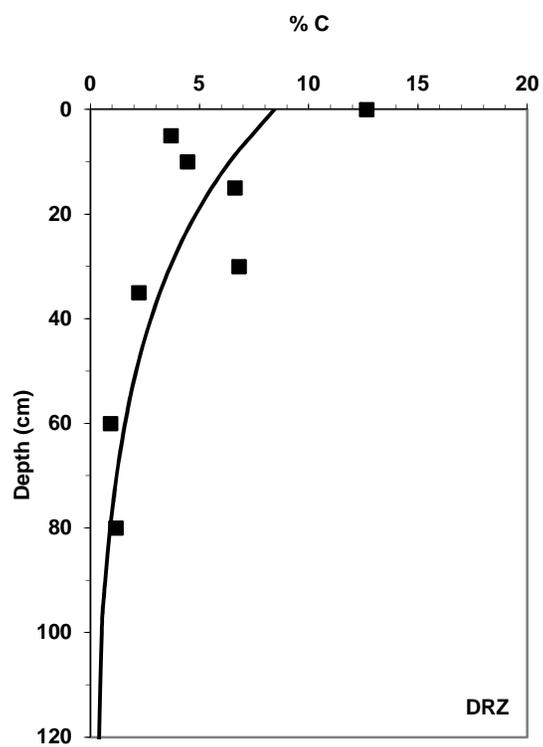
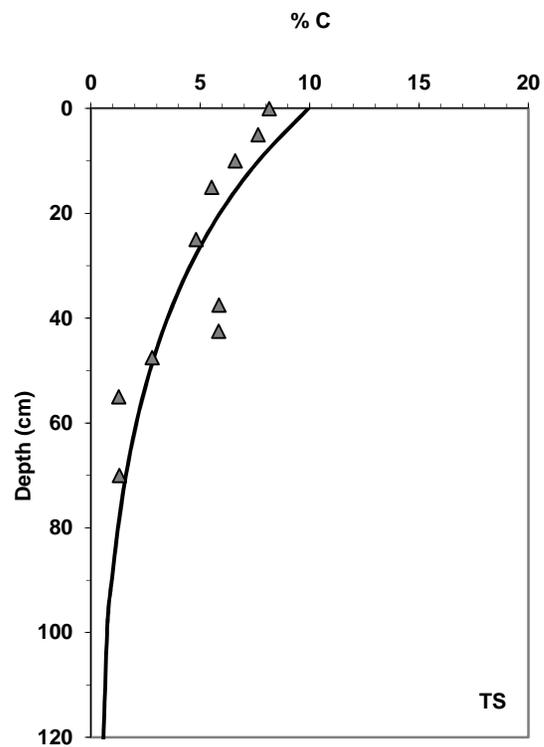
$$\ln(C) = -kz + \ln(C_0)$$

Burford and Bremner (1975) and Beauchamp et al. (1980) report a total organic C content of 0.5% as a critical value where the denitrification capacity of soil begins to significantly increase. The depth where this occurs at TS, UTZ, and DRZ is 115, 88, and 102 cm, respectively.

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**Figure C.1.** Soil carbon profiles for TS, DRZ, and UTZ. Profiles are shown with the linear fit of log transformed values of carbon with depth used to model the exponential decay function.

