Winter 2017

Nitrogen dynamics and retention in the river network of a tropical forest, Luquillo Mountains, Puerto Rico

Richard Brereton
University of New Hampshire, Durham

Follow this and additional works at: https://scholars.unh.edu/dissertation

Nitrogen dynamics and retention in the river network

of a tropical forest, Luquillo Mountains, Puerto Rico

BY

RICHARD L. BRERETON

BA, Colorado College, 2008

DISSERTATION

Submitted to the University of New Hampshire

in Partial Fulfillment of

the Requirements for the Degree of

Doctor of Philosophy

in

Earth & Environmental Science

December, 2017

COMMITTEE PAGE

This dissertation has been examined and approved in partial fulfillment of the requirements for the degree of

Doctor of Philosophy in Earth and Environmental Science by:

Dr. William H. McDowell, Professor,
Department of Natural Resources and the Environment
(Dissertation Director)

Dr. Heidi Asbjornsen, Associate Professor,
Department of Natural Resources and the Environment

Dr. John L. Campbell, Research Ecologist,
USDA Forest Service Northern Research Station

Dr. Wilfred M. Wollheim, Associate Professor,
Department of Natural Resources and the Environment

Dr. Adam S. Wymore, Research Assistant Professor,
Department of Natural Resources and the Environment

On April 20th, 2017

Original approval signatures are on file with the University of New Hampshire Graduate School.

ACKNOWLEDGEMENTS

DEDICATION

To Morgan and Clay

and to my parents, who instilled in me the joy of figuring things out.

ABSTRACT
NITROGEN DYNAMICS AND RETENTION IN THE RIVER NETWORK
OF A TROPICAL FOREST, LUQUILLO MOUNTAINS, PUERTO RICO

by

Richard Brereton

University of New Hampshire, December, 2017

This dissertation identifies gaps in the scientific understanding of nutrient cycling, particularly nitrogen (N) cycling, in streams and riparian zones of tropical montane forests, and addresses several of those gaps with original field-based research using study watersheds in the Luquillo Mountains of Puerto Rico as the model system. The Luquillo Mountains have features typical of mature montane tropical forests, such as high background N concentrations in streams and groundwater relative to streams in other biomes. As a USDA Forest Service Experimental Forest, the Luquillo Mountains are accessible to researchers and have abundant monitoring and experimental datasets from which to build hypotheses and experimental approaches.

Chapter 1 is a review of the state of the literature on biological response to nutrients, particularly N, in streams of the Luquillo Mountains. This chapter also includes a gap analysis of research questions that are of greatest importance to the environmental regulatory and management community, that have not yet been addressed.

Chapter 2 looks in-depth at ammonium (NH_4^+) cycling in headwater streams. Headwater streams in tropical forests are typically light- and organic matter-limited in their demand for

nutrients. Ammonium can serve as both a nutrient and an energy source, leading to a hypothesized high demand that can be compared across streams using ambient uptake velocity (v_f). This study experimentally enriched headwater streams with transient NH_4^+ pulses to determine NH_4^+ demand and mechanisms for uptake. Ambient v_f ranged from 0 to 2.9 mm min^{-1}, lower than other tropical streams and streams in other biomes in the literature. Though demand was relatively low, areal uptake rate in the streambed was high due to high background NH_4^+ concentrations. When compared with streams in other geological regions of the Luquillo Mountains, the streams in this study stand out for their sandy substrate and their low phosphorus concentrations, suggesting that NH_4^+ removal pathways may be limited by nutrients or habitat for NH_4^+-oxidizing microorganisms.

Chapter 3 focuses on N cycling in riparian zones. Riparian zones are widely understood as nitrogen N cycling hotspots, but significant gaps remain in our understanding of the complex biogeochemistry of NH_4^+ transformations in riparian groundwater. Tropical forest watersheds in particular have distinctive N biogeochemistry that is still poorly understood. This study was the first to examine in-situ NH_4^+ cycling in a riparian aquifer, using push-pull tests PPTs to experimentally enrich groundwater with NH_4^+ and trace NH_4^+ removal from solution, and the first to apply the Damkohler number to riparian NH_4^+ dynamics to measure the balance between residence time and reaction rate. The rate constant k for NH_4^+ retention during the five PPTs ranged from 0.13 to 0.68 hr^{-1}; the residence time ranged from 27 to 512 days; and the Damköhler number ranged from 72 to 11620, indicating that nearly complete removal of added NH_4^+ would occur over transport from the PPT well to the stream. Low dissolved oxygen availability and lack of net nitrate production indicate that nitrification was not the dominant pathway of NH_4^+ removal. Iron was abundant in surface riparian soils in the form of HCl-extractable Fe(III), and

declined to near zero at the depth of the PPTs accompanied by abundant HCl-extractable Fe(II), suggesting that Fe(II) production and NH_4^+ oxidation could be coupled. Sorption-desporption reactions and the associated equilibrium potentially explain why high background NH_4^+ concentrations persisted before and after the PPTs, though we expect that NH_4^+ pulse experiments like those conducted here saturate abiotic storage within soil and provide a determination of biotic NH_4^+ removal.

TABLE OF CONTENTS

CHAPTER 1: RESPONSES OF AQUATIC BIOTA TO NUTRIENTS IN STREAMS OF THE LUQUILLO MOUNTAINS

A scientific review and research gap analysis

1.1 INTRODUCTION

Site description

The Luquillo Experimental Forest (LEF) encompasses 11,500 ha within El Yunque National Forest in northeastern Puerto Rico (Lat. 18.3º N, Lon. 65.8º W). Steep, mountainous terrain dominates LEF, with deep valleys separated by high, knife-like ridges. The highest point in LEF is El Toro Peak at an elevation of 1065m, while the lowest elevations ~200m. Rainfall is high with a strong elevation gradient, ranging from 2500 mm/yr at lower elevations to ≥4500 mm/yr at upper elevations. Air temperature ranges from 15-30ºC year-round (McDowell et al. 2012).

LEF is predominantly forested, with four tropical rainforest types present at different elevations or topographic positions. Tabonuco forest dominates at 300-550 m elevation while colorado forest dominates from 550-800 m and elfin forest dominates above 800 m. Sierra palm forests are found in valley bottoms and ridge tops at any elevation (Murphy et al. 2012). Land use in LEF has been closely monitored and tightly controlled since the forest's establishment, prior to which low-intensity agriculture and forestry were practiced in the lower elevations. In recent decades, many areas bordering LEF have experienced urban or suburban development, while other areas have been allowed to regenerate forest cover following cessation of agriculture (Gould et al. 2012).

Four main watersheds drain LEF: the Rio Mameyes in the north, the Rio Fajardo in the east, the Rio Icacos/Blanco in the south, and the Rio Espiritu Santo in the west (Figure 1). Due to the abundant year-round rainfall, a dense network of perennial headwater streams drain this landscape of steep mountain flanks. Hurricanes have strongly influenced the hydrology, biota, and nutrient cycling of streams at LEF. Streams in LEF are extremely flashy and have steep, often rocky or boulder-strewn channels (Murphy and Stallard 2012).

Rocks underlying LEF are mostly Cretaceous, volcaniclastic, sedimentary, and marine-deposited, with intrusions of the Eocene Rio Blanco quartz diorite. Contact metamorphism surrounding these intrusions produced harder hornfels-type rock that underlay the highest peaks (Murphy et al. 2012). Watersheds with quartz diorite bedrock have lower channel slopes and sandy, meandering channels. Soils on the quartz diorite bedrock consist of loam Inceptisols with high quartz content, while soils on the volcaniclastic bedrock largely consist of clay and clay loam Inceptisols and Oxisols with very little quartz. Flow paths through the volcaniclastic-derived soils tend to be shallow due to high abundance of occluding clays (McDowell et al. 2012).
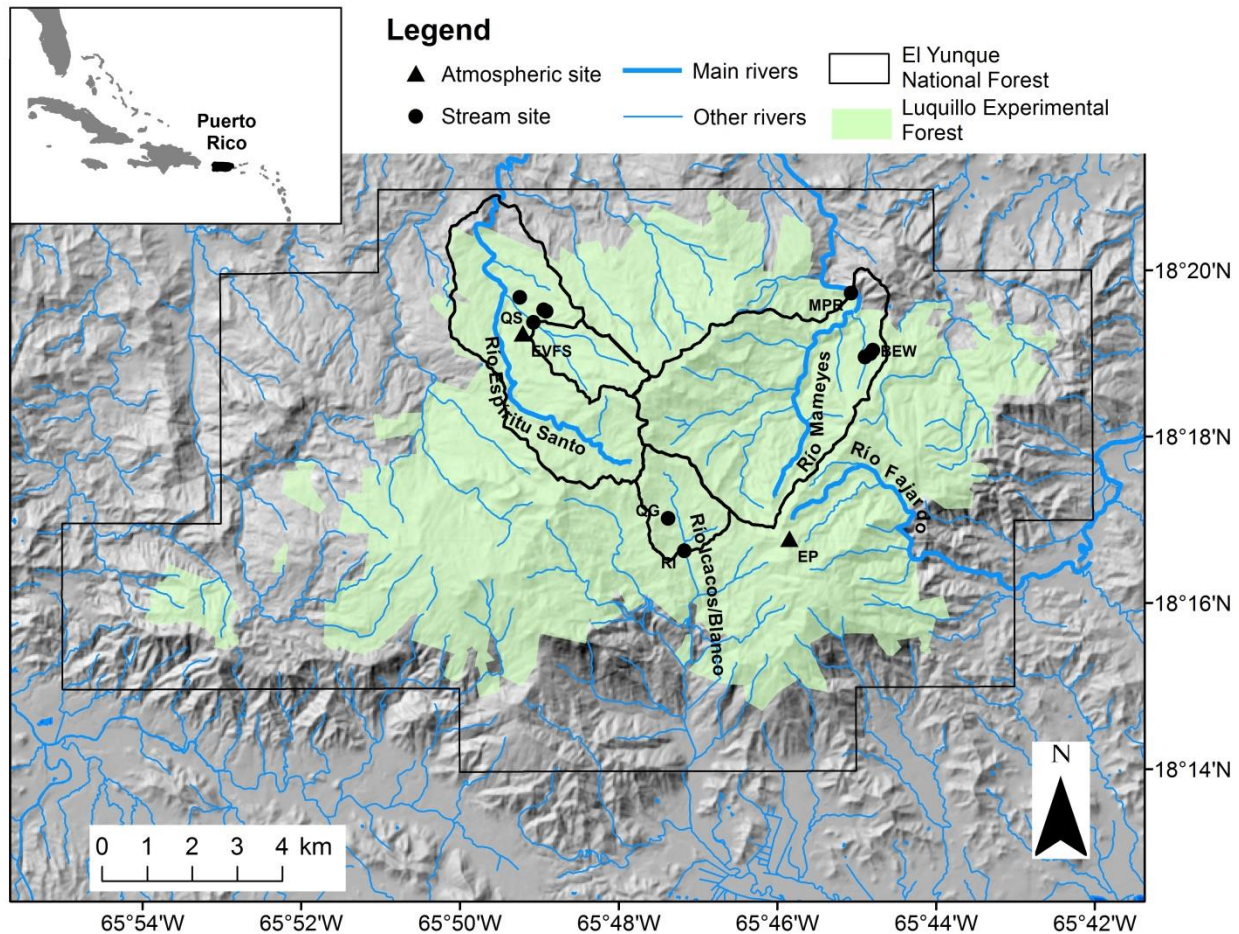
Figure 1. The Luquillo Experimental Forest, including locations of major reference watersheds (BEW= Bisley Experimental Watersheds; MPR = Rio Mameyes-Puente Roto; RI = Rio Icacos; QG = Quebrada Guaba; QS = Quebrada Sonadora) and atmospheric input monitoring sites (EP = East Peak; EVFS = El Verde Field Station).

Research History

The United States Forest Service established LEF in 1956 to broaden the scope of research in tropical forestry, although natural history and forestry studies had been conducted at this site since the early 20th century. The Tropical Forest Experiment Station, later renamed the International Institute of Tropical Forestry (IITF), was established in 1939 to provide a scientific basis for reforestation following several destructive hurricanes in the 1920s and 30s (Wadsworth 1995). LEF-based work on sustainable forest production, including pine and mahogany

plantations, produced important contributions to the literature on tropical forestry worldwide (Wadsworth 1995; Lugo et al. 2006; Lugo and Heartsill Scalley 2014).

Studies of ecological structure and function at LEF, including water quality, hydrology, and watershed management, emerged from earlier forestry research (Brown et al. 1983). Continuous stream gauging began in 1968, though some gauging was conducted sporadically beginning in the 1940s. Stream hydrology and chemistry studies were first carried out in the 1960s (Cuevas and Clements 1975). Modern ecological research in LEF also began in the 1960s, with five years of monitoring and experimentation on ecosystem processes as part of the Rain Forest Project supported by the Atomic Energy Commission (Odum 1970). IITF expanded from its original focus on forestry to conduct a broad range of continuing ecological and watershed studies in LEF.

The United States Geological Survey (USGS) has a long record of LEF research in hydrology and stream chemistry. Intermittent water quality monitoring has taken place at USGS stations since the 1960s. As part of the Water, Energy and Biogeochemical Budgets (WEBB) program, USGS has published stream monitoring data from three LEF reference watersheds (Rio Mameyes, Rio Icacos, and Quebrada Guaba), along with two nearby watersheds with agricultural land use histories, in a 15-year study of watershed solute and sediment flux.

In 1988 LEF became a member site of the National Science Foundation's Long Term Ecological Research (LTER) network. As an LTER site, LEF established regular stream nutrient monitoring as part of a program of multi-scale and integrated ecosystem studies. In addition, LEF has been a member site in the National Science Foundation's Critical Zone Observatory

(CZO) network since 2009, which has included studies of stream solutes, sediments, and nutrients.

Access to Data and Publications

For more details about the work at Luquillo LTER, including detailed site information and a comprehensive list of publications, see the Luquillo LTER website: http://www.lternet.edu/sites/luq/. Many data sets collected by LEF studies are on the Luquillo LTER online repository: https://portal.lternet.edu/nis/home.jsp.. Long-term daily streamflow and weather data from LEF that are formatted for ready comparison with other EFR and LTER sites may be downloaded at http://climhy.lternet.edu/. Forest Service publications and related journal articles can be downloaded on the IITF website http://www.fs.usda.gov/iitf/. The Luquillo CZO website also has a data repository at http://criticalzone.org/luquillo/data/datasets/ and a list of publications.

1.2 Biological Responses to Stream Nitrogen and Phosphorus

Issues of concern

Tropical and neotropical forests have undergone the highest rates of deforestation and land use change of any forest type since the mid-twentieth century (FAO & JRC 2012). Land use change in Puerto Rico over this period has largely run counter to trends elsewhere in the tropics, with substantial reforestation occurring on Puerto Rico's abandoned agricultural areas (Gould et al. 2012). Urban and suburban development has also proceeded since the mid-20th century so abandoned agricultural lands have either been urbanized or have naturally afforested with a mix of native and exotic species that together constitute a novel ecosystem (Lugo and Helmer 2004; Morse et al. 2014). LEF streams thus represent rare and valuable forested reference streams

against which to compare streams draining urbanized watersheds and regenerating forests in Puerto Rico and elsewhere the tropics (Heartsill-Scalley and Lopez-Marrero 2014).

Urban development of watersheds downstream of LEF has caused increased N and P loading, as well as changes to other factors that may impact stream biota, including light availability, temperature, dissolved oxygen [DO], dissolved organic carbon [DOC], suspended sediment, channel structure, and stream flow (Santos-Román et al. 2003, de Jesús-Crespo and Ramírez 2011, Potter et al. 2014, Ramírez et al. 2014). Urban wastewater discharge from publicly owned treatment works and suburban/rural septic tank discharge are major vectors of N and P enrichment of streams (Figueroa-Nieves et al. 2014). Excessive N and P loading can degrade water quality for beneficial uses such as drinking water, recreation, and aquatic ecosystem integrity.

Drinking water quality is of great concern for Puerto Rico's headwater streams, because streams that drain forested uplands are a major source of the island's clean drinking water. For example, more than 20% of Puerto Rico's population relies for drinking water primarily on streams leaving LEF (Scatena and Johnson 2001), and use of those sources has intensified recently. Diversion of flow from streams leaving LEF has increased from 54% in the 1990s to 70% in 2004 (Crook et al. 2007).

In addition to threatening local drinking water, excessive N and P loads can also degrade water quality in sensitive downstream estuaries and coastal ecosystems such as mangrove forests, seagrass beds, and coral reefs, which are important nurseries for many fish stocks and attractions for the tourism industry.

Atmospheric deposition of N in precipitation, dust, and aerosols is a concern in many regions. Although downwind of the Atlantic Ocean, there is concern that Puerto Rico might receive atmospherically deposited nutrients from global or long-range transport from distant sources such as dust originating from the Sahara Desert (McDowell et al. 1990, Pett-Ridge 2009).

The effects of global climate change on streams are also of concern in LEF. Hurricane-derived forest disturbance drives changes in nitrogen loading in LEF watersheds, and climate models predict intense hurricanes to become more frequent in the North Atlantic as climate warms (Knutson et al. 2010, Emanuel 2013).

Climate change may also cause increasing impacts on LEF streams from drought, a concern that was recently highlighted in the drought of 2015. Recent hydro-meteorological studies in the region show that wetter conditions prevailed in recent years in Hispaniola (Comorazamy et al. 2013), and in Puerto Rico daily rainfall increased slightly from 2001-2013 (Van Beusekom et al. 2015). Despite recent wetter conditions in the region, coupled climate-water cycle models predict a high likelihood that drought will become more frequent and severe in the Caribbean region due to climate change (Prudhomme et al. 2015). Drought has complex impacts on stream biota, including indirect impacts via changing nutrient status. In dry periods, streams can experience low flows with concentrating effects on nutrients as flow volume decreases, even if nutrient inputs remain constant. Reduced flows can also directly alter habitat for aquatic organisms and decrease water available for transpiration by riparian plants.

Findings from studies

Overall N and P conditions in streams

Nitrate-N, NH_4^+-N, total dissolved nitrogen, and PO_4^{3-}-P have been monitored in LEF watersheds on a weekly basis since the 1980s, with Sonadora, Toronja, and Icacos beginning in 1983, Bisley 1-3 in 1988, and Mameyes-Puente Roto and Prieta in 1989 (McDowell and Asbury 1994, Schaefer et al. 2000).

Nitrogen concentration and flux in LEF streams were higher than in most temperate forested reference watersheds, which is common in tropical and subtropical forests (Lewis et al. 1999, Hedin et al. 2009, Brookshire et al. 2012). Nitrate concentrations were particularly high (≥ 100 ug NO_3^--N/L) compared to other forested reference streams. Nitrate concentration was unrelated to discharge, indicating biotic control (McDowell and Asbury 1994, Shanley et al. 2011). Nitrate flux was controlled by total runoff, which in turn is a function of the elevation gradient of precipitation (McDowell and Asbury 1994). Dissolved P concentrations and export were low to moderate, a common feature of other tropical forest streams. Iron and aluminum oxides in soils were shown to bind PO_4^{3-} strongly in soils, immobilizing P and delaying transport to streams via leaching (Liptzin and Silver 2009).

LEF is upwind of sources of atmospheric pollution local to Puerto Rico, which makes it an ideal site to monitor regional-to-global-scale atmospheric N inputs. The National Atmospheric Deposition Program (NADP) has been monitoring wet deposition of N since 1985. Stallard (2001) and Ortiz-Zayas et al. (2006) both reported a significant increase in wet N deposition through 2003, but since then N deposition has not increased (Stallard 2012). At current rates (2-3.5 kg/ha/yr), wet deposition of N is not considered a major vector of stream enrichment in

Puerto Rico, but if it increased in the future, atmospheric deposition of N might become an issue for the region's streams.

Data on dry N and P deposition from windborne dust or aerosols are lacking for LEF and Puerto Rico in general, although throughfall has been extensively studied. Throughfall washes the products of dry deposition off the forest canopy, and at LEF it is significant enriched in NO_3^--N, NH_4^+-N, and P above ambient levels in rain water (McDowell et al. 1990, McDowell 1998, Heartsill Scalley et al. 2007).

Phosphorus enrichment is not considered a concern in headwater streams such as those within LEF. Deposition of dust carried from the Sahara desert across the Atlantic Ocean by the Trade Winds has been estimated to be a significant input of P to LEF watersheds, totaling 0.23 kg P/ha/yr or slightly larger than the average input from bedrock weathering (Pett-Ridge 2009). This source is strongly seasonal, peaking in June and July (Stallard 2012). Although it is unclear how future P delivery from Saharan dust may be affected by climate change, at current levels this source is not a major vector of P enrichment to streams.

Ability of streams to regulate N and P dynamics

Organisms in LEF streams actively cycled N between the water column and benthic food webs. Uptake of the inorganic forms NH_4^+ and NO_3^- has been studied in detail. A $^{15}NH_4^+$ stable isotope enrichment study showed that NH_4^+ uptake was dominated by nitrification rather than assimilation (Merriam et al. 2002). A plateau addition of trace amounts of $^{15}NH_4^+$ continuously added over a 42-day period to one LEF stream, Bisley 3, provided detailed accounting for the fate of added ^{15}N. Rapid stream NH_4^+ uptake occurred, 60% of which was due to nitrification. Over the study period, 65% of the isotopic tracer was exported in all forms, but only 1% was

exported as $^{15}NH_4^+$, indicating that the stream actively processed 99 per cent of the added $^{15}NH_4^+$. Fifty percent of added ^{15}N was exported as NO_3^-, 8% as DON, and 6% as particulate N (5% during a single storm event). Of the 45% of added ^{15}N that was retained, most was in fine benthic organic matter (FBOM) and epilithon (10% and 6%, respectively, of the total amount added). Less than 1% was retained in leaves, wood, insects, shrimp, and crabs combined.

Potter et al. (2010) conducted stable isotopic $^{15}NO_3^-$ additions lasting 24 hours in three LEF streams to quantify NO_3^- uptake and denitrification. Uptake was dominated by autotrophic assimilation rather than heterotrophic assimilation. Denitrification was a significant pathway, averaging a third of total uptake. Although less is known about P dynamics than N dynamics in LEF streams, Benstead et al. (2010) reported significant uptake of PO_4^{3-} in Bisley 3 and Prieta streams.

Consumers in higher trophic levels exert indirect influences on N and P dynamics in LEF streams. The most important consumers in LEF stream food webs are decapods: three genera of freshwater shrimp (*Atya* spp., *Xiphocaris elongata*, and *Macrobrachium* spp.) and one freshwater crab (*Epilobocera sinuatifrons*) (Covich and McDowell 1996; Zimmerman and Covich 2003, Perez-Reyes et al. 2015, Perez-Reyes et al. 2016). There are numerous examples of streams dominated by shrimp and crabs in tropical landscapes (Boulton et al. 2008, Jacobsen et al. 2008, Rincón & Covich 2014). Wright and Covich (2005) found that LEF shrimp fill important ecological niches that are often filled by insect taxa in temperate streams such as shredders, scrapers, filterers and predators. In LEF streams, *Atya* and *X. elongata* exerted top-down control over the benthic resource base, with indirect impact on N and P (Pringle et al. 1999; Crowl et al. 2001, Benstead et al. 2010). Grazing by *Atya* and detrital processing by *X. elongata* prevented accumulation of N and P in autotrophic biomass or FPOM standing stock. This grazing released

nutrients for uptake and assimilation by autotrophs, and increased leaf litter decay rates. Greathouse et al. (2006) further observed that reaches of streams above dams that excluded shrimp had far greater benthic organic matter and N standing stocks than did streams with shrimp.

Fish and snails are also important consumers in LEF streams, except upstream of cascades and steep waterfalls that act as barriers to their upstream passage (Covich et al. 2009, Crowl et al. 2012, McDowell et al. 2012). Mountain mullet (*Agonostomus monticola*) are present up to ~400 m elevation and prey on shrimp, possibly releasing grazing pressure on autotrophs. Goby (*Sicydium plumieri*) can be found up to ~700 m elevation because they are more capable of climbing dispersal barriers using suction-cup-shaped pectoral appendages, and are important algivores. Herbivorous neritid snails (*Neritina* spp.) can climb the steepest inclines but generally are not found upstream of waterfalls, possibly owing to short life spans and the energetic demands of migration (Pyron and Covich 2003). Surveys have found the highest densities of shrimp upstream of the ranges of fish and snails (Greathouse and Pringle 2006, Covich et al. 2009, Hein et al. 2011).

N and P effects on LEF stream food webs and ecosystem processes

To test N and P limitation on biofilm growth, Tank and Dodds (2003) deployed artificial substrates that diffused N and P, both separately and in combination, into LEF streams and monitored chlorophyll *a* production by epilithic and epixylic biofilms. Bisley showed low chlorophyll *a* production in all treatments and no additional biofilm growth with added N or P; the P enrichment showed less growth than the control. Among LEF streams, only Bisley has been tested for N and P limitation of biofilms. No studies of the response of submerged or riparian vegetation to nutrients have been conducted at LEF.

Studies have also found no effects on decomposition and DOM uptake in response to increased N and P availability in LEF streams. Tank and Dodds (2003) studied fungal biomass production on wooden disks under N and/or P enrichment conditions. They found that ergosterol, a fungal biomarker, did not increase with N or P enrichment, providing evidence that wood-decomposing fungi are not limited by these nutrients. In a Costa Rican tropical forest stream, however, Rosemond et al. (2002) studied rates of leaf litter breakdown in a number of streams that constituted a natural P concentration gradient. They found that leaves broke down faster in the higher P streams, suggesting P-limitation of heterotrophic activity. No such study or experimental analysis has yet been conducted at LEF.

In LEF streams, high NO_3^- concentrations have been attributed to high nitrification rates in soils and streams (Merriam et al. 2002). The watersheds on quartz diorite bedrock had high NH_4^+ concentrations in groundwater flowing through riparian zones, potentially supplying the stream with ample substrate for nitrification, but volcaniclastic watersheds had nearly undetectable levels of NH_4^+ in riparian groundwater. Riparian zones removed up to 90% of N from groundwater before it reached streams (McDowell et al. 1992, Chestnut and McDowell 2000). Where NH_4^+ was available to stream biota, nitrifying microorganisms using NH_4^+ as an energy source were able to compete with microorganisms using NH_4^+ as a nutrient (Merriam et al. 2002).

Studies of other factors

Periods of drought in LEF have complex impacts on stream biota and food webs, causing accelerated rates of leaf senescence in terrestrial vegetation and litter delivery to streams, as well as accumulation in streams of litter and organic debris due to a lack of flushing. During the 1994 drought, pool depth decreased and aquatic habitat contracted, causing crowding and disrupting

reproductive capacity among shrimp (Covich et al. 2003). Although these impacts on biota might be expected to alter stream N and P dynamics, no changes in LEF stream N and P concentrations were reported during the droughts of 1989, 1991, or 1994. Preliminary results from the drought of 2015 also suggest no change in N and P concentrations (W. McDowell unpublished data).

Frequent high flows in LEF streams might also be expected to affect biotic response to N and P. For example, scouring might limit periphyton biomass and thereby limit periphyton nutrient uptake, but one study observed no decrease in periphyton biomass after high flows in an LEF stream (Ortiz-Zayas et al. 2005). Relationships between discharge and concentration have been used in recent years to improve predictions of solute flux at high flows, as well as to make inferences about watershed sources and sinks of solutes and suspended materials. Discharge has been found not to control NO_3^- concentration in LEF watersheds (McDowell and Asbury 1994, Shanley et al. 2011). Instead of showing a physical dilution or flushing response to discharge, NO_3^- is thought to be under biotic control in LEF watersheds, likely a result of both terrestrial and aquatic immobilization (McDowell and Asbury 1994, Shanley et al. 2011).

Light availability at the stream surface was low (0.3 mol/m^2/d) beneath LEF's dense canopy (Tank and Dodds 2003). Consistent with low available light, undisturbed streams were highly heterotrophic. Even low elevation reaches were predominantly heterotrophic, despite having wider channel widths that permitted more light to reach the benthic surface (Ortiz-Zayas et al. 2005). In wider reaches, strong grazing pressure by shrimp may have limited autotropic standing crop, rather than available light.

Food webs in LEF streams depend heavily on litter inputs from riparian vegetation as a source of energy (Covich and McDowell 1996). High nitrification rates in Bisley have led to the

hypothesis that NH_4^+ is an important additional energy source to overall benthic metabolism and that energy demand regulates NH_4^+ uptake in these tropical forested streams (Merriam et al. 2002).

Temperatures in LEF streams range from 17-30° C. Stream temperatures generally decrease with altitude and show much less seasonal and diurnal variation than temperate forest streams (Brown et al. 1983, McDowell and Asbury 1994, Ortiz-Zayas et al. 2005, Stallard and Murphy 2012).

Reference Watersheds and Monitoring Sites

All LEF study watersheds have been formally designated as reference watersheds. They have been managed for minimal on-site human disturbance since LEF was established in 1956, and before that the only land use was scattered, low-intensity agriculture, forestry, and mining. As a result of this land use history, these LEF study basins are increasingly rare and valuable examples of well-studied reference conditions against which streams elsewhere in Puerto Rico (and in the wet tropics worldwide) may be compared.   All of the research findings from LEF streams reported in this chapter are based on work done in these LEF reference streams.

Reference watersheds at LEF were selected on northeastern, northern and southern flanks of the Luquillo Mountains to encompass the full range of environmental conditions present. These conditions, particularly rainfall, vary with aspect in response to the prevailing Northeast Trade winds.

The Rio Mameyes watershed drains 1782 ha on the steep northeastern slope of the Luquillo Mountains, at 83-1050 m elevation. Mameyes was 99% forested when it was acquired by USFS in the 1930s, although parts of the basin had previously experienced selective logging

14

and small-scale agriculture at lower elevations (Murphy et al. 2012). Agriculture was largely abandoned following a destructive 1899 hurricane (Scatena 1989). USGS has continuously measured discharge and rainfall at the watershed outlet since 1967.

The Bisley experimental watersheds, within the Rio Mameyes basin, have been managed as reference watersheds since they were acquired by the Forest Service in 1934. Bisley 1 watershed has an area of 7 ha; Bisley 2 an area of 6 ha; and Bisley 3 an area of 35 ha. Three gaged streams, two meteorological stations, soil and vegetation plots, and groundwater monitoring wells are located within the Bisley watersheds. Groundwater chemistry and hydrology in the riparian zone of Bisley 3 have been monitored intermittently 1985-present. Rainfall and throughfall chemistry were monitored regularly at Bisley during 1988-2007.

Three reference watersheds are located at the El Verde research area within the Rio Espiritu Santo basin on the northern flank of the Luquillo Mountains: Quebrada Sonadora, Quebrada Toronja, and Quebrada Prieta. Their areas are: Sonadora, 262 ha; Toronja, 51 ha; and Prieta, 31 ha. The National Atmospheric Deposition Program (NADP) has monitored wet deposition chemistry at the El Verde Field Station since 1985.

Rio Icacos is a 326 ha reference watershed located high on the Luquillo Mountains southern flank at 620-832 m. It is dominated by Colorado forest with abundant Sierra Palm forest on ridges and in riparian zones. The Icacos basin had very little logging and no agriculture before acquisition by the USFS (Brown et al. 1983). USGS has measured stream discharge continuously since 1968 and precipitation continuously since 1989. Stream chemistry has been monitored on a weekly basis since the 1980s, and frequent event-based sampling were conducted by the USGS WEBB project in the 1990s and early 2000s. Groundwater chemistry and hydrology in the

riparian zone of a small tributary to the Rio Icacos have been monitored intermittently 1985-2010 and quarterly 2011-present. Quebrada Guaba, another sub-watershed of Rio Icacos, has been monitored with weekly, continuous and event-based sampling. At East Peak (1030 m), which flanks the Rio Icacos watershed to the east, meteorological data, wet deposition chemistry, and airborne dust chemistry are monitored continuously.

Over the period 1987-2007, Bisley 2, Bisley 3 and Sonadora all showed significant decreasing trends in monthly flow-weighted nitrate concentrations (Argerich et al. 2013). During 1997- 2007, stream nitrate in these three watersheds, plus Bisley 1, also significantly decreased.

Cross-site and regional studies

Streams in LEF have been included in numerous cross-site, regional, and global studies of stream nutrient flux because LEF has one of the most detailed stream chemistry records of any tropical/subtropical forest. LEF data were used directly to develop N and P criteria for water quality regulations across Puerto Rico. Sotomayor et al. (2011) made recommendations for N and P criteria using a USGS water chemistry data archive from 57 stations across Puerto Rico that included water chemistry from six forested reference sites across Puerto Rico, three of which are LEF reference watersheds (Icacos, Mameyes-Puente Roto, and Sonadora).

Comparisons of developed vs. undeveloped watersheds in Puerto Rico have used LEF data. Santos-Roman et al. (2003) used water quality monitoring data from 15 sites across Puerto Rico, including two within LEF, and demonstrated that N and P concentrations were lower (and overall water quality conditions were better) in forested watersheds than in urban watersheds. Stallard and Murphy (2012) compared stream chemistry findings from the USGS WEBB project for Icacos, and Mameyes, with two watersheds with historical or current agricultural influence.

16

Concentrations and yields of NO_3^-, NH_4^+, and PO_4^{3-} were higher in the developed watersheds, which the authors attributed to agricultural activities and domestic waste disposal.

Biological communities in urban streams with high N and P, draining watersheds outside the boundaries of LEF, have been compared to LEF streams. In the Rio Piedras watershed, which drains the southeastern San Juan metropolitan area, macroinvertebrate communities shifted with increasing urban density from sensitive taxa like mayflies (Ephemeroptera) and caddisflies (Trichoptera) to tolerant taxa like midges (Diptera: Chironomidae) (Ramírez et al. 2009; de Jesús-Crespo and Ramírez 2011). These studies did not measure shrimp or crab populations, but reported sightings in the urban watersheds. Native diadromous (migratory between salt- and freshwater) fish species such as *A. monticola* persisted in urban streams, where there are no physical barