AN ABSTRACT OF THE DISSERTATION OF

Jay P. Zarnetske for the degree of Doctor of Philosophy in Water Resources Science presented on September 7, 2011.

Title: Hydrological and Biogeochemical Dynamics of Nitrate Production and Removal at the Stream – Ground Water Interface.

Abstract approved:

Roy D. Haggerty

The feedbacks between hydrology and biogeochemical cycling of nitrogen (N) are of critical importance to global bioavailable N budgets. Human activities are dramatically increasing the amount of bioavailable N in the biosphere, which is causing increasingly frequent and severe impacts on ecosystems and human welfare. Streams are important features in the landscape for N cycling, because they integrate many sources of terrestrially derived N and control export to downgradient systems via internal source and sink processes. N transformations in stream ecosystems are typically very complex due to spatiotemporal variability in the factors controlling N biogeochemistry. Thus, it is difficult to predict if a particular stream system will function as a net source or sink of bioavailable N. A key location for N transformations in stream ecosystems is the hyporheic zone, where stream and ground waters mix. The hyporheic zone can be a source of bioavailable N via nitrification or a sink via denitrification. These N transformations are regulated by the physical and biogeochemical conditions of hyporheic zones. Natural heterogeneity in streams leads to unique combinations of both the physical and biogeochemical conditions which in turn result in unique N source and sink conditions.

This dissertation investigates the relationships between physical and biogeochemical controls and the resulting fate of bioavailable N in hyporheic zones. The key physical factor investigated is the supply rate of solutes which is a function of
transport processes - advection and dispersion, and transport conditions - hydraulic conductivity and flowpath length. Different physical conditions result in different characteristic residence times of water and solutes in hyporheic zones. The key biogeochemical factors investigated are the dynamics of oxygen (O\textsubscript{2}), labile dissolved organic carbon (DOC), and inorganic bioavailable N (NH\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3}\textsuperscript{−}). This dissertation uses \textsuperscript{15}N isotope experiments, numerical modeling of coupled transport of the bioavailable N species, O\textsubscript{2} and DOC, and a suite of geophysical measurements to identify the key linkages between hydrological and biogeochemical controls on N transformations in hyporheic zones. Specifically, it was determined that the conditions governing the fate of hyporheic N are both the physical transport and reaction kinetics – the residence time of water and the O\textsubscript{2} uptake rate. An important scaling relationship is developed by relating the characteristic timescales of residence time and O\textsubscript{2} uptake. The resulting dimensionless relationship, the Damköhler number for O\textsubscript{2}, is useful for scaling different streams hyporheic zones and their role on stream N source – sink dynamics. More generally, these investigations demonstrate that careful consideration and quantification of hydrological processes can greatly inform the investigation of aquatic biogeochemical dynamics and lead to the development of process-based knowledge. In turn, this process-based knowledge will facilitate more robust approaches to quantifying and predicting biogeochemical cycles and budgets.
Hydrological and Biogeochemical Dynamics of Nitrate Production and Removal at the Stream – Ground Water Interface

by
Jay P. Zarnetske

A DISSERTATION
submitted to
Oregon State University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy

Presented September 7, 2011
Commencement June 2012

APPROVED:

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Dean of the Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

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Jay P. Zarnetske, Author
ACKNOWLEDGMENTS

This dissertation and my PhD were primarily funded by the U. S. National Science Foundation through research grant no. EAR-0409534 and education grant no. DGE-0333257. Completion of this dissertation would not have been possible without the support of the Oregon State University Ecosystem Informatics and Water Resources Graduate Programs. I especially thank the directors of both programs, Julia Jones and Mary Santelmann, for supporting my science, my training, and career-development. I also thank the Klopfenstein Farm for generously granting access to the Drift Creek research sites. The Klopfenstein’s willingness to share their land has greatly benefited my research, Oregon State University, and the science developed through my dissertation.

My deepest thanks go to my major advisor, Roy Haggerty. Roy has always showed me optimism when things went wrong, shared sound advice with me when I needed it most, and encouraged me to push my limits so I could explore new and challenge questions. I also whole-heartedly thank Steve Wondzell whose help has lead to some of the greatest discoveries in my ability to conduct and communicate science. Many thanks also to my committee members, Stan Gregory and Vrushali Bokil, who have added breadth and strength to my training and my science, especially a deep appreciation for interdisciplinary approaches to ecosystem studies. I thank my graduate council representative, Dr. Keith Levien, for his generous time and service. I also want to thank key research collaborators who have shared their experience, knowledge, and research with me, namely, Michael Gooseff, Michelle Baker, and Breck Bowden for they introduced me to stream hydrology and biogeochemistry - subjects I find endlessly fascinating and challenging to study. Past and present lab mates, especially Alba Argerich and Ricardo González-Pinzón, you have always provided me with a laugh, a fact, or a cup of coffee when I needed it.

I want to thank my research “partners,” hyporheic zone and nitrogen cycling, for continually finding ways to challenge and inspire me. You were not easy to get to
know, and at times I still do not understand you, but I expect we (‘‘HZ-N-JZ’’) have a long, fruitful future ahead of us.

Last, but certainly not least, I share my appreciation and love for my family and friends. Mom, dad, Kirsten, Scott, and Becky, I thank you for always supporting my decisions and providing help whenever I needed it. Thank you to my friends whom I have come to know and love while in Corvallis – your friendship and support is genuinely cherished. Phoebe and Kenai…words are not enough… thank you for your boundless love, support, enthusiasm, and patience for me. We did it!

I could not have completed my journey through graduate school without any of the people mentioned here. I have learned so much from each of you. You all have and continue to inspire me.
CONTRIBUTION OF AUTHORS

Chapter 2: Dr. Steven Wondzell assisted with the collection and interpretation of the data.

Chapter 3: Dr. Steven Wondzell and Dr. Michelle Baker assisted with the interpretation of the data.

Chapter 4: Dr. Steven Wondzell assisted with the interpretation of the data.

Chapter 5: Dr. Steven Wondzell assisted with the interpretation of the data. Dr. Vrushali Bokil provided input on the implementation of the numerical modeling. Ricardo González-Pinzón provided input on the uncertainty and sensitivity analysis of the numerical model.
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DEDICATION

This dissertation is dedicated to my parents, John and Diane Zarnetske.
Hydrological and Biogeochemical Dynamics of Nitrate Production and Removal at the Stream – Ground Water Interface
1 – General Introduction

1.1. Context of Dissertation

1.1.1. Bioavailable Nitrogen

Nitrogen (N) is an essential nutrient for ecosystems. The biologically-available forms of N are typically ammonium, nitrous oxides, and organic nitrogen, and these bioavailable forms of N are often a limiting nutrient in many terrestrial and aquatic ecosystems [National Research Council, 2000]. Humans have dramatically altered the global bioavailable N budgets. Humans are now accountable for more than doubling the global bioavailable N created each year with humans producing >157 Tg yr\(^{-1}\) and all other natural processes only producing 125 Tg yr\(^{-1}\) [Galloway et al., 2004]. These anthropogenic increases in bioavailable N are primarily associated with the increased use of cultivated N-fixing leguminous plants, industrial produced fertilizers, and fossil fuels. The rate at which humans add bioavailable N to the global budget is also dramatic with rates 5 times greater than that of observed atmospheric CO\(_2\) increases over the last 50 years [Davidson and Seitzinger, 2006].

Human production and use of bioavailable N has played an important role in meeting the needs of global food and energy production, but there are major consequences for this anthropogenic alteration to the global N budget. Namely, human produced bioavailable N is rapidly accumulating in ecosystems across the planet – terrestrial, freshwater, and marine. The result is that excess bioavailable N is occurring in ecosystems previously limited by N. This excess bioavailable N causes a cascade of effects that ultimately lead to major disturbances to ecosystems and human welfare [Galloway et al., 2003]. For example, bioavailable N alters the global carbon cycle [Gruber and Galloway, 2008] which has consequences for global ecosystem productivity and climate change. In aquatic systems it leads to eutrophication events in many freshwater and marine environments that cause degraded water quality [Smith, 2003] leading to hypoxia and fishery deaths [Rabalais, 2002]. As the bioavailable N
continues to increase so do the frequency and intensity of these detrimental consequences, especially in aquatic ecosystems [Diaz and Rosenberg, 2008]. Streams and river networks are acutely sensitive and important to the dynamics of bioavailable N because they rapidly convey and concentrate excess N from terrestrial environments to downgradient aquatic, groundwater, and coastal marine environments. However, stream networks can also regulate these N inputs through biogeochemical processes, thereby mitigating the negative impacts to downgradient water bodies.

A key biogeochemical process that regulates the global bioavailable N budget is denitrification. Denitrification is the microbially-mediated process by which bioavailable nitrate (NO\textsubscript{3}\textsuperscript{-}) and nitrite (NO\textsubscript{2}\textsuperscript{-}) is reduced to an inert form of N (N\textsubscript{2}) that can return to the atmosphere. More specifically, denitrifying microbes use these nitrous oxides as terminal electron acceptors in anaerobic respiration, so denitrification is a process that predominately occurs in anoxic environments (Table 1.1).

Denitrification is very important to the biosphere because it represents the primary pathway to reducing excess global bioavailable N [Davidson and Seitzinger, 2006]. However, after nearly a century of research on denitrification, the role it plays at any spatial or temporal scale in the local, regional, or global N cycle is still not known. Estimates of denitrification are the least certain component the global N budget [Seitzinger et al., 2006], and therefore inhibit the ability to fully understand the local, regional, and global N cycle, including how excess N affects ecosystems and management practices. Consequently, there is a great need to focus research efforts on improving estimates of denitrification in the environment [Groffman et al., 2006; Boyer et al., 2006].

1.1.2. Stream – Groundwater Interactions and Bioavailable Nitrogen Cycling

Streams are important features in the landscape for the N cycling, because they integrate many sources of terrestrially derived N and control N export to downgradient systems via internal N source and sink processes (e.g., mineralization of organic forms of N and denitrification of NO\textsubscript{3}\textsuperscript{-}, respectively). As a result, there is a need to
determine the key factors controlling sources and sinks of reactive N in stream ecosystems. Unfortunately, N transformations in aquatic ecosystems are typically very complex and are coupled in both space and time. Thus, it is difficult to predict if a particular component of a stream system will function as a net source or sink of bioavailable N, and over which temporal and spatial scales it will function.

Research has established that headwater and mid-network streams are most effective at regulating downstream N exports [Alexander et al., 2000; Peterson et al., 2001]. These same headwater to mid-network streams are also where stream - ground water (hyporheic) flux is greatest relative to surface water flux [Anderson et al., 2005]. The hyporheic zone is the region adjacent to the stream channel where stream water and ground waters mix (Figure 1.1). Hyporheic zones are known to be key N transformation locations in streams [e.g., Duff and Triska, 1990, Holmes et al., 1994]. Hyporheic zones function this way because they possess strong hydrologic and biogeochemical gradients [Jones and Holmes, 1996; Baker et al., 2000]. These gradients lead to different redox conditions, which in turn control the conditions under which many biogeochemical reactions can occur [Hedin et al., 1998]. In particular, redox conditions control when and where the dominant NO₃⁻ source process - nitrification, and the dominant NO₃⁻ sink process - denitrification, can occur (Table 1.1). Nitrification represents the chemoautotrophic oxidation of NH₄ to NO₃⁻, where the NH₄ is the result of mineralization of dissolved organic forms of N. Unfortunately, hyporheic-N dynamics are not well enough established to identify when and where denitrification will be the dominant fate of NO₃⁻ versus when and where NO₃⁻ production via nitrification will dominate in a system.

The physical and biogeochemical conditions of hyporheic zones regulate N biogeochemistry. The physical factor is the supply rate of solutes which is a function of advection, dispersion, hydraulic conductivity, and flowpath length. Different physical conditions result in different residence times of water and solutes in hyporheic zones. The biogeochemical factors are the oxygen (O₂), labile dissolved
organic carbon (DOC), organic nitrogen (DON), inorganic nitrogen (NH$_4^+$ and NO$_3^-$), temperature, and pH. Primarily, nitrifying microbes require O$_2$ and DON, while denitrifiers require anoxic reducing conditions, a DOC source to serve as an electron donor, and a supply of NO$_3^-$ to respire. In many systems, nitrification and denitrification are tightly coupled because nitrification consumes O$_2$ while producing NO$_3^-$, and both anoxic conditions and NO$_3^-$ availability will stimulate denitrification [e.g., Duff and Triska, 1990, Holmes et al., 1996]. Natural heterogeneity in streams leads to unique combinations of both the physical and biogeochemical conditions which in turn result in unique N source and sink conditions. This heterogeneity makes it difficult to identify a priori the function of a stream hyporheic zone, so it is important to identify and account for the key components of the hyporheic N cycle. Accounting for these key components can be complicated to do in the field and among streams [Böhlke et al., 2009], because measuring denitrification is very complex and measurements are hard to scale across time and space [Galloway et al., 2004]. Currently, studies of these hyporheic N dynamics are limited to site-specific descriptive models and hyporheic controls on N flux through streams and catchments is not known. Therefore, there is a need to identify new approaches for assessing the role of the hyporheic zones on the fate of stream NO$_3^-$ that account for the complexity and are scalable across different systems.

1.2. **OBJECTIVES AND HYPOTHESES OF DISSERTATION**

This dissertation seeks to identify the relationships between the physical transport and biological reaction controls on bioavailable NO$_3^-$ transformations in hyporheic zones. Currently, there is a large disconnect between known coupling of physical and biological controls on the fate of hyporheic N, and how investigations of hyporheic N are conducted - most investigations ignore either the physics or the biology or are unable to connect the two factors in a robust way. The limited amount of research that does connect physical and biological processes ignores much of the
important complexity, such as representing the spatial and temporal coupling of nitrification and denitrification in systems. Furthermore, attempts to incorporate denitrification into stream models do not mechanistically represent the hyporheic zone or incorporate empirical hyporheic field observations. This dissertation helps to fill this knowledge gap through field experiments and numerical modeling studies that directly address coupled physical transport and biological reaction controls on hyporheic N. This dissertation applies a multidisciplinary framework and methods that include isotopes, mass balance, and numerical modeling. The experiments and the models developed in this dissertation were done with the intent to inform new integrated hydroecological conceptual models, address the role of the hyporheic zone across different stream systems and scales, and provide robust data sets for future analysis and modeling efforts.

The specific objectives and hypotheses (H) of this dissertation are summarized below:

**OBJECTIVE 1:** Understand how the physical residence time and flow path length interact with the biophysical substrate conditions (NO₃⁻, DO, and DOC) to control hyporheic NO₃⁻ dynamics.

(H₁) Stream nitrification and denitrification is controlled by the hyporheic residence time and flowpath length.

(H₂) Hyporheic nitrification and denitrification are coupled in time and space.

(H₃) Longer residence times and flowpath lengths will produce reducing conditions that will promote denitrification, while shorter residence times and flowpath lengths will produce oxidizing conditions that will promote nitrification.

(H₄) Hyporheic denitrification is limited by labile (biologically useful) DOC availability, especially at long residence times when DO is depleted.

**OBJECTIVE 2:** Quantify the influence of hyporheic denitrification on the whole-reach scale of a stream using multi-scale measurements.
(H5) Independent and complimentary measurements of hyporheic and reach-scale transport and biophysical conditions can be linked via estimates of hydrologic transport and denitrification reaction kinetics, specifically the characteristic hyporheic transport and denitrification reaction time scales.

**Objective 3:** Understand what combinations of physical and biophysical conditions will promote the hyporheic zone functioning as a net source (nitrification) or sink (denitrification) at the whole-reach scale. Develop scaling relationships using transport and reaction kinetics of the key factors controlling the fate of hyporheic N. Use these scaling relationships to estimate the net nitrification or denitrification function of any stream hyporheic zone.

(H6) Many physical and biological factors control nitrification and denitrification, but there are only a few factors that exert first-order controls on net hyporheic N transformations and function. These key factors include the hydrologic residence time, the oxygen removal rate, and the availability of labile organic carbon, because hydrology supplies solutes to the microbes and oxygen and labile carbon control microbial denitrification potentials.

1.3. **Organization of Dissertation**

The organization of this dissertation is such that each chapter addresses the above objectives and hypothesis while building upon each preceding chapter. More specifically, the dissertation starts by presenting research that focuses on process-based field experimental studies and then finishes with the development and implementation of scalable mechanistic modeling studies informed by the field experiments.

Chapter 2: *Dynamics of Nitrate Production and Removal as a Function of Residence Time in the Hyporheic Zone.* This chapter addresses the need to quantify the coupling of hyporheic hydrology and biogeochemical conditions and their role in creating stream N sources and sinks, namely nitrification and denitrification. Until the
linkages between hyporheic hydrology and N biogeochemistry are recognized, it will not be possible to identify hyporheic controls on N dynamics at reach and catchment scales. In this study I couple reactive $^{15}$N tracers (\(^{15}\text{N-NO}_3^-\)) and conservative tracers simultaneously to track hydrologic and NO$_3^-$ conditions in a hyporheic zone. This study relates hyporheic nitrification and denitrification controls to residence times (Objective 1: H1, H2, H3), which will ultimately help to upscale denitrification measurements to reach and network scales in a way that is linked quantitatively to hyporheic exchange.

Chapter 3: Labile Dissolved Organic Carbon Supply Limits Hyporheic Denitrification. Key factors in the fate of N traveling through a hyporheic zone are the organic carbon conditions (substrate quality and quantity) in the stream and hyporheic zone. Though the previous studies on coupled hyporheic DOC and N dynamics consistently indicate that the type and quality of DOC in a hyporheic system influences denitrification rates, none have both isolated the role of labile DOC supply on denitrification and made direct measurements in a hyporheic environment. Therefore, I evaluate the role of labile DOC on denitrification along a redox gradient that forms along hyporheic flowpaths in a gravel bar. I combined $^{15}\text{NO}_3^-$ tracing techniques with an addition of labile DOC to examine the role of DOC in controlling denitrification (Objective 1: H4).

Chapter 4: Coupling Multi-Scale Observations to Evaluate Hyporheic Nitrate Removal at the Reach-Scale of an Upland Agricultural Stream. This chapter evaluates how common scale-dependent measurements of denitrification can be utilized to make key estimates of the influence of hyporheic dynamics at the whole-reach scale (Objective 2: H5). The common methods used for hyporheic and reach measurements are all valuable within their own spatial and temporal resolution. However, there is need to link them to address the multiple spatial and temporal scales that control N cycling in streams. Therefore, I present a new method that links a set of independent and complimentary multi-scale estimates of hyporheic and whole-reach denitrification.
I then use this method to estimate the amount of whole-reach denitrification occurring in the hyporheic zone of an experimental stream.

Chapter 5: Coupling Hyporheic Nitrification-Denitrification: Evaluating Net Nitrate Source-Sink Dynamics as a Function of Transport and Reaction Kinetics. In this chapter, I develop and apply a numerical 1D, multispecies, reactive N transport model to address Objective 3 and test H6. The model was used to evaluate the coupling of physical transport conditions (advection, dispersion, and residence time) and biogeochemical redox conditions with multiple Monod kinetics for O\textsubscript{2}, NH\textsubscript{4}, NO\textsubscript{3}, and DOC. I used a dimensionless, steady-state form of the model to simulate the observed O\textsubscript{2}, NH\textsubscript{4}, NO\textsubscript{3}, and DOC concentrations profiles observed in the well-characterized hyporheic zone presented in Chapters 1 through 3. Additional stochastic numerical experiments were performed to evaluate the net NO\textsubscript{3}\textsuperscript{−} source or sink function of hyporheic exchange across a large range of hydrologic and biogeochemical kinetic conditions. Global sensitivity analyses were used to identify the key model parameters governing model behavior and performance.

Chapter 6: Conclusions. This chapter presents a synthesis of the dissertation by discussing the key scientific findings and merits of the dissertation while highlighting the next research objectives needed to mechanistically understand and ultimately manage how bioavailable N cycles in stream environments.

The information presented in this dissertation is fundamental to linking point-scale observations to large-scale measurements and modeling of hyporheic bioavailable N source-sink dynamics. It provides important linkages between the fields of hydrology and biogeochemistry. This connection between traditional scientific disciplines should help to further our holistic understanding of the interplay between the hydrology, biogeochemistry, and ecology of stream systems.
Table 1.1: Stoichiometry of key redox reactions included in the cycling of stream nitrogen [modified from Hedin et al., 1998].

<table>
<thead>
<tr>
<th>Reaction processes</th>
<th>General stoichiometric reaction equation</th>
<th>Free energy $\Delta G^0$ (kJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic respiration (reductive)</td>
<td>CH$_2$O + O$_2$ → CO$_2$ + H$_2$O</td>
<td>-501</td>
</tr>
<tr>
<td>Nitrification (oxidative)</td>
<td>O$_2$ + (½)NH$_4^+$ → (½)NO$_3^-$ + H$^+$ + (½)H$_2$O</td>
<td>-181</td>
</tr>
<tr>
<td>Denitrification (reductive)</td>
<td>CH$_2$O + (4/5) NO$_3^-$ + (4/5) H$^+$ → (7/5) H$_2$O + (2/5) N$_2$ + CO$_2$</td>
<td>-476</td>
</tr>
</tbody>
</table>
The hyporheic zone is the region where stream water exchanges with groundwater (delineated with blue dashed lines). Hyporheic exchange operates over many spatial and temporal scales (red dashed lines). Hyporheic exchange is typically induced by variations in the hydraulic head of a stream system. The head gradients are associated with abrupt changes in streambed slope (see inset image of riffle example) or stream meanders and preferential flowpaths. The hyporheic zone at the stream bed and banks represents a unique environment (ecotone) because they are where watershed groundwater (yellow arrows) and surface water interact [figure modified from Tonina and Buffington, 2009].
2 – Dynamics of Nitrate Production and Removal as a Function of Residence Time in the Hyporheic Zone

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Journal of Geophysical Research - Biogeosciences
116, G01025
DOI: 10.1029/2010JG001356
ABSTRACT

Biogeochemical reactions associated with stream nitrogen cycling, such as nitrification and denitrification, can be strongly controlled by water and solute residence times in the hyporheic zone (HZ). We used a whole-stream steady-state $^{15}$N-labeled nitrate ($^{15}$NO$_3^-$) and conservative tracer (Cl$^-$) addition to investigate the spatial and temporal physiochemical conditions controlling the denitrification dynamics in the HZ of an upland agricultural stream. We measured solute concentrations ($^{15}$NO$_3^-$, $^{15}$N$_2$ (g), as well as NO$_3^-$, NH$_3$, DOC, DO, Cl$^-$), and hydraulic transport parameters (head, flow rates, flowpaths, and residence time distributions) of the reach and along HZ flowpaths of an instrumented gravel bar. HZ exchange was observed across the entire gravel bar (i.e., in all wells) with flowpath lengths up to 4.2 m and corresponding median residence times greater than 28.5 h. The HZ transitioned from a net nitrification environment at its head (short residence times) to a net denitrification environment at its tail (long residence times). NO$_3^-$ increased at short residence times from 0.32 to 0.54 mg-N L$^{-1}$ until a threshold of 6.9 h and then consistently decreased from 0.54 to 0.03 mg-N L$^{-1}$. Along these same flowpaths, declines were seen in DO (from 8.31 to 0.59 mg-O$_2$ L$^{-1}$) and DOC (from 3.0 to 1.7 mg-C L$^{-1}$). The rates of the DO and DOC removal and net nitrification were greatest during short residence times while the rate of denitrification was greatest at long residence times. $^{15}$NO$_3^-$ tracing confirmed that a fraction of the NO$_3^-$ removal was via denitrification as $^{15}$N$_2$ was produced across the entire gravel bar HZ. Production of $^{15}$N$_2$ across all observed flowpaths and residence times indicated that denitrification microsites are present even where nitrification was the net outcome. These findings demonstrate that the HZ is an active nitrogen sink in this system and that the distinction between net nitrification and denitrification in the HZ is a function of residence time and exhibits threshold behavior. Consequently, incorporation of HZ exchange and water residence time characterizations will improve mechanistic predictions of nitrogen cycling in streams.
2.1 Introduction

Surplus nitrogen adversely affects aquatic systems, contributing to extensive surface and ground water degradation, which is a persistent and growing global problem [Schlesinger et al., 2006; Diaz and Rosenberg, 2008]. Stream ecosystems can be important locations of N retention along the continuum between terrestrial and ocean environments. Research has established that headwater and mid-network streams are most effective at regulating downstream nitrogen exports [Peterson et al., 2001; Alexander et al., 2000; Mulholland et al., 2008]. These same small streams are also where stream-ground water (hyporheic, HZ) flux is greatest relative to surface flux [Anderson et al., 2005]. Previous work clearly shows that HZ exchange can regulate nitrogen [Holmes et al. 1996; Wondzell and Swanson, 1996; Hill et al., 1998]. However, the linkages between HZ hydrology and stream nitrogen export are poorly understood and there is no clear mechanistic representation of HZ controls on nitrogen flux through streams [Duff and Triska, 2000; Böhlke et al., 2009]. Hence, there is a need to quantify the coupling of HZ hydrology and biogeochemical conditions and their role in creating stream nitrogen sources and sinks. Until the linkages between HZ hydrology and nitrogen biogeochemistry are established, it will be unclear how the HZ influences nitrogen dynamics at reach and catchment scales. In this study we move toward this mechanistic understanding of nitrogen fate and transport by observing and relating the HZ residence time scales to N transformations.

There are many processes that temporarily remove or relocate inorganic N from stream water (e.g., sorption onto substrate, assimilation into plants or microbes), but denitrification is the primary mechanism by which inorganic N is permanently removed from the stream system. Consequently, denitrification has been the source of significant research because of its potential role in regulating the downstream transport of inorganic N. Denitrification in streams is primarily regulated by redox conditions, NO$_3^-$ concentrations, and labile dissolved organic carbon (DOC) availability [Holmes et al., 1996; Duff et al., 1996; Baker et al., 1999]. The HZ is known to create strong
gradients in each of these conditions regulating nitrogen cycling [Triska et al., 1993; Jones and Holmes, 1996; Hill et al., 1998; Hedin et al., 1998]. Consequently, the HZ has been identified as a potential hotspot for denitrification in aquatic systems [McClain et al., 2003]. However, the HZ is not simply a net sink (via denitrification), it can also be a source (via nitrification) of nitrate where ever nitrification exceeds denitrification [Jones et al., 1995]. The nitrate produced in the HZ can fuel primary production in surface waters [Valett et al., 1994; Henry and Fisher, 2003].

The role each HZ plays in regulating downstream nitrogen export is variable in space and time [e.g., Wondzell, 1996]. This is due to the temporal and spatial variation in substrate and transport limitations on nitrogen transformations. For example substrate limitations acting on denitrification, such as the type and quantity of nitrogen and DOC entering the HZ, can vary significantly in time [e.g., seasonally; Kaplan and Newbold, 2000]. On the other hand, physical transport conditions of the HZ, which are a function of energy gradients and hydraulic conductivity, will regulate the rate at which nitrogen, dissolved oxygen (DO), and DOC are supplied to the sediment [Baker et al., 2000]. For example, DO exerts a strong control on nitrogen dynamics in the HZ, and research has shown that DO dynamics are related to water residence time in the HZ [Triska et al., 1993; Findlay et al., 1995; Valett et al., 1996; Morrice et al., 2000]. Recently, research has started to integrate physical transport and biogeochemical approaches to assess HZ denitrification as a function of HZ residence time [e.g., Gu et al., 2007; Clilverd et al., 2008; Pinay et al., 2008]. The use of $^{15}$N tracers has also advanced our understanding of aquatic N cycling. In particular, Böhlke et al. [2004, 2009] and Mulholland et al. [2004, 2008] demonstrated the usefulness of the field $^{15}$N tracer approach for determining denitrification rates of streams at the reach scale. Böhlke et al. [2004, 2009], in particular, showed that the key sources of uncertainty in measuring reach denitrification with traditional mass balance approaches – nitrification and nitrate uptake can be accounted for with the use of $^{15}$N tracer approach. Although many advances about nitrogen cycling in streams have
resulted from this $^{15}$N tracer work, these studies were unable to account for the entire nitrogen budget in streams. Böhlke et al. [2004] and Mulholland et al. [2004, 2008] suggest that a portion of the unaccounted for nitrogen may be due to benthic and hyporheic nitrogen retention and removal processes. Recently, Böhlke et al. [2009] demonstrated with whole stream $^{15}$NO$_3^-$ addition experiments that between 14 and 97% of whole stream denitrification was attributed to HZ denitrification.

The objective of this study was to assess the substrate and transport conditions controlling net HZ denitrification. We hypothesize that biogeochemical reactions associated with stream nitrogen cycling, such as nitrification and denitrification are strongly controlled by water residence times in the HZ. To test the hypothesis, we conducted a whole-stream steady-state $^{15}$NO$_3^-$ and a conservative tracer experiment in an upland agricultural stream to measure the in situ spatial and temporal hydraulic and biogeochemical conditions controlling HZ nitrification and denitrification. We show here that $^{15}$NO$_3^-$ tracing techniques characterize HZ denitrification and that the conditions conducive to net denitrification vary with subsurface residence times. Ultimately, relating HZ denitrification controls to residence times will help to upscale denitrification measurements to reach and network scales in a way that is linked quantitatively to transient storage.

2.2 METHODS

2.2.1 Study Site

The study site consists of a 303 m reach containing an instrumented gravel bar hyporheic zone on Drift Creek (Figure 2.1), a 3rd-order stream within the Willamette River basin in western Oregon, USA (44.9753°N, 122.8259°W). The drainage area above the study reach is 6517 ha, and has mixed land-use dominated by agriculture (lower catchment) and forestry (upper catchment). The catchment population is predominantly rural with septic-systems, another potential source of N in the study stream. Annual precipitation is 1190 mm and comes primarily during the winter as
rain. Base flow discharge gradually decreases to an annual minimum (< 50 L s\(^{-1}\)) in early September. The study reach was modified by channelization in the past, as were many of the streams in this agricultural region. The channelized stream is incised into competent bedrock consisting of andesite flow breccias and is now separated from an active flood plain. The incised active channel is 10-20 m wide and is bounded by steep banks 3-5 m high. The alluvial thickness above bedrock (as depth to refusal) varies from 0 to ≥ 1.5 m. Consequently, the reach has a limited and constrained hyporheic zone. There is a thin riparian corridor on each side of the stream (< 15 m) that separates the stream from the annually-rotated crop fields (Figure 2.1C). The riparian zone is primarily vegetated by deciduous trees, grasses, and thick stands of Himalayan Blackberry (*Rubus armeniacus*). This riparian vegetation provides extensive canopy cover over the stream during the summer base-flow period. The study reach has a slope of 0.007 m m\(^{-1}\) and the morphology is primarily a planebed channel with occasional pool-riffle sequences [see Montgomery and Buffington, 1997 for definitions of channel types]. The stream bed consists of poorly sorted sand, gravel, cobbles, and boulders (Figure 2.1D).

The hyporheic zone study site is a lateral gravel bar approximately 6.1 m by 4 m (Figure 2.1B, D). This gravel bar is adjacent to a riffle on one side and connected to the bedrock channel bank on the other side. The gravel bar separates two pools and spans a head loss across the riffle of 0.13 m. The alluvium comprising this gravel bar was uniformly 1.2 m thick. The observed and modeled subsurface exchange across this gravel bar primarily occurs along lateral flowpaths from the head to the tail of the bar (Figure 2.1B). This gravel bar was instrumented with a well network (\(n = 11\)) of 3.8 cm-I.D. schedule 40 PVC wells screened 0.2 – 0.4 m below ground surface. Chloride (Cl\(^{-}\)) tracer tests conducted prior to the experiment demonstrated that all wells were connected to stream water and that well water originated from the stream and not from the groundwater aquifer. This lack of groundwater inflows reduces the uncertainty of \(^{15}\)N tracing interpretation in this HZ system; groundwater inflow of
nitrogen is a common source of uncertainty seen in other systems [e.g., Böhlke et al. 2004]. Background stream and hyporheic biogeochemistry are presented in Table 2.1.

2.2.2 Field Procedures

We performed a whole-stream steady-state $\delta^{15}$NO$_3^-$ and conservative tracer (Cl$^-$) injection on 23-24 August 2007 when discharge was relatively stable. Following methods from Mulholland et al. [2004], an injection solution of $\delta^{15}$NO$_3^-$ (as 99% enriched K$^{15}$NO$_3^-$) and Cl$^-$ (as NaCl$^-$) was released at a constant rate (154 mL min$^{-1}$) using a peristaltic pump at the head of the reach for 27.5 h starting at 14:28 h (Geopump Series I, Geotech Environmental Equipment, Denver, Colorado, USA, note that the use of trade names in this publication is for reader information and does not imply endorsement by the U.S. Department of Agriculture of any product or service). The amount of K$^{15}$NO$_3^-$ introduced to the reach was calculated to produce a target $\delta^{15}$N enrichment of 10 000‰ in the stream water NO$_3^-$. The Cl$^-$ mass addition target was to elevate the background stream Cl 400 % and generate an electrical conductivity increase of 50 %. The solution was injected into a turbulent riffle sufficiently upstream of the first sampling location to guarantee complete lateral and vertical channel mixing at all downstream sampling locations. The K$^{15}$NO$_3^-$ addition produced a 3% increase in ambient stream N-NO$_3^-$. Electrical conductivity was used to measure the real-time Cl$^-$ transport through the stream and hyporheic zone. The electrical conductivity measurements were taken every 60 s in all 11 wells and in the stream water at the head and tail of the gravel bar. These electrical conductivity measurements were made with 13 multiplexed, in situ, CS547A conductivity and temperature probes connected to a CR1000 (Campbell Scientific, Logan, Utah, USA). The Cl$^-$ transport at the end of the experimental reach was captured via automated sampling every 10 minutes until plateau and then every 2 h during plateau (ISCO model 3700, Lincoln, Nebraska, USA) and subsequently field measured with a electrical conductivity meter (YSI model 63, Yellow Springs, Ohio, USA). These electrical conductivity measurements were used to characterize the
solute transport dynamics including flow rates, flow paths, and residence times as well as to inform the timing of the sampling regime described below.

The water sampling regime consisted of collecting multiple rounds of stream and hyporheic samples during the two phases of the experiment – pre injection and plateau (steady-state). For each location (11 wells plus stream water at the gravel bar head), repeated sampling occurred during the pre-injection ($n=3$) and plateau ($n=5$) periods (Figure 2.2). The plateau sampling period was initiated at 22.5 h after injection when all hyporheic wells demonstrated near steady-state electrical conductivity. Repeated hyporheic samples were collected approximately every 1 h during the plateau period.

Water samples were collected for key solute concentrations and $\delta^{15}$N enrichments relevant to the respiratory denitrification process ($\delta^{15}$NO$_3^-$, $\delta^{15}$N$_2$ (g), as well as NO$_3^-$, NH$_3$, DOC, DO, Cl$^-$, and specific ultraviolet absorption (SUVA$_{254}$). Hyporheic well samples were collected with a field peristaltic pump (Masterflex L/S, Vernon Hills, Illinois, USA) [Woessner, 2007]. All water samples were immediately filtered through ashed Whatman GF/F glass fiber filters (0.7 μm pore size) into acid washed HDPE bottles (60 mL for nutrient chemistry and 1 L for $^{15}$N isotope samples). Following filtering, nutrient chemistry samples and isotope samples were stored on ice in the field and later refrigerated at 4°C or frozen in the laboratory until processed and analyzed. DO concentrations were measured in situ with a calibrated YSI DO Meter (Model 52) at all locations prior to collecting each round of samples. Samples were also collected for $\delta^{15}$N$_2$O (g), but were unable to be analyzed due to technical problems at the stable isotope laboratory. However, denitrification in freshwater and near shore marine system sediments consists almost entirely of N$_2$ production with N$_2$O/N$_2$ production ratios generally between <0.001 and <0.05 [Seitzinger, 1998; Mulholland et al., 2004], so $^{15}$N$_2$ by itself is capable of characterizing the majority of the denitrification dynamics.
The $^{15}$N gas collection for each sample occurred in the field and followed procedures adapted from Hamilton and Ostrom [2007]. A peristaltic pump was used to collect 80 mL water samples into a 140 mL plastic syringe (Becton-Dickinson, Franklin Lakes, NJ, USA) fitted with stopcocks. All visible bubbles were expelled so that there was no headspace. To avoid any atmospheric N contamination, sample syringes were submerged under water in a processing tub kept at stream temperature. An underwater transfer of 40 mL high purity He was added to each sample syringe. Sample syringes were then gently shaken for 10 min to permit equilibration of the N$_2$ (g) into the He headspace. Following equilibration, approximately 14 mL of headspace gas was then injected into preevacuated 12 mL exetainers (Labco Limited, Wycombe, UK). Exetainers were preevacuated by pumping them down to a pressure of $< 50$ mtorr using a Welch vacuum pump (Model DirectTorr 8905, Skokie, Illinois, USA) and then stored underwater in He purged DI water-filled centrifuge tubes until sample collection. Sample-filled exetainers were then returned to their zero headspace He purged DI water-filled centrifuge tubes for storage until analysis.

Following the experiment (5-6 Sept. 2007) and during similar stable low flow conditions, we collected detailed thalweg surface water and channel surface topography data for the reach using a Topcon total station (Model GTS-226, Livermore, California, USA) and standard surveying methods with spatial resolution of $x \leq 1$ m, $y \leq 1$ m, $z \leq 0.01$ m for the greater reach and $x \leq 0.1$ m, $y \leq 0.1$ m, $z \leq 0.005$ m for the instrumented gravel bar site.

2.2.3 Laboratory Procedures

Stream and hyporheic samples were analyzed for NO$_3$-N, NH$_3$-N, DOC, and Cl$^-$ at the Cooperative Chemical Analytical Laboratory (Corvallis, USA). The NO$_3$-N and NH$_3$-N measurements were made by a Technicon Auto-Analyzer II. The NO$_3^-$ and NH$_3$ nutrient analyses were performed following standard colorimetric methodology and had detection limits of 0.001 mg L$^{-1}$ and 0.01 mg L$^{-1}$, respectively. The concentration of total DOC was determined with a Shimadzu TOC-VCSH
Combustion Analyzer (Tokyo, Japan; detection limit = 0.05 mg L\(^{-1}\)). The Cl\(^{-}\) was determined by ion chromatography (Dionex 1500, Sunnyvale, California, USA; detection limit = 0.01 mg L\(^{-1}\)). SUVA values were determined by dividing the UV absorbance measured at \(\lambda=254\) nm by the DOC concentration and are reported in the units of liter per milligram carbon per meter [Weishaar et al., 2003].

The \(\delta^{15}\)N content of the stream and hyporheic water NO\(_3^-\) was determined by methods adapted from Sigman et al. [1997] and Mulholland et al. [2004], which are briefly summarized below. Prior to \(\delta^{15}\)N analysis, \(^{15}\)NO\(_3^-\) samples with blanks and standards were processed in the following manner. First, a volume of each sample (0.25 – 1 L; processing volume is dependent on N content of each sample) was stripped of its dissolved NH\(_4^+\) and had its NO\(_3^-\) concentrated. Second, the concentrated sample NO\(_3^-\) was captured on a prepared filter via a reduction/diffusion/sorption procedure (full reduction of NO\(_3^-\) to NH\(_4^+\), which is then converted to NH\(_3\) that diffuses into the headspace and ultimately gets captured on the acidified sorption filter). After complete transfer of NO\(_3^-\) to the sample filter, the samples were sealed and sent for \(^{15}\)NO\(_3^-\) analysis. All \(\delta^{15}\)NO\(_3^-\) and \(\delta^{15}\)N-gas samples were analyzed by the Marine Biological Laboratory Stable Isotope Facility (MBL, Woods Hole, Massachusetts, USA). Replicate analyses of the water and gas samples show the precision of \(\delta^{15}\)NO\(_3^-\) and \(\delta^{15}\)N isotope measurements is +/− 80.0 ‰ and +/− 0.2 ‰, respectively.

### 2.2.4 Parameter Calculations

Electrical conductivity breakthrough curves (as a measure of Cl\(^{-}\) transport) at the head of the gravel bar and in each well were used to measure the median residence time of the study reach and the HZ water in each well. The median residence time was calculated as the time required to raise the EC in the well to one half the plateau concentration. In the case of the wells, median residence times were calculated based on the observed Cl\(^{-}\) arrival at the gravel bar, not the start of the injection.
To estimate reactive solute transport through and removal by the HZ, we compared the observed NO$_3^-$, NH$_3$, DO, and DOC concentrations to the conservative tracer concentrations at steady-state conditions [after Morrice et al., 2000]. In the absence of biological or chemical removal, the reactive compounds and Cl$^-$ transport should be identical. Based upon this assumption, we calculated the NO$_3^-$, NH$_3$, DO, and DOC concentrations according to the measured Cl$^-$ concentrations observed at each well:

$$R_{pred,x,t} = R_{inj,x} \left[ \frac{C_{x,t=0}}{C_{x,t=0}} \right] + R_{x,t=0} \left[ 1 - \frac{C_{x,t=0}}{C_{x,t=0}} \right]$$  \hspace{1cm} (1)$$

where, $R$ is the solute concentration of interest (NO$_3^-$, NH$_3$, DO, or DOC), $C$ is the conservative tracer concentration (Cl$^-$), and subscripts $pred$, $inj$, $x$, $t$ represent predicted well concentration at plateau, injection concentration in the stream, well location, and sample time period, respectively. We then calculated the difference between the measured and predicted reactive solute concentrations for each well during the plateau conditions. Reactive solute removal occurs when the observed concentration is less than the predicted concentration and production occurs when the observed concentration is greater than the predicted concentration.

2.3 RESULTS

2.3.1 Stream Hydrology and Chemical Conditions During Experiment

Stream flow conditions were relatively stable over the experiment with a mean flow of 22 L s$^{-1}$ and a variance of ± 2.2 L s$^{-1}$. This variance in flow did not create any measurable change in the stage of the stream along the reach, near the gravel bar, or in the heads of the gravel bar wells. Surveying of channel topography and geometry yielded a reach mean depth, $d$, of 0.23 m and a mean wetted width, $w$, of 5.21 m. Repeated measurements of the head at wells (before plateau sampling disturbances)
reflected stable surface water elevations as there was no detectible variation during the experiment. Stream and HZ water temperature ranged between 14.1 and 16.5° C during the injection period. Measured surface water nutrient and chemistry conditions were stable across the experiment and did not show diel patterns with NO₃⁻ (0.318-0.325 mg-N L⁻¹), NH₃ (0.021-0.024 mg-N L⁻¹), DOC (2.95-3.45 mg-C L⁻¹), DO (8.10-8.51 mg-O₂ L⁻¹), and pH (6.65-6.85).

2.3.2 Spatial Dynamics of Hyporheic Transport and N Transformation Conditions

Chloride plateau concentration conditions were achieved in all 11 hyporheic wells, demonstrating good connectivity with surface water. Nominal flowpath lengths (Figure 2.3) ranged from 0.5 m (H1) to 4.2 m (K3). The mean DO decreased from 8.31 to 0.59 mg-O₂ L⁻¹ along HZ flowpaths (Figure 2.4A) and the mean DOC decreased from 3.01 to 1.7 mg-C L⁻¹ (Figure 2.4D). The DOC SUVA254 concentrations were more spatially variable than DOC, indicating that different locations within the HZ had different quantities of aromatic DOC (Figure 2.4D, contour map), but did generally decrease along flowpaths (3.22 to 0.94 L mg-C⁻¹ m⁻¹). Along flowpaths, DO and DOC removal rates were largest in the first 2 m of the flowpaths but continued across the entire gravel bar. In contrast, the N-species did not consistently decrease along flowpaths. Nitrate increased at the proximal end of the flowpaths (< 0.55 m) from 0.34 to 0.54 mg-N L⁻¹ and then decreased along the remainder of the flowpaths from 0.54 to 0.02 mg-N L⁻¹ (Figure 2.4B). Similarly, NH₃ increased from 0.02 to 0.11 mg-N L⁻¹ at the proximal end of the flowpaths and then decreased from 0.11 to 0.01 mg-N L⁻¹ along the remainder of the flowpaths (Figure 2.4C).

Tracing of $^{15}$NO₃⁻ confirmed that a fraction of the NO₃⁻ removal was via denitrification as $^{15}$N₂ was produced across the entire gravel bar HZ (Figure 2.4F). Production of $^{15}$N₂ occurred along all portions of the flowpaths, even portions characterized by net nitrification (elevated NO₃⁻ and NH₃). Importantly, there was no consistent spatial gradient in the $^{15}$NO₃ enrichment from proximal to distal ends of the
HZ flowpaths (Figure 2.4E) which, if present, would indicate non-steady state dynamics. Therefore the range of $^{15}$NO$_3^-$ enrichment is a function of steady-state hydrologic and biological conditions. The gravel bar plateau $^{15}$NO$_3^-$ enrichment ranged from 4 260 ‰ (well I3) to 6 805 (well J2), while the stream water ranged from 9 935 to 10 092 ‰.

2.3.3 Temporal Dynamics of Hyporheic Transport and N Transformation

Tracing Cl$^-$ transport through the HZ generated median residence times ranging from 3.8 to 28.5 h (Figure 2.3). The shortest residence times were generally associated with the head of the gravel bar while the longest were located at the tail of the gravel bar. However, the longest median residence time (28.5 h) was observed at a mid-bar well, J1. Comparison of residence times to measured biogeochemical conditions (Figure 2.5) indicates that residence times less than 6.9 h were associated with a net dominance of oxic conditions and aerobic microbial processes (O$_2$ respiration and nitrification) while residence times beyond 6.9 h were associated with a net dominance of hypoxic-anoxic conditions and anaerobic microbial processes (denitrification). More specifically, the greatest rates of DO, DOC, and SUVA$_{254}$ reduction corresponded with the greatest rates of NH$_3$ and NO$_3^-$ production (Figure 2.5), all of which co-occurred during the first 6.9 h of observed transport. Beyond 6.9 h of residence time, DO, DOC, and SUVA$_{254}$ continue to decrease gradually to a minimum of 0.51 mg-O$_2$ L$^{-1}$, 1.66 mg-C L$^{-1}$, and 0.94 L mg-C$^{-1}$ m$^{-1}$, respectively. Further, SUVA$_{254}$ indicates that the highest fraction of labile DOC occurred when residence times were smallest, and that labile DOC was largely depleted by the time water attained larger residence times (Figure 2.5D).

The concentration of $^{15}$N$_2$ increased along proximal portions of flow paths with residence times < 3.8 h, reaching a peak at the most distal portions of the flow paths where residence times exceeded 22 h (Figure 2.5F). The concentration of NO$_3^-$ (mg-N L$^{-1}$) also increased along the proximal portions of flow paths where residence times were less than 6.9 h, indicating concurrent nitrification and denitrification throughout
the proximal portion of the gravel bar (Figure 2.5). Increases in NH$_3$ were concurrent with consumption of DO and DOC (Figure 2.6). Comparison of DO and DOC concentrations with the conservative transport of Cl$^-$ demonstrates that DO and DOC show net loss over all residence times (Figure 2.6). Conversely, NH$_3$ shows net production until the very longest residence time of 28.5 h when it shows net loss, while NO$_3^-$ shows net production until 18.2 h followed by net loss.

2.4 DISCUSSION

We utilized a whole-stream steady-state $^{15}$NO$_3^-$ and conservative tracer (Cl$^-$) addition to observe spatial and temporal hydraulic and physiochemical conditions controlling NO$_3^-$ dynamics in a HZ. Our results illustrate that nitrification and denitrification: 1. create non-linear NO$_3^-$ dynamic along HZ transport, 2. are not exclusively segregated processes in space and time, and 3. are strongly controlled by water and solute residence times in the HZ. From these findings we are able to confirm and build upon earlier conceptual frameworks [Jones and Holmes, 1996; Valett et al., 1996; Hedin et al., 1998] that relate HZ nitrification and denitrification dynamics along flow paths and with residence times.

2.4.1 Spatial and Residence Time Dynamics of Hyporheic N

Nitrate production vs. removal can be site- and scale-dependent, and the hyporheic biogeochemistry in our study shows general spatial patterning in net N transformation processes consistent with earlier studies [e.g., Holmes et al., 1994; Pinay et al., 1994; Holmes et al., 1996]. The upgradient end of the HZ flowpaths is dominated by oxic conditions and is a net NO$_3^-$ production hot spot, while the middle and downgradient parts of the flowpaths are anoxic and are a net NO$_3^-$ removal hot spot (Figure 2.4). At the scale of the entire gravel bar, however, this HZ is a net NO$_3^-$ removal hot spot for the stream. In contrast, other, smaller HZ units in the stream may be production hotspots because of their shorter flow paths and residence times. The
residence time can be used to mark where this HZ turns from one redox condition to another - from net NO$_3^-$ production to net NO$_3^-$ removal (Figure 2.7). In this HZ, a water residence time of 6.9 h marks the threshold that separates these conditions. This observed threshold supports the HZ N transformation conceptual framework put forth by Jones and Holmes [1996] and Hedin et al. [1998]. The creation of this residence time threshold is complex and is a function of: 1. HZ water temperature (as it controls microbial activity and DO in water), 2. concentration of DO across the HZ (controlled by biological oxygen demand and advected supply), 3. HZ DOC supply and quality, 4. amount of NO$_3^-$ in the HZ system, and 5. the physical hydraulics subsumed in the physical residence time of water (e.g., head gradient, hydraulic conductivity, advection, and dispersion).

As shown in Figure 2.7, at times shorter than the threshold, transport and substrate conditions promote mineralization of stream sourced DON or particulate organic matter and subsequent nitrification with denitrification (N$_2$ production) limited to microsite reactions [Sheibley et al., 2003]. As residence times increase, the extent of anaerobic water in the HZ grows and the effective nitrification rate decreases until its contribution to net NO$_3^-$ production is negligible. At the threshold residence time, both processes are co-occurring and this is likely the location where the greatest rates of denitrification will be observed. Beyond the threshold, the HZ is dominated by net denitrification, and concentrations of both NH$_3$ and NO$_3^-$ decrease rapidly as denitrification is not substrate (labile DOC) limited and NO$_3^-$ production rapidly decreases. Finally, at much longer residence times, the rate of denitrification will decrease due to an increasing DOC substrate limitation and lack of NO$_3^-$, even though redox conditions remain appropriate to carry out denitrification.

Similar nitrification-denitrification coupling has been observed in stream HZs where DON-rich anoxic groundwater flows into oxic stream sediments [e.g., Hedin et al., 1998; Sheibley et al., 2003]. The thermodynamic framework of Hedin et al. [1998] clearly illustrates that microbial redox processes, including nitrification-
denitrification, will be tightly coupled in the riparian and hyporheic environments where solutes exchange across oxic-anoxic boundaries. Our study illustrates this tight coupling occurs in space and time as shown by the spatial distribution of N species (Figure 2.4) and the residence time threshold between net nitrification and denitrification (Figure 2.7). This suggests that a thermodynamic approach is useful and should be coupled to physical transport dynamics in order to better understand the spatial and temporal distribution of different hyporheic redox environments.

Earlier studies [e.g., Valett et al. 1996; Pinay et al. 2008] indicate that biologically mediated N fluxes through the HZ are explicitly a function of HZ flowpath length and implicitly a function of residence time [i.e., space for time; see Jones and Holmes, 1996 and references therein]. In this study, $^{15}$N tracing explicitly shows that there is a relationship between N fluxes and HZ residence time. Earlier studies by Valett et al. [1996] and Pinay et al. [2008] collected measures of HZ solute transit times during their N addition experiments and found that NO$_3^-$ uptake and denitrification were the dominant N transformations processes. However, they did not observe the coupling of nitrification and denitrification (i.e., nitrification preceding denitrification along increasing residence times). In their studies, NO$_3^-$ uptake and denitrification processes occurred across all measured transport times achieving maximums during the first 1.7 h of travel time. Conversely, our study shows a different N removal pattern - one that is more complex, with nitrification occurring at short residence times and denitrification at late residence times (Figure 2.6, 2.7). A main difference between our study and these earlier studies is the use of $^{15}$NO$_3^-$ to trace N dynamics under near ambient NO$_3^-$ conditions. Valett et al. [1996] and Pinay et al. [2008] elevated NO$_3^-$ concentrations in their HZ studies (6.4- to 24-fold NO$_3^-$ increase, respectively). NO$_3^-$ transformations are concentration-dependent, and elevated NO$_3^-$ conditions can increase rates of HZ uptake and denitrification [Jones and Holmes, 1996]. Consequently, the use of $^{15}$NO$_3^-$ allowed us to observe different NO$_3^-$ transformations under approximately ambient concentrations.
In addition to NO$_3^-$ removal via denitrification, bacterial assimilation is an important N retention process and may account for a significant fraction of the observed DOC and NO$_3^-$ removal across the HZ. Following Sobczak et al. [2003], using the observed DOC loss across the HZ = 1.35 mg-C L$^{-1}$, and assuming a microbial growth efficiency of 50\% and a microbial C:N = 7:1, a potential 0.68 mg-C L$^{-1}$ and 0.1 mg-N L$^{-1}$ could be assimilated into microbial biomass. Under these assumptions, microbial assimilation can account for 21\% of the NO$_3^-$ loss across the gravel bar HZ. This microbial assimilation of NO$_3^-$ is supported by the lower $^{15}$N enrichment values observed in the HZ $^{15}$NO$_3^-$ compared to the stream surface waters (Figure 2.4). Both microbial assimilation and denitrification will act to lower $^{15}$N enrichment of the HZ $^{15}$NO$_3^-$ pool. After accounting for assimilation of NO$_3^-$, the respiratory denitrification may account for as much as 79\% of the total NO$_3^-$ removed from stream water flowing through the HZ of this gravel bar.

DOC quantity and quality also clearly depend on residence time. This is expected given the role DOC plays as a substrate for the observed nitrification and denitrification. Previous work has demonstrated a strong positive relationship between DOC loss and bacterial productivity along hyporheic flowpaths [Sobczak and Findlay, 2002]. The concurrent declines in DOC and DO concentration with increasing flowpath length and residence times indicates strong aerobic metabolism in this gravel bar HZ. Similar observations of DOC declines were also seen along flowpath length in the controlled mesocosms experiments of Sobczak et al. [2003]; they found that the DOC dynamics were a function of rapid microbial utilization of the bioavailable fractions of the DOC followed by conservative transport of the unavailable DOC fraction. In this study, we are able to relate these DOC spatial dynamics to transport times. In doing so, we see the same conservative transport of the less labile fraction of the DOC at longer flowpaths and residence times (Figure 2.5D); however some labile DOC must be present at later residence times to provide necessary substrate for denitrification.
2.4.2 \(^{15}\text{N} \) Tracing Shows Overlapping N Process Domains

At the organismal level, microbial denitrification requires anoxic conditions. However, it is well known that denitrification can occur in anoxic biofilms within bulk conditions that are oxic [e.g., Holmes et al., 1996]. Hence, it is possible for denitrification and nitrification to proceed concurrently in a small volume of the HZ. Our observation of \(^{15}\text{N}_2\) production at wells exhibiting net NO\(_3^-\) production (Figure 2.7) indicates that over short residence times the water has encountered denitrification microsites in less mobile pore water. When low-flow water samples were collected from wells, water and solutes were accessed from both mobile and immobile domains, as seen in other groundwater well sampling regimes [e.g., Harvey et al., 2000]. In the case of our study, at short residence times, the advection-dominated mobile domain likely supports the aerobic processes and the diffusion-dominated immobile domain supports anaerobic processes. In contrast, at long residence times both mobile and immobile domains become anoxic as the DO is utilized along the flowpath. The use of \(^{15}\text{N} \) tracing permitted us to see that a HZ water sample contains signals from multiple distinct N transformation domains. This overlapping domain complexity can be accounted for with a residence time distribution perspective, because the residence time distribution of sample volume integrates the effect of different process domains encountered during hyporheic transport (e.g., large subsurface heterogeneity and gradients created by advective-dispersive transport, mixing flowpaths, or substrate patchiness).

Future work should explore the role of these overlapping process domains in light of other possible explanations that we are unable to address with the current study. For example, labile DOC subsurface heterogeneity can also generate spatial and temporal N transformation heterogeneity. Another issue is that rapid localized redox condition changes could be created while drawing the low-flow water sample. During the sampling, an otherwise anoxic region in the HZ around the well may have more oxic water drawn into the well via the pumping of upgradient preferential
flowpaths. In this case, a denitrification signal from the anoxic pore waters surrounding the well will be combined with the upgradient waters properties such as higher DO concentrations and nitrification.

2.5 CONCLUSIONS

Results from the coupled conservative and $^{15}$N reactive tracer experiment provide definitive evidence of in situ denitrification occurring in the HZ. Further, the comparison between conservative tracer and reactive $^{15}$N tracers enable us to relate the fate and transport of DO, DOC, and NO$_3^-$ to water residence times. In this hyporheic zone, short residence times were dominated by aerobic metabolic processes such as the rapid utilization of DO and DOC and the production of ammonia and nitrate (ammonification and nitrification, respectively). However, a clear denitrification signal, concurrent in both space and time, was also observed during short residence times indicating anaerobic microsites. Beyond a residence time threshold of 6.9 h in this HZ, the anaerobic metabolic process of denitrification dominated the system, and resulted in a net removal of nitrate from the stream. Thus, this HZ was a hot spot for nitrogen transformation, where hot spots of nitrate production and removal were distinguished by residence time.

In this gravel bar HZ, the combination of $^{15}$N tracing with a relatively elegant, highly instrumented hydrologic system (Figure 2.1), we see that residence time helps make N transformation relationships more clear (Figure 2.7). The actual spatial and temporal location of the threshold between net N transformation process domains is expected to vary between representative HZ units given their exact combination of hydrologic and upgradient biogeochemical and substrate characteristics. Further, this threshold is likely to vary in time with daily and seasonal changes in hydraulic, temperature, and water chemistry conditions. Ultimately, relating hyporheic denitrification controls to residence times will help to upscale denitrification
measurements to stream reach and network scales in a way that is linked quantitatively to transient storage.
ACKNOWLEDGMENTS

Support for this project was provided via NSF grant EAR-0409534 and EAR-0409591 to RH, SMW, and MAB and NSF grant DGE-0333257 and OSU Institute for Water and Watersheds (IWW) grant to JPZ. Further support was provided by the Hollis M. Dole Environmental Geology Foundation at OSU. Any opinions, findings and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the NSF. We thank the associate editor and two anonymous reviewers whose comments improved this manuscript. Special thanks to: V. Adams, S. Baxter, P. Zarnetske, A. Argerich, and B. Burkholder for field/lab assistance; L. Ashkenas and S. Thomas for advising JPZ on stable $^{15}$N handling; M. Otter of MBL’s Stable Isotope Laboratory for analyzing $^{15}$N samples; and C. Jones and K. Motter of CCAL and OSU IWW Collaboratory for help with analyzing general water chemistry.
Table 2.1: Background stream and hyporheic biogeochemistry (mean of 3 observations before injection ± 1 standard error).

<table>
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<tr>
<th>Site</th>
<th>DO (mg-O₂ L⁻¹)</th>
<th>NO₃⁻ (mg-N L⁻¹)</th>
<th>NH₃ (mg-N L⁻¹)</th>
<th>DOC (mg-C L⁻¹)</th>
<th>δ¹⁵N-NO₃⁻ (% vs. AIR)</th>
<th>δ¹⁵N-N₂ (% vs. AIR)</th>
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<tbody>
<tr>
<td>Stream</td>
<td>8.31 ± 0.43</td>
<td>0.32 ± 0.01</td>
<td>0.02 ± 0.02</td>
<td>3.01 ± 0.15</td>
<td>2.19 ± 0.61</td>
<td>3.00 ± 0.37</td>
</tr>
<tr>
<td>G1</td>
<td>2.07 ± 0.05</td>
<td>0.54 ± 0.06</td>
<td>0.11 ± 0.02</td>
<td>2.07 ± 0.15</td>
<td>5.48 ± 3.33</td>
<td>1.20 ± 0.61</td>
</tr>
<tr>
<td>H1</td>
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<td>0.43 ± 0.05</td>
<td>0.05 ± 0.01</td>
<td>2.18 ± 0.14</td>
<td>5.35 ± 2.27</td>
<td>0.80 ± 0.61</td>
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<tr>
<td>H2</td>
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<td>0.27 ± 0.03</td>
<td>0.06 ± 0.01</td>
<td>2.01 ± 0.17</td>
<td>5.00 ± 3.26</td>
<td>0.50 ± 1.01</td>
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<tr>
<td>H3</td>
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<td>2.01 ± 0.20</td>
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<td>I1</td>
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<td>1.70 ± 0.11</td>
<td>10.25 ± 2.96</td>
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Figure 2.1: A. Map of the Drift Creek study site showing tracer injection site and the gravel bar hyporheic site. B. Map of the hyporheic study site showing locations of wells (dots with cross hairs) and water potentiometric surface during the injection experiment. Stream briefly bifurcates near gravel bar (i.e., not a tributary confluence) and water chemistry is the same across channel. Dotted arrow indicates a single representative simulated flowpath between the head and tail of the gravel bar. C. Photo of experimental reach looking downstream. D. Photo of hyporheic study site looking upstream showing a detailed view of the gravel bar including the wells (white pipes). Photos were taken during summer base flow conditions.
Figure 2.2: The stream at gravel bar and representative distal HZ well (K2) electrical conductivity (EC) breakthrough curves showing the timing of the repeated pre-injection and plateau sampling events. Gaps in well EC data represent times when EC probes were disturbed via sampling.
Figure 2.3: Nominal flowpath length and median residence time for each hyporheic zone well.
Figure 2.4: Spatial steady-state hyporheic zone biogeochemical and $^{15}$N conditions: A. dissolved oxygen (DO, mg-O$_2$ L$^{-1}$), B. nitrate (NO$_3^-$, mg-N L$^{-1}$), C. ammonia (NH$_3$, mg-N L$^{-1}$), D. total dissolved organic carbon (DOC, mg-C L$^{-1}$) with SUVA$_{254}$ contours (interval equals 0.1 L mg$^{-1}$ m$^{-1}$), E. $\delta^{15}$N-nitrate ($\delta^{15}$NO$_3^-$, o/oo vs AIR), and F. $\delta^{15}$N-dinitrogen ($\delta^{15}$N$_2$, o/oo vs AIR). Maps present spatially interpolated mean values generated from repeated samples ($n$=5) collected during tracer plateau conditions. Stream water values did not vary between the head and tail of the gravel bar.
Figure 2.5: Steady-state hyporheic zone biogeochemical and $^{15}$N conditions relative to median residence time: A. dissolved oxygen (DO), B. nitrate (NO$_3^-$), C. ammonia (NH$_3$), D. total dissolved organic carbon (DOC) with SUVA$_{254}$, E. $\delta^{15}$N-nitrate ($\delta^{15}$NO$_3^-$), and F. $\delta^{15}$N-dinitrogen ($\delta^{15}$N$_2$). Each data point represents the mean values generated from repeated samples ($n$=5, error bars = ± 2 standard error) collected during tracer plateau conditions. Stream water values are shown as a median residence time = 0 h.
Figure 2.6: Apparent net production and removal based upon conservative transport relative to median residence time for: A. dissolved oxygen (DO), B. nitrate (NO$_3^-$), C. ammonia (NH$_3$), and D. total dissolved organic carbon (DOC). Positive values represent production, negative values represent removal, and the zero line represents conservative transport of compound. Each data point represents the mean values generated from repeated samples ($n = 5$, error bars = ± 2 standard error) collected during tracer plateau conditions.
Figure 2.7: Conceptual model showing a continuum between net hyporheic nitrification and denitrification conditions (labeled lines) as a function residence time. Note that the conceptual model is overlaid on the observed steady-state nitrification-denitrification species, dissolved organic carbon, and dissolved oxygen conditions: A. dissolved oxygen (DO, black circles), B. nitrate (NO$_3^-$, blue diamonds), C. ammonia (NH$_3$, gray triangles), D. total dissolved organic carbon (DOC, orange squares), and E. $\delta^{15}$N-dinitrogen ($\delta^{15}$N$_2$, red circles). Each data point represents the normalized mean values generated from repeated plateau samples ($n=5$, error bars = ± 2 standard error, values were normalized by maximum observed concentration or $^{15}$N enrichment).
3 – Labile Dissolved Organic Carbon Supply Limits Hyporheic Denitrification

Jay P. Zarnetske, Roy Haggerty, Steven M. Wondzell, and Michelle A. Baker

Journal of Geophysical Research - Biogeosciences
Conditionally Accepted
ABSTRACT

We used an in situ ¹⁵N-labeled nitrate (¹⁵NO₃⁻) and acetate (AcO⁻) well-to-wells injection experiment to determine how the availability of labile dissolved organic carbon (DOC) as AcO⁻ influences microbial denitrification in the hyporheic zone (HZ) of an upland (3rd-order) agricultural stream. A 48-h steady-state injection of a conservative tracer, chloride, and ¹⁵NO₃⁻ was used to quantify ambient HZ denitrification via ¹⁵N₂ production. Following ambient plateau measurements of denitrification during the first 24 h, a second conservative tracer, bromide, and the labile DOC source, AcO⁻, were co-injected for an additional 24 h to measure HZ denitrification under increased DOC supply. Conservative tracers were observed at 4 of the 9 down-gradient wells. Receiving wells had HZ median residence times of 7.0 to 13.1 h, nominal flowpath lengths of 0.7 to 3.7 m, and hypoxic conditions (<1.5 mg-O₂ L⁻¹). All 4 receiving wells demonstrated ¹⁵N₂ production during ambient conditions, indicating that the HZ was an environment with active denitrification. The AcO⁻ addition stimulated denitrification as evidenced by significant δ¹⁵N₂ increases by factors of 2.7 to 26.1 in receiving wells, and significant decreases of NO₃⁻ and DO in the two wells most hydrologically connected to the injection. The rate of nitrate removal in the HZ increased from 218 kg ha⁻¹ yr⁻¹ to 521 kg ha⁻¹ yr⁻¹ under elevated AcO⁻ conditions. In all receiving wells, increases of bromide and ¹⁵N₂ occurred without concurrent increases in AcO⁻ indicating that 100% of AcO⁻ was retained or lost in the HZ. These results support the assertion that denitrification in anaerobic portions of the hyporheic zone is limited by labile DOC supply.

3.1 INTRODUCTION

There are many environments where denitrification occurs, but studies have shown that stream systems are particularly efficient at removing and retaining excess nitrogen (N) [Seitzinger et al., 2006] with headwater and mid-network streams being the most effective in regulation of downstream N exports [Peterson et al., 2001;
Alexander et al., 2000; Mulholland et al., 2008]. Characteristic of these headwater and mid-network streams is the prominence of stream water – groundwater (hyporheic, HZ) exchange flux relative to surface water flux [Anderson et al., 2005]. Hyporheic exchange is also known to strongly influence N transformations and cycling in streams by increasing solute residence times and solute contact with reactive biofilms [Duff and Triska, 1990; Holmes et al., 1994; Jones et al., 1995; Wondzell and Swanson, 1996; Valett et al., 1996; Hedin et al., 1998; Hill et al., 1998]. Therefore, it follows that HZ exchange can exert a primary hydrologic control on the export of N from small to mid-network watersheds.

A key factor in the fate of N traveling through a HZ (Figure 3.1) is the organic carbon (C) conditions (substrate quality and quantity) in the stream and HZ [Baker et al., 1999; Kaplan and Newbold, 2000; Sobczak and Findlay, 2002]. In streams, the predominant form of C is dissolved organic carbon (DOC) [Fisher and Likens, 1973] and only a fraction of that DOC is readily labile (i.e., bioavailable) [Swank and Caskey, 1982]. For hyporheic systems, either surface or groundwaters enriched with DOC are advected into hyporheic systems and fuel aerobic and anaerobic hyporheic metabolism [Findlay, 1995; Jones et al., 1995; Baker et al., 2000].

As reviewed in Duff and Triska [2000 and references therein], the occurrence of denitrification in the HZ is complex and is not just related to DOC and NO$_3^-$ availability. Denitrification along HZ flowpaths is also a function of 1. the concentration of DO across the HZ which is controlled by biochemical oxygen demand and advected supply, 2. HZ water temperature because it controls microbial activity and DO saturation in water, and 3. the hydraulics that drive the physical transport and residence time of water such as the head gradient, hydraulic conductivity, advection, and dispersion. Of these factors, the biogeochemical conditions controlling hyporheic denitrification primarily vary by the amount of NO$_3^-$ and quality and quantity of DOC present in the system [Findlay, 1995; Kaplan and Newbold, 2000]. If both NO$_3^-$ and labile DOC are abundant then denitrification rates
can be large [e.g., Holmes et al., 1996, Storey et al., 2004]. Spatial patterning of DOC availability along hyporheic flowpaths adds further to the complexity. DOC and DO are expected to decline rapidly at the head of the HZ flowpaths as microbial processes preferentially utilized DO and labile fractions of DOC [Vervier and Naiman, 1992; Sobczak et al., 2003; Zarnetske et al., 2011]. Further along the HZ flowpaths the labile DOC availability can become depleted leading to DOC quality limitations on denitrification [Sobczak et al., 2003; Zarnetske et al., 2011]. Also, HZ DOC sources from stream and ground water vary with discharge and season [Vervier and Naiman, 1992; Baker and Vervier, 2004] which, in turn, vary HZ metabolism and denitrification.

The use of stable isotope ($^{15}$N) tracers has greatly advanced understanding of aquatic N cycling by allowing the tracing of N through ecosystem pathways experiencing different physical and biological conditions. For example, Böhlke et al. [2004; 2009] and Mulholland et al. [2004, 2008] demonstrated the usefulness of the $^{15}$N tracer approach for determining denitrification rates of streams at the reach scale. More recently, subsurface $^{15}$N tracing has been successfully used to study hyporheic processes associated with reactive N transport [Clilverd et al., 2008; Zarnetske et al., 2011]. Direct hyporheic $^{15}$N addition enables the distinction between microbial assimilation and retention pathways and the respiratory denitrification pathway (i.e., microbial N$_2$ production), which was not possible in previous hyporheic coupled C-N studies that relied on C and N mass-balance approaches [e.g., Hedin et al., 1998; Baker et al., 1999; Sobczak et al., 2003]. On the other hand, previous studies have directly measured HZ denitrification (via acetylene block) across spatial and temporal ranges of DOC and NO$_3^-$ conditions and found that a positive correlation exists between the quantity of labile DOC and N$_2$O production [Baker and Vervier, 2004; Arango et al., 2007]. Though the previous studies on coupled hyporheic DOC and N dynamics consistently indicate that the type and quality of DOC in a hyporheic system
influences denitrification rates, none have both isolated the role of labile DOC supply on denitrification and made direct measurements in a hyporheic environment. We evaluate the role of labile DOC on denitrification along a redox gradient that forms along hyporheic flowpaths in a gravel bar. We combined \(^{15}\text{NO}_3^-\) tracing techniques with an addition of labile DOC to examine the role of DOC in controlling denitrification. The previous work at this study site demonstrated that denitrification and DOC dynamics were closely coupled [Zarnetske et al., 2011]. Under base flow conditions, DOC persisted across all residence times, but denitrification rates and DOC uptake decreased beyond threshold flowpath lengths and residence times (solid curves, Figure 3.2). These spatial patterns of DOC in the HZ showed preferential loss of labile DOC at the head of flowpaths, leaving the less labile fraction to be transported to more distal portions of the flowpath. We utilized these patterns to test the hypothesis that denitrification in the HZ is limited by the supply of labile DOC to the hypoxic-anoxic portions of HZs. We expected that adding labile DOC to the middle of these hyporheic flow paths would increase metabolic processing rates downgradient of the addition such that DO will decrease, \(\text{N}_2\) production will increase, and overall \(^{15}\text{NO}_3^-\) mass export will decrease (dashed curves, Figure 3.2).

3.2 Methods

3.2.1 Study Site

The study site consists of an instrumented gravel bar in Drift Creek (Figure 3.3A), a 3rd-order stream within the Willamette River basin in western Oregon, USA (44.9753°N, 122.8259°W). The drainage basin is 6517 ha, and has mixed land-use dominated by agriculture (lower catchment) and forestry (upper catchment). The basin’s population is primarily rural and residences are serviced by septic-systems, another potential source of N in the study stream. Annual precipitation is 1190 mm and comes predominantly during the winter as rain. Base flow discharge gradually decreases to an annual minimum (< 50 L s\(^{-1}\)) in early September. The study reach was
modified by channelization in the past, as were many of the streams in this agricultural region. The channelized stream is incised into competent bedrock (andesite flow breccias) and is now separated from its flood plain. The active channel is 5-20 m wide and is bounded by steep banks 3-5 m high. The alluvial thickness above bedrock (as depth to refusal) varies from 0 to ≥ 1.5 m, which constrains the extent of the hyporheic zone. The study reach containing the instrumented gravel bar has a slope of 0.007 m m⁻¹ and the morphology is primarily a planebed channel with occasional pool-riffle sequences [see Montgomery and Buffington, 1997 for definitions of channel types].

The stream bed and instrumented gravel bar consists of poorly sorted sand, gravel, cobbles, and boulders.

The hyporheic zone study site is a lateral gravel bar approximately 6.1 m by 4 m (Figure 3.3B) with a riffle on one side and connected to the bedrock channel bank on the other side. The gravel bar separates two pools and spans a head loss across the riffle of 0.135 m. The alluvium comprising this gravel bar was uniformly 1.2 m thick. This gravel bar was instrumented with a well network (n = 11) of 3.8-cm-I.D. schedule 40 PVC wells screened 0.2 – 0.4 m below ground surface. The observed and modeled subsurface exchange across this gravel bar primarily occurs along lateral flowpaths from the head to the tail of the bar (Figure 3.3B). A previous investigation demonstrated that all wells are connected to stream water and that well waters come from the stream and not the local groundwater aquifer [Zarnetske et al., 2011]. Based upon multiple tracer tests at the study site, the hydraulic conductivity of the gravel bar is 4.03 to 6.63 m d⁻¹.

3.2.2 Tracer Experiment

A 48 h steady-state well injection of a conservative tracer, Cl⁻, and, ¹⁵NO₃⁻ was used to quantify ambient HZ denitrification via ¹⁵N₂ production. Following ambient plateau measurements of denitrification during the first 24 h, a second conservative tracer, Br⁻, and labile DOC source, acetate (AcO⁻), were co-injected for an additional 24 h to measure HZ denitrification under increased labile DOC supply (see
experimental design, Figure 3.4). AcO$^-$ was selected because 1. it is naturally produced and consumed in many aquatic systems, including HZs [Chapelle and Bradley, 1996; Baker et al., 1999], 2. it is highly bioavailable to microbes [Drake, 1994], 3. it is highly soluble (NaAcO solubility at 20° C = 464 g L$^{-1}$), and 4. has demonstrated low sorption potentials in natural stream sediments (e.g., $K_d \approx 0$, [Baker et al., 1999]). Further, AcO$^-$ was used as a pure DOC source so as to avoid the confounding factor of using more complex natural DOC sources (e.g., leaf leachate) which contains additional nutrients [Sobczak et al., 2003].

The injection experiment was performed on 1-3 September 2008 when stream discharge and hyporheic head gradients were stable. The injection consisted of two steady-state experimental periods – “Pre-DOC” and “DOC.” During the Pre-DOC period, an injection solution of $^{15}$NO$_3^-$ (as 99% enriched K$^{15}$NO$_3$) and Cl$^-$ (as NaCl$^-$) was released at a constant rate (3 mL min$^{-1}$) using a metering pump (FMI QG150, Fluid Metering, Inc., Syosset, NY, USA; note that the use of trade names in this publication is for reader information and does not imply endorsement by the U.S. Department of Agriculture of any product or service) into the upgradient injection well, H1, for 47.75 h starting at 14:45 on 1 September. During the DOC period, an injection solution of conservative tracer Br$^-$ (as KBr) and NaAcO was released at a constant rate (6 mL min$^{-1}$) into H1 with a second FMI QG150 metering pump for 21.17 h starting at 17:20 on 2 September.

Based upon pilot studies of dilution factors between the injection site and the downgradient receiving wells, the injected solutes needed to be substantially more concentrated than the ambient HZ conditions. The $^{15}$NO$_3^-$ introduced at the injection site, H1, had a concentration of 30.7 mg L$^{-1}$, which was calculated to produce a maximum hyporheic $\delta^{15}$N enrichment plume of 40 000‰ in the HZ water NO$_3^-$ leaving the injection site. The Cl$^-$ injection concentration was 4 367 mg L$^{-1}$ based upon an addition target to elevate the background HZ Cl$^-$ by 1 260 % and generate clearly detectable electrical conductivity (EC) increase in downgradient HZ flowpaths.
The AcO\textsuperscript{−} injection concentration was 504 mg L\textsuperscript{−1}, while the Br\textsuperscript{−} injection concentration was 50 mg L\textsuperscript{−1}. During the entire injection time, the injection well water column was continually mixed via closed-system peristaltic pumping to ensure full solute mixing and equal concentration release across the entire screened well section (0.2-0.4 m below ground surface). The low injection pump rates during the experiment did not create a detectable increase in head elevation in well H1.

We used EC to measure the real-time Cl\textsuperscript{−} transport between the source well and downgradient receiving wells. The EC measurements were taken every 60 s in 8 wells (G1, H1, H2, I1, J1, J2, J3, K2, K3) and in the stream water at the head and tail of the gravel bar to detect if stream EC varied during the experiment. These EC measurements were made with 10 multiplexed, in situ, CS547A conductivity and temperature probes connected to a CR1000 (Campbell Scientific, Logan, Utah, USA). These EC measurements were used to characterize the conservative solute transport dynamics including flow rates, flow paths, and residence times as well as to inform the timing of the sampling regime described below.

The water sampling regime consisted of collecting multiple rounds of hyporheic samples during the three phases of the experiment – 1. Pre-injections, 2. Pre-DOC plateau (\textsuperscript{15}NO\textsubscript{3}\textsuperscript{−} and Cl\textsuperscript{−} steady-state), and 3. DOC plateau (AcO\textsuperscript{−} and Br\textsuperscript{−} steady-state). For each location (9 wells plus stream water at the gravel bar head), repeated sampling occurred during the pre-injections (n = 3), Pre-DOC plateau (n = 5), and during DOC amendment (n = 5) periods (Figure 3.4). The Pre-DOC plateau sampling period was initiated at 18.5 h after \textsuperscript{15}NO\textsubscript{3}\textsuperscript{−} and Cl\textsuperscript{−} injection started when all hyporheic wells demonstrated steady-state EC. Following the Pre-DOC Cl\textsuperscript{−} transport times, the DOC amendment sampling period was initiated at 18.5 h after AcO\textsuperscript{−} and Br\textsuperscript{−} injected started. Repeated hyporheic samples were collected approximately every ~2 h during each respective plateau period. All collected samples were analyzed for solutes relevant to denitrification and tracing injection solutes (δ\textsuperscript{15}NO\textsubscript{3}, δ\textsuperscript{15}N\textsubscript{2}, as well as concentrations of NO\textsubscript{3}\textsuperscript{−}, AcO\textsuperscript{−}, total DOC, DO, Cl\textsuperscript{−}, and Br\textsuperscript{−}). Hydraulic transport
parameters (head, flow rates, flowpaths, and residence times) were also measured along the instrumented HZ.

Hyporheic well samples were collected with a field peristaltic pump (Masterflex L/S, Vernon Hills, Illinois, USA) [Woessner, 2007]. All water samples were immediately filtered through ashed Whatman GF/F glass fiber filters (0.7 μm pore size) into acid washed HDPE bottles (60 mL for nutrient chemistry and 1 L for δ^{15}N isotope samples). Following filtering, nutrient chemistry samples and isotope samples were stored on ice in the field and later refrigerated at 4°C or frozen in the laboratory until processed and analyzed. DO concentrations were measured in situ with a calibrated YSI DO Meter (Model 52) at all locations prior to collecting each round of samples. Samples were also collected for δ^{15}N_{2O} (g), but could not be analyzed due to technical problems at the stable isotope laboratory. Nonetheless, denitrification in freshwater and near shore marine system sediments consists almost entirely of N_{2} production with N_{2}O/N_{2} production ratios generally between < 0.001 and < 0.05 [Seitzinger, 1998; Mulholland et al., 2004], so δ^{15}N_{2} by itself is capable of characterizing the majority of the denitrification dynamics.

The δ^{15}N gas collection for each sample occurred in the field and followed procedures adapted from Hamilton and Ostrom [2007]. A low-flow peristaltic pump was used to collect 80 mL water samples into a 140 mL plastic syringe (Becton-Dickinson, Franklin Lakes, NJ, USA) fitted with stopcocks. All detectable bubbles were expelled to create a zero headspace. Sample syringes were submerged under water in a processing tub kept at stream temperature to avoid atmospheric N contamination. An underwater transfer of 40 mL high purity He was added to each sample syringe. Equilibration of the N_{2} (g) into the He headspace of each sample was achieved by gently agitating the syringes for 10 min. After equilibration, 14 mL of headspace gas was injected into preevacuated 12 mL exetainers (Labco Ltd., Wycombe, UK). Preevacuation of exetainers was achieved by pumping them down to a pressure of < 50 mTorr using a Welch vacuum pump (Model DirectTorr 8905,
Skokie, Illinois, USA). All exetainers were stored underwater in He purged DI water-filled centrifuge tubes until sample collection. All sample-filled exetainers were returned to their zero headspace He purged DI water-filled centrifuge tubes for storage until analysis.

During the tracer experiment period, we collected detailed surface water and channel surface topography data around the instrumented gravel bar site using a Topcon total station (Model GTS-226, Livermore, California, USA). The applied standard surveying methods had a spatial resolution of $x \leq 0.1$ m, $y \leq 0.1$ m, $z \leq 0.005$ m for the instrumented gravel bar site.

### 3.2.2.3 Laboratory Procedures

Stream and hyporheic samples were analyzed for NO$_3^-$-N, DOC-C, AcO$^-$ (CH$_3$COO$^-$), Cl$^-$, and Br$^-$ at the Oregon State University Institute for Water and Watersheds Collaboratory (Corvallis, USA). The NO$_3^-$-N measurements were made by a Technicon Auto-Analyzer II using standard colorimetric methodology with detection limits of 0.001 mg L$^{-1}$. The concentration of total DOC was determined with a Shimadzu TOC-VCSH Combustion Analyzer (Tokyo, Japan; detection limit = 0.05 mg L$^{-1}$). The AcO$^-$, Cl$^-$, and Br$^-$ were determined by ion chromatography (Dionex 1500, Sunnyvale, California, USA; detection limit = 0.01 mg L$^{-1}$).

The $^{15}$N content of the stream and hyporheic water NO$_3^-$ was determined by methods adapted from Sigman et al. [1997] and Mulholland et al. [2004]. These methods are briefly summarized below. Prior to $^{15}$N analysis, $^{15}$NO$_3^-$ samples with blanks and standards were processed as follows: 1. a volume of each sample (0.25 – 1 L; processing volume is dependent on N content of each sample) was stripped of its dissolved NH$_4^+$ and had its NO$_3^-$ concentrated, 2. the concentrated sample NO$_3^-$ was captured on a prepared filter via a reduction/diffusion/sorption procedure (full reduction of NO$_3^-$ to NH$_4^+$, which is then converted to NH$_3$ that diffuses into the headspace and ultimately gets captured on the acidified sorption filter), and 3. after complete transfer of NO$_3^-$ to the sample filter, the samples were sealed and sent for
$^{15}$NO$_3^-$ analysis. All $^{15}$NO$_3^-$ and $^{15}$N-gas samples were analyzed by the Marine Biological Laboratory Stable Isotope Facility (MBL, Woods Hole, Massachusetts, USA). Data are reported using delta notation, where

$$\delta^{15}N = 1000 \times \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right)$$

and $R$ is the ratio of $^{15}$N:$^{14}$N in the sample or standard (atmospheric gas). Replicate analyses of the water and gas samples show the precision of $\delta^{15}$NO$_3^-$ and $\delta^{15}$N$_2$ isotope measurements is ± 80.0 ‰ and ± 0.2 ‰, respectively.

3.2.4 Parameter and Statistical Calculations

For all downgradient wells receiving a detectable EC increase from the injection site, the EC breakthrough curves (as a measure of Cl$^-$ transport) were used to calculate the median residence time of the HZ water flowpaths. The median residence time was calculated as the time required to raise the EC in the well to one half the plateau EC. The nominal flowpath length was measured as linear distance between the injection site and each downgradient well.

The connectivity of the receiving wells to the injection well was based on the conservative tracer (Cl$^-$ and Br$^-$) transport during the experiment. The hydrologic connectivity ($D$) to the injection well at steady state, i.e., the fraction of water arriving at the receiving well that originated at the injection, was calculated as:

$$D_{x,t} = \frac{c_{x,t} - c_{x,t=0}}{c_{inj} - c_{x,t=0}},$$

where $c$ is the concentration of the conservative tracer Cl$^-$, $x$ is the well location, $t$ is the time of sample, and $inj$ is the injection site. In the absence of biological or chemical removal pathways, the reactive compounds and conservative tracer transport should be identical. Based upon this assumption, we used $D$ to calculate the predicted
reactive solute concentrations for NO$_3^-$, DOC, and AcO$^-$ in each of the receiving wells before and during the AcO$^-$ augmentation period as:

$$S_{\text{pred},x,t} = S_{\text{inj}} D_{x,t} + S_{x,t=0} (1 - D_{x,t}) ,$$

where $S_{\text{pred}}$ is the predicted concentration of the solute of interest at steady-state. We then calculated the difference between the measured and predicted reactive solute concentrations for each well during the AcO$^-$ augmentation plateau conditions. NO$_3^-$, DOC, and AcO$^-$ removal occurs when the observed concentration is less than the predicted concentration and production occurs when the observed concentration is greater than the predicted concentration [Hedin et al., 1998; Baker et al., 1999]. Further, if the difference between predicted and observed NO$_3^-$ concentration increases following AcO$^-$ augmentation, it indicates increased rates of NO$_3^-$ removal along the HZ flowpath when AcO$^-$ was added to the system.

We compared changes in concentrations of solutes for the Pre-DOC and DOC treatment periods using a paired t-test (significance at $p = 0.05$, df = 4). We treated repeated samples within a well as independent replicates. We ran paired t-tests to determine how strongly means of Pre-DOC and means of DOC differed in each well. We excluded wells H3, I1, I3, J1, and J3 because they did not receive measurable concentrations of the added solutes.

### 3.3 RESULTS

**3.3.1 Background Stream and Hyporheic Biogeochemical Conditions**

Stream flow conditions were relatively stable over the experiment with a mean flow of 32 L s$^{-1}$ and a variance of ± 3.2 L s$^{-1}$. This variance in flow did not create any detectable change in the measured head across the gravel bar during the experiment. Stream and HZ water temperature ranged between 12.8 and 15.8$^\circ$ C during the injection experiment. Measured stream surface water nutrient and chemistry
conditions were stable during the experiment with mean and 1 standard deviation \((n = 13)\) values of \(\text{NO}_3^-\) \((0.61 \pm 0.01 \text{ mg-N L}^{-1})\), \(\text{AcO}^-\) \(< 0.01 \pm 0.005 \text{ mg L}^{-1}\), \(\text{DOC}\) \((1.94 \pm 0.36 \text{ mg-C L}^{-1})\), \(\text{DO}\) \((10.62 \pm 0.98 \text{ mg-O}_2 \text{ L}^{-1})\), \(\text{Cl}^-\) \((3.60 \pm 0.25 \text{ mg L}^{-1})\), \(\text{Br}^-\) \((0.010 \pm 0.005 \text{ mg L}^{-1})\) and pH \((6.8 \pm 0.2)\).

Background hyporheic conditions collected immediately prior to the start of the injection experiment showed flow and biogeochemical conditions consistent with the previous study by Zarnetske et al. [2011]. HZ flowpaths originated at the head of the gravel bar and are discharged to the stream at the tail of the gravel bar (Figure 3.3B). The biogeochemical conditions showed that the proximal ends of HZ flowpaths were characterized as oxic while distal ends were anoxic and indicated net \(\text{NO}_3^-\) removal (Table 3.1). The supply of \(\text{DOC}\) and \(\text{DO}\) to the HZ is primarily from stream water advecting into the sediment at the head of the gravel bar (Figure 3.5E & 3.5G, respectively). The \(\text{DO}\) and \(\text{DOC}\) removal rates were greatest in the first 2 m of HZ transport, but removal persisted across the entire gravel bar. While the patterns were similar along the HZ flowpaths of the earlier study [Zarnetske et al., 2011; solid curves in Figure 3.2], there were differences. No \(\text{AcO}^-\) was detected in the stream or hyporheic water above the detection limit of 0.01 mg L\(^{-1}\). Both \(\text{Cl}^-\) and \(\text{Br}^-\) were present before the experiment, but did not vary between stream and hyporheic waters or among wells (Table 3.1). Relative to the previous study, the background \(\text{DOC}\) conditions were lower in stream and HZ waters and a strong nitrification region at the head of the HZ flowpaths was not present.

### 3.3.2 Solute Transport and Retention: Pre-DOC Addition

Four of the nine downgradient wells were hydrologically connected to the H1 well injection site as determined by a significant increase \((p < 0.05\); t-test; \(df = 4\)) in \(\text{Cl}^-\) during the first Pre-DOC injection plateau period (H2, J2, K2, K3; Figures 3.5 & 3.6). The steady-state hydrologic connectivity \((D)\) to the injection was 0.013 at J2, 0.0020 at K2, and 0.0090 at K3 (Table 3.2). (Note that the tracer injection rate was very low and was diluted into a large volume of HZ water flowing through the gravel
bar. Still, the relatively high concentration of conservative and $^{15}$N tracers resulted in distinct breakthroughs, despite the apparent low connectivity). The H2 well was connected to the injection well, but the level of connectivity varied between Pre-DOC and DOC periods thereby failing the experimental assumption of steady-state flow dynamics between the experimental injection periods as shown in Section 3.3.3. EC did not vary significantly between Pre-DOC and DOC periods in the other capture wells (data not shown but see Cl$^-$ in Figure 3.5A & 3.5B). Nominal flowpath lengths from the injection site to the receiving wells ranged from 2.18 m (J2) to 3.66 m (K3). The median hyporheic transport times from the injection site to J2, K2, and K3 were 9.8, 11.3, and 13.3 h, respectively (Table 3.2).

The level of conservative tracer Cl$^-$ connectivity consistently corresponded with the level of $^{15}$NO$_3^-$ reaching the receiving wells ($\delta^{^{15}}$NO$_3^-$‰ = 721.8 * [Cl$^-$ mg L$^{-1}$] – 2248, $r^2 = 0.999$, n = 30), so the $^{15}$NO$_3^-$ tracer spatial plume is the same as the Cl$^-$ plume shown in Figure 3.5. Nitrate concentrations were differentially altered by the K$^{^{15}}$NO$_3$ addition. The two most connected wells showed increased NO$_3^-$ concentration (J2 increased from 0.41 to 0.50 mg-N L$^{-1}$ and K3 increased from 0.43 to 0.49 mg-N L$^{-1}$) and there was no change in the least connected well K2 (Table 3.2 & Figure 3.5G). The DOC in three connected wells was lower than background conditions. During this period no AcO$^-$ was detected in any of the repeated sampling rounds (Table 3.2). $^{15}$NO$_3^-$ tracer enrichment and $^{15}$N$_2$ production via denitrification were also detected at significant but varying levels in each of the receiving wells (Figures 3.5, 3.6).

### 3.3.3 Solute Transport and Retention: Post-DOC Addition

The steady-state conditions of $^{15}$NO$_3^-$ and Cl$^-$ tracer addition was confirmed as we detected no significant differences ($p > 0.05$; t-test, df = 4; Figure 3.6) between the Pre-DOC and DOC Cl$^-$ concentrations at 3 of the 4 connected wells (J2, K2, K3). Well H2 had a significantly higher Cl$^-$ concentration and $^{15}$NO$_3^-$ enrichment during the DOC period compared to the Pre-DOC period (Figure 3.7). Therefore, H2 is not used
for comparison between Pre-DOC and DOC treatment periods of the experiment. Of the connected wells, H2 is located adjacent and closest to the injection site (nominal flowpath length = 0.67 m; Figure 3.5). The second conservative tracer, Br−, injected with the AcO− showed the same dilution and downgradient tracer plume behavior as the Cl− tracer. The injected AcO− during the DOC addition period formed a plume from the injection site that behaved similar to the conservative tracers (Figure 3.5). However, the elevated total DOC concentrations did not persist along the flowpaths and were near Pre-DOC conditions at the most distal downgradient receiving well, K2. Accounting for the AcO− injected into the HZ and dilution along the flowpaths to each well showed that all of the AcO− was retained in the HZ, with as much as 6.36 mg-AcO− L−1 being retained along flowpath between wells H1 and J2 (Table 3.2).

The DO, NO3−, and δ15N2 conditions changed significantly during the DOC addition. The DO in the receiving wells all showed significantly decreased concentrations under elevated labile DOC conditions creating more anoxic conditions in the three receiving wells (p < 0.05; t-test; df = 4; Figure 3.6). The mean NO3− concentration decreased in all three receiving wells, with highly significant decreases in NO3− seen in the two most connected wells (p < 0.001 for K3 and J2, Figure 3.6). This decrease in NO3− resulted in overall NO3− conditions lower than the background conditions, and after accounting for dilution showed that NO3− was retained along the flowpaths at levels between 0.05 and 0.37 mg-N L−1. The δ15NO3− enrichment did not vary significantly following the addition of AcO−, but the δ15N2 signature increased significantly in all receiving wells with δ15N2 enrichment increasing up to 26.1 times that of the Pre-DOC levels (p < 0.001; t-test; df = 4; Figure 3.6).

3.4 DISCUSSION

This study shows that labile DOC limits denitrification along hyporheic flowpaths and that NO3− losses can be attributed in part to denitrification in this HZ. The tracing of the denitrification pathway of 15NO3− to 15N2 under ambient and
elevated AcO\(^-\) (as labile DOC) conditions confirm earlier theoretical- and observation-based conclusions regarding the role of HZ DOC quantity and quality on denitrification dynamics in hyporheic systems [Hedin et al., 1998; Baker et al., 1999; Hill et al., 2000; and Sobczak and Findlay, 2002].

The HZ conditions and steady-state experimental design allowed us to evaluate our hypotheses about the role of labile DOC in HZ denitrification (Figure 3.2). The post-DOC conditions clearly show that the addition of labile DOC to this HZ increased metabolic processing rates downgradient of the addition such that the additional labile DOC was utilized and removed rapidly (Figure 3.5). The utilized labile DOC stimulated additional aerobic respiration as seen in the increased DO deficit conditions (Figure 3.6). Consequently, the increased anaerobic conditions and elevated supply of carbon substrate, lead to significant increases in denitrification rates (Figure 3.6). Overall, the addition of labile DOC resulted in a significant increase in the total NO\(_3^-\) mass removed by this gravel bar HZ (Figure 3.6).

### 3.4.1 Hyporheic Zone as a Stream DOC Sink

This study shows that the HZ is a sink for stream DOC and that in the HZ, the labile fraction of the DOC is preferentially utilized and retained. We observed a DOC gradient across the gravel bar flowpaths. At the head of the gravel bar, the hyporheic water DOC concentrations are similar to the DOC of surface waters, but hyporheic DOC concentration consistently declined along the flowpaths (Figure 3.5). Spatial declines of HZ DOC along flowpaths are consistently seen in gravel bar hyporheic investigations where the major source of HZ DOC is stream water [e.g., Vervier and Naiman, 1992; Findlay, 1993]. We also know that, in this gravel bar, the lability of DOC declines along the flowpath and at a rate greater than the decline of total DOC [Zarnetske et al., 2011]. This labile DOC pattern is also consistent with previously documented natural and experimental hyporheic systems where DOC quality was measured [e.g., Sobczak and Findlay, 2002; Sobczak et al., 2003].
A portion of the DOC advected through the gravel bar is utilized for microbial aerobic and anaerobic respiration, as shown by the rapid depletion of DO and the ambient levels of denitrification occurring along the flowpaths (Figure 3.5 and 3.6). We also know that despite the large addition of AcO$^-$ to this gravel bar, no above-background AcO$^-$ concentrations were detected at any downgradient points before or during the AcO$^-$ addition. The 100% retention of the AcO$^-$ across a 24 h plateau injection period (i.e., the DOC, as AcO$^-$, uptake capacity was not achieved) combined with the known low sorption potential and high solubility of AcO$^-$ [e.g., Baker et al., 1999] indicates that in this HZ, metabolism is strongly limited by DOC supply even under the elevated DOC conditions of the experiment. The controlled experimental observations of DOC limitation on HZ metabolism in this study mirror the findings of Jones [1995] and Baker et al. [2000] who observed that natural variations in stream-sourced DOC supply correlated with HZ metabolism and N transformation rates.

3.4.2 Labile DOC Supply Controls Hyporheic NO$_3^-$ Dynamics

The HZ denitrification rates are DOC substrate-limited in this gravel bar as documented along the extended flowpaths leading to wells J2, K2, and K3, where all other conditions for denitrification are present except for a sufficient source of labile DOC to serve as an electron donor. This was proven under controlled, steady-state conditions where just a change in the labile DOC availability resulted in more extensive anaerobic conditions and increased denitrification rates (both NO$_3^-$ concentrations decreased and $\delta^{15}$N$_2$ signature increased significantly (Figure 3.5 and 3.6)).

The HZ DOC and NO$_3^-$ dynamics fit the conceptual model of Findlay [1995], which hypothesizes that the influence of the HZ at the reach scale is a function of two variables – the rate of hydrologic exchange in and out of the HZ and the rate of biogeochemical processes in the HZ. In this system, we see that denitrification is limited by the amount of labile DOC supplied via hydrologic exchange from the DOC-rich surface waters. No lateral hillslope or groundwater inflow has been detected at
this gravel bar site. Thus, surface water is the primary source of DOC to this HZ at summer low flow. Consequently, the denitrification in this system will vary primarily as a function of the quantity and quality of the DOC in the surface water and the hydrologic conditions promoting hyporheic exchange across the gravel bar. During a prior investigation on the same gravel bar [Zarnetske et al., 2011], the HZ had a much greater NO$_3^-$ removal efficiency with approximately 99% of the NO$_3^-$ removed along flowpaths traversing the gravel bar versus only 57% in this study. During the previous study, hydrologic exchange conditions were similar (i.e., head gradient, hydraulic conductivity, and residence time) as were the ambient stream NO$_3^-$ concentrations (0.54 mg L$^{-1}$ and 0.61 mg L$^{-1}$, respectively), but a key difference was that stream DOC concentration entering the HZ was much higher – 3.01 mg L$^{-1}$ in the previous study versus 1.94 mg L$^{-1}$ in the current study. The larger flux of DOC to the head of the gravel bar during the earlier study also stimulated greater DO consumption rates. These higher DO consumption rates lead to the development of anoxic conditions occurring over shorter flowpath lengths than the present study. Therefore, a larger portion of the HZ had reducing conditions conducive to denitrification in the Zarnetske et al. [2011] study. However, after adding the labile DOC source to the HZ in this study we see that the gravel bar becomes more anoxic and NO$_3^-$ removal efficiency almost doubled from the Pre-DOC to the DOC periods (Table 3.2 and Figure 3.6).

The addition of the labile DOC along mid-flowpaths also changed the transport of advected stream DOC through the gravel bar. There was higher total DOC concentrations in receiving wells J2 and K3 during the acetate addition period (Figure 3.6). The acetate addition did not directly add to the measured total DOC concentrations as no acetate was recovered in the down gradient wells. However, the acetate may have indirectly increased downgradient total DOC concentrations in two ways. First, the acetate is preferentially consumed over many other stream-sourced DOC compounds [Hall and Meyer, 1998], which could decrease the uptake rate of
stream-sourced DOC. Second, the physical adsorption of hyporheic DOC is concentration-dependent [Findlay and Sobczak, 1996] and leads to DOC immobilization [Fiebig and Lock, 1991]. The subsurface acetate addition may have filled many of the DOC sorption sites with acetate. Thus decreasing the number of available DOC sorption sites along the flowpaths, and decreasing the uptake rate of stream-sourced DOC. Together the preferential uptake of acetate and decrease in available DOC sorption sites, in effect, would shunt the stream-sourced DOC further down the flowpaths resulting in the higher total DOC concentrations in J2 and K3 during the acetate injection period.

We conclude that, with sufficient upgradient supply of labile DOC entering HZ environments, the HZ can play very significant role in regulating downstream NO$_3^-$ export in Drift Creek. To demonstrate this point, we compare ambient NO$_3^-$ removal rates to the DOC augmented removal rates at the gravel bar site. A calibrated groundwater flow model of the HZ site shows a minimum of 226 L m$^{-2}$ d$^{-1}$ of hyporheic exchange flow occurs under the hydrologic conditions of the DOC experiment [Chapter 4]. Given this hydrologic exchange flow and the background observed NO$_3^-$ retention rates, the NO$_3^-$ mass removal rate at this gravel bar at this time is 218 kg ha$^{-1}$ yr$^{-1}$. This removal rate is within the range of previous surface water - groundwater exchange studies (e.g., 10 - 39 kg ha$^{-1}$ yr$^{-1}$, Lowrance et al., [1997]; up to 6600 kg ha$^{-1}$ yr$^{-1}$, Hedin et al., [1998]). In comparison to the NO$_3^-$ and DOC values seen in these previous studies, the ambient NO$_3^-$ and DOC in our system was an order of magnitude lower for both N and C constituents. The decreased availability of NO$_3^-$ and DOC alone can limit the potential for total NO$_3^-$ removal via denitrification compared to the previous investigations. However, by artificially increasing only the supply of labile HZ DOC to our receiving wells (i.e., not the entire gravel bar), we were able to increase the NO$_3^-$ removal rate by 303 kg ha$^{-1}$ yr$^{-1}$ to a total of 521 kg ha$^{-1}$ yr$^{-1}$. This is an increase of 139% above background removal rates.
The injection of labile DOC in this experimental gravel bar is akin to groundwater – surface water environments where a DOC-rich groundwater or riparian flowpath converges with other hyporheic flowpaths. Hedin et al. [1998] and Hill et al. [2000] observed high denitrification rates where hydrologic conditions promoted mixing of DOC-rich groundwaters with DOC-poor waters that contained NO$_3^-$. Therefore, the formation of these denitrification hot spots is governed by the complex groundwater hydraulics that mix waters containing DOC and NO$_3^-$ [McCain et al., 2003]. In this gravel bar HZ, the ambient DOC gradient is the result of an imbalance between the hydrologic transport and biogeochemical reaction kinetics - the advected supply rate is less than the biochemical demand rate for labile DOC. By experimentally manipulating the labile DOC gradient with an acetate addition we altered the balance between transport and reaction kinetics and created a denitrification hot spot and altered total DOC transport in this HZ.

This experiment also indicates that anthropogenic (intentional and unintentional) or natural additions of labile DOC to a HZ system will facilitate greater denitrification if NO$_3^-$ supply is not limiting. Similar to groundwater remediation practices (e.g. denitrification walls sensu Schipper and Vojvodic-Vukovic [1998]), strategic additions of a labile DOC source to the near stream environment could mitigate NO$_3^-$ flux into a stream system [Pfenning and McMahon, 1996, Hedin et al., 1998], but that would require extensive understanding of the complex hydrologic flowpaths and flow rates leading to the denitrification sites.

3.5 CONCLUSIONS

We showed that hyporheic environments are an important sink for both DOC and reactive N in freshwater ecosystems and that the fate of reactive N, in this case denitrification, is tightly coupled to C dynamics. The in situ steady-state labile DOC and $^{15}$NO$_3^-$ addition experiment definitively showed that denitrification is occurring in
the HZ of this upland agricultural stream and that the availability of labile C strongly limits the overall denitrification potential.

In this study we looked at a known denitrification hot spot (gravel bar) and created a denitrification hot moment [sensu McCain et al., 2003] by augmenting the supply of labile DOC. The prospect of identifying these denitrification hot spots and hot moments in aquatic ecosystems and denitrification models is improving, especially with improved and growing available high temporal and spatial resolution data sets [Groffman et al., 2009]. We echo Gruber & Galloway [2008]: C and N cycles are coupled in river systems and they should be evaluated and modeled as coupled processes. The use of total DOC in hyporheic denitrification models is likely to overestimate denitrification potentials, especially if all the DOC is considered available for microbial processes. This study indicates that future hyporheic denitrification investigations should measure and account for the labile fraction of DOC and not just the total DOC.
ACKNOWLEDGMENTS

Support for this project was primarily provided by a NSF Ecosystem Informatics IGERT fellowship (grant DGE-0333257) to JPZ, research grants from the OSU Institute for Water and Watersheds and a North American Benthological Society Endowment Fund to JPZ, and the NSF grants EAR-0409534 and EAR-0409591 to RH, SMW, and MAB. Additional support was provided by the Hollis M. Dole Environmental Geology Fund at OSU. Any opinions, findings and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the NSF. We thank the Harry Klopfenstein Farm for generously granting access to the Drift Creek research sites. Special thanks to: V. Adams, P. Zarnetske, and J. Yin for field assistance, and C. Jones and K. Motter of CCAL and OSU IWW Collaboratory for help with analyzing general water chemistry.
Table 3.1: Ambient stream and hyporheic temperature and biogeochemical parameters (reported as means, n = 3), and nominal transport distance from the H1 injection site to each well ("-" and "+" denote up-gradient and down gradient of H1).

<table>
<thead>
<tr>
<th>Location</th>
<th>Temperature (°C)</th>
<th>DO (mg-O₂ L⁻¹)</th>
<th>NO₃⁻ (mg-N L⁻¹)</th>
<th>DOC (mg-C L⁻¹)</th>
<th>Acetate (mg-AcO⁻ L⁻¹)</th>
<th>Cl⁻ (mg L⁻¹)</th>
<th>Br (mg L⁻¹)</th>
<th>Nominal distance from injection (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stream</td>
<td>12.8</td>
<td>10.62</td>
<td>0.612</td>
<td>1.94</td>
<td>&lt;0.01</td>
<td>3.596</td>
<td>0.011</td>
<td>-0.5</td>
</tr>
<tr>
<td>G1</td>
<td>13.1</td>
<td>4.24</td>
<td>0.433</td>
<td>1.54</td>
<td>&lt;0.01</td>
<td>3.497</td>
<td>0.011</td>
<td>-1.54</td>
</tr>
<tr>
<td>H1 (Inj Site)</td>
<td>13.2</td>
<td>4.31</td>
<td>0.453</td>
<td>1.14</td>
<td>&lt;0.01</td>
<td>3.485</td>
<td>0.010</td>
<td>0</td>
</tr>
<tr>
<td>H2</td>
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<td>2.5</td>
<td>0.447</td>
<td>1.15</td>
<td>&lt;0.01</td>
<td>3.528</td>
<td>0.014</td>
<td>+0.67</td>
</tr>
<tr>
<td>H3</td>
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<td>0.83</td>
<td>0.37</td>
<td>0.85</td>
<td>&lt;0.01</td>
<td>3.818</td>
<td>0.016</td>
<td>+1.5</td>
</tr>
<tr>
<td>I1</td>
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<td>0.477</td>
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<td>3.537</td>
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</tr>
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<td>0.394</td>
<td>0.91</td>
<td>&lt;0.01</td>
<td>3.471</td>
<td>0.021</td>
<td>+1.75</td>
</tr>
<tr>
<td>J1</td>
<td>14.1</td>
<td>0.76</td>
<td>0.317</td>
<td>0.8</td>
<td>&lt;0.01</td>
<td>3.421</td>
<td>0.018</td>
<td>+1.97</td>
</tr>
<tr>
<td>J2</td>
<td>14</td>
<td>0.88</td>
<td>0.413</td>
<td>0.84</td>
<td>&lt;0.01</td>
<td>3.461</td>
<td>0.012</td>
<td>+2.18</td>
</tr>
<tr>
<td>J3</td>
<td>13.5</td>
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<td>0.45</td>
<td>0.81</td>
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<td>3.500</td>
<td>0.012</td>
<td>+2.83</td>
</tr>
<tr>
<td>K2</td>
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<td>0.7</td>
<td>0.348</td>
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<td>&lt;0.01</td>
<td>3.440</td>
<td>0.011</td>
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<td>K3</td>
<td>13.7</td>
<td>1.31</td>
<td>0.43</td>
<td>0.86</td>
<td>&lt;0.01</td>
<td>3.479</td>
<td>0.010</td>
<td>+3.66</td>
</tr>
</tbody>
</table>
Table 3.2: Steady-state DOC augmentation period transport times, well hydrologic connectivity, and solute responses for the 3 wells hydrologically connected to H1. Note: Solute background values are means \((n = 3)\) and Pre-DOC and DOC period values are means \((n = 5)\).

<table>
<thead>
<tr>
<th>Hyporheic Condition</th>
<th>Location</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K2</td>
<td>K3</td>
<td>J2</td>
<td></td>
</tr>
<tr>
<td>Connectivity to injection, (D) ((\cdot))</td>
<td>0.002</td>
<td>0.009</td>
<td>0.013</td>
<td></td>
</tr>
<tr>
<td>Median transport time from injection (h)</td>
<td>11.3</td>
<td>13.3</td>
<td>9.8</td>
<td></td>
</tr>
<tr>
<td>(\text{NO}_3^-) (mg-N L(^{-1}))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Background</td>
<td>0.35</td>
<td>0.43</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>Pre-DOC</td>
<td>0.35</td>
<td>0.49</td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td>with DOC Predicted, (S)</td>
<td>0.40</td>
<td>0.73</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td>Observed</td>
<td>0.35</td>
<td>0.38</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>Retention</td>
<td>-0.05</td>
<td>-0.35</td>
<td>-0.37</td>
<td></td>
</tr>
<tr>
<td>(\text{DO}) (mg-O(_2) L(^{-1}))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Background</td>
<td>0.70</td>
<td>1.31</td>
<td>0.88</td>
<td></td>
</tr>
<tr>
<td>Pre-DOC</td>
<td>0.93</td>
<td>1.38</td>
<td>1.06</td>
<td></td>
</tr>
<tr>
<td>with DOC Predicted, (S)</td>
<td>0.80</td>
<td>1.34</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>Observed</td>
<td>0.79</td>
<td>1.18</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td>Retention</td>
<td>-0.01</td>
<td>-0.16</td>
<td>-0.13</td>
<td></td>
</tr>
<tr>
<td>(\text{Acetate}) (mg-AcO(^-) L(^{-1}))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Background</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Pre-DOC</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>with DOC Predicted, (S)</td>
<td>0.80</td>
<td>4.77</td>
<td>6.36</td>
<td></td>
</tr>
<tr>
<td>Observed</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Retention</td>
<td>-0.80</td>
<td>-4.77</td>
<td>-6.36</td>
<td></td>
</tr>
</tbody>
</table>
Figure 3.1: Illustration showing a simplified longitudinal cross-sectional view of a stream–hyporheic (HZ) environment with commonly observed microbially mediated pathways for nitrate (NO$_3^-$) transformations. These pathways were observed in the present study’s hyporheic system (Zarnetske et al., 2011). Surface waters supply NO$_3^-$ and dissolved organic nitrogen (DON) and carbon (DOC) to the HZ. In aerobic regions of the HZ, DON can be mineralized to NH$_4^+$ which can be transformed via nitrification to create additional NO$_3^-$.

In aerobic regions, DON and NO$_3^-$ can be retained in the HZ via microbial assimilation processes. DOC and NO$_3^-$ entering anaerobic portions of the HZ can be utilized for denitrification, which produces dinitrogen oxide (N$_2$O) and dinitrogen (N$_2$). The N$_2$O and N$_2$ can degas out of the stream system and return to the atmosphere (ATM).
Figure 3.2 Steady-state denitrification dynamics at the study site hyporheic zone (solid curves, modified from Zarnetske et al., 2011) showing the hypothesized response of denitrification (dashed curves) to an addition of labile dissolved organic carbon (DOC). The shaded regions represent a net change in the abundance of a solute due to the addition of labile DOC. Note that the labile fraction of the total DOC is presented as the upper shaded region under the DOC curve with the remainder of the mass representing less bioavailable forms of DOC. Overall, we hypothesize that the addition of labile DOC to the HZ will significantly increase dinitrogen ($N_2$) production, decrease total nitrate ($NO_3^-$) concentration and mass transported through the system, and facilitate additional dissolved oxygen (DO) consumption.
Figure 3.3: A. Map of the Drift Creek study site showing lateral gravel bar hyporheic site. B. Map of the hyporheic study site showing locations of wells (dots with cross hairs), hyporheic injection site, and water potentiometric surface during the injection experiment. Note: Stream briefly bifurcates near gravel bar (i.e., not a tributary confluence) and water chemistry is the same across channel. Dotted arrow indicates a single representative simulated advective flowpath from the injection well, H1, to the stream.
Figure 3.4: Graphical depiction of tracer experiment showing the Pre-DOC and DOC injection periods and the sampling regime.
Figure 3.5: Spatial biogeochemical and $\delta^{15}$N signatures during steady-state Pre-DOC and DOC augmentation periods of the experiment. Reported values are the means for each period ($n = 5$).
Figure 3.6: Change in solute concentrations for the Pre-DOC and DOC treatment periods in receiving wells. K2, K3, and J3 demonstrate steady-state conditions and are sorted from left to right by increasing levels of connectivity to injection. Note: *, **, *** denotes t-test significant difference of means at $p < 0.05$, $p < 0.01$, $p < 0.001$, respectively, with df = 4. Axis scales vary between plots.
Figure 3.7: Change in solutes concentrations for the Pre-DOC and DOC treatment periods in well H2. Conservative (Cl) and $^{15}$NO$_3$ tracer shifts demonstrate non-steady state connectivity to the H1 injection site between the Pre-DOC and DOC treatment periods. Note: *** denotes t-test significant difference of means at $p < 0.001$, respectively, with df = 4. Axis scales vary between plots.
4 – Coupling Multi-Scale Observations to Evaluate Hyporheic Nitrate Removal at the Reach Scale of an Upland Agricultural Stream

Jay P. Zarnetske, Roy Haggerty, and Steven M. Wondzell
ABSTRACT

Excess nitrate (NO₃⁻) in stream systems is a growing and persistent problem for both aquatic and coastal ecosystems, and denitrification represents the primary removal process for NO₃⁻ in streams. Hyporheic zones, where stream and ground waters mix, can have high denitrification potentials; however, their role on reach- and network-scale NO₃⁻ removal is unknown because it is difficult to estimate. In this study we utilize independent and complementary multi-scale measurements of denitrification and total NO₃⁻ uptake to quantify the role of hyporheic NO₃⁻ removal in a 303 m reach of a third-order agricultural stream in western Oregon, USA. The reach scale NO₃⁻ dynamics were characterized with steady-state ¹⁵N- NO₃⁻ tracer addition experiments and solute transport modeling, while the hyporheic conditions were measured via in situ hyporheic biogeochemical and groundwater modeling. Further, we present a method to link these independent multi-scale measurements. Overall, we found that hyporheic NO₃⁻ removal (rate coefficient $\lambda_{HZ} = 0.0069$ h⁻¹) accounted for 17% of the observed total reach NO₃⁻ uptake, and 32% of the reach denitrification estimated from the ¹⁵N experiments. The primary limitations of hyporheic denitrification at the reach-scale were labile dissolved organic carbon availability (low hyporheic SUVA₂₅₄) and the limited size of the hyporheic zone due to anthropogenic channelization (sediment thickness ≤ 1.5 m). Linking multi-scale methods provided rarely possible estimates of hyporheic influence on stream NO₃⁻ dynamics. However, it also demonstrates that the traditional reach-scale tracer experimental designs and subsequent transport modeling [sensu Stream Solute Workshop, 1990] cannot be used alone to directly investigate the role of the hyporheic zone on reach-scale water and solute dynamics.
4.1 Introduction

Nitrate ($\text{NO}_3^-$) is an essential nutrient in stream ecosystems. However, anthropogenic alterations to the global bioavailable nitrogen budgets, landscape use, and hydrologic cycles have resulted in excess $\text{NO}_3^-$ in many aquatic ecosystems [Seitzinger et al., 2006; Gruber and Galloway, 2008]. These excess $\text{NO}_3^-$ stream loads create many ecological disturbances that negatively impact downgradient freshwater and coastal systems [e.g., Sala et al., 2000; Smith, 2003; Diaz and Rosenberg, 2008]. Thus there is a need to understand and manage the sources and sinks of $\text{NO}_3^-$ in stream ecosystems because streams connect the terrestrial systems to the marine systems [Boyer et al., 2006]. There are many $\text{NO}_3^-$ sources to streams (e.g., fertilizer runoff, wastewater discharge, organic nitrogen mineralization), but there is only one significant $\text{NO}_3^-$ sink – denitrification [Seitzinger et al., 2006]. Denitrification is the dissimilatory reduction of $\text{NO}_3^-$ and nitrite ($\text{NO}_2^-$) to nitrous oxide ($\text{N}_2\text{O}$) and dinitrogen ($\text{N}_2$) gases. Denitrification occurs in many locations within stream ecosystems, but a known hot spot for denitrification is the hyporheic zone, where stream water and ground waters mix. The hyporheic zone is a key location for denitrification because it creates strong physical transport and biological reaction gradients, which in turn, promote redox gradients and denitrification [Duff and Triska, 2000]. However, the role of hyporheic denitrification at the reach-scale is rarely accounted for in nutrient cycling studies, and therefore it is not known if hyporheic denitrification comprises a substantial amount of the $\text{NO}_3^-$ removal at the reach- and network-scale.

The removal of $\text{NO}_3^-$ in hyporheic zones and stream reaches is temporally variable and is a function of complex interdependent factors, such as, hydrology and biogeochemistry [McCain et al., 2003; Seitzinger et al., 2006]. Consequently, methods for measuring and modeling hyporheic and reach $\text{NO}_3^-$ removal and denitrification are difficult to implement and result in high uncertainty [Groffman et al., 2006; Birgand et al., 2007; Böhlke et al., 2009]. A key barrier to evaluating the
role of hyporheic $\text{NO}_3^-$ removal at larger scales is that hyporheic studies tend to evaluate spatial scales that are much smaller than the length of the reach and time scales that are much longer than the water residence time of the reach. Consequently, the scientific techniques developed to measure hyporheic processes and stream reach processes are very different.

Hyporheic $\text{NO}_3^-$ removal investigations are primarily conducted by extracting sediment from the stream and then measuring denitrification potentials of the sediment under different biogeochemical and physical conditions. The denitrification potential of sediment is typically investigated via sediment assays (e.g., acetylene block technique [Yoshinari and Knowles, 1976]) or intact flow through sediment cores (e.g., $\text{NO}_3^-$ concentration gradients and modeling; [e.g., Nielsen, 1992; Sheibley et al., 2003; Smith et al., 2006]). This type of sediment denitrification measurements usually measure dynamics over timescales much greater than 1 h. These sediment extraction techniques are useful for making benthic measurements, but they unnaturally alter hyporheic flow conditions and are only representative of a small portion of the hyporheic zone. Some investigators utilize hyporheic wells systems to less invasively measure $\text{NO}_3^-$ removal dynamics across larger spatial scales [e.g., Valett et al., 1996; Baker et al., 1999], but these dynamics can be confounded by unknown subsurface heterogeneity and very long transport timescales.

Reach scale $\text{NO}_3^-$ removal investigations are primarily conducted via $\text{NO}_3^-$ addition and tracer experiments [sensu the methods of Stream Solute Workshop, 1990]. In these studies, nutrient concentration dynamics are observed along the experimental reach and/or at the end of the reach. The reach concentration observations are then used to model and infer coupled hydrological and biological processes affecting the transport of the nutrients. These nutrient addition experiments are capable of measuring the integrated effects of hydrologic and biological process controls on $\text{NO}_3^-$ uptake in streams [e.g., Peterson et al., 2001]. Additionally, the nutrient addition studies lead to the development of the stream nutrient spiraling
concept for NO$_3^-$ and other nutrients [Webster and Patten, 1979; Newbold et al., 1981]. Nutrient spiraling provides a unifying conceptual model for how an element (e.g., N or P) is transported and cycled in a stream ecosystem via coupled hydrologic and biological pathways. An important metric used in the nutrient spiraling concept is the uptake length (commonly referred to as $S_w$). In the case of NO$_3^-$, $S_w$ is the average channel distance a NO$_3^-$ molecule travels before being taken up by a physical or biological retention or removal process (e.g., biological utilization and/or denitrification). This metric of nutrient spiraling is used to make nutrient processing comparisons between widely varying stream systems [e.g., Peterson et al., 2001; Mulholland et al., 2008]. However, the fact that $S_w$ integrates both hydrological and biological processes means that it cannot be used to isolate individual hydrological or biological processes controlling NO$_3^-$ dynamics. For example, it cannot distinguish if role of net denitrification on the observed $S_w$ or where in the stream the NO$_3^-$ uptake is occurring. The use of $^{15}$N-NO$_3^-$ (here after referred to $^{15}$NO$_3^-$) helped to overcome some of the limitation of $S_w$, because individual atoms of N could be tracked through the different biological compartments of a stream system, including stream denitrification rates [Mulholland et al., 2004]. Despite the advancement in many stream tracer techniques since the Stream Solute Workshop [1990], the reach scale experiments and resulting metrics, including, $S_w$, are still unable to determine if the observed NO$_3^-$ removal occurred in the main channel or in the hyporheic zone.

Multi-scale measurements made in the hyporheic zone and the stream need to be integrated in order to effectively evaluate the role of the hyporheic NO$_3^-$ removal at the reach scale. Few studies exist that relate measurements of total hyporheic denitrification at the reach scale by utilizing independent and complementary multiscale methods [see Böhlke et al., 2009]. Consequently, in this study, we use and link independent and complimentary measurements and models of hyporheic- and reach-scale dynamics to quantify the role of hyporheic denitrification on NO$_3^-$ uptake in an experimental reach of an upland agricultural stream. We used a highly instrumented
gravel bar to make multiple in situ biogeochemical measurements under near ambient NO$_3^-$ (i.e., < 3% above background) and natural hydraulic conditions. The hydrologic conditions of the gravel bar were characterized with tracer studies and groundwater flow modeling. The hyporheic measurements reduce some of the bias and uncertainty inherent to the process of trying to scale up point-sourced sediments sample measurements [Böhlke et al., 2009]. The reach scale NO$_3^-$ dynamics were estimated via a $^{15}$NO$_3^-$ addition experiment (sensu the Lotic Intersite Nitrogen Experiment, LINX [Mulholland et al., 2004]). The $^{15}$NO$_3^-$ study was used to quantify reach scale total NO$_3^-$ uptake and denitrification rates, which is then directly related to the hyporheic NO$_3^-$ denitrification and transport rates.

4.2 METHODS

4.2.1 Study Site

The study site shown in Figure 4.1A consists of a 303 m reach containing an instrumented hyporheic zone on Drift Creek, a 3$^{rd}$-order stream within the Willamette River basin in western Oregon, USA (44.9753°N, 122.8259°W). The catchment upgradient of the study site is 6517 ha. The catchment consists of mixed land-use with primarily agriculture and residences served by septic-system. Annual precipitation predominantly comes during the winter months and amounts to 1190 mm. After the winter rainy season ends, the base flow gradually decreases to an annual minimum in early September.

The channel morphology of the experimental reach was modified in the past by channelization. The modified reach has a slope of 0.0071 m m$^{-1}$ and the active-channel is predominantly that of pool-riffle with alluvium consisting of poorly sorted sand, gravel, cobbles, and boulders. The stream is channelized into competent bedrock and separated from the flood plain. The alluvium above the bedrock surface is limited to a thickness of 0 to $\leq$ 1.5 m. This limited alluvium constrains the extent of the hyporheic zone in the experimental reach. At the time of the experiment, the
stream had a mean depth, of 0.33 m, mean wetted width of 5.21 m, and a very small gain in discharge (1.2 % increase) due to lateral inflows and groundwater exchange [Zarnetske et al., 2011].

The hyporheic zone study site is a lateral gravel bar approximately 6.1 m by 4 m, which is proximal to a riffle on the south side and a bedrock outcrop on the north side (Figure 4.1B). This is the same hyporheic zone system described in detail in Zarnetske et al. [2011] and Zarnetske et al. [conditionally accepted]. The head drop from the head of the gravel bar to the pool below was 0.19 m during the study. This head loss induces lateral hyporheic flowpaths that originates at the head of the gravel bar and terminates in the downgradient pool [Zarnetske et al., 2011]. The alluvial thickness at the gravel bar site is 1.2 m ± 0.25 m. This gravel bar has a well network consisting of 11 3.8-cm-diameter wells screened from 0.2 – 0.4 m below ground surface. Each well is connected to stream water originating from the head of the gravel bar.

The stream and hyporheic background biogeochemical conditions are presented in Table 4.1. These conditions permitted the $^{15}\text{NO}_3^-$ enrichment of the NO$_3^-$ for 27.5 h, which was sufficient injection time to create steady-state (i.e., plateau) values in all measured solute reservoirs in the experimental reach and instrumented hyporheic zone.

4.2.2 Field Experimental Procedures

The field and laboratory procedures used to collect the data for this study are presented in detail in Zarnetske et al. [2011], and are briefly summarized below. We performed a whole-stream steady-state $^{15}\text{NO}_3^-$ and conservative tracer (Cl$^-$) injection to measure reach and hyporheic zone NO$_3^-$ dynamics during summer base flow conditions (August 23 – 24, 2007). Following adapted methods from Mulholland et al. [2004], an injection solution of $^{15}\text{NO}_3^-$ (as 99% enriched K$^{15}\text{NO}_3$) and Cl$^-$ (as NaCl) was released at a constant rate into a riffle at the head of the reach for 27.5 hours starting 14:28 h 23 August 2007. The K$^{15}\text{NO}_3$ addition rate was estimated based upon
targets of $\delta^{15}\text{N}$ enrichment of 10 000‰ in the stream water $^{15}\text{NO}_3^-$ and < 3% increase in ambient $\text{NO}_3^-$ concentration. The Cl$^-$ mass addition target was to elevate the background stream Cl$^-$ 400% and generate a 50% increase above ambient specific conductivity (SC) conditions. The solution was injected into a turbulent riffle sufficiently upstream of the first sampling location to guarantee compete lateral and vertical channel mixing at all downstream sampling locations.

SC measured the Cl$^-$ transport through the stream and hyporheic zone. The SC measurements were collected in all 11 gravel bar wells and in the stream water at the head and base of the gravel bar. The SC measurements were collected every 60 s, which is sufficient to capture the details of the Cl$^-$ breakthrough curves. These SC measurements were used as input data for stream and hyporheic solute transport models that quantified transport conditions, including advection rates, residence time characteristics, and mass exchange rates between stream and hyporheic zones (see details in Sections 4.2.3.2 and 4.2.4.2).

The water sampling regime consisted of collecting multiple rounds of stream and hyporheic samples during the pre-injection (background) and steady-state (plateau) phases of the experiment. Repeated sampling occurred during the pre injection ($n = 3$) and plateau ($n = 5$) periods at each hyporheic zone location (11 wells and stream water at the gravel bar head). The plateau sampling period was started 22.5 h after injection started, which is when all hyporheic wells demonstrated near steady-state SC conditions. Repeated hyporheic and stream samples were collected approximately every 1 h during the plateau period. In addition, 6 longitudinal samples were collected in duplicate for each sampling period at locations 4.4 above and 22, 84, 110, 183, and 303 m below the injection site so that reach scale $\text{NO}_3^-$ uptake and denitrification parameters could be calculated following Mulholland et al. [2004]. All water samples were analyzed for key solute concentrations and $\delta^{15}\text{N}$ enrichments relevant to the respiratory denitrification process ($^{15}\text{NO}_3^-$, $^{15}\text{N}_2$). The laboratory
procedures for the stream and hyporheic NO$_3^-$, Cl$^-$, and the $^{15}$N content of the stream and hyporheic water NO$_3^-$ and N$_2$ are presented in Zarnetske et al. [2011].

Immediately following the experiment and during similar stable base flow conditions, we collected detailed thalweg surface water and channel surface topography data for the reach using a Topcon total station (Model GTS-226, Livermore, California, USA) and standard surveying methods with spatial resolution of $x \leq 1$ m, $y \leq 1$ m, $z \leq 0.01$ m for the greater reach and $x \leq 0.1$ m, $y \leq 0.1$ m, $z \leq 0.005$ m for the instrumented gravel bar site. Additionally, hydraulic conductivities ($K$) for the gravel bar hyporheic zone were based upon the geometric mean value of 22 slug tests (2 tests per well) analyzed with the Bouwer and Rice method [Bouwer and Rice, 1976]. These survey and $K$ data were used to parameterize the numerical groundwater flow model to simulate hyporheic exchange across the gravel bar site so that total water and NO$_3^-$ flux could be calculated based upon the experimental and model results.

4.2.3 Reach Parameter Calculations

4.2.3.1 Reach Nitrate Conditions

The reach NO$_3^-$ uptake and denitrification conditions were estimated following the $^{15}$NO$_3^-$ tracer experiment methods of Mulholland et al. [2004], which are summarized in this section. At longitudinal sampling stations along the reach (Figure 4.1), specific discharge was calculated from the dilution of the Cl$^-$ tracer during plateau conditions. These discharge measurements were used to calculate the tracer $^{15}$NO$_3^-$ flux at each station by multiplying the discharge by the background-corrected $^{15}$NO$_3^-$ concentrations. The fractional NO$_3^-$ total uptake rate per unit distance ($k_{tot}$, m$^{-1}$) was then calculated from the regression of ln (tracer $^{15}$NO$_3^-$ flux) versus distance from the $^{15}$N injection station. The inverse of the regression slope is the uptake length of NO$_3^-$ ($S_w$; m) [Newbold et al., 1981]. From $S_w$, the mass-transfer velocity of NO$_3^-$, $V_f$ (cm s$^{-1}$), was calculated as the specific discharge ($Q/w$) divided by $S_w$. The mass of NO$_3^-$ taken up from the water column per unit streambed area and
time is the areal uptake of NO$_3^-$ ($U$; mg m$^{-2}$ s$^{-1}$) is computed as the product of $V_f$ and the mean ambient NO$_3^-$ concentration.

The fractional denitrification rate per unit length ($k_{den}$; m$^{-1}$) was estimated by an N$_2$ production rate model [Mulholland et al., 2004]. The model of N$_2$ production was fit to the longitudinal pattern in the fluxes of tracer $^{15}$N as N$_2$ collected during injection plateau. The flux of $^{15}$N$_2$ at each longitudinal station was calculated by multiplying background-corrected $^{15}$N$_2$ by the specific station discharge. The reaeration rates of N$_2$ ($k_{ar}$, m$^{-1}$) used in the model were generated using the surface renewal model [Owens, 1974]. The measured reaeration rate for the model was $k_{ar} = 0.023$ m$^{-1}$. These denitrification rates represent a minimum value because we were unable to account for N$_2$O production in the estimates [see Zarnetske et al., 2011]; however, denitrification in freshwater sediments is almost entirely N$_2$ production with N$_2$O:N$_2$ production ratios generally between $<0.001$ and $<0.05$ [Seitzinger, 1998; Mulholland et al., 2004]. Thus, $^{15}$N$_2$ alone is able to characterize the large majority of denitrification in the system.

4.2.3.2 Reach Hydrologic Conditions

We used a 1D solute transport model to provide optimized parameters for reach-representative estimates of advection, dispersion, and transient storage for our solute addition experiment. This 1D solute transport model (STAMMT-L) is described by Haggerty and Reeves [2002a] and Haggerty et al. [2002b]. We briefly describe the governing equations below and shown how they relate to the well-known OTIS solute transport model [Bencala and Walters, 1983; Runkel, 1998]. The model applies a user-specified residence time distribution to the general 1D advection-dispersion transport equation. The transport equation for a system that is initially tracer-free with no longitudinal inputs is:

$$\frac{\partial c}{\partial t} = -v \frac{\partial c}{\partial x} + D \frac{\partial^2 c}{\partial x^2} - \beta_{tot} \frac{\partial}{\partial t} \int_0^t c(\tau) g^*(t - \tau) d\tau$$  \hspace{1cm} (1)
where \( v \) is the mean in-stream advection velocity (m h\(^{-1}\)), \( D \) is the longitudinal dispersion (m\(^2\) h\(^{-1}\)), \( \beta_{\text{tot}} \) is the ratio of the mass in the immobile zone to that in the mobile zone at equilibrium, \( c \) is the conservative tracer concentration in the stream (mg L\(^{-1}\)), and \( \tau \) is a lag time (h). In the last term of equation (1), \( g^*(t) \) is convolved with the stream concentration to represent exchange with the transient storage zone, including the hyporheic zone, following an appropriate residence time distribution. The probability density function that the tracer remains in storage after a time, \( t \), is defined as:

\[
g^*(t) = \alpha_2 e^{-\alpha_2 t} \tag{2}
\]

for an exponential residence time distribution where \( \alpha_2 \) is the first-order rate coefficient (h\(^{-1}\)). This form of the model is equivalent to the standard first-order model provided by Bencala and Walters [1983] and implemented numerically by Runkel [1998] in the U.S. Geological Survey OTIS model. The OTIS model has a storage-exchange rate \( \alpha \) that is equivalent to the product of \( \alpha_2 \) and \( \beta_{\text{tot}} \), where \( \beta_{\text{tot}} \) is equal to the ratio of storage area (\( A_s \)) to stream cross-sectional area (\( A \)) from the OTIS model. Further characterization of the transient storage conditions can be described by the mean storage residence time, \( \tau_{\text{stor}} \) (h), which represents the average time that a water particle spends within a storage zone and is calculated as \( 1/\alpha_2 \) for exponential residence time distribution simulations.

Parameters were estimated by STAMMT-L using a non-linear least squares algorithm [Marquardt, 1963] that minimized the sum of square errors on the logarithms of the tracer concentrations. For all simulations, we calculated the root mean squared error (RMSE) as defined by Bard [1974] between the observed and simulated data. A RMSE value of 0 indicates a perfect fit of the simulated values to the observations. Optimization runs for the tracer test were completed using the exponential residence time distribution method and the simulation that generated the
lowest RMSE was accepted as the best model. The model parameters $v$, $D$, $\beta_{tot}$, and $\alpha_2$ were calculated in the optimization process.

4.2.4 Hyporheic Parameter Calculations

4.2.4.1 Hyporheic Nitrate Conditions

SC breakthrough curves (as a measure of Cl$^-$ transport) at the head of the gravel bar and in each well were used to measure the median residence time of the hyporheic water in each well. The median residence time was calculated as the time required to increase the SC in the well to one half the plateau value. In the case of the wells, median residence times were calculated based on the observed SC arrival at the gravel bar, not the start of the injection. The measured NO$_3^-$ concentrations were then related to these median residence times, so that NO$_3^-$ removal rates can be calculated.

We assume that in our hyporheic system, the observed NO$_3^-$ removal dynamics are primarily due to denitrification as evidenced by the $^{15}$N$_2$ production seen in the gravel bar during the experiment [Zarnetske et al., 2011]. Therefore, we refer to observed decreases in NO$_3^-$ as apparent denitrification. The apparent denitrification rate, $k_{HZ}$ (h$^{-1}$), can be calculated from the observed steady-state hyporheic NO$_3^-$ concentration profiles as:

$$C_{NO_3} = e^{-k_{HZ} t_{med}}$$

where $C_{NO_3}$ is the concentration of NO$_3^-$ (mg-N L$^{-1}$), and $t_{med}$ is the median residence time (h$^{-1}$) of water in the hyporheic zone as measured from the conservative tracer (Cl$^-$) breakthrough curve.

4.2.4.2 Hyporheic Hydrologic Conditions

A ground water flow model (MODFLOW) was used to simulate 3D hyporheic exchange flow (HEF) through the gravel bar and estimate total water and solute flux conditions. The model domain was defined in space by the gravel bar and sediment...
thickness surveys and was partitioned into 0.050 m horizontal grid cells. The model had 5 layers generating cells matching the vertical resolution (0.20 m) of the screened well sampling location. The stream was modeled as a spatially variable constant head boundary condition established by the measured surface water elevations. For all sediment layers, anisotropy of $K$, was incorporated such that $K_{x,y}$ (horizontal) was an order of magnitude greater than $K_z$ (vertical) [Freeze and Cherry, 1979]. The assigned $K$ value of $4.7 \times 10^{-5}$ m s$^{-1}$ was based upon geometric mean value of 22 variable head tests in the gravel bar following the tracer experiment. Effective porosity was set to 0.30. Groundwater and lateral subsurface inflow or outflow was considered negligible and was not incorporated into the model design because there is no evidence of either occurring over this segment of the stream reach. The hyporheic flow was modeled with a steady-state simulation because stream surface flow did not vary sufficiently across the experiment period to detect a change in the wetted perimeter of the gravel bar. The model solves for the distribution of hydraulic heads within the model domain and, subsequently, the velocities and travel times in the hyporheic zone [Kasahara and Wondzell, 2003; Wondzell et al., 2009].

The ground water flow model was also used to calculate the hyporheic – stream exchange rate, $a_{HZ}$ (h$^{-1}$), the mean residence time in the hyporheic zone, $\tau_{HZ}$ (h), and total HEF (L h$^{-1}$). The residence times in the gravel bar were partitioned into 1 h residence time intervals, and the specific HEF, $q_{HEF}$ (L h$^{-1}$) was calculated for each interval [sensu Kasahara and Wondzell, 2003 and Wondzell et al., 2009]. The mean hyporheic residence time, $\tau_{HZ}$, is the mean of the $q_{HEF}$ distribution normalized by total HEF. The $a_{HZ}$ is $1/\tau_{HZ}$.

4.2.5 Relating Hyporheic and Reach Nitrate Dynamics

In order to quantify the influence of the hyporheic NO$_3^-$ removal on the reach NO$_3^-$ removal rates, we present a method that links the individual transport and reaction parameters generated from the hyporheic and reach tracer experiments and solute transport modeling. We did this following the suggestions of Runkel [2007]
and Botter et al. [2010], where they show that the effective NO$_3^-$ removal rate at the reach scale, $\lambda_{tot}$ (h$^{-1}$), can be represented by the sum of the NO$_3^-$ removal rate in the water column, $\lambda_{stream}$ (h$^{-1}$), and in the hyporheic zone, $\lambda_{HZ}$ (h$^{-1}$):

$$\lambda_{tot} = \lambda_{stream} + \lambda_{HZ}$$  \hspace{1cm} (4)

The studies of Runkel [2007], Botter et al. [2010] and Argerich et al. [in press] also showed that $\lambda_{HZ}$ can be estimated from the hydrologic transport and solute reaction kinetics estimated in Section 4.2.4. Thus, we calculated $\lambda_{HZ}$ as:

$$\lambda_{HZ} = \beta_{tot} \left( \frac{k_{HZ} * a_{HZ}}{k_{HZ} + a_{HZ}} \right)$$  \hspace{1cm} (5)

where $\beta_{tot}$ and $a_{HZ}$ in this expression of $\lambda_{HZ}$ come from the reach and groundwater transport modeling and are used to scale the effect of the hyporheic zone size and exchange rates, respectively, on the reach NO$_3^-$ removal dynamics. From the reach hydrologic transport and spatially explicit NO$_3^-$ reaction kinetics quantified in Section 4.2.3, we represented $\lambda_{tot}$ as:

$$\lambda_{tot} = k_{tot} * v$$  \hspace{1cm} (6)

where the reach mean velocity, $v$, is used to transform $k_{tot}$ to a temporally explicit NO$_3^-$ removal rate. Further, we calculated the NO$_3^-$ removal rate in the water column as:

$$\lambda_{stream} = \lambda_{tot} - \lambda_{HZ}$$  \hspace{1cm} (7)

We assumed that the $\lambda_{HZ}$ is primarily the result of hyporheic denitrification as indicated by significant hyporheic N$_2$ production observed in the Drift Creek hyporheic zone [Zarnetske et al., 2011]. Thus, we also related the $\lambda_{HZ}$ to the measured
reach $k_{den}$ generated from the reach $^{15}$N$_2$ production model (Section 4.2.3). Similar to $\lambda_{tot}$, we calculated the reach denitrification rate, $\lambda_{den}$, as:

$$\lambda_{den} = k_{den} \times v$$

(8)

The different removal rates were related to each other by taking the ratio of the hyporheic-scale removal rate, $\lambda_{HZ}$ to the reach-scale total NO$_3^-$ removal rate, $\lambda_{tot}$, and the denitrification removal rate, $\lambda_{den}$.

4.3 Results

4.3.1 Reach Conditions

Stream chemistry was stable during the experiment and reflected the pre-injection conditions in Table 4.1. The water temperature varied by 2.4 °C during the injection period (minimum = 14.1; maximum = 16.5°C). Measured surface water nutrient and chemistry conditions during the experiment were NO$_3^-$ (0.318-0.325 mg-N L$^{-1}$), NH$_3$ (0.021-0.024 mg-N L$^{-1}$), DOC (2.95-3.45 mg-C L$^{-1}$), DO (8.10-8.51 mg-O$_2$ L$^{-1}$), and pH (6.65-6.85). The $^{15}$NO$_3^-$ addition experiment demonstrated that Drift Creek had a total effective NO$_3^-$ removal rate, $\lambda_{tot}$, for the reach of 6.0 x 10$^{-4}$ m$^{-1}$, which results in an uptake length, $S_u$, of 1667 m and an uptake velocity, $V_f$, of 4.0 x 10$^{-4}$ cm s$^{-1}$ (Table 4.2). The stream denitrification rate, $k_{den}$, was 3.3 x 10$^{-4}$ m$^{-1}$, which is equivalent of 50% of total NO$_3^-$ removal rate from stream water. Reach NO$_3^-$ uptake and denitrification models presented good simulation fits for observed $^{15}$NO$_3^-$ dynamics ($k_{tot}$ : $R^2$ = 0.85, Figure 4.2; and $k_{den}$ : SSE = 1.36, Figure 4.3).

Stream flow conditions were uniform during the experiment with a mean flow of 22.0 L s$^{-1}$ and a standard deviation of ± 2.2 L s$^{-1}$. This minor variability in discharge did not induce measurable changes in the stage of the stream along the reach. The reach solute transport modeling showed good agreement between the simulated and observed conservative tracer data (RMSE = 0.02, Figure 4.4). The
estimated reach transport parameters from the STAMMT-L model are \( v = 67.7 \text{ m h}^{-1} \), \( D = 13.9 \text{ m}^2 \text{ h}^{-1} \), \( \tau_{stor} = 0.49 \text{ h} \), \( \alpha_2 = 2.03 \text{ h}^{-1} \), and \( \beta_{tot} = 0.17 \) (Table 4.2).

### 4.3.2 Hyporheic Conditions

The stream water and solutes advected into the gravel bar were altered along the hyporheic flowpaths. The mean DO decreased from 8.31 to 0.59 mg-O\(_2\) L\(^{-1}\) along HZ flowpaths and the mean DOC decreased from 3.01 to 1.7 mg-C L\(^{-1}\) (Table 4.1). The DOC SUVA\(_{254}\) concentrations generally decreased along flowpaths (3.22 to 0.94 L mg-C\(^{-1}\) m\(^{-1}\)). The DO and DOC removal rates were largest in the first 2 m of the flowpaths but continued across the entire gravel bar. Overall, NO\(_3^-\) decreased along the flowpaths from a peak value of 0.54 down to 0.02 mg-N L\(^{-1}\) (Figure 4.5). The first-order NO\(_3^-\) removal model (Equation 3) was able to capture the behavior of the observed hyporheic NO\(_3^-\) conditions (\(R^2 = 0.83\), Figure 4.5) and yielded an apparent denitrification rate, \(k_{HZ}\), of 0.094 h\(^{-1}\) (Table 4.2).

Repeated measurements of the head elevations in the wells (before plateau sampling disturbances) reflected stable hydraulic head conditions as there was no detectible variation during the experiment. Tracing Cl\(^-\) transport through the HZ generated median residence times ranging from 3.8 to 28.5 h (Table 4.1). The hyporheic exchange model provided a good simulation fit to observed head conditions during the experimental period (Figure 4.6). The groundwater flow modeling shows the head gradient (Figure 4.7) and flowpaths during this study are consistent with the tracer-based arrival and residence times at each of the wells (Table 4.1 and Figure 4.8). The result of the hyporheic exchange model showed 61 L h\(^{-1}\) of HEF at the gravel bar site, which is equivalent to 4.6% of the main channel flow. The mean residence time in the hyporheic zone was 14.05 h and the mass exchange rate, \(\alpha_{HZ}\), was 0.071 h\(^{-1}\) (Table 4.2; Figure 4.9). The resulting hyporheic NO\(_3^-\) removal rate, \(\lambda_{HZ}\), calculated from the groundwater flow parameters and observed steady-state NO\(_3^-\) dynamics was 6.9 x 10\(^{-3}\) h\(^{-1}\) (Table 4.2).
4.4 **DISCUSSION**

Interpretations of the HZ NO$_3^-$ removal at the reach scale must be done cautiously because there are many sources of uncertainty with linking multi-scale measurements. Consequently, it is difficult to know if the observed role of the HZ on NO$_3^-$ dynamics in Drift Creek is either a true reflection of the physical and biological dynamics of the system or a reflection of the inherent uncertainty associated with making multi-scale NO$_3^-$ removal measurements. This same issue was recently encountered by Böhlke *et al.* [2009] who synthesized multi-scale measurement and modeling (e.g., sediment cores, reach N mass budgets, $^{15}$N injections) to assess the role of denitrification in streams. Böhlke *et al.* [2009] found that it was very difficult to reconcile and connect the different scales of the measurements. Consequently, we discuss both the hydroecological processes and methodological issues that control our estimates of hyporheic NO$_3^-$ removal at the reach-scale.

4.4.1 **Key Hydroecological Controls on Hyporheic NO$_3^-$ Removal Estimates**

The estimated Drift Creek HZ NO$_3^-$ removal rates accounted for 32% of the total reach denitrification and 17% of total reach NO$_3^-$ uptake. These removal rates demonstrate that even a volumetrically constrained HZ can be an important regulator of stream NO$_3^-$ during summer base flow conditions. This is in agreement with the synthesis of extensive multi-scale investigations conducted in two streams of the upper Mississippi River basin. In this synthesis, Böhlke *et al.* [2009] showed that hyporheic zones account for 14-97% of the total denitrification occurring at the reach level. This wide range of HZ denitrification can be attributed to many different local hydroecological factors that vary in space and time [Duff and Triska, 2000] including, but not limited to, stream flow conditions, amount of hyporheic exchange flow, the water residence times in hyporheic zones, the amount of labile DOC available, water temperature, the structure and abundance of hyporheic microbial communities, and the amount of NO$_3^-$ in the system. Each of these factors is expected to be very different between the upper Mississippi River basin and the upper Willamette River basin.
Given the limitations of the data between the two sites, a comparison between all of these factors is not possible. However, a known key difference is the abundance of NO$_3^-$ in the streams of the two basins. The amount of stream NO$_3^-$ in the Böhlke et al. [2009] studies ranged between 1.4 and > 14 mg L$^{-1}$, while Drift Creek is known to vary between 0.1 and 0.6 mg L$^{-1}$. The much greater availability of NO$_3^-$ in the Mississippi streams alone will stimulate much stronger denitrification rates in hyporheic zones of the Böhlke et al. [2009] studies, because denitrification rates increase with stream NO$_3^-$ availability [Seitzinger, 1988; Phenning and McMahon, 1997; Smith et al., 2006; Mulholland et al., 2008].

The Drift Creek hyporheic zone is known to be a net denitrification hotspot [Zarnetske et al., 2011], however, its role on the total reach NO$_3^-$ removal was limited to 17% under the experimental base flow conditions. Typically, this large difference between denitrification and total NO$_3^-$ uptake would indicate large differences in the biological pools utilizing the NO$_3^-$. For example, a third-order, N-limited stream in the nearby Oregon Cascade Mountains has most of the inorganic N uptake occurring in the bryophytes, epixylon, and fine benthic organic material, while denitrification was negligible despite having a large hyporheic zone [Ashkenas et al., 2004]. Similarly, the strongly N-limited streams of Antarctica have large hyporheic water flux relative to the main channel, but these streams also demonstrated that the majority (~74%) of the NO$_3^-$ uptake occurred within the main channel organisms while the transient storage zones, including hyporheic zones, accounted for the other ~ 26% [McKnight et al., 2004; Runkel, 2007]. Drift Creek is less N-limited than the other Oregon and Antarctic streams, but there are still many biological pools rapidly utilizing and retaining the NO$_3^-$ in the reach as shown by the water column uptake rate of 0.034 h$^{-1}$. Quantifying the role of each of the individual biological pools of N in Drift Creek was not a part of this study, but they seem to be similar to the Cascade and Antarctic N-limited streams, in that, the main channel NO$_3^-$ retention and removal is important to reach-scale NO$_3^-$ uptake.
There are known biogeochemical and physical limitations on hyporheic denitrification in Drift Creek. A key limiting factor for hyporheic denitrification is the availability of labile dissolved organic carbon (SUVA\textsubscript{254} decreases along flowpaths: Table 4.1; Zarnetske et al., [conditionally accepted]) and this may restrict the impact of the hyporheic zone on reach NO\textsubscript{3}^- dynamics. From a purely physical perspective, the hyporheic zone of the experimental reach is constrained by the stream channelization and the thin sediment thickness (0 to 1.5 m). This constrained HZ will limit the volume of reactive sediment where NO\textsubscript{3}^- removal can occur and the ratio of hyporheic exchange flow to surface water flow ($\beta_{tot}$ or $A_s/A$), which in turn will limit the influence of the hyporheic exchange at the reach-scale [Zarnetske et al., 2008; Tonina and Buffington, 2011).

4.4.2 Key Methodological Controls on Hyporheic NO\textsubscript{3}^- Removal Estimates

An inherent issue with the scale of our hyporheic NO\textsubscript{3}^- measurements is that we assume that the gravel bar study site is representative of the hyporheic processes occurring across the entire reach. Typically, hyporheic NO\textsubscript{3}^- transformations are made with even smaller representative hyporheic volumes. For example, denitrification rates are based on techniques such as laboratory sediment-assay methods (e.g., acetylene block technique, [Yoshinari and Knowles, 1976]) and flow-through core and column experiments [e.g., Nielsen, 1992; Sheibley et al., 2003; Smith et al., 2006]. Therefore, our measurement of minimally disturbed in situ hyporheic exchange and N transformations across many cubic meters of sediment is a more integrated and robust measurement of natural hyporheic processes than the above-mentioned methods. The lack of lateral and groundwater and solutes in the experimental reach further minimizes the natural variability which typically limits the ability to scale up local measurements [Alexander et al., 2007]. Still hyporheic denitrification depends on many factors, and one key factor at Drift Creek is the role of hyporheic residence time [Zarnetske et al., 2011]. In Drift Creek, net nitrification and net denitrification rates are controlled by residence time, where short hyporheic residence times ($< 6.9$ h)
during the 2007 base flow conditions lead to net nitrification and longer residence times lead to net denitrification. The local variation of hyporheic exchange within the reach will generate different residence time distributions than that observed in the instrumented gravel bar. Therefore, we acknowledge that our estimates will be biased toward high hyporheic denitrification estimates if the residence times in the gravel bar are greater than the true mean residence times of the entire Drift Creek hyporheic zone. Conversely, we may have underestimated the role of hyporheic denitrification if our selected gravel bar has characteristic residence times less than the mean residence time of the entire Drift Creek hyporheic zone.

We used reach-scale $^{15}$N tracer experiments to measure NO$_3^-$ removal and denitrification rates because the $^{15}$N tracer method is a robust and commonly used measurement technique. However, the method still has many sources of uncertainty. The strengths of using $^{15}$N additions in an NO$_3^-$ removal experiment is that it allows the actual $^{15}$N in the $^{15}$NO$_3^-$ molecules to be traced through a system, including the denitrification pathway via $^{15}$N$_2$ production, while minimally altering the ambient NO$_3^-$ conditions in the ecosystem [e.g., Mulholland et al., 2004; Mulholland et al., 2008]. The key source of uncertainty in the $^{15}$NO$_3^-$ tracer method is associated with the measurements of the physical transport conditions needed to estimate NO$_3^-$ uptake and denitrification [Böhlke et al., 2009]. As in Böhlke et al. [2009] most $^{15}$NO$_3^-$ uptake investigations calculate their physical transport parameters, including the apparent hyporheic − surface water transport parameters ($A_s$ and $\alpha$) from a reach-scale 1D solute transport model (e.g., OTIS [Runkel, 1998]). In our study, we get closer to a better representation of the hyporheic zone and reach transport timescale by quantifying the actual hyporheic exchange flow ($q_{HEF}$) and the mass rates ($\alpha_{HZ}$), rather than relying on one master reach-scale $\alpha$ to account for exchange between the surface and subsurface environments. Also our methods for quantifying the hyporheic zone are based upon the advected hyporheic exchange and not the diffusion dominated microscale (< 1 cm) where stream channel water can interact with denitrifying biofilms at the stream-HZ
interface [Sørensen et al., 1998]. These main channel biofilms locations plus water column based denitrification may account for the other 68% of NO$_3^-$ removal seen at the reach-scale ($\lambda_{\text{stream}}$, Table 4.2).

Another source of uncertainty coming from the estimates of hydrologic conditions is associated with the “window of detection” for reach-scale tracer experiments [Wagner and Harvey, 1997; Harvey and Wagner, 2000]. The “window of detection” of reach tracer experiment may not be able to detect tracers that enter long residence pathways, such as hyporheic flow paths. Consequently, the subsequent transport modeling of the stream tracer dynamics [sensu Stream Solute Workshop, 1990] may not be sensitive to the long exchange time scales and the entire volume of the hyporheic zone. This means that reach tracer test typically underestimate the volume of the storage zones ($A_s$), including the hyporheic zone, and the long exchange timescales ($\alpha_{HZ}$). Therefore, these reach tracer experiments can artificially decrease the influence of transient storage and hyporheic zones, which are typically the most biogeochemically reactive environments in streams [Baker et al., 2000; McCain et al., 2003]. Inspection of the modeled residence times of the gravel bar hyporheic zone, and the reach scale mean residence time in Drift Creek indicate that there is a window of detection problem in our study, because they are significantly different ($\tau_{HZ} = 14.05$ h versus $\tau_{stor} = 0.49$ h). This indicates that the reach scale methods at Drift Creek may not detected much of the hyporheic NO$_3^-$ transformation processes because they operated over timescales significantly longer than that of the observed reach transport timescales. Figure 4.9 shows that 11% of the hyporheic flow occurs at timescales longer than the timescales of the $^{15}$N plateau sampling period used to calculate the $\lambda_{tot}$ and $\lambda_{den}$ (i.e., 22.5 - 27.5 h after start of injection). So even though we sampled at plateau conditions, approximately 11% of the hyporheic flowpaths had not yet returned to the main channel and therefore would not influence the reach-scale measurements. In terms of spatial scales, inspection of equation (5) shows that the $\lambda_{HZ}$ is very sensitive (i.e., directly proportional) to the reach measured $\beta_{tot}$ (i.e., $A_s/A$), and
the $q_{HEF}$ timescales demonstrate that the reach scale tracer experiments are unable to see the full hyporheic portion of $A_s$. Therefore, the calculated apparent $\lambda_{HZ}$ is most likely an underestimate of the true $\lambda_{HZ}$ in Drift Creek. Zarnetske et al. [2011] showed net denitrification rates occurring over timescales $> 6.9$ h in the Drift Creek hyporheic zone during summer base flow, so the $\sim 11\%$ of $q_{HEF}$ not observed during the main channel plateau sampling was dominated by strong denitrification potentials.

4.5 **CONCLUSIONS**

We used independent and complementary methods to make seldom possible estimates of HZ denitrification controls on the reach scale NO$_3^-$ conditions. Under the summer base flow conditions at Drift Creek, we estimated that the hyporheic zone accounted for 17% of the total reach NO$_3^-$ uptake and 32% of the total reach denitrification. Anthropogenic alterations to the stream, namely channelization, have simplified and reduced the spatial extent of the biogeochemical active hyporheic zone thereby reducing its influence on the reach-scale NO$_3^-$ removal rates.

The coupling of reach dynamics with *in situ* hyporheic dynamics in this study emphasizes the potential role of the hyporheic zone as a regulator of aquatic N dynamics, however, it also emphasizes many methodological limitations that introduce uncertainty to these estimates of N removal pathways. Namely, the methods that integrate biogeochemical reaction kinetics with physical transport kinetics at the reach scale, strongly bias estimates toward shorter characteristic timescales more indicative of the main channel conditions. This timescale bias may underestimate the true impact of the hyporheic zone on biogeochemical processes. Thus, this study is another instance that brings into question the validity of using the traditional reach-scale tracer experimental designs and subsequent transport modeling [sensu Stream Solute Workshop, 1990] to investigate the role of the hyporheic zone on water and solute dynamics.
ACKNOWLEDGMENTS

This research was supported by NSF grant EAR-0409534 to RH and SMW, and NSF grant DGE-0333257 and OSU Institute for Water and Watersheds (IWW) grant to JPZ. Further support was provided by the Hollis M. Dole Environmental Geology Foundation at OSU. Any opinions, findings and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the NSF. We thank the Harry Klopfenstein Farm for generously granting access to the Drift Creek research sites. We extend special thanks to: V. Adams, S. Baxter, P. Zarnetske, A. Argerich, and B. Burkholder for field/lab assistance; L. Ashkenas and S. Thomas for advising JPZ on stable 15N handling; M. Otter of MBL’s Stable Isotope Laboratory for analyzing 15N samples; and C. Jones and K. Motter of CCAL and OSU IWW Collaboratory for help with analyzing general water chemistry.
Table 4.1: Background stream and hyporheic biogeochemistry (mean of three observations before injection ± 1 standard error) with measured median residence time of surface water to each hyporheic well.

<table>
<thead>
<tr>
<th>Site</th>
<th>Median travel time (h)</th>
<th>DO (mg-O₂ L⁻¹)</th>
<th>NO₃⁻ (mg-N L⁻¹)</th>
<th>NH₃ (mg-N L⁻¹)</th>
<th>DOC (mg-C L⁻¹)</th>
<th>SUVA₂54 (L mg⁻¹ m⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stream</td>
<td>NA</td>
<td>8.31 ± 0.43</td>
<td>0.32 ± 0.01</td>
<td>0.02 ± 0.02</td>
<td>3.01 ± 0.15</td>
<td>3.22 ± 0.18</td>
</tr>
<tr>
<td>G1</td>
<td>6.87</td>
<td>2.07 ± 0.05</td>
<td>0.54 ± 0.06</td>
<td>0.11 ± 0.02</td>
<td>2.07 ± 0.15</td>
<td>1.88 ± 0.17</td>
</tr>
<tr>
<td>H1</td>
<td>3.80</td>
<td>3.27 ± 0.09</td>
<td>0.43 ± 0.05</td>
<td>0.05 ± 0.01</td>
<td>2.18 ± 0.14</td>
<td>1.25 ± 0.17</td>
</tr>
<tr>
<td>H2</td>
<td>16.20</td>
<td>1.30 ± 0.03</td>
<td>0.27 ± 0.03</td>
<td>0.06 ± 0.01</td>
<td>2.01 ± 0.17</td>
<td>1.96 ± 0.21</td>
</tr>
<tr>
<td>H3</td>
<td>16.32</td>
<td>0.72 ± 0.06</td>
<td>0.33 ± 0.03</td>
<td>0.05 ± 0.01</td>
<td>2.01 ± 0.20</td>
<td>1.20 ± 0.23</td>
</tr>
<tr>
<td>I1</td>
<td>14.90</td>
<td>1.09 ± 0.05</td>
<td>0.25 ± 0.04</td>
<td>0.08 ± 0.01</td>
<td>1.94 ± 0.15</td>
<td>1.79 ± 0.15</td>
</tr>
<tr>
<td>I3</td>
<td>18.17</td>
<td>0.93 ± 0.09</td>
<td>0.13 ± 0.00</td>
<td>0.07 ± 0.01</td>
<td>1.98 ± 0.10</td>
<td>1.31 ± 0.11</td>
</tr>
<tr>
<td>J1</td>
<td>28.45</td>
<td>0.70 ± 0.06</td>
<td>0.07 ± 0.02</td>
<td>0.01 ± 0.01</td>
<td>1.66 ± 0.12</td>
<td>0.94 ± 0.13</td>
</tr>
<tr>
<td>J2</td>
<td>18.95</td>
<td>0.61 ± 0.09</td>
<td>0.13 ± 0.01</td>
<td>0.02 ± 0.01</td>
<td>1.79 ± 0.10</td>
<td>1.27 ± 0.11</td>
</tr>
<tr>
<td>J3</td>
<td>21.40</td>
<td>0.51 ± 0.05</td>
<td>0.11 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>1.76 ± 0.08</td>
<td>1.81 ± 0.12</td>
</tr>
<tr>
<td>K2</td>
<td>21.03</td>
<td>0.65 ± 0.05</td>
<td>0.08 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>1.71 ± 0.06</td>
<td>1.01 ± 0.07</td>
</tr>
<tr>
<td>K3</td>
<td>22.70</td>
<td>0.59 ± 0.10</td>
<td>0.09 ± 0.01</td>
<td>0.02 ± 0.01</td>
<td>1.70 ± 0.11</td>
<td>1.05 ± 0.12</td>
</tr>
</tbody>
</table>
Table 4.2: Multi-scale physical transport and NO$_3^-$ reaction parameters for the hyporheic- and reach-scale experiments and modeling.

<table>
<thead>
<tr>
<th>Hyporheic and Stream Parameters</th>
<th>Units</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hyporheic Zone (Gravel Bar)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Physical Parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean residence time, $\tau_{HZ}$</td>
<td>h</td>
<td>14.05</td>
</tr>
<tr>
<td>mass transfer rate, $\alpha_{HZ}$</td>
<td>h$^{-1}$</td>
<td>0.071</td>
</tr>
<tr>
<td><strong>Reaction Parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>apparent denitrification rate, $k_{HZ}$</td>
<td>h$^{-1}$</td>
<td>0.094</td>
</tr>
<tr>
<td>NO$<em>3^-$ uptake rate, $\lambda</em>{HZ}$</td>
<td>h$^{-1}$</td>
<td>0.0069</td>
</tr>
<tr>
<td><strong>Reach</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Physical Parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean advection velocity, $v$</td>
<td>m h$^{-1}$</td>
<td>67.7</td>
</tr>
<tr>
<td>dispersion, $D$</td>
<td>m$^2$ h$^{-1}$</td>
<td>13.9</td>
</tr>
<tr>
<td>mean storage residence time, $\tau_{stor}$</td>
<td>h</td>
<td>0.49</td>
</tr>
<tr>
<td>mass transfer rate, $\alpha_2$</td>
<td>h$^{-1}$</td>
<td>2.03</td>
</tr>
<tr>
<td>ratio of storage area to stream area, $\beta_{tot}$</td>
<td>--</td>
<td>0.17</td>
</tr>
<tr>
<td><strong>Reaction Parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO$<em>3^-$ uptake rate per length, $k</em>{tot}$</td>
<td>m$^{-1}$</td>
<td>6.0 x 10$^{-4}$</td>
</tr>
<tr>
<td>fractional denitrification rate, $k_{den}$</td>
<td>m$^{-1}$</td>
<td>3.3 x 10$^{-4}$</td>
</tr>
<tr>
<td>NO$_3^-$ uptake length, $S_w$</td>
<td>m</td>
<td>1667</td>
</tr>
<tr>
<td>NO$_3^-$ mass-transfer velocity, $V_f$</td>
<td>cm s$^{-1}$</td>
<td>4.0 x 10$^{-4}$</td>
</tr>
<tr>
<td>NO$<em>3^-$ uptake rate, $\lambda</em>{tot}$</td>
<td>h$^{-1}$</td>
<td>0.041</td>
</tr>
<tr>
<td>denitrification rate, $\lambda_{den}$</td>
<td>h$^{-1}$</td>
<td>0.022</td>
</tr>
<tr>
<td>NO$<em>3^-$ water column uptake rate, $\lambda</em>{stream}$</td>
<td>h$^{-1}$</td>
<td>0.034</td>
</tr>
</tbody>
</table>

**Hyporheic Zone Versus Reach**

| $\lambda_{HZ}$ $\lambda_{tot}$ | -- | 0.17 |
| $\lambda_{HZ}$ $\lambda_{den}$ | -- | 0.32 |
Figure 4.1: Drift Creek experimental reach (a) and instrumented gravel bar site (b). The open circles in (a) are the longitudinal sampling locations for the whole-reach experiment, and the circles with cross-hairs in (b) are the hyporheic well locations. The white polygon on the gravel bar site represents the spatial domain of the hyporheic exchange model.
Figure 4.2: The Drift Creek ln\(^{15}\text{NO}_3^-\) flux versus distance downstream from the injection site during the steady-state addition experiment. The slope of the regression line is the total fractional NO\(_3^-\) uptake rate \((k_{\text{tot}})\) and the inverse of the slopes is the NO\(_3^-\) uptake length \((S_{\text{u}})\).
Figure 4.3: Plot of the $^{15}$N$_2$ flux (solid circles) versus the distance from the injection site. The solid line shows the best-fit solution of the denitrification model to the observed data (sum of squared error = 1.36 between simulated and observed data).
Figure 4.4: The reach-scale conservative tracer (specific conductivity, SC) breakthrough curve (OBS, gray diamonds) with the optimal model fit (SIM, solid line) for the reach (RMSE = 0.021).
Figure 4.5: Steady-state hyporheic zone NO$_3^-$ conditions relative to mediana residence time. Each data point represents the mean values generated from repeated samples ($n = 5$, error bars = ± 2 standard error) collected during tracer plateau conditions.
Figure 4.6: Results from best-fit groundwater model between simulated and observed heads in the hyporheic wells of Drift Creek. Points close to the 1:1 line indicate good agreement between observed and simulated data.
Figure 4.7: Plan view of the Drift Creek hyporheic exchange model showing the steady-state head gradient across the gravel bar (wells are shown as black circles and the head contours = 0.01 m).
Figure 4.8: Hyporheic flow path simulations for different residence times, $\tau_{HZ}$, in the Drift Creek hyporheic zone. Note: flowpaths are shown in blue with the residence time defined termination point shown as a red circle.
Figure 4.9: The distribution of specific hyporheic exchange flow ($q_{HEF}$) across the hyporheic residence times, $\tau_{HZ}$, in the experimental gravel bar site.
5 – Coupling Hyporheic Nitrification-Denitrification: Evaluating Net Nitrate Source-Sink Dynamics as a Function of Transport and Reaction Kinetics

Jay P. Zarnetske, Roy Haggerty, Steven M. Wondzell, Vrushali A. Bokil, and Ricardo González-Pinzón
ABSTRACT

The fate of biologically-available nitrogen (N) and carbon (C) in stream ecosystems is controlled by the coupling of physical transport and biogeochemical reaction kinetics. However, determining the relative role of physical and biogeochemical controls at different temporal and spatial scales is difficult. The hyporheic zone (HZ), where ground water–stream water mix, can be an important location controlling N and C transformations because it creates strong gradients in both the physical and biogeochemical conditions that control redox biogeochemistry. We evaluated the coupling of physical transport and biogeochemical redox reactions by linking an advection, dispersion, and residence time model with a multiple Monod kinetics model simulating the concentrations of oxygen (O$_2$), ammonium (NH$_4$), nitrate (NO$_3$), and dissolved organic carbon (DOC). The model successfully simulated the O$_2$, NH$_4$, NO$_3$ and DOC concentration profiles observed in the HZ at our study site. We then used global Monte Carlo sensitivity analyses with a nondimensional form of the model to examine coupled nitrification-denitrification dynamics across many scales of transport and reaction conditions. Results demonstrated that the residence time of water in the HZ and the uptake rate of O$_2$ from either respiration and/or nitrification determined whether the HZ was a source or a sink of NO$_3$ to the stream. We further show that the net NO$_3$ source or sink function of a HZ is determined by the ratio of characteristic transport time to the characteristic reaction time of O$_2$ (i.e., the Damköhler number, $Da_{O2}$), where HZs with $Da_{O2}$ < 1 will be net nitrification environments and HZs with $Da_{O2}$ >> 1 will be net denitrification environments. Our coupling of the hydrologic and biogeochemical limitations of N transformations across different temporal and spatial scales within the HZ allows us to explain the widely contrasting results of previous investigations of HZ N dynamics which variously identify the HZ as either a net source or sink of NO$_3$. Our model results suggest that only estimates of residence times and O$_2$ uptake rates are necessary to predict whether a HZ will be either a net source or sink of NO$_3$. 
5.1 INTRODUCTION

5.1.1 Background

Bioavailable forms nitrogen (N), such as nitrate (NO$_3$), are necessary for aquatic ecosystem productivity, and the availability of reactive N often limits ecosystem productivity [Jones and Holmes, 1996]. However, human alterations to the global N budgets have more than doubled the bioavailable supply of reactive N over the last century which in turn has caused increasingly negative impacts on water quality and aquatic ecosystems (e.g., biodiversity loss [Sala et al., 2000]; water quality degradation [Smith, 2003], accelerated global carbon and N cycling [Gruber and Galloway, 2008]; increased hypoxic events [Diaz and Rosenberg, 2008]). Streams in particular are important points in the landscape for the reactive N cycle, because they integrate many sources of N and control N export to downgradient systems via internal N source and sink processes (e.g., mineralization of organic forms of nitrogen and denitrification of NO$_3$, respectively). Consequently, there is a need to determine the key factors controlling sources and sinks of reactive N in stream ecosystems.

Unfortunately, N transformations in aquatic ecosystems are typically very complex and are couple multiple N species in both space and time. Thus, it is difficult to predict if a particular component of an aquatic system will function as a net source or sink, and at what temporal and spatial scales it will function over. In this study, we focus on how and why stream – ground water (hyporheic, HZ) interactions are important locations for coupled N transformations in the stream systems, and how they can function as both a source or sink of NO$_3$ to downgradient aquatic systems.

HZs are where stream and ground waters come together and mix in aquatic ecosystems. HZs are known to be key N transformation locations in streams [e.g., Duff and Triska, 1990, Holmes et al., 1994]. HZs function this way because they possess strong hydrologic and biogeochemical gradients [Jones and Holmes, 1996; Baker et al., 2000a]. These gradients lead to different redox conditions, which in turn control the conditions under which many biogeochemical reactions can occur [Hedin
et al., 1998]. In particular, redox conditions control where and when the dominant NO$_3$ source process - nitrification, and the dominant NO$_3$ sink process - denitrification, can occur. Both nitrification and respiratory denitrification are facilitated by microbes. Nitrification represents the chemoautotrophic oxidation of NH$_4$ to NO$_3$. Denitrification is the reduction of dissolved NO$_3$ to dinitrogen gas (N$_2$), which, in turn, can return to the atmosphere (Table 5.1). Denitrification is a particularly important N transformation in stream systems, because it represents the one true sink of NO$_3$ in aquatic ecosystems. Therefore, there is much interest in identifying when and where denitrification will be the dominant fate of NO$_3$ versus when and where NO$_3$ production via nitrification will dominate in a system.

There are both physical and biogeochemical conditions of HZs that regulate N biogeochemistry. The physical factor is the supply rate of solutes which is a function of advection, dispersion, hydraulic conductivity, and flowpath length. These physical conditions result in different characteristic residence times of water and solutes in HZs. The biogeochemical factors are the oxygen (O$_2$), labile carbon, organic nitrogen (DON), inorganic nitrogen (NH$_4$ and NO$_3$), temperature, and pH. Primarily, nitrifying microbes require O$_2$ and DON, while denitrifiers require anoxic reducing conditions, a DOC source to serve as an electron donor, and a supply of NO$_3$ to respire. In many systems, nitrification and denitrification are tightly coupled because nitrification consumes O$_2$ while producing NO$_3$, and both anoxic conditions and NO$_3$ availability will stimulate denitrification [e.g., Duff and Triska, 1990, Holmes et al., 1996]. Natural heterogeneity in streams leads to unique combinations of both the physical and biogeochemical conditions which in turn result in unique N source and sink conditions. This heterogeneity makes it hard to identify a priori the function of an HZ, so it is important to identify and account for the key components of the HZ N cycle. Accounting for these key components can be difficult to do in the field and across stream systems. Consequently, there is a need to identify new approaches for
assessing the role of the HZ on the fate of NO$_3$ that account for the complexity and are scalable across different systems.

Recent meta-analysis findings by Seitzinger et al. [2006] and experimental studies by Zarnetske et al. [2011] showed that net nitrification and denitrification are coupled and related to residence time in the HZ, where net nitrification dominates short residence times and net denitrification dominates long residence times (Figure 1). This linking of NO$_3$ dynamics to residence time helps simplify some of the above-stated complexities, while offering an explanation why previous studies of HZ NO$_3$ dynamics showed inconsistent HZ functioning - as either a source of NO$_3$ via nitrification or sink of NO$_3$ via denitrification. For example, Holmes et al. [1994] showed that short residence time oxic HZ flowpaths within the desert N-limited stream function as net nitrification systems, while much longer residence time anoxic HZ flowpaths of a more temperate N-rich river function as a net denitrification system [Pinay et al., 2009]. Larger synoptic sampling of spatially diverse stream ecosystems also shows heterogeneity in whether the stream sediment would function as net source or sink of nitrate given catchment setting and land use type [Inwood et al., 2005; Arango and Tank, 2008]. Accounting for the differences in residence time dynamics should collapse some of the variability seen between systems with respect to HZ N source-sink processes [Seitzinger et al., 2006, Gu et al., 2007, Zarnetske et al., 2011].

5.1.2 Objectives and Conceptual Framework of Study

Our goal is to identify a general theoretical framework to predict the NO$_3$ source and sink potentials of a given stream HZ. To do this we seek to identify the key subset of physical and biogeochemical parameters controlling N transformations in HZs (see Section 5.1.1). Building upon the existing literature, we pose the hypothesis that the net source or sink function of a HZ will be primarily a function of the characteristic residence time scales of water and solute in the HZ and the characteristic reaction (uptake) rate time scales of O$_2$. In other words, the potential function of an HZ as a source or sink of NO$_3$ will be primarily controlled by the
supply and demand rates of O$_2$, because O$_2$ controls the redox conditions which regulate where and when nitrification and denitrification occur [Seitzinger, 1988; Hedin et al., 1998]. Dissolved oxygen is critical to this hypothesis because it is known that O$_2$ availability in saturated sediment strongly inhibits denitrification rates [e.g., Terry and Nelson, 1975; van Kessel, 1977; Christensen et al., 1990]. Further, we focus on the O$_2$ uptake (respiration) rate because it is regulated by the labile DOC availability in the system, where low labile DOC availability will limit O$_2$ respiration rates [Pusch and Schwoerbel, 1994; Baker et al., 2000]. Therefore, O$_2$ uptake rates subsume some of the complex dynamics of labile DOC in a system. Additionally, the logistics of directly measuring or modeling water residence times and oxygen dynamics in HZ systems is easier than that of NO$_3$ and DOC (e.g., field and experimental tracer tests, groundwater flow models, and O$_2$ measurement instruments). Therefore, we hypothesize that the Damköhler number for O$_2$, $Da_{O2}$, (ratio of oxygen reaction rate time scales, $V_{O2}$ [T$^{-1}$], to water residence time scales, $\tau$ [T], where $\tau = L/v$, $L$ is the length of the flowpath, and $v$ [LT$^{-1}$] is the advected water velocity), in an HZ system will be a good indicator of the HZ potential function as a net nitrification or denitrification point in the landscape.

$$Da_{O2} = \frac{V_{O2}}{\tau}$$  \hspace{1cm} (1)

The Damköhler number is a useful concept for hydrochemical processes that are a function of both transport and reaction rates, because it is a simple dimensionless number that compares role of reaction and transport processes within and across systems [Domenico and Schartz, 1998; Ocampo et al., 2006; Gu et al., 2007; Green et al., 2009]. However, no previous study has attempted to use this scaling approach to identify when and where coupled N source and sink transformations, such as nitrification and denitrification, will occur. Therefore, we expand on our $Da_{O2}$ hypothesis to explore different conceptual HZ conditions and the resulting HZ
functioning as a net nitrification or denitrification system. First we need to define the
HZ function as a net source via nitrification or denitrification by calculating the
fraction change in NO\textsubscript{3} mass between the initial NO\textsubscript{3} concentrations at the head, \(N_{in}\),
and tail of the HZ flowpath, \(N_{out}\):

\[
R_N = \frac{N_{out}}{N_{in}}
\]  \hspace{1cm} (2)

Thus, \(R_N (0 < R_N < \infty)\), where a net denitrification system is \((0 < R_N < 1)\) and a net
nitrification system is \((R_N > 1)\). Next we can relate the \(R_N\) to the \(Da_{O2}\) of a system or
flowpath (Figure 5.2), such that we see the characteristic \(Da_{O2}\) of an HZ will control
the aerobic and anaerobic domains in the system, and therefore the domains over
which net nitrification and denitrification can exist. For example, net denitrification
will be inhibited at values of \(Da_{O2} < 1\), because this region of a system is where the
physical supply rate timescale of O\textsubscript{2} (i.e., \(\tau = L/v\)) is greater than the demand rate
timescale of O\textsubscript{2} (i.e., \(V_{O2}\)), and therefore will be oxic. This \(Da_{O2} < 1\) domain will
promote nitrification if NH\textsubscript{4} is present, while also inhibiting denitrification. A value
of \(Da_{O2} = 1\) represents a critical point in the system when the physical supply
timescale is equal to the biological demand timescale, and therefore represents the
point in a system where O\textsubscript{2} is exhausted and anaerobic conditions will influence the
biogeochemical processes. Lastly, all values of \(Da_{O2} > 1\) represent points in a system
where demand exceeds the supply, and therefore will be anaerobic and have the
potential to experience net denitrification if NO\textsubscript{3} and labile DOC are present.

5.1.3 Approach of Study

We developed and applied a numerical 1D, multispecies, reactive N transport
model to test the central hypothesis and conceptual model of this study (Section 5.1.2).
The model was used to evaluate the coupling of physical transport conditions
(advection, dispersion, and residence time) and biogeochemical redox conditions with
multiple Monod kinetics for $O_2$, $NH_4$, $NO_3$, and DOC. We used a dimensionless, steady-state form of the model to simulate the observed $O_2$, $NH_4$, $NO_3$, and DOC concentrations profiles observed in a well-characterized HZ of an agricultural stream ([Zarnetske et al., 2011]; shown in Figure 5.2). The basic transport modeling approach and governing equations are similar to the denitrification study of Gu et al. [2007], however, in this study we move beyond investigating just denitrification by incorporating nitrification ($NO_3$ source) dynamics and coupling it with denitrification ($NO_3$ sink) dynamics.

The dimensionless, steady-state form of the model was verified with the Zarnetske et al. [2011] data, and calibration was based upon simultaneously optimizing the model performance on all state-variables ($O_2$, $NH_4$, $NO_3$, and DOC). Next global Monte Carlo sensitivity analyses were used to identify the key model parameters governing model behavior and performance for the Zarnetske et al. [2011] HZ conditions. To specifically evaluate the general $NO_3$ source-sink hypothesis and conceptual model in Figure 5.1 we conducted a subsequent Monte Carlo sensitivity analysis of all possible model parameter combinations seen in the literature. The resulting Monte Carlo simulations were used to evaluate the key parameters governing the likelihood of simulating a net source or sink system as defined by the objective function of $R_N$.

5.2 METHODS

5.2.1 Study Site

The field and experimental data used in this modeling study was generated during prior solute tracer experiments in an instrumented gravel bar HZ on Drift Creek (Figure 5.3A), which is a third-order stream in the Willamette River basin of western Oregon, USA (44.9753°N, 122.8259°W). The catchment above the gravel bar site has mixed land use which is dominated by agricultural land and rural residential properties with septic systems near the site and forestry in the headwaters. The experimental
conditions under which the data were collected were stable base flow conditions of 22 Ls⁻¹. Of note, this gravel bar is underlain by bedrock and disconnected from a riparian aquifer source, so all HZ exchange through the gravel bar is stream water that originates at the head of the gravel bar and terminates at the tail of the gravel bar (Figure 5.3B). The stream water nutrient and chemical concentrations at the site were stable over the course of the experiment: NO₃⁻ (0.318-0.325 mg-N L⁻¹), NH₃ (0.021-0.024 mg-N L⁻¹), DOC (2.95-3.45 mg-C L⁻¹), O₂ (8.10-8.51 mg-O₂ L⁻¹), and pH (6.65-6.85). For additional details on the field study site and experimental observations used in this study refer to Zarnetske et al. [2011].

5.2.2 Model Development

5.2.2.1 Model Formulation with Transport and Reaction Kinetics

5.2.2.1.1 Transport Processes in Model

The transport of reactive solutes along hyporheic flowpaths was modeled with a 1D advection-dispersion model with multiple Monod biological reactions. This general modeling approach of using both physical transport and reaction kinetics has been used to successfully model reactive NO₃⁻ reduction and transport in HZ environments [Sheibley et al., 2003; Gu et al., 2007]. The basic form of the mathematical model is:

\[
\frac{∂c_i}{∂t} = D \frac{∂^2 c_i}{∂x^2} - v \frac{∂c_i}{∂x} + R_i
\]  

(3)

where \(C_i\) is the concentration of the \(i\)th solute (ML⁻³), \(D\) is the physical dispersion coefficient (L²T⁻¹), \(v\) is the physical advected velocity (LT⁻¹), and \(R_i\) is the biological reaction rate term (ML⁻³T⁻¹) representing the uptake kinetics of the \(i\)th solute due to all biogeochemical processes. The \(v\) values for the Drift Creek modeling in this study were taken from the Zarnetske et al. [2011] study which calculated \(v\) from the observed HZ breakthrough curves (BTC) of Cl⁻. Dispersion in this study is assumed
to be $D = \alpha_L v$, where $\alpha_L$ is the dispersivity, because dispersion effects are not the focus of this study. The dispersivity was set as $\alpha_L = 0.02L$, which is representative of how $\alpha_L$ scales with $L$ in groundwater systems [Neumann, 1990; Gelhar et al., 1992].

5.2.2.1.2 Reaction Processes in Model

The biological reactions modeled are aerobic respiration, nitrification, and denitrification (Table 5.1). These biologically mediated reactions are represented with multiple Monod kinetics in the transport model. Monod kinetics represents a chain of enzymatically mediated reactions with a limiting step described by Michaelis-Menten kinetics. Therefore, the reaction can be limited by multiple factors – substrates, electron acceptors, and nutrient availability. Multiple Monod kinetics are preferred over other kinetic models (e.g., instantaneous or zero- and first-order kinetics), because the Monod model does not assume that a biological reaction is instantaneous and it can capture multiple-order (zero-, first-, and mixed-order) behavior of biological reactions [Bekins, et al., 1998]. The multiple Monod model was also selected because it is not known a priori when and where a particular reaction is rate-limited by substrate or nutrient availability. Following Molz et al. [1986] and Gu et al. [2007] formulations, the multiple Monod kinetics are governed by the concentration of substances linked with a reaction – the terminal electron donors and acceptors associated with respiration, nitrification, and denitrification. The general mathematical form of the Monod model used in this study is:

$$
R_i = \frac{dC_i}{dt} = \frac{V_{\text{max}} X}{I} \left( \frac{C_{ED}}{K_{ED} + C_{ED}} \right) \left( \frac{C_{EA}}{K_{EA} + C_{EA}} \right)
$$

where, $R_i$ is the biological reaction for the $ith$ solute, $C_i$ is the concentration of the $ith$ solute, $V_{\text{max}}$ is the maximum specific microbial uptake rate ($T^{-1}$), $I$ is a noncompetitive factor (e.g., oxygen inhibits denitrification), $X$ is the microbial biomass concentration ($ML^{-3}$), $C_{ED}$ and $C_{EA}$ are the concentrations of the solutes involved in the reaction, $K_{ED}$
and $K_{EA}$ are the half-saturation constants (ML$^{-3}$), and the subscripts $ED$ and $EA$ designate the electron donor substrate and the electron acceptor, respectively. Given that the model will be used to assess steady-state conditions in the HZ, we do not consider biomass concentration ($X = 1$), growth, or transport within the model domain (i.e., biomass is at steady-state and attached to the solid particles, [Gu et al., 2007]). Therefore we adopt a macroscopic modeling approach which describes biogeochemical reactions without considering the distribution of the biomass within the pore space. The macroscopic approach is used widely in simulating reactive transport processes [Bekins, 1998]. The microbial biomass is included in the model as a lumped parameter in the reaction terms of the governing equations (i.e., $V_{max}$ in equation 4). The advantage of this formulation is that it does not require assumptions that cannot be verified by direct observations.

5.2.2.1.3 Inhibiting Reactions in Model

There are two N transforming reactions in the model – nitrification and denitrification that are limited by the availability of O$_2$ in the system. Nitrification requires O$_2$ and denitrification is noncompetitively inhibited by O$_2$. In this case, the noncompetitive inhibition arises from the fact that O$_2$ is thermodynamically advantageous over NO$_3$ as an electron acceptor [Hedin et al., 1998]. Therefore, a noncompetitive uptake inhibition model needs to be used for $I$ in equation (4). Segel [1975] provides a general mathematical form for modeling uptake inhibition for a noncompetitive situation such as the inhibition of denitrification by O$_2$. This general form is:

$$I = 1 + \frac{C^I}{K^I}$$

where, $C^I$ (ML$^{-3}$) is the concentration of the inhibiting substance and $K^I$ (ML$^{-3}$) is the inhibition constant for the reaction. Upon inspection of equation (5), it is seen that
when \( C^d \ll K^d \) there is little to no inhibition because \( I \approx 1 \). Conversely, inhibition can be very large when \( C^d \gg K^d \) because \( I \to \infty \) under these conditions.

### 5.2.2.1.4 Carbon Dynamics in the Model

As seen in Table 5.1, labile DOC (shown as CH\(_2\)O) is an essential component of aerobic respiration and denitrification and therefore is represented in model. DOC in the model domain can originate in two ways: 1. advected into the HZ with the O\(_2\), NH\(_4\), and NO\(_3\), or 2. generated \textit{in situ} via dissolution of particulate organic carbon (POC) located within the HZ sediment. Previous hyporheic studies have shown that POC can be advected into the HZ pore space [Marmonier et al., 1995] or entrapped during flood events that mobilize the streambed [Metzler and Smock, 1990]. Further, recent studies by Gu et al. [2007] and Peyrard et al. [2011] showed that \textit{in situ} sources of DOC are necessary to explain observed denitrification rates (i.e., advection supplied DOC was inadequate to fuel the observed denitrification). Groundwater studies have shown that \textit{in situ} POC sources, such as buried organic matter, release DOC as a kinetic process [Robertson and Cherry, 1995]. Investigators of groundwater and hyporheic POC dissolution [e.g., Jardine et al., 1992; MacQuarrie et al., 2001; Gu et al., 2007] have used the following kinetic dissolution model that simulates the generation of DOC \textit{in situ}:

\[
\frac{dC_p}{dt} = \alpha(k_d C - C_p)
\]  

where, \( C_p \) is the amount of POC mass in the sediment \( (M_{POC} M_{sediment}^{-1}) \), \( C \) is the DOC concentration in the water, \( \alpha \) is a first-order mass transfer coefficient \( (T^{-1}) \), and \( k_d \) is a linear distribution coefficient for the HZ sediment \( (L^3 M_{sediment}^{-1}) \). The observed POC of the sediment from the Drift Creek site is 2.27% [Nabelek, 2009].
5.2.2.1.5 Governing Equations of Model

The overall coupled nitrification and denitrification dynamics and 1D reactive solute transport modeled in this study are described by five coupled equations, four for each dissolved species (oxygen, ammonium, nitrate, DOC) and one for the dissolution of POC.

Dissolved Oxygen:

\[
\frac{\partial O_2}{\partial t} = D \frac{\partial^2 O_2}{\partial x^2} - v \frac{\partial O_2}{\partial x} - V_{O_2}y_0_2 \left( \frac{C}{K_C + C} \right) \left( \frac{O_2}{K_{O_2} + O_2} \right) \\
-V_{O_2} (1 - y_{O_2}) \left( \frac{NH_4}{K_{NH_4} + NH_4} \right) \left( \frac{O_2}{K_{O_2} + O_2} \right)
\] (7)

Ammonium:

\[
\frac{\partial NH_4}{\partial t} = D \frac{\partial^2 NH_4}{\partial x^2} - v \frac{\partial NH_4}{\partial x} - V_{NH_4} y_{NH_4} \left( 1 - \frac{K^I}{K^I + O_2} \right) \left( \frac{NH_4}{K_{NH_4} + NH_4} \right) \left( \frac{O_2}{K_{O_2} + O_2} \right) \\
-V_{NH_4} (1 - y_{NH_4}) \left( \frac{NH_4}{K_{NH_4} + NH_4} \right) \left( \frac{C}{K_C + C} \right)
\] (8)

Nitrate:

\[
\frac{\partial N}{\partial t} = D \frac{\partial^2 N}{\partial x^2} - v \frac{\partial N}{\partial x} + V_{NH_4} y_{NH_4} \left( 1 - \frac{K^I}{K^I + O_2} \right) \left( \frac{NH_4}{K_{NH_4} + NH_4} \right) \left( \frac{O_2}{K_{O_2} + O_2} \right) \\
-V_N \left( \frac{K^I}{K^I + O_2} \right) \left( \frac{C}{K_C + C} \right) \left( \frac{N}{K_N + N} \right)
\] (9)
Dissolved Organic Carbon:

\[
\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} - v \frac{\partial C}{\partial x} + \alpha(k_d C - C_p) - V_{O_2}y_{O_2} \left( \frac{C}{K_C + C} \right) \left( \frac{O_2}{K_{O_2} + O_2} \right) - V_{NH_4} \left( 1 - y_{NH_4} \right) \left( \frac{NH_4}{K_{NH_4} + NH_4} \right) \left( \frac{C}{K_C + C} \right) - V_N \left( \frac{K^I}{K^I + O_2} \right) \left( \frac{C}{K_C + C} \right) \left( \frac{N}{K_N + N} \right)
\]

where, \(O_2, NH_4, N,\) and \(C\) are concentrations of dissolved oxygen, ammonium, nitrate, and dissolved organic carbon \([\text{ML}^{-3}]\); \(v\) is linear velocity \([\text{LT}^{-1}]\); \(D\) is dispersion coefficient \([\text{L}^2 \text{T}^{-1}]\); \(V_{O_2}, V_{NH_4}\), and \(V_N\) are the maximum specific uptake rate of the substrate for carbonaceous and nitrification \(O_2\), nitrification and biological demand, and denitrification, respectively \([\text{T}^{-1}]\); \(K_{O_2}, K_{NH_4}, K_N\) and \(K_C\) are the half-saturation constant for \(O_2, NH_4, NO_3,\) and \(DOC\), respectively \([\text{ML}^{-3}]\); \(K^I\) is the noncompetitive inhibition concentration of \(O_2\) that separates nitrification of \(NH_4\) and denitrification of \(NO_3\), respectively \([\text{ML}^{-3}]\); \(y_{O2}\) is the partition (or yield) coefficient for general aerobic respiration and nitrification \([-\] based upon stoichiometry (ratio of secondary substrate to primary substrate consumed (e.g., 0.4 mg \(O_2\cdot CH_2O\)/mg \(O_2\cdot NH_4\) [Lee et al., 2006]); and \(y_{NH_4}\) is the partition (or yield) coefficient for nitrification and general biological uptake \([-\] based upon stoichiometry (ratio of secondary substrate to primary substrate consumed (e.g., 0.6 mg/mg; [Lee et al., 2006]).

5.2.2.1.6 Nondimensional Governing Equations of Model

The nondimensional forms of models can permit a model and its parameters to be evaluated across different system conditions [Bahr and Rubin, 1987]. Therefore, we used the nondimensional form of the model equations to examine the role of rate-limiting processes across different hyporheic conditions and between systems [sensu
Gu et al., 2007]. The nondimensional forms of the governing equations from Section 5.2.2.1.4 are:

**Dissolved Oxygen:**

\[
\frac{\partial O_2^*}{\partial \tau} = \frac{1}{Pe} \frac{\partial^2 O_2^*}{\partial \chi^2} - \frac{\partial O_2^*}{\partial \chi} - Da_{O_2} y_{O_2} \left( \frac{C^*}{K^*_C + C^*} \right) \left( \frac{1}{K^*_O_2 + O_2^*} \right) \\
- Da_{O_2} \left( 1 - y_{O_2} \right) \left( \frac{NH_4^*}{K^*_NH_4 + NH_4^*} \right) \left( \frac{1}{K^*_O_2 + O_2^*} \right)
\]

(11)

**Ammonium:**

\[
\frac{\partial NH_4^*}{\partial \tau} = \frac{1}{Pe} \frac{\partial^2 NH_4^*}{\partial \chi^2} - \frac{\partial NH_4^*}{\partial \chi} \\
- Da_{NH_4} y_{NH_4} \left( 1 - \frac{K^*_i}{K^*_i + O_2^*} \right) \left( \frac{1}{K^*_NH_4 + NH_4^*} \right) \left( \frac{O_2^*}{K^*_O_2 + O_2^*} \right) \\
- Da_{NH_4} \left( 1 - y_{NH_4} \right) \left( \frac{1}{K^*_NH_4 + NH_4^*} \right) \left( \frac{C^*}{K^*_C + C^*} \right)
\]

(12)

**Nitrate:**

\[
\frac{\partial N^*}{\partial \tau} = \frac{1}{Pe} \frac{\partial^2 N^*}{\partial \chi^2} - \frac{\partial N^*}{\partial \chi} + Da_{NH_4} y_{NH_4} \left( 1 - \frac{K^*_i}{K^*_i + O_2^*} \right) \left( \frac{1}{K^*_NH_4 + NH_4^*} \right) \left( \frac{O_2^*}{K^*_O_2 + O_2^*} \right) \\
- Da_N \left( \frac{K^*_i}{K^*_i + O_2^*} \right) \left( \frac{C^*}{K^*_C + C^*} \right) \left( \frac{1}{K^*_N + N^*} \right)
\]

(13)
Dissolved Organic Carbon:

\[
\frac{\partial C^*}{\partial \tau} = \frac{1}{Pe} \frac{\partial^2 C^*}{\partial x^2} + \frac{\partial C^*}{\partial x} + \frac{1}{Pe} \left( \frac{C^*}{K_C^* + C^*} \right) \left( \frac{1}{K_O^* + O^*_2} \right) - \frac{1}{K_N^* + N^*} \left( \frac{1}{K^*_N + N^*} \right) - \frac{1}{K^*_N + N^*} \left( \frac{1}{K^*_N + N^*} \right)
\]

Particulate Organic Carbon:

\[
\frac{d C_o}{d \tau} = D a_C \left( k_d C^* - k_d o \right)
\]

where, \( x/L; \tau = tv/L; Pe = vL/D; D a_{O2} = V_{O2} L/V; D a_{NH4} = V_{NH4} L/V; D a_N = V_N L/V; \)
\( D a_C = aL/v; O^*_2 = O_2/O_2^0; NH_4^* = NH_4/NH_4^0; N^* = N/N_0; DOC^* = DOC/DOC_0; \)
\( K^*_I = K_I/O_2^0; K^*_O2 = K_{O2}/O_2^0; K^*_NH4 = K_{NH4}/NH_4^0; K^*_N = K_N/N_0; \)
\( a \) is relative distance \([-\]; \( \tau \) is relative time \([-\]; \( L \) is flowpath length \([L]\); \( Pe \) is the Peclet number \([-] \); \( Da \) is the Damköhler number where subscripts \( O_2, NH_4, N, C \) are dissolved oxygen, ammonium, nitrate, and carbon, respectively \([-\]; \( O^*_2 \) is the \( O_2 \) relative concentration \([-\]; \( NH_4^* \) is the ammonium relative concentration \([-\]; \( N^* \) is the nitrate relative concentration \([-\]; \( K^*_I \) is the relative inhibition constant distinguishing between nitrification and denitrification \([-\]; \( K^*_O2 \) is the relative half saturation \( O_2 \) constant \([-\]; \( K^*_NH4 \) is the relative half saturation ammonium constant \([-\]; \( K^*_N \) is the relative half saturation nitrate constant \([-\]; \( K^*_C \) is the relative half saturation DOC constant \([-\]; \( y_{O2} \) is the partition (yield) coefficient for general carbonaceous respiration and nitrification \([-\]); \( y_{NH4} \) is the partition (yield) coefficient for nitrification and general biological uptake \([-\]); and \( k_d \) is the initial distribution coefficient.

5.2.2.1.7 Assumptions of Model

The key assumptions used in deriving the transport and reaction terms in the model can be summarized as follows:
1. Dispersion scales with the length of the system such that $D = \alpha_L v$, where $\alpha_L$ is the dispersivity and $\alpha_L = 0.02L$, which is representative of how $\alpha_L$ scales with $L$ in groundwater systems [Neumann, 1990; Gelhar et al., 1992].

2. Multiple Monod kinetics is appropriate within a macroscopic reactive transport modeling approach and biomass growth and transport is not considered (i.e., biomass is at steady-state and attached to the solid particles) per the macroscopic approach and steady-state conditions under which the model is used [Bekins et al., 1998].

3. The dissolution of POC and the re-sorption of DOC are described by reversible first-order kinetics [Jardine et al., 1992].

4. The entire fraction of DOC is labile and bioavailable for the reactions.

5. We use NH$_4$ to represent the mineralization product of DON, and do not have a NH$_4$ source term. We do not include DON as a source term or state-variable because there are many poorly defined intermediary reactions and pathways between DON and NH$_4$. However, it is well documented that NH$_4$ is readily converted to NO$_3$ in a single process – nitrification, so we represent the NH$_4$ as the direct source term for NO$_3$. Therefore, we represent DON mineralization, by setting the the initial NH$_4$ concentration to the peak observed NH$_4$ concentration observed in the Drift Creek HZ.

6. We assume that stoichiometric relationships represent the partitioning of O$_2$ demand between aerobic respiration and nitrification and NH$_4$ demand via nitrification and biological uptake [Lee et al., 2006].

5.2.2.2 Numerical Solution

The steady-state approaches employed in this study follow the basic numerical scheme put forth by Gu et al. [2007], which eliminates the time-domain. Adopting a similar numerical solution approach allows for more direct comparisons between the Gu et al. [2007] denitrification study and the present study.
A second-order centered finite-difference approximation was used to discretize the spatial derivatives in the steady-state model for first and second-order spatial derivatives. We let $U$ represent a state variable in the model (e.g., $U = O_2^*$) and develop the general steady-state form of the model. This general form is:

\[ \frac{\partial u}{\partial \tau} = 0 = \frac{1}{p_c} \frac{\partial^2 u}{\partial x^2} - \frac{\partial u}{\partial x} - RU \quad (16) \]

\[ U(0) = U_o \quad \text{(Inlet Boundary Condition)} \]

\[ \left. \frac{\partial u}{\partial x} \right|_{x=1} = 0 \quad \text{(Outlet Boundary Condition)} \]

where, $R$ represents all of the multiple Monod kinetic terms which are evaluated explicitly at the old values. A Dirichlet-type condition is used at the inlet of the model domain and a Neumann-type boundary condition at the outlet which is also estimated by a second-order approximation.

We let $dx = h > 0$ represent the space step on $[0, 1]$ and define $x_j = (j - 1)h$. Let $U_j^h \approx U(x_j), j = 1, 2 \ldots n$. The model domain is defined as:

\[ dx = h \]

\[ x_1 \quad x_2 \quad x_3 \ldots \quad x_j \quad x_{n-1} \quad x_n \quad x_{n+1} \]

0 \quad 1

The discrete scheme is constructed by using a second-order centered finite-difference approximation to the spatial derivatives:

\[ 0 = \frac{1}{p_c} \frac{\partial^2 u}{\partial x^2} \bigg|_{x_j} - \frac{\partial u}{\partial x} \bigg|_{x_j} - RU \bigg|_{x_j} \quad (17) \]

\[ 0 = \frac{1}{p_c} \left[ \frac{U_{j+1}^h - 2U_j^h + U_{j-1}^h}{h^2} \right] - \left[ \frac{U_{j+1}^h - U_{j-1}^h}{2h} \right] - RU_j^h \quad (18) \]

\[ 0 = U_{j+1}^h - 2U_j^h + U_{j-1}^h - \left( \frac{p_c h}{2} \right) (U_{j+1}^h - U_{j-1}^h) - RPe \, h^2 U_j^h \quad (19) \]
Discretization of the inlet boundary condition is:

\[ U_1^h = U_0 \]  

To discretize the Neumann boundary condition \( \frac{\partial u}{\partial x} \bigg|_{x=1} = 0 \) at the outlet, we introduce a fictitious point \( x_{n+1} \) and discretized both the Neumann condition and equation (16) at the point \( x_n \) as:

\[
0 = \frac{1}{Pe} \left[ \frac{U_{n+1}^h - 2U_n^h + U_{n-1}^h}{h^2} \right] - \frac{[U_{n+1}^h - U_{n-1}^h]}{2h} - RU_n^h \tag{21}
\]

\[
0 = \left[ \frac{U_{n+1}^h - U_{n-1}^h}{2h} \right] \tag{22}
\]

We can eliminate \( U_{n+1}^h \) from these equations to get:

\[
0 = \frac{1}{Pe} \left[ \frac{2U_{n-1}^h - 2U_n^h}{h^2} \right] - RU_n^h \tag{23}
\]

\[
0 = 2U_{n-1}^h - (2 + Pe \, h^2)U_n^h \tag{20c}
\]

We discretize the equations for each state variable using the scheme in equations (20), and collect discrete equations for all state variables. We get a large system of algebraic linear equations with a tridiagonal coefficient matrix. We solve the system using the Gaussian elimination method \([Hornberger and Wiberg, 2005]\). The system has the general form of \( Ax = B \), where \( A \) is a tridiagonal coefficient matrix containing the algebraic linear equations (size: \( 4n \times 4n \)), \( x \) is a vector containing the numerical solutions for all state variables (size: \( 4n \)), and \( B \) is the right hand side (RHS) vector (size: \( 4n \)). These are as defined as follows:
where $T$ is a tridiagonal matrix containing the algebraic equations (size: $n \times n$) and boundary conditions for each state variable. So,

\[
T = \begin{bmatrix}
1 & \frac{p e h}{2} & 0 & \ldots & \ldots & 0 \\
(1 + \frac{p e h}{2}) & -(2 + RPe h^2) & \left(1 - \frac{p e h}{2}\right) & 0 & \vdots \\
0 & \ddots & \ddots & \ddots & \vdots & 0 \\
\vdots & 0 & \left(1 + \frac{p e h}{2}\right) & -(2 + RPe h^2) & \left(1 - \frac{p e h}{2}\right) \\
0 & \ldots & 0 & 2 & -(2 + RPe h^2)
\end{bmatrix}
\]

(25)

\[
x = \begin{bmatrix}
O^h_1, \ O^h_2, \ldots, O^h_n, \ NH^h_{41}, \ NH^h_{42}, \ldots, NH^h_{4n}, N^h_1, N^h_2, \ldots, N^h_n, C^h_1, \ C^h_2, \ldots, C^h_n
\end{bmatrix}^T
\]

(26)

\[
\begin{array}{c|c}
\text{index} & \text{entry} \\
\hline
1 & 1 \\
\vdots & 0 \\
\vdots & \vdots \\
n - 1 & 0 \\
n & 1 \\
\vdots & 0 \\
\vdots & \vdots \\
B = RHS = 2n - 1 & 0 \\
2n & 1 \\
\vdots & 0 \\
\vdots & \vdots \\
3n - 1 & 0 \\
3n & -(RPe h^2) \\
\vdots & \vdots \\
4n - 1 & -(RPe h^2) \\
4n & 1 \\
\end{array}
\]

(27)
We used the numerical Peclet criteria, $Pe_2$, to assess and control the numerical dispersion and stability when selecting the spacing of the nodes in the model domain. The $Pe_2$ is given as:

$$Pe_2 = \frac{v \Delta x}{D} \leq 2$$

(28)

where, $\Delta x$ is the selected average nodal spacing [Huyakorn and Pinder, 1983].

### 5.2.2.3 Boundary and Initial Conditions of Model

Boundary and initial conditions were selected based upon the stream and hyporheic conditions observed in the Zarnetske et al. [2011] study. Initial conditions for the dissolved solute concentrations throughout the model were set to 0 mg L$^{-1}$. The initial POC was set to 2.27% based upon previous measurements of the site sediment [Nabelek, 2009] The Dirichlet type boundary condition (i.e., specified concentration) was used to represent the stream sourced solutes entering the model domain (i.e., head of lateral hyporheic flowpaths). A Neumann type boundary condition was used to represent advective transport of solutes out of the model domain (i.e., tail of lateral hyporheic flowpaths). Using the Neumann type boundary conditions assumes that the rate at which solute mass exits the flowpath via dispersion is negligible and can be ignored in the model. Therefore the boundary conditions are:

$$C_i (0, t) = C_{i,o},$$

$$\frac{\partial C_i}{\partial x} (L, t) = 0, \text{ when } t \geq 0,$$

where $C_i$ is the concentration of the $i$th solute, and $C_{i,o}$ is the $i$th solute concentration at the head of the hyporheic flowpath (i.e., the upgradient stream concentration).

### 5.2.3 Model Sensitivity Analysis and Parameter Estimation

The overall parameter estimation procedure involved: 1. fixing the known parameters values seen at the Drift Creek HZ, 2. using a Monte Carlo based global
sensitivity analysis to systematically identify the subset of key sensitive parameters for model optimization, and 3. calibrating the model to the Drift Creek observations by assigning the mean literature values to insensitive parameters and inversely optimizing the model by sampling the remaining sensitive parameter values from the range of literature values.

5.2.3.1 Global Sensitivity Analysis

Model parameter ranges and values were selected based upon findings from the study site or published studies (Table 5.2). As seen in Table 5.2, the model requires many input parameters that are not known a priori. Therefore, we evaluate the parameter space with a Monte Carlo based regional sensitivity analysis (RSA, \cite{Hornberger and Spear, 1981}) to isolate the key parameters to include in model optimization. The physical transport parameters of advection and dispersion were not included in the sensitivity analysis because they were measured in the field. The reaction kinetic parameters based upon stoichiometric relationships ($\gamma_{O2}$ and $\gamma_{nh}$) were also fixed to the literature value and not included in the sensitivity analysis. The remaining reaction kinetic parameters (Table 5.2) were included in the sensitivity analysis.

The basic RSA approach uses the observed data from Drift Creek to identify the reaction parameters by systematically minimizing a model objective function to isolate the set of parameter values that optimally describes the observed data. In this RSA, we use the Nash-Sutcliffe Efficiency (NSE, \cite{Nash and Sutcliffe, 1970}) as the objective function, $R_{NSE}$. The $R_{NSE}$ is calculated as:

\[
R_{NSE,i} = 1 - \left[ \frac{\sum_{i=1}^{n}(Y_{i}^{obs} - Y_{i}^{sim})^2}{\sum_{i=1}^{n}(Y_{i}^{obs} - Y_{i}^{mean})^2} \right]
\]

(29)

where, $R_{NSE,i}$ is the NSE for the $i$th state variable, $n$ is the total number of observations, $Y_{i}^{obs}$ is the $i$th observation for the normalized solute concentration being
evaluated, $Y_i^{sim}$ is the $i$th simulation value for the normalized solute concentration being evaluated, $Y_i^{mean}$ is the mean of the normalized observed values for the solute concentration being evaluated. The $R_{NSE}$ is an appropriate objective function in cases where there are non-linear dynamics which is indicative of the natural dynamics of NO$_3$ in HZs and our model system [Moriasi et al., 2007]. The $R_{NSE}$ is a normalized statistic that calculates the relative magnitude of the residual variance ("noise") compared to the measured data variance ("information"). The $R_{NSE}$ demonstrates how well the observed versus simulated data fits the 1:1 line. The $R_{NSE}$ can range from $-\infty$ to 1. The model accuracy improves as $R_{NSE} \rightarrow 1$. When the $R_{NSE} = 0$, it indicates that the model prediction is as accurate as the mean of the observed data. In the case of $-\infty < R_{NSE} < 0$, it indicates that the observed mean is a better predictor than the model.

We then calculate the mean of the 4 $R_{NSE}$ values generated for the O$_2$, NH$_4$, N, and DOC simulations, $\bar{R}_{NSE}$. The $\bar{R}_{NSE}$ is used to evaluate the total model performance because it better represents the goodness of the simulation across all state-variables and the coupled system dynamics. We transformed the observed data from Zarnetske et al. [2011] in two ways before conducting the sensitivity analysis and parameter estimations: 1. we normalized the concentrations of all solutes by their initial condition (i.e., $C_o/C$), and 2. we increased the number of observations across the spatial domain from $n = 12$ to $n = 21$ via linearly interpolating across the observed data. The use of normalized concentrations in the $\bar{R}_{NSE}$ calculation gives equal weight to all of the individual calculated $R_{NSE}$.

The specific steps of the RSA used in this study are summarized below [for more detail see Hornberger and Spear, 1981; Hornberger et al., 1985; Wagener and Kollat, 2007]:

1. Parameters from the model were selected for inclusion in the RSA (Table 5.2).
2. Literature values were used to identify the range of each parameter in the RSA (Table 5.2).
3. Uniform distribution bounded by the literature values were created for each parameter.
4. A series of Monte Carlo simulations were run ($n \geq 10000$), where each simulation involves randomly sampling and recording a set of parameters from their uniform distributions.
5. For each simulation, the simulation data was evaluated against the observed data by calculating and recording the $\overline{R_{NSE}}$ for that simulation.
6. The sampled parameter set for the simulation was then systematically partitioned into most behavioral to most non-behavioral groups, where behavioral means the parameter set produced a good model fit to the observations ($\overline{R_{NSE}} \rightarrow 1$) and non-behavioral means the parameter set produced a poor model fit to the observations ($\overline{R_{NSE}} \rightarrow -\infty$). We applied the Freer et al. [1996] RSA methodology within the Monte Carlo Analysis Toolbox (MCAT, [Wagener and Kollat, 2007]) to divide the parameter populations into 10 bins of equal size according to their $\overline{R_{NSE}}$ value.
7. The cumulative distributions of each binned parameter set were generated. The separation between the distributions of the most behavioral (best-fit) and most non-behavioral (worst-fit) curves indicates a statistical difference between the properties of the behavioral and non-behavioral parameter values. If the separation is large between the two distributions, then the parameter is deemed sensitive because its value is strongly correlated with the model performance.
9. The separation between the distribution curves of a parameter was quantified by using the Kolmogorov-Smirnov two-sample test [Kottegoda and Rosso, 1997]. The Kolmogorov-Smirnov two-sample test yields the maximum distance between the distributions, $\Delta_{RNSE}$, where the $\Delta_{RNSE}$ ranges between 0 and 1 and a $\Delta_{RNSE}$ close to 0 indicates an insensitive parameter.
10. The $\Delta_{RNSE}$ for each parameter is used to heuristically rank it relative to the other parameters in the sensitivity analysis with the most sensitive parameters having the largest $\Delta_{RNSE}$ and the least sensitive parameters having the smallest $\Delta_{RNSE}$. 


5.2.3.2 Parameter Estimation

Based upon the Global RSA (Section 5.2.3.2), only the 5 most sensitive parameters were included in the parameter estimation procedure to reduce the likelihood of encountering model equifinality. The 4 insensitive parameters were set to the mean literature value during the parameter estimation procedure. The mean literature values were used because there is a limited range of published values available for each parameter in the model. Using the \( R_{NSE} \) again as the model objective function, we create a Monte Carlo simulation set to identify the best performing simulations and their associated parameter values. The parameter ranges are then updated (reduced) to reflect the parameter ranges identified as having the most optimal \( R_{NSE} \), and then another Monte Carlo simulation set generated to further constrain the parameter ranges. The subsequent optimal \( R_{NSE} \) are compared to the previous optimal \( R_{NSE} \), and if the objective function is reduced, the procedure is repeated until a Monte Carlo simulation set produces the same \( R_{NSE} \) or the identical parameter set is obtained. The model parameter set has converged on an optimal solution when no further improvement in the objective function was achieved through further iterations [Zheng and Bennet, 1995].

5.2.3.3 Model Uncertainty

The Generalized Likelihood Uncertainty Estimation (GLUE) method was combined with the Monte Carlo simulations to address the issue of model equifinality and quantify the uncertainty of the selected best-fit model. The GLUE is a statistical method commonly applied in hydrology to quantify the uncertainty of model predictions using a Monte Carlo stochastic simulation set [Beven and Binley, 1992]. The GLUE analysis is briefly summarized below [for details see Beven and Binley, 1992, Beven and Freer, 2001].

The Monte Carlo simulation set provides an estimate of the statistics for the system state variables. In the GLUE method, each specific model simulation and its
specific parameter set is weighted by the likelihood of the same parameter set. This weighting is done for all time (or space) steps in the simulation. Then for each point in time (or space), a cumulative frequency distribution of solutions are created using the model objective function \( R_{NSE} \), where the simulations and associated parameter sets are rated by the likelihood that they fit the observed data. At this point, confidence intervals of these simulations are assigned and created by linearly interpolating the simulated solution space within selected confidence limits. In this analysis, we assigned confidence intervals of 0.05 and 0.95 and the GLUE analysis was implemented within MCAT [Wagener and Kollat, 2007].

5.2.3.4 Hyporheic N Source-Sink Sensitivity Analysis

We used another global Monte Carlo sensitivity analysis to evaluate which model parameters were most important in controlling whether the hyporheic system generated a net increase (source) or decrease (sink) of NO\(_3\) along the flowpath. To do this we follow the above described RSA method (Section 5.2.3.1) but we redefined the objective function to represent the fractional change of NO\(_3\) in the system, \( R_N \) (equation 2). For this RSA, we included all 10 reaction parameters plus the advection rate so that residence time \( (\tau = L/v) \) can be evaluated. The Monte Carlo simulations \( (n = 10000) \) were generated by randomly sampling parameter sets from the literature bounded uniform distributions for the reaction parameters and a uniform distribution of residence times between 0 and 1400 h. The RSA and subsequent Kolmogorov-Smirnov two-sample test yielded a parameter list ranking the sensitivity of each parameter.

5.3 RESULTS

5.3.1 Drift Creek Model and Sensitivity Analysis

The model captures the overall behavior of the steady-state NO\(_3\), NH\(_4\), O\(_2\), and DOC dynamics observed in the Drift Creek hyporheic zone. The coupling of
nitrification and denitrification in the model effectively simulated the nonlinear NO\textsubscript{3} concentration profiles seen along the hyporheic flowpaths. The DOC persistence along the flowpath was simulated with the POC dissolution model when coupled with the advected DOC sources. The optimal model fit for all 4 solutes had a $\overline{R_{\text{NSE}}} = 0.85$ (Figure 5.4), and the calibrated reaction parameter values are shown in Table 5.2.

The GLUE analysis demonstrated that the level of model uncertainty varies between the solutes and for each solute along the flowpath with the greatest uncertainty occurring along mid flowpath lengths where nitrification and denitrification are coupled (Figure 5.5). The uncertainty with the NO\textsubscript{3} concentration profile was greatest, which is to be expected given that the fate of NO\textsubscript{3} is coupled to all the other solutes. For example, much of the NO\textsubscript{3} model uncertainty is associated with the observed autocorrelation between the specific uptake rates of NH\textsubscript{4} and O\textsubscript{2}, $V_{\text{NH}4}$ and $V_{\text{O2}}$, respectively (Figure 5.6). The roles of $V_{\text{NH}4}$ and $V_{\text{O2}}$ in the model are similar in that they both consume O\textsubscript{2}. Therefore, they both control the onset of anaerobic and denitrification conditions, but only $V_{\text{NH}4}$ directly increases NO\textsubscript{3} in the system making it more influential in modeling the observed nitrification. At larger length and time scales in the model, the uncertainty of the NO\textsubscript{3} predictions decreases because the $V_{\text{NH}4}$ and $V_{\text{O2}}$ are limited by O\textsubscript{2} availability and therefore exert less influence on the NO\textsubscript{3} conditions (Figure 5.5). The RSA sensitivity analysis of the Drift Creek model illustrated that the hyporheic zone was particularly sensitive to the nitrification parameters of $V_{\text{NH}4}$, and the half-saturation coefficient, $K_{\text{NH}4}$ ($\Delta_{\text{RNSE}} = 0.76$ and 0.67, respectively, Table 5.3). This sensitivity to the nitrification conditions is expected given the strong nitrification signal observed along the short residence times of hyporheic exchange at Drift Creek. The DOC mass exchange rate from POC, $\alpha$, was also a key parameter ($\Delta_{\text{RNSE}} = 0.47$) controlling the models ability to simulate the biogeochemistry along flowpaths.
5.3.2 \textit{NO}_3 \textit{Source-Sink Sensitivity Analysis}

The global $R_N$ based sensitivity analysis showed that the uptake rate of $O_2$ and the advection rate were the two most influential parameters on the fate of inorganic $N$ in the simulated hyporheic systems ($\Delta R_N$ for $V_{O2} = 0.5$, and $v = 0.65$, Table 5.4 and Figure 5.7). The simulations were also sensitive to the denitrification and nitrification rates, but to a lesser degree ($\Delta R_N$ for $V_N = 0.4$ and $V_{NH4} = 0.38$), while the other parameters were relatively insensitive ($\Delta R_N < 0.26$).

The Monte Carlo simulations conditioned on the Drift Creek initial and boundary conditions were conducted for the two most sensitive hyporheic parameters (physical advection rate or residence time, $\tau$, and the biological $O_2$ uptake rate, $V_{O2}$). The Monte Carlo simulations sampled across known literature range for $V_{O2}$ and the arbitrary residence time range of 0 - 1400 h. This resulting $R_N$ response surface shows the possible combinations of NO$_3$ source-sink dynamics for these two key hyporheic parameters (Figure 5.8). The $R_N$ response illustrates that a net nitrification outcome can only occur at $V_{O2} < 1.15$ h$^{-1}$ across the sampled residence times. Conversely, a net denitrification outcome dominates most of the $V_{O2}$ and residence time parameter space. The maximum net denitrification occurs when the largest possible residence time and $V_{O2}$ are combined in the model.

The stochastic Monte Carlo simulations were conditioned on the Drift Creek initial and boundary conditions, but also represent the random sampling of 11 parameters (physical and biogeochemical parameters) across known literature ranges. This allowed us to simulate a larger range of possible hyporheic conditions effecting NO$_3$ source-sink dynamics. The simulations yielded a wide range of net nitrification and net denitrification hyporheic systems. The resulting $R_N$ for all 10000 resulting $Da_{O2}$ values shows dynamics similar to our original hypothesis for hyporheic $N$ dynamics, except that the onset of denitrification was at $Da_{O2}$ values greater than 1 (Figure 5.9). This shift in $Da_{O2}$ is associated with the additional residence time...
needed for denitrification to reduce the NO$_3$ generated via nitrification at earlier residence times.

5.4 DISCUSSION

Here we present the key findings from the Drift Creek model (Section 5.4.1), and then discuss the more general conceptual framework of hyporheic NO$_3$ source and sink processes that are illustrated by the stochastic numerical experiments (Section 5.4.2).

5.4.1 Drift Creek Dynamics

5.4.1.1 Drift Creek Hyporheic N Transformation

The Drift Creek model results closely matched the observed hyporheic N transformations and illustrate a hyporheic system where both nitrification and denitrification are tightly coupled. The two most significant reaction kinetic parameters in this complex biogeochemical system were the nitrification rate and the half-saturation constant for nitrification ($V_{NH_4}$ and $K_{NH_4}$, respectively; Table 5.3). These parameters are essential for capturing the observed nonlinearity in the NO$_3$ concentration profile, particularly the increase in NO$_3$ observed along shorter flowpaths and residence times (Figure 5.2 and 5.4). Furthermore, the nitrification rate contributes to the decrease of O$_2$ availability and the onset of redox conditions conducive to denitrification (Table 5.1). Therefore, this study supports the assertion that denitrification will be tightly coupled to nitrification conditions in streams where potential nitrification rates are high [Jones and Holmes, 1996; Kemp and Dodds, 2002; Sheibley et al., 2003].

The nitrification and denitrification rates observed at Drift Creek were high compared to many other saturated environments ($V_{NH_4} = 2.1$ h$^{-1}$ and $V_N = 2.9$ h$^{-1}$, respectively). For example, many soils and lake sediments have nitrification rates < 0.4 h$^{-1}$ and denitrification rates < 1.2 h$^{-1}$ (see Table 5.2 references). However, the Drift
Drift Creek rates are more typical of other hyporheic and riparian investigations, especially those that account for transport and reaction kinetics in their modeling approach [Sheibley et al., 2003; Gu et al., 2007]. Thus, this study further illustrates that hyporheic zones are biogeochemical hotspots in the landscape because they are places where hydrologic and biological processes create strong redox gradients [McClain et al., 2003].

5.4.1.2 Drift Creek N Transformations Coupled to C

Drift Creek nitrification-denitrification model also indicates that N transformations were strongly coupled to C dynamics as simulations were sensitive to the DOC supply from the POC source. Specifically, the amount of POC did not limit the denitrification in the system, but the mass transfer rate of DOC, $a$, from the POC source did limit denitrification and the optimality of the overall model (Table 5.3). Previous investigations showed that the quantity of POC is important to heterotrophic metabolism in stream sediments [e.g., Findlay and Sobczak, 1996; Fischer et al., 1996], but our simulations indicate that it is the ability of that POC to generate bioavailable DOC that is the real control on HZ N transformations.

The importance of DOC supply in N transformations is expected based upon the stoichiometry of heterotrophic metabolic processes and many previous experimental and modeling studies [see review in Duff and Triska, 2000]. The DOC serves as the electron donor in the reductive processes of aerobic respiration and denitrification (Table 5.1), so it is expected that DOC quality and availability will be a strong control on many HZ metabolic and redox conditions [Hedin et al., 1998]. Modeling by Peyrard et al. [2011] also showed that observed denitrification rates could not be adequately simulated without in situ POC generating DOC along the hyporheic flowpaths. Further, experimental work by Baker et al. [1999] and Zarnetske et al. [conditionally accepted], used labile DOC additions and redox sensitive solutes to prove that HZ metabolic rates, including denitrification, are
strongly limited by DOC supply, especially along flowpaths with long residence times.

Some investigators have not observed C limitations on hyporheic N transformations [e.g., Hill et al., 1998; Storey et al., 2004; Gu et al., 2007]. The denitrification modeling study by Gu et al. [2007] did not show a DOC limitation on observed denitrification rates of Cobb Mill Creek, a lowland sand-bedded stream in Virginia, USA. Interestingly, the present study and the Gu et al. [2007] study have many similarities including similar reactive transport modeling approaches, C kinetic parameter values (α and k_d), and sediment POC content (2-3% POC), and yet, POC sourced DOC is essential to fuel denitrification in Drift Creek and not in Cobb Mill Creek. There are two possible differences between the study systems: 1. the POC supply versus removal rates to the hyporheic zone and 2. the lability of the DOC derived from the in situ POC. We can estimate the importance of the hyporheic POC supply rate by calculating the maximum dissolution rate of POC. The maximum removal via dissolution to DOC can be solved as e^{-αt}. The result is that, in a 30 day period, ~40% of the POC at Drift Creek would be depleted while ~30% would be depleted from Cobb Mill Creek. As noted by Gu et al. [2007], their stream experiences frequent flow events that mobilize the sand bed material allowing POC to be buried and replenish depleted POC stocks. On the other hand, annual snowmelt drives the major bed-mobilizing flow events at Drift Creek, which means that POC burial alone may be insufficient to replenish hyporheic POC. Alternative mechanisms of hyporheic POC supply may be occurring at the site, such as advected transport and plant root exudation, and this may alter the type of POC in the HZ. The lability of the DOC at the Gu et al. [2007] site is unknown, but the lability of the DOC at the Drift Creek site decreases along the flowpath [Zarnetske et al., 2011] and is known to limit HZ denitrification rates [Zarnetske et al., conditionally accepted]. If the lability of the DOC derived from the sediment POC sources is different between the two sites, that contrast alone could explain the different roles DOC supply had on N transformations.
between the two studies. Therefore, the complexities associated with quality and quantity of DOC in fueling HZ metabolic processes requires further study and consideration in future studies.

Our model takes a simplified approach to modeling the hyporheic DOC source conditions – assuming all DOC is bioavailable and that it can be sourced in situ via POC dissolution. While our approach to modeling DOC dynamics is adequate for the purpose of this modeling study and many others [e.g., Jardine et al., 1992; MacQuarrie et al., 2001], there are still many unknown processes and pathways for DOC dynamics in HZs that require further investigation. Consequently, further improvements in modeling the hyporheic DOC availability and utilization should improve our ability to model coupled C and N transformations and identify the different roles DOC plays between systems (e.g., this study and the Gu et al., [2007] study).

5.4.2 Transport and Reaction Kinetics Control N Source-sink Dynamics

The dimensionless Damköhler number for dissolved O$_2$ presents a useful framework from which to investigate and estimate the net nitrification or denitrification potential of a hyporheic system. The effectiveness of combining the physical hydrological conditions with the O$_2$ uptake conditions to predict NO$_3$ source and sink processes in this study is not surprising. From a thermodynamic perspective it makes sense that $Da_{O2}$ would help define NO$_3$ source and sink systems, because the microbial redox reaction energy released during the respiration of NO$_3$ is second only to O$_2$ in microbial respiration processes (Free energy: aerobic respiration = 501kJ and denitrification = 476kJ [Hedin et al., 1998]). Therefore the use of O$_2$ dynamics as a first-order approximation of the onset of denitrification is theoretically justified. However, while the use of $Da_{O2}$ to predict other redox reactions maybe possible, it becomes less certain because the thermodynamic advantage of microbes to utilize other terminal electron acceptors (e.g., reducing Mn(IV), Fe(III), SO$_4$) is much less than O$_2$. 
5.4.2.1 Nitrification Dynamics and Limitations

A system or flowpath with $D_{\text{a}O_2} < 1$ means that net nitrification will be reaction rate limited, because it $O_2$ supply is not limiting. The reaction rate may be limited by other factors such as the number of reaction sites available, the amount of DON and NH$_4$ available, the temperature, or pH. At $D_{\text{a}O_2} > 1$ nitrification will become transport limited by the availability of $O_2$ and not the other physiochemical factors, because the supply rate of $O_2$ is less than biological demand. Revisiting Figure 5.1, we can see that net nitrification can occur over the entire $D_{\text{a}O_2}$ domain if the right combination of reaction substrate is available. For example, the hypothetical maximum nitrification system would contain: 1. a large source $O_2$, 2. a large source of DON to mineralize, 3. a low amount of ambient of NO$_3$ such that denitrifier communities are not recruited to the system, and 4. a low amount of labile DOC such that aerobic respiration of $O_2$ would be limited leaving more $O_2$ for nitrification.

5.4.2.2 Denitrification Dynamics and Limitations

A system or flowpath with $D_{\text{a}O_2} < 1$ means that net denitrification will be transport rate limited, because denitrification will be inhibited by the $O_2$ supply. In this domain of $D_{\text{a}O_2} < 1$, there may be adequate NO$_3$ and labile DOC available to carry out denitrification, but the bulk of the water will be oxic and have the potential to fuel nitrification. In this $D_{\text{a}O_2} < 1$ domain, denitrification would be restricted to microsites where anaerobic conditions can develop [Holmes et al., 1996; Zarnetske et al., 2011], however, the net effect of denitrification in this $D_{\text{a}O_2}$ domain will be limited. Conversely, at points in a system where $D_{\text{a}O_2} > 1$, denitrification potentials will dominate and will become substrate limited because $O_2$ inhibition will no longer exist. At $D_{\text{a}O_2} > 1$, substrate and chemical factors such as NO$_3$ and labile DOC availability, temperature, and pH will become the dominant controls on denitrification rates.
5.4.2.3 **Coupled Nitrification and Denitrification dynamics**

Nitrification and denitrification were strongly coupled along a set of flowpaths in the Drift Creek HZ (Figure 5.2 and 5.4) and in the stochastic modeling results (Figure 5.9). As seen in Drift Creek, by tracking O$_2$ and NO$_3$ dynamics of a water traveling along an flowpaths (i.e., create a distribution of $Da_{O2}$ going from low $Da_{O2}$ to high $Da_{O2}$; see dotted line in Figure 5.1 and transparent box on Figure 5.8) that parcel of water can experience coupled nitrification and denitrification. Initially the parcel of water travels along the head of the flowpaths (low $Da_{O2}$ values) and will experience nitrification which consumes O$_2$ and increases NO$_3$ mass. Therefore, the nitrification of that parcel of water promotes conditions ideal for denitrification – anoxia and high NO$_3$ availability. So as the parcel of water spends more time in the system as it travels toward the distal end of the flowpaths (high $Da_{O2}$ values), denitrification will start to dominate and NO$_3$ mass will decrease. In the stochastic hyporheic simulations, the onset of net denitrification is not at the originally hypothesized $Da_{O2} = 1$, but at values closer to $Da_{O2} = 10$ (Figure 5.9). This can be explained by the process of coupled nitrification and denitrification, because net denitrification ($R_N < 1$) cannot occur until the additional NO$_3$ mass generated during nitrification is denitrified. This additional denitrification will require additional time in the reactive system resulting in larger $Da_{O2}$ values. If a stream had the unusual conditions of little to no DON or NH$_4$, such as the streams in Antarctica [Gooseff et al., 2004; Koch et al., 2010], then we might expect to see net denitrification occurring closer to $Da_{O2} = 1$.

The numerical modeling and sensitivity analyses also indicate that future nitrification-denitrification modeling efforts many not require all of the reaction kinetic parameters involved in the present study. For example, across the stochastic hyporheic simulations, the half-saturation constants of the Monod kinetics and the O$_2$ inhibition term were less influential than the effective reaction rate constants in dictating the nitrification and denitrification dynamics (Table 5.4). This suggests that
if the goal is to predict net nitrification or denitrification conditions of a hyporheic system, simpler expressions of the reaction kinetics (e.g., zero- and first-order kinetic models) may be capable of capturing the basic behavior of nitrification and denitrification while reducing the parsimony of the model. For example, inspection of equation 4 shows that, if we can assume \( C_i >> K_i \) in our study system, the Monod kinetic model reduces to the zero-order model \( R_i \approx V_i \), where the subscript \( i \) represents a solute concentration of either an electron donor, \( ED \) or electron acceptor, \( EA \). Alternatively, if we know \( C_i << K_i \) in the study system, the Monod kinetic term becomes the first-order model \( R_i \approx V_i C_i \). These simplifying kinetic assumption of \( C_i << K_i \) was successfully employed by Sheibley et al. [2003] to simulate the inorganic N transformations in hyporheic profusion cores of an N-limited system, albeit on limited data sets and without comparison to alternative forms of the Monod kinetic model.

There are additional factors not directly accounted for in the numerical modeling of this study and the \( DaO2 \) approach, such as actual labile DOC supply, temperature, and pH. These additional factors will ultimately dictate how tightly coupled in space and time the nitrification and denitrification domains are in a system [Jones and Holmes, 1996; Duff and Triska, 2000]. Still the \( DaO2 \) approach seems to indirectly capture most of this complexity and offers a simplified framework for assessing the role of stream hyporheic zones on N cycling.

5.5 Conclusions

We demonstrate in this study that the characteristic hyporheic transport and reaction rate timescales will determine when and where net nitrification and denitrification will occur in a system and when each process is either transport or reaction rate limited. The Drift Creek site was a particularly useful hyporheic system to model, because it contains an observable continuum from nitrification to denitrification conditions. The nitrification-denitrification model successfully simulated the observed Drift Creek N transformation conditions and demonstrated that
the key conditions controlling hyporheic nitrification and denitrification were the nitrification rate, DOC supply, and the O$_2$ uptake rate. The model was also useful in a more expansive investigation of how HZ systems can operate as a NO$_3$ source or sink. The stochastic models of widely-varying hyporheic transport and reaction rate conditions showed that the key controls on the fate of inorganic nitrogen and hyporheic redox conditions is primarily governed by the residence time of the solute in the system and the O$_2$ uptake rate. Further, these two parameters can be related to each other in a useful way via the dimensionless Damköhler number for O$_2$, which is simply the ratio of the characteristic residence time scale and O$_2$ uptake time scale. This $Da_{O2}$ is a useful scaling approach for evaluating different streams hyporheic zones and flowpaths to each other, and may help make predictions about the hyporheic functioning as either a source or a sink of inorganic N in a given stream.
ACKNOWLEDGMENTS

Support for this project was provided by a NSF Ecosystem Informatics IGERT fellowship (grant DGE-0333257) to JPZ, research grants from the OSU Institute for Water and Watersheds and a North American Benthological Society Endowment Fund to JPZ, and the NSF grants EAR-0409534 and EAR-0409591 to RH, and SMW. Further support was provided by the Hollis M. Dole Environmental Geology Foundation at OSU. Any opinions, findings and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the NSF. We thank the Harry Klopfenstein Farm for generously granting access to the Drift Creek research sites. We extend special thanks to: V. Adams, S. Baxter, P. Zarnetske, A. Argerich, and B. Burkholder for field/lab assistance, and C. Jones and K. Motter of CCAL and OSU IWW Collaboratory for help with analyzing general water chemistry.
Table 5.1 Stoichiometry of redox reactions in the reactive transport model [modified from *Hedin et al.*, 1998].

<table>
<thead>
<tr>
<th>Reaction processes</th>
<th>General stoichiometric reaction equation</th>
<th>Free energy $\Delta G^0$ (kJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic respiration</td>
<td>$\text{CH}_2\text{O} + \text{O}_2 \rightarrow \text{CO}_2 + \text{H}_2\text{O}$</td>
<td>-501</td>
</tr>
<tr>
<td>(reductive)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrification</td>
<td>$\text{O}_2 + (\frac{1}{2})\text{NH}_4^+ \rightarrow (\frac{1}{2})\text{NO}_3^- + \text{H}^+ + (\frac{1}{2})\text{H}_2\text{O}$</td>
<td>-181</td>
</tr>
<tr>
<td>(oxidative)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Denitrification</td>
<td>$\text{CH}_2\text{O} + (4/5)\text{NO}_3^- + (4/5)\text{H}^+ \rightarrow (7/5)\text{H}_2\text{O} + (2/5)\text{N}_2 + \text{CO}_2$</td>
<td>-476</td>
</tr>
<tr>
<td>(reductive)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 5.2 Model parameters used in this study with literature sources, showing parameter ranges used in the sensitivity analyses and the calibrated values for Drift Creek.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>units</th>
<th>Literature Range</th>
<th>Drift Creek Model</th>
<th>$R_{NSE}$ Sensitivity Analysis</th>
<th>$R_{N}$ Sensitivity Analysis</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Physical Parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$L$</td>
<td>cm</td>
<td>500+</td>
<td>500</td>
<td>500</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>$v$</td>
<td>cm h$^{-1}$</td>
<td>17.1+</td>
<td>17.1</td>
<td>0.01-100</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>$D$</td>
<td>cm$^2$ h$^{-1}$</td>
<td>0.02$L_v$</td>
<td>0.02$L_v$</td>
<td>0.02$L_v$</td>
<td>25, 26</td>
<td></td>
</tr>
<tr>
<td><strong>Reaction Parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_O2$</td>
<td>h$^{-1}$</td>
<td>0.75-10.0</td>
<td>1.6*</td>
<td>0.75-10.0</td>
<td>0.75-10.0</td>
<td>16, 21, 22, 23</td>
</tr>
<tr>
<td>$V_{NH4}$</td>
<td>h$^{-1}$</td>
<td>0.36-4.2</td>
<td>2.1*</td>
<td>0.36-4.2</td>
<td>0.36-4.2</td>
<td>18, 20, 21, 22</td>
</tr>
<tr>
<td>$V_N$</td>
<td>h$^{-1}$</td>
<td>0.26-10</td>
<td>2.9†</td>
<td>0.26-10</td>
<td>0.26-10</td>
<td>3, 11, 15, 17, 18, 20, 21, 22</td>
</tr>
<tr>
<td>$K_{O2}$</td>
<td>mg L$^{-1}$</td>
<td>0.2-5.8</td>
<td>4.5*</td>
<td>0.2-5.8</td>
<td>0.2-5.8</td>
<td>5, 8, 14, 16, 17, 19, 20, 21, 22, 23</td>
</tr>
<tr>
<td>$K_C$</td>
<td>mg L$^{-1}$</td>
<td>1.0-10.0</td>
<td>5.6†</td>
<td>1.0-10.0</td>
<td>1.0-10.0</td>
<td>5, 8, 14, 17, 21, 22</td>
</tr>
<tr>
<td>$K_{NH4}$</td>
<td>mg L$^{-1}$</td>
<td>0.1-1.1</td>
<td>1.0*</td>
<td>0.1-1.1</td>
<td>0.1-1.1</td>
<td>1, 2, 7, 9, 13, 21</td>
</tr>
<tr>
<td>$K_N$</td>
<td>mg L$^{-1}$</td>
<td>0.21-3.1</td>
<td>1.4†</td>
<td>0.21-3.1</td>
<td>0.21-3.1</td>
<td>3, 5, 8, 11, 14, 15, 17, 21, 22</td>
</tr>
<tr>
<td>$K'$</td>
<td>mg L$^{-1}$</td>
<td>0.2-1.0</td>
<td>0.6†</td>
<td>0.2-1.0</td>
<td>0.2-1.0</td>
<td>4, 5, 6, 8, 14, 17, 21, 22</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>h$^{-1}$</td>
<td>$1 \times 10^5 - 1 \times 10^3$</td>
<td>$1 \times 10^4$*</td>
<td>$1 \times 10^5 - 1 \times 10^3$</td>
<td>$1 \times 10^5 - 1 \times 10^3$</td>
<td>10, 17, 22</td>
</tr>
<tr>
<td>$k_d$</td>
<td>L$^3$ kg$^{-1}$</td>
<td>50</td>
<td>50†</td>
<td>50</td>
<td>5.0-100</td>
<td>12, 17, 22</td>
</tr>
<tr>
<td>$y$</td>
<td>--</td>
<td>0.4</td>
<td>0.4†</td>
<td>0.4</td>
<td>0.4</td>
<td>21</td>
</tr>
<tr>
<td>$y_{NH4}$</td>
<td>--</td>
<td>0.6</td>
<td>0.6†</td>
<td>0.6</td>
<td>0.6</td>
<td>21</td>
</tr>
</tbody>
</table>

$R_{NSE}$ 0.85

Table 5.3: The Drift Creek model parameter sensitivity ranking based upon the regional sensitivity analysis with the objective function $\overline{R}_{NSE}$. The $\Delta_{RNSE}$ represents the parameter sensitivity as determined by the statistical Kolmogorov-Smirnov test of the RSA results.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sensitivity Rank</th>
<th>$\Delta_{RNSE}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{NH4}$</td>
<td>1</td>
<td>0.73 *</td>
</tr>
<tr>
<td>$K_{NH4}$</td>
<td>2</td>
<td>0.67 *</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>3</td>
<td>0.47 *</td>
</tr>
<tr>
<td>$K_{O2}$</td>
<td>4</td>
<td>0.32 *</td>
</tr>
<tr>
<td>$V_{O2}$</td>
<td>5</td>
<td>0.28 *</td>
</tr>
<tr>
<td>$K_I$</td>
<td>6</td>
<td>0.17 +</td>
</tr>
<tr>
<td>$K_N$</td>
<td>7</td>
<td>0.12 +</td>
</tr>
<tr>
<td>$V_N$</td>
<td>8</td>
<td>0.09 +</td>
</tr>
<tr>
<td>$K_C$</td>
<td>9</td>
<td>0.09 +</td>
</tr>
</tbody>
</table>

*used in model calibration for Drift Creek conditions
+fixed at mean literature value for Drift Creek model
Table 5.4: Hyporheic nitrification-denitrification parameter sensitivity ranking based upon the regional sensitivity analysis with the objective function of $R_N$, the N fractional change (i.e., NO$_3$ production or removal). The $\Delta_{RN}$ represents the parameter sensitivity as determined by the statistical Kolmogorov-Smirnov test of the RSA results.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sensitivity Rank</th>
<th>$\Delta_{RN}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$v$</td>
<td>1</td>
<td>0.65</td>
</tr>
<tr>
<td>$V_{O2}$</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>$V_N$</td>
<td>3</td>
<td>0.4</td>
</tr>
<tr>
<td>$V_{NH4}$</td>
<td>4</td>
<td>0.38</td>
</tr>
<tr>
<td>$K_C$</td>
<td>5</td>
<td>0.26</td>
</tr>
<tr>
<td>$K_N$</td>
<td>6</td>
<td>0.25</td>
</tr>
<tr>
<td>$a$</td>
<td>7</td>
<td>0.23</td>
</tr>
<tr>
<td>$k_d$</td>
<td>8</td>
<td>0.2</td>
</tr>
<tr>
<td>$K_{NH4}$</td>
<td>9</td>
<td>0.15</td>
</tr>
<tr>
<td>$K_I$</td>
<td>10</td>
<td>0.12</td>
</tr>
<tr>
<td>$K_{O2}$</td>
<td>11</td>
<td>0.11</td>
</tr>
</tbody>
</table>

*used in model calibration for Drift Creek conditions
+fixed at mean literature value for Drift Creek model
Figure 5.1: A conceptual model showing how net nitrification and net denitrification potential ($R_N$) is a function of the Damköhler number, $Da_{O2}$ (ratio of characteristic hydrologic transport timescale to biological $O_2$ uptake timescale). The gray area represents the hypothesized $R_N$ domain for all combinations of hyporheic conditions controlling nitrification and denitrification. The dashed line within the domain represents the conditions observed along hyporheic flowpaths at Drift Creek in this study and in Zarnetske et al. [2011].
Figure 5.2: Nitrification and denitrification dynamics as a function of residence time in the Drift Creek hyporheic zone [modified with permission from Zarnetske et al., 2011]. The reported data represent steady-state concentrations of dissolved oxygen ($O_2$, green circles), nitrate ($NO_3$, blue diamonds), and total dissolved organic carbon (DOC, orange squares). Each point presents the normalized mean values generated from repeated steady-state samples collected along hyporheic flowpaths ($n=5$, error bars $= \pm 2$ standard error, values were normalized by initial observed concentration seen at the head of the flowpath).
Figure 5.3: (A) Map of the Drift Creek Study site showing the gravel bar hyporheic site used in Zarnetske et al. [2011] and in the modeling study. (B) Map of the gravel bar hyporheic zone showing locations of wells (circles with cross hairs) and the water potentiometric contours. The dashed arrow indicates an individual representative simulated flowpath between the head and tail of the gravel bar.
Figure 5.4: Observed (open-circles) versus the best-fit model simulations (lines). The $R_{NSE}$ for the optimal simulations presented is 0.85.
Figure 5.5: Observed solute concentrations (dots) with the GLUE generated model confidence intervals (0.05 and 0.95) for each solute. The dCFL subplots show how the normalized level of uncertainty varies across the spatial domain of the Drift Creek model.
Figure 5.6: The cumulative parameter distributions of 9 reaction kinetic parameters included in the Regional Sensitivity Analysis of the Drift Creek model.
Figure 5.7: Cumulative parameter distributions of 11 physical transport and reaction kinetic parameters included in the Regional Sensitivity Analysis of the stochastic hyporheic simulations.
Figure 5.8: The $R_N$ response surface for oxygen uptake rate, $V_O2$, and residence time in the HZ, $\tau$, showing the net nitrification domain ($R_N > 1$) in blue and net denitrification domain ($R_N < 1$) in red. The transparent box on the $R_N$ response surface shows the observed parameter domain measured during field investigations of the Drift Creek hyporheic zone.
Figure 5.9: The relationship between $R_N$ and $D_{aO_2}$ based upon 10,000 stochastically generated simulations using biogeochemical reaction parameter sampling values bounded by known literature value ranges. Net nitrification simulations are shown as blue dots and net denitrification simulations are shown as red dots. Note the general agreement with the hypothesized solution space shown in Figure 5.1.
6 – Conclusion

This dissertation uses $^{15}$N isotope tracing, numerical modeling, and a suite of other geophysical methods to investigate two key, coupled catchment processes - hyporheic exchange and bioavailable N dynamics. This research identifies hydrologic-biogeochemical linkages and quantifies rates of N transformations with respect to transport rates. This research elucidates the role of the hyporheic zone as a “hotspot” in whole-stream N transformation. This research impacts the broader stream nutrient and water quality research communities because it offers a way to account for previously immeasurable hyporheic biogeochemical interactions. Furthermore, it develops process-based scaling relationships that will enable the investigation of complex hyporheic N transformations across variable spatial and temporal scales that are inherent to stream ecosystems.

6.1 Central Themes and Key Discoveries of Dissertation

The fundamental conclusion of this dissertation is that the investigation and comprehension of denitrification processes requires linking disciplines and spatiotemporal scales. Under this over-arching conclusion, this dissertation presents three major themes for investigating denitrification in stream and hyporheic systems. The first theme is that multidisciplinary methodological approaches are essential to the study of aquatic denitrification. The second theme is that careful consideration and quantification of hydrologic and geomorphic processes can greatly inform the investigation of denitrification occurring in streams and hyporheic zones. The third theme is that the development of process-based knowledge concerning how different environmental variables control denitrification potentials. This process-based knowledge will facilitate more efficient and robust approaches to quantify and potentially manage denitrification across stream systems. Below I present the key findings of each study included in this dissertation and how the studies tie into the three themes of conducting denitrification research.
In Chapters 2, I used whole-stream steady-state $^{15}$N-labeled nitrate ($^{15}$NO$_3^-$) and conservative tracer addition experiments and hydrogeology measurements to investigate the spatial and temporal physiochemical conditions controlling the denitrification dynamics in the hyporheic zone of an upland agricultural stream (Drift Creek, Oregon, USA). I used these conservative and reactive tracers to inform hydrologic transport and biologically-mediated nitrification and denitrification reactions, thus demonstrating that residence time controls where and when nitrification and denitrification occurs in a hyporheic zone. The hyporheic zone flowpaths transitioned from a net nitrification environment at short residence times to a net denitrification environment at long residence times. Under the experimental conditions there was a residence time threshold of 6.9 h that separated the net nitrification and denitrification domains. Thus, this hyporheic zone was a hot spot for nitrogen transformation, where hot spots of nitrate production and removal were distinguished by residence time. This study implies that accounting for hyporheic exchange and water residence time will help upscale hyporheic denitrification measurements to stream networks scales in a way that is mechanistically linked to quantifiable hydrologic proprieties.

In Chapter 3, I build on Chapter 2 and use our understanding of hydrology to investigate a key biogeochemical control on hyporheic N transformations – the availability of labile dissolved organic carbon (DOC). Again, I utilized $^{15}$N and conservative tracers to observe the denitrification in the hyporheic system of Drift Creek, but this time I experimentally manipulated the availability of the labile DOC supply. By carefully considering the hydrology with hydrogeology measurements and water tracers, I was able to isolate the biogeochemical control of labile DOC availability on denitrification in hyporheic zones. I showed that labile DOC will stimulate denitrification, especially in regions of the hyporheic zone that have strong reducing environments. In Drift Creek, the addition of labile DOC dramatically altered the hyporheic function on stream N dynamics with hyporheic NO$_3^-$ removal rates.
increasing from 218 kg ha\(^{-1}\) yr\(^{-1}\) to 521 kg ha\(^{-1}\) yr\(^{-1}\). In other words, I took a known denitrification hot spot in the stream and created a denitrification hot moment by augmenting the supply of labile DOC. By leveraging my understanding of the hydrology with hydrogeology measurements and water tracers, I was able to isolate the biogeochemical control of labile DOC availability on denitrification in hyporheic zones. Overall, this study clearly emphasizes that aquatic C and N cycles are coupled, and that the quantity and quality of DOC in a stream system will be an important factor for future consideration in process-based experiments and models of stream denitrification.

Chapter 4 presents one of the first robust estimates of the total hyporheic influence on whole-reach scale denitrification. For this study, I used independent and complimentary measurements of aquatic denitrification and hydrologic transport kinetics. These measurements were made at multiple scales (e.g., point, plot, and reach). I utilized the detailed hyporheic and whole-reach field NO\(_3\)\(^-\) experiments from Chapter 2 in Drift Creek, and developed subsequent groundwater and stream transport models to derive scalable reaction and transport rates. This study showed that 17% of the total reach NO\(_3\)\(^-\) uptake and 32% of the total reach denitrification occurred in the Drift Creek hyporheic zone. In addition to the potential biogeochemical limitations on hyporheic denitrification, observations of the actual hydrogeology and geomorphic setting also helped explain the observed denitrification rates. For example, a limitation on the influence of hyporheic denitrification was the constrained vertical and lateral extent of the hyporheic zone through which stream water exchanged and NO\(_3\)\(^-\) removal could occur.

The use of multi-scale measurements in Chapter 4 also emphasizes many methodological limitations that introduce uncertainty to these estimates of NO\(_3\)\(^-\) removal pathways. Namely, the methods that integrate NO\(_3\)\(^-\) biogeochemical reaction kinetics with physical transport kinetics at the reach scale bias NO\(_3\)\(^-\) removal estimates toward shorter characteristic timescales more indicative of the main channel. This
timescale bias may result in systematic underestimates of the true impact of hyporheic exchange on biogeochemical processes. Therefore, new methods must be developed to investigate the different spatial and temporal scales of stream denitrification.

Each of the above Chapters represents novel combinations of the most promising methodological techniques and tools we have to investigate aquatic denitrification. Therefore, the data sets generated in these investigations are robust and have the potential to provide further insight into denitrification dynamics via additional analyses. However, establishing broad conclusions from these data sets must be done with caution because they were all collected within one stream system. Using these observation-based studies alone may lead to exaggerated conclusions about hyporheic N cycling dynamics due to pseudoreplication, so the data should be used in conjunction with additional modeling studies to reduce the potential influence of pseudoreplication. Toward this goal, Chapter 5 builds on all prior chapters by developing a numerical modeling approach to investigate hyporheic NO$_3^-$ source and sink function that accounts for residence time (Chapter 2), different N and DOC conditions (Chapter 3), and is capable of scaling across space and time (Chapter 4). This modeling investigation focused on the scaling issues of predicating if a stream hyporheic exchange will be a source via nitrification or a sink via denitrification, given different combinations of physical transport and biogeochemical reaction kinetics. In Drift Creek the key conditions controlling hyporheic nitrification and denitrification were the nitrification rate, DOC supply, and the oxygen (O$_2$) uptake rate, which is supported by the field experiments in Chapters 2 and 3. I also use the model for a more expansive, general investigation of how hyporheic systems can operate as a NO$_3^-$ source or sink to the stream. Stochastically generated models of widely-varying hyporheic transport and reaction rate conditions showed that there are a couple of key controls on the fate of bioavailable N and hyporheic redox conditions. The key conditions governing the fate of hyporheic N are the residence time of solutes and the O$_2$ uptake rate. An important hyporheic scaling relationship was developed by
relating these two kinetic parameters to each other. The parameters were related to each other via the dimensionless Damköhler number for O$_2$, which is the ratio of the residence time scale and to the O$_2$ uptake time scale. This Damköhler relationship was a useful scaling approach for evaluating different stream hyporheic zones, and represents a new elegant approach to investigate the complexities of stream N source-sink dynamics. It may also be incorporated into future modeling studies that attempt to make predictions about the stream hyporheic functioning as either a source or a sink of bioavailable N.

6.2 **KEY OUTSTANDING ISSUES ARISING FROM DISSERTATION**

This dissertation provides new insights into the properties of hyporheic processes and their role on stream N cycling. However, this dissertation also raises many outstanding issues about stream-hyporheic dynamics and N cycling. These issues are primarily associated with the scaling of denitrification across spatiotemporal heterogeneity.

A key issue not addressed in the above studies is temporal variability. Streams are dynamic systems that are typically characterized by temporal variations in hydrological and biogeochemical properties. So how do changes in stream hydrologic conditions (e.g., stream flow rates and stage) alter hyporheic exchange rates and residence times that control denitrification potentials? Or, how do variations in stream and groundwater chemistry (e.g., seasonal changes in NO$_3^-$, DOC, and temperature) affect the redox conditions that ultimately control denitrification? And, how do these physical and ecological conditions interact - do they vary in a way such that they interact to control stream N conditions? For example, Chapter 2 and 3 clearly showed that denitrification in Drift Creek is dependent upon the residence time distribution of hyporheic exchange and the availability of labile DOC. Thus, seasonal flow variations in Drift Creek that result in shorter hyporheic residence times would decrease the
hyporheic denitrification rates. Alternatively, if a summer rain event induced a pulse of highly labile DOC in the stream, hyporheic denitrification would likely increase.

Fundamentally there is context dependence to the temporally-varying response of hyporheic N dynamics, but the findings of Chapter 5 are encouraging in that they show us a way forward to study streams as dynamical N cycling systems. Future investigations can measure how the characteristic timescales of transport and O\textsubscript{2} uptake vary across different hydrologic and biogeochemical regimes. This will provide a temporally-varying estimate of the Damköhler number for a stream, which will provide estimates of when a stream hyporheic zone functions as a source or a sink of N in the stream.

The other main question is associated with the large scale spatial variability of hydrologic and biogeochemical conditions controlling N transformations. Individual streams exist as a part of a network, where there is a continuum from the smallest streams in the headwaters to the largest streams draining into the ocean. The question that arises about the role of hyporheic N transformations, is where along this stream network continuum does hyporheic exchange control stream N cycling? Further, are there places along the stream network that are optimal for hyporheic denitrification? For example, Drift Creek is a third-order (i.e., mid-network) stream with known hyporheic exchange that is surrounded by land uses that results in regionally high stream NO\textsubscript{3}\textsuperscript{-} concentrations. As a result, denitrification potentials are not strongly limited by hyporheic transport or reaction rates. However, what happens to denitrification potentials as you move upgradient into the headwaters or downgradient into the larger-order streams?

The findings of this dissertation indicate that we can start to address spatial heterogeneity in N transformations by focusing on the variation of hyporheic residence time distributions, hyporheic exchange relative to the main channel, and labile carbon across the stream network. In Drift Creek, we are likely to find very different hyporheic denitrification influence as we move upgradient or downgradient of the
third-order reach. The steep headwaters of Drift Creek are forested and have few human or natural sources of NO$_3^-$ and labile DOC, as well as potentially short hyporheic residence times, so their denitrification rates will be reaction-limited despite high rates of hydrologically-driven hyporheic exchange. Conversely, the denitrification in larger rivers downgradient of Drift Creek will be limited despite regionally high NO$_3^-$ and labile DOC concentrations, because hyporheic exchange rates are low and will limit the flux of water and solutes to denitrification sites. Thus, as with the temporal heterogeneity issue, the role of hyporheic denitrification on streams has a spatial context that needs to be quantified and compared across systems.

In summary, the findings of this dissertation help to identify the key measurements and scaling relationships required to develop comparisons of bioavailable N conditions between sites and across time. Furthermore, this dissertation presents a new coupled hydrologic-biogeochemical modeling framework to address complex aquatic N cycling dynamics. Still, there are many issues to be resolved about when and where hyporheic dynamics matter to stream N cycling. To address this outstanding issues, future investigations of aquatic N cycling, especially nitrification and denitrification, should keep the central themes of this investigation in mind. First, use a multi-disciplinary, multi-scale approach to investigations. Second, use the physical hydrologic and geomorphic properties of a system to inform and reduce the uncertainty of estimating complex biogeochemical processes. Lastly, seek to develop process-based relationships between the variables controlling nitrification and denitrification so that scalable relationships can be identified, quantified, and modeled across stream systems.
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