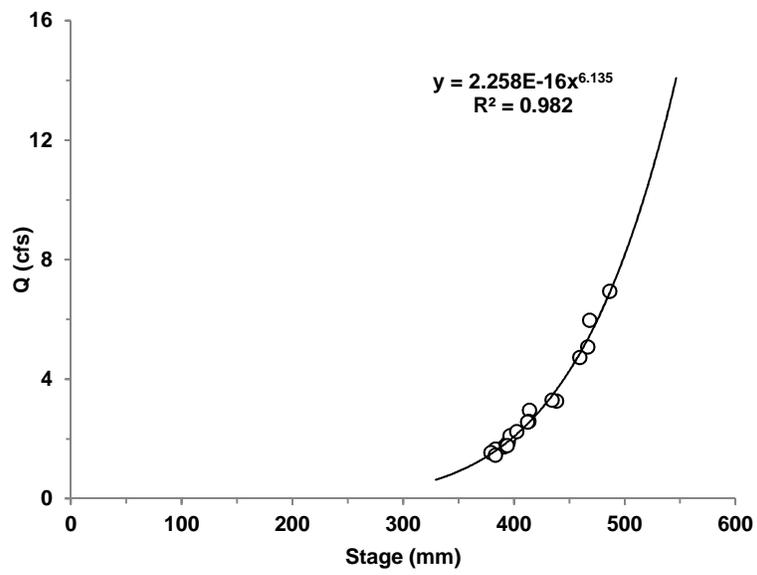


## APPENDIX D

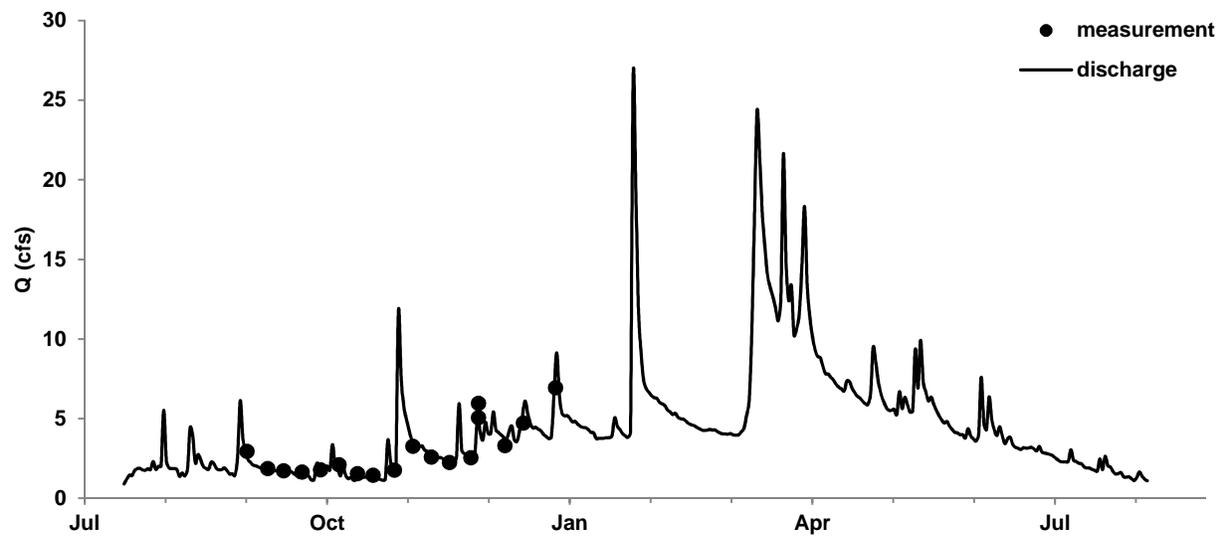
### STREAM DISCHARGE AND NITRATE CONCENTRATION

#### **STREAM DISCHARGE**

A streamgage was established at the southern catchment outlet. Stream stage (height of the water surface) was recorded using a capacitance water level logger housed in a slotted and screened PVC pipe within the stream channel. Periodic measurements of discharge were made immediately upstream of the streamgage using a Marsh-McBirney Flo-Mate 2000 electromagnetic velocity flow meter (Hach Company, Loveland, CO, USA). A stage-discharge relation was developed using 18 discharge measurements made over a 6-month period to capture a sufficient range of stages and streamflows (Figure D.1). The stage-discharge relation was applied to the stage record to obtain a continuous record of discharge (Figure D.2).



**Figure D.1.** Stage-discharge relation, modeled as a power function, for the southern valley catchment.



**Figure D.2.** Continuous record of discharge for the southern valley catchment from July 2009 to August 2010. Solid circles represent flow measurements used to develop the stage-discharge relation.

## CONCENTRATION-DISCHARGE REALTIONSHIP

A hyperbolic dilution model was used to characterize the apparent nitrate concentration-discharge relationship in the southern catchment stream (Johnson et al., 1969; Salmon et al., 2001):

$$C = \frac{\alpha_1}{1 + \alpha_2 Q} + \alpha_3$$

where  $C$  is the concentration of  $\text{NO}_3\text{-N}$  ( $\text{mg L}^{-1}$ ),  $Q$  is the discharge (cfs), and  $\alpha_1$ ,  $\alpha_2$ , and  $\alpha_3$  are constants for the stream at this specific monitoring location.  $\alpha_1$  corresponds to the concentration of  $\text{NO}_3\text{-N}$  in groundwater (or soil water) from which the stream originates,  $\alpha_2$  is related to the curvature of the function, and  $\alpha_3$  corresponds to the concentration of  $\text{NO}_3\text{-N}$  in water that mixes with the stream. To estimate  $\alpha_1$  and  $\alpha_3$ , we looked at the behavior of the function when  $Q$  approaches zero or infinity:

At the highest flow,  $Q \rightarrow \infty$ , therefore  $(\alpha_2 Q) \rightarrow \infty$ , and it follows that  $(\frac{\alpha_1}{1 + \alpha_2 Q}) \rightarrow 0$ , so  $C = \alpha_3$

At the lowest flow,  $Q \rightarrow 0$ , therefore  $(\alpha_2 Q) \rightarrow 0$ , and it follows that  $(\frac{\alpha_1}{1 + \alpha_2 Q}) \rightarrow \alpha_1$ , so  $C = \alpha_1 + \alpha_3$

Physical interpretation allows us to make some assumptions about  $\alpha_1$  and  $\alpha_3$ . At the highest possible flow—in a dilution controlled system—the concentration will never be less than zero, so  $\alpha_3 \geq 0$ . At the lowest possible flow, concentration of stream water should be equal to that of the source water, so  $\alpha_1 = C_0$ . As a first approximation, we assume that  $\alpha_1$  is equal to the mean  $\text{NO}_3\text{-N}$  concentration found in the shallow groundwater ( $6.239 \text{ mg L}^{-1}$ ; see Chapter 4), and that  $\alpha_3$  is equal to a concentration of zero. A value of 0.199 for  $\alpha_2$  was determined by fitting the model curve through the observed data and minimizing the sum of squared residuals.

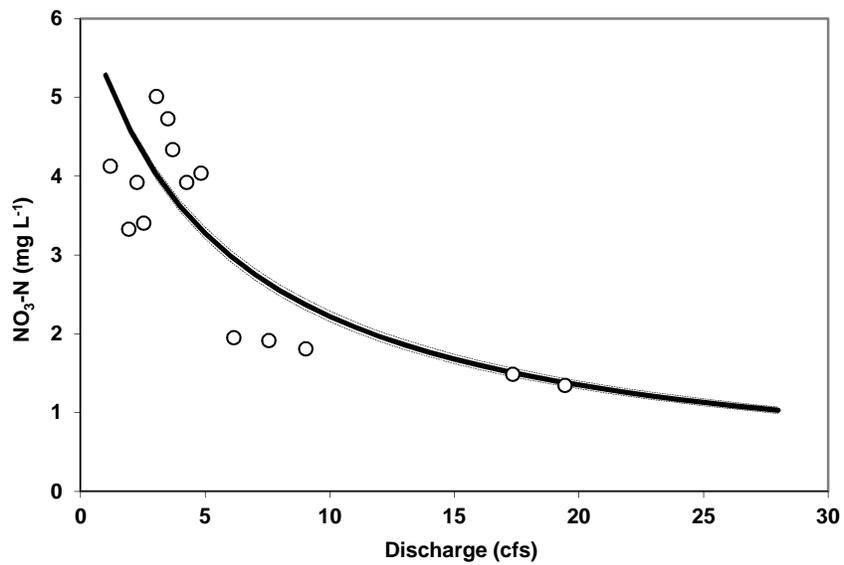
Graphical representation of the model is shown with observed nitrate-discharge measurements ( $R^2=0.62$ , Figure D.3):

$$C = \frac{6.24}{1 + 0.20Q}$$

#### REFERENCES

Johnson, N.M., G.E. Likens, F.H. Bormann, D.W. Fisher, and R.S. Pierce. 1969. A working model for the variation in stream water chemistry at Hubbard Brook Experimental Forest, New Hampshire. *Water Resour. Res.* 5:1353-1363.

Salmon, C.D., M.T. Walter, L.O. Hedin, and M.G. Brown. 2001. Hydrological controls on chemical export from an undisturbed old-growth Chilean forest. *J. Hydrol.* 253:69-80.



**Figure D.3.** Hyperbolic dilution model used to estimate in-stream nitrate concentration as a function of discharge. Open circles represent paired measurements used to develop the concentration-discharge relationship (solid line). Multiple cross validation was used to estimate uncertainty in the model (dashed lines).

## APPENDIX E

### LIST OF PUSH-PULL DATA

#### FIRST YEAR (All sites – Chapters 3 and 4)

DATE	WELL	RATE	ERR	N <sub>2</sub>	N <sub>2</sub> O	NO <sub>3</sub> -N REM	DOC	NO <sub>3</sub> -N	DO	TEMP
11/29/2007	TS1	77.6	47.6	76.5	1.1	0.07	3.90	0.58	6.05	3.8
4/19/2008	TS1	116.7	93.6	107.0	9.6	0.11	3.19	0.17	6.83	15.3
11/29/2007	TS2	0.4	0.1	0.0	0.4	0.00	3.70	0.20	8.22	4.2
4/19/2008	TS2	378.6	208.2	361.9	16.7	0.36	3.41	0.04	2.35	13.4
5/26/2008	TS2	171.6	53.2	141.0	30.6	0.16	4.55	0.14	2.53	13.6
6/24/2008	TS2	154.5	58.5	148.7	5.8	0.15	4.62	0.05	2.80	15.3
7/22/2008	TS2	2.7	0.2	0.0	2.7	0.00	5.73	0.13	3.10	18.1
11/13/2007	DRZ1	327.4	81.9	327.2	0.2	0.20	1.24	2.29		
4/12/2008	DRZ1	434.2	200.2	434.2	0.0	0.27	0.76	4.06	5.89	9.0
5/19/2008	DRZ1	29.6	26.6	0.0	29.6	0.02	0.72	4.07	5.75	9.6
6/17/2008	DRZ1	0.8	0.0	0.0	0.8	0.00	0.97	7.88	3.73	14.8
7/23/2008	DRZ1	25.1	2.8	0.0	25.1	0.02	0.96	13.98	2.92	16.7
8/20/2008	DRZ1	184.1	111.0	138.7	45.3	0.11	1.07	8.91	2.82	18.4
4/12/2008	DRZ2	418.2	266.2	417.6	0.5	0.26	0.61	7.39	3.37	8.7
5/19/2008	DRZ2	125.6	73.9	95.7	29.9	0.08	0.91	10.27	3.55	9.7
6/17/2008	DRZ2	1364.2	313.9	1021.8	342.4	0.84	2.16	0.94	4.70	15.8
1/9/2008	DRZ3	279.6	181.3	279.3	0.2	0.18	1.02	7.76	5.30	8.5
4/13/2008	DRZ3	289.5	111.7	288.7	0.8	0.19	0.92	7.03	3.03	9.8
5/20/2008	DRZ3	795.2	108.8	742.4	52.8	0.52	1.41	4.37	3.27	12.0
6/18/2008	DRZ3	3495.6	253.7	2527.3	968.3	2.28	2.77	1.27	2.90	17.0
1/9/2008	DRZ4	60.7	29.6	58.1	2.7	0.04	2.33	1.47	4.75	7.3
4/13/2008	DRZ4	16.2	1.9	0.0	16.2	0.01	0.96	6.55	2.95	7.9
5/20/2008	DRZ4	779.0	80.7	542.8	236.2	0.51	1.84	0.41	2.25	11.1
6/18/2008	DRZ4	4227.2	150.1	4018.7	208.5	2.76	2.54	0.01	2.45	16.9
4/14/2008	DRZ5	336.9	117.1	332.1	4.8	0.15	1.21	5.42	2.52	10.3
5/21/2008	DRZ5	3599.1	210.7	2705.1	894.0	1.59	2.98	0.13	3.18	11.2
4/14/2008	DRZ6	86.9	84.3	0.0	86.9	0.04	1.25	5.22	3.08	9.7
5/21/2008	DRZ6	2325.0	175.5	1727.4	597.6	1.02	2.36	0.17	2.78	10.7
4/5/2008	UTZ1	40.4	40.2	0.0	40.4	0.03	1.07	6.00	5.75	9.1
5/27/2008	UTZ1	651.6	130.1	650.7	0.9	0.52	1.64	9.66	4.54	11.5
6/25/2008	UTZ1	192.4	116.5	161.3	31.1	0.15	1.63	4.28	2.78	18.0

7/24/2008	UTZ1	190.3	77.9	156.7	33.5	0.15	1.41	5.05	2.17	18.2
4/5/2008	UTZ2	112.7	49.4	112.7	0.0	0.09	0.89	9.35	5.92	7.9
5/27/2008	UTZ2	61.4	61.4	61.4	0.0	0.05	1.11	16.26	5.44	10.7
6/25/2008	UTZ2	518.7	108.8	513.2	5.5	0.42	1.12	9.53	2.98	17.7
7/24/2008	UTZ2	201.7	101.9	0.0	201.7	0.16	1.42	3.69	2.36	18.1
1/10/2008	UTZ3	294.2	157.4	294.2	0.0	0.27	0.79	5.11	6.74	8.1
4/6/2008	UTZ3	3.2	3.2	3.2	0.0	0.00	1.15	3.49	5.79	10.1
5/28/2008	UTZ3	3.0	0.4	0.0	3.0	0.00	1.37	10.18	4.67	13.1
6/26/2008	UTZ3	55.7	38.0	0.0	55.7	0.05	1.64	3.82	3.23	18.6
1/10/2008	UTZ4	122.6	68.9	122.6	0.0	0.11	0.68	15.30	7.76	7.3
4/6/2008	UTZ4	202.9	160.6	202.9	0.0	0.19	0.63	7.20	6.02	9.4
5/28/2008	UTZ4	84.7	38.9	84.5	0.2	0.08	1.25	19.01	5.15	12.8
6/26/2008	UTZ4	227.7	73.7	219.1	8.6	0.21	1.89	7.11	3.11	18.2
4/7/2008	UTZ5	375.0	119.8	374.7	0.3	0.25	0.98	17.16	5.68	8.3
5/29/2008	UTZ5	84.9	43.9	80.1	4.8	0.06	1.42	15.29	3.07	14.8
4/7/2008	UTZ6	0.0	0.0	0.0	0.0	0.00	0.74	17.45	6.36	7.6
5/29/2008	UTZ6	349.1	132.7	324.6	24.4	0.23	1.22	3.34	4.20	14.5

RATE = denitrification rate ( $\mu\text{g N kg soil}^{-1} \text{d}^{-1}$ )

ERR = standard error (same units as RATE)

$\text{N}_2$  =  $\text{N}_2$  component (same units as RATE)

$\text{N}_2\text{O}$  =  $\text{N}_2\text{O}$  component (same units as RATE)

$\text{NO}_3\text{-N REM}$  = denitrification rate expresses as nitrate removal rate ( $\text{mg N L}^{-1} \text{d}^{-1}$ )

DOC = dissolved organic carbon ( $\text{mg L}^{-1}$ )

$\text{NO}_3\text{-N}$  = nitrate-nitrogen ( $\text{mg N L}^{-1}$ )

DO = dissolved oxygen ( $\text{mg L}^{-1}$ )

TEMP = temperature ( $^{\circ}\text{C}$ )

PH = pH

## SECOND YEAR (Riparian sites – Chapter 2)

DATE	WELL	RATE	ERR	N <sub>2</sub>	N <sub>2</sub> O	NO <sub>3</sub> -N REM	DOC	NO <sub>3</sub> -N	DO	TEMP	PH
3/26/2009	DRZ1	55.6	55.5	55.5	0.0	0.03	0.67	7.83	6.64	7.3	
4/25/2009	DRZ1	617.0	84.0	617.0	0.0	0.38	0.67	8.10	6.39	13.3	7.17
5/13/2009	DRZ1	52.6	36.9	52.6	0.0	0.03	0.95	7.86	4.74	12.1	7.02
6/1/2009	DRZ1	0.0	0.0	0.0	0.0	0.00	1.19	7.83	3.03	11.4	6.87
7/6/2009	DRZ1	234.0	122.5	233.8	0.2	0.14	0.98	8.55	2.77	15.1	7.08
8/4/2009	DRZ1	43.0	42.2	42.3	0.7	0.03	0.90	10.42	2.59	17.4	6.96
9/1/2009	DRZ1	2.0	0.0	0.0	2.0	0.00	0.87	11.23	2.27	17.6	7.05
10/19/2009	DRZ1	2.3	0.2	0.0	2.3	0.00	1.38	4.15	2.58	10.9	7.02
11/17/2009	DRZ1	48.8	41.3	47.9	1.0	0.03	1.42	7.34	3.48	9.8	6.98
12/27/2009	DRZ1	27.7	25.4	26.1	1.6	0.02	1.40	9.72	4.71	7.0	6.99
3/26/2009	DRZ2	33.3	33.3	33.3	0.0	0.02	0.76	8.34	6.16	6.7	
4/25/2009	DRZ2	426.6	216.4	421.9	4.7	0.26	1.22	7.83	4.96	13.5	6.70
5/13/2009	DRZ2	559.9	417.1	558.2	1.7	0.34	1.49	6.80	4.31	12.4	7.05
6/1/2009	DRZ2	281.2	105.6	274.4	6.8	0.17	1.07	6.08	2.12	12.5	6.97
7/6/2009	DRZ2	889.3	380.7	749.8	139.4	0.54	1.10	6.19	1.88	16.6	6.82
8/4/2009	DRZ2	1536.7	142.4	918.6	618.2	0.94	1.78	4.96	1.87	18.8	6.88
9/1/2009	DRZ2	503.8	76.4	67.3	436.4	0.31	1.96	6.91	2.02	17.2	6.80
11/17/2009	DRZ2	108.5	86.1	85.8	22.7	0.07	1.85	9.45	3.52	8.6	6.95
12/27/2009	DRZ2	99.7	97.3	96.4	3.3	0.06	1.64	10.25	4.71	5.4	7.01
3/27/2009	DRZ3	324.6	77.1	323.4	1.2	0.21	1.44	3.42	3.16	9.5	
4/26/2009	DRZ3	955.5	66.2	950.7	4.9	0.62	1.18	1.64	2.04	15.5	6.53
5/14/2009	DRZ3	1184.6	173.5	1130.3	54.3	0.77	2.08	1.31	2.43	12.4	6.78
6/2/2009	DRZ3	3318.4	411.9	2795.8	522.6	2.17	3.62	1.27	1.76	15.4	6.82
7/7/2009	DRZ3	4176.5	1084.3	2079.8	2096.7	2.73	6.14	0.97	1.68	16.8	6.83
8/5/2009	DRZ3	3538.1	669.1	2350.5	1187.6	2.31	6.41	1.31	2.50	19.4	6.82
3/27/2009	DRZ4	1178.3	143.2	881.0	297.3	0.77	1.15	6.61	2.50	9.1	
4/26/2009	DRZ4	2967.4	271.5	2553.5	413.9	1.94	1.72	4.68	2.45	13.7	6.89
5/14/2009	DRZ4	2449.3	235.9	2139.8	309.5	1.60	2.07	1.15	2.33	11.2	6.87
6/2/2009	DRZ4	7493.9	243.5	7045.3	448.5	4.89	2.47	0.04	1.62	14.6	6.94
7/7/2009	DRZ4	6797.8	776.4	5138.5	1659.4	4.44	4.87	0.04	1.75	17.5	6.77
8/5/2009	DRZ4	10238.3	273.6	8276.7	1961.6	6.69	5.31	0.01	1.78	17.9	6.70
3/29/2009	UTZ1	162.7	127.7	162.5	0.1	0.13	0.85	12.65	5.61	8.9	
4/28/2009	UTZ1	435.4	141.9	435.3	0.2	0.35	1.19	14.00	5.17	12.1	7.09
5/16/2009	UTZ1	379.8	20.4	379.5	0.3	0.31	1.37	11.94	3.70	12.5	7.09
6/3/2009	UTZ1	170.1	169.7	169.7	0.5	0.14	1.65	8.36	2.74	12.8	7.05
7/8/2009	UTZ1	817.0	234.7	810.7	6.3	0.66	1.61	6.90	2.27	14.8	7.03
8/7/2009	UTZ1	214.0	43.2	188.9	25.1	0.17	2.04	2.96	1.68	17.5	6.90

9/2/2009	UTZ1	76.3	8.0	0.0	76.3	0.06	1.79	1.01	1.89	17.5	6.91
11/18/2009	UTZ1	130.4	68.7	129.1	1.3	0.10	2.17	0.98	2.41	9.1	7.06
3/29/2009	UTZ2	0.0	0.0	0.0	0.0	0.00	0.63	13.20	8.52	7.8	
4/28/2009	UTZ2	0.0	0.0	0.0	0.0	0.00	1.00	12.78	7.86	11.8	6.91
5/16/2009	UTZ2	0.0	0.0	0.0	0.0	0.00	1.33	14.98	6.30	12.1	6.86
6/3/2009	UTZ2	1124.0	243.1	1124.0	0.0	0.90	1.59	15.87	4.90	13.1	6.88
7/8/2009	UTZ2	1018.3	122.8	1018.1	0.2	0.82	1.27	15.36	3.40	15.2	6.95
8/7/2009	UTZ2	886.1	236.8	872.8	13.3	0.71	1.36	6.74	1.17	17.3	6.94
9/2/2009	UTZ2	146.9	65.8	123.8	23.1	0.12	1.46	1.02	1.88	18.1	6.86
11/18/2009	UTZ2	132.8	67.0	132.6	0.2	0.11	1.30	2.90	2.77	8.2	7.01
12/15/2009	UTZ2	107.3	60.3	107.3	0.0	0.09	0.79	5.11	4.75	7.3	
3/30/2009	UTZ3	544.6	333.4	544.5	0.1	0.51	1.32	13.04	4.79	6.4	
4/29/2009	UTZ3	678.3	504.5	677.4	0.9	0.63	1.04	13.53	4.74	10.8	6.89
5/17/2009	UTZ3	1097.5	238.1	1078.7	18.8	1.02	1.60	13.32	4.12	10.7	7.05
6/4/2009	UTZ3	642.9	113.0	606.8	36.1	0.60	1.85	10.70	2.85	14.5	6.96
7/9/2009	UTZ3	321.3	120.2	118.9	202.3	0.30	2.23	6.90	2.38	17.3	7.01
8/8/2009	UTZ3	274.4	236.3	240.3	34.1	0.25	1.52	5.43	3.60	21.0	7.08
3/30/2009	UTZ4	0.1	0.0	0.0	0.1	0.00	1.24	16.14	6.31	10.8	
4/29/2009	UTZ4	928.0	472.0	927.6	0.4	0.86	1.32	15.98	7.12	5.9	7.10
5/17/2009	UTZ4	315.8	189.1	315.3	0.5	0.29	1.82	17.05	5.01	10.4	6.91
6/4/2009	UTZ4	690.8	227.7	687.0	3.8	0.64	1.54	14.65	3.12	14.9	6.84
7/9/2009	UTZ4	317.9	189.5	314.2	3.7	0.30	1.59	12.81	2.39	16.7	6.93
8/8/2009	UTZ4	1045.7	601.3	1035.9	9.8	0.97	1.70	5.98	1.88	19.1	6.86

Note: In second year, DRZ1 and UTZ1 were DO-amended push-pulls, with dosing DO adjusted to  $\sim 2 \text{ mg L}^{-1}$ ; DRZ4 and UTZ4 were C-amended push-pulls, with dosing DOC adjusted to  $\sim 128 \text{ mg L}^{-1}$ . In chapter 2, U1 and U2 correspond to UTZ2 and UTZ3 listed above, respectively. Likewise, D1 and D2 correspond to DRZ2 and DRZ3, respectively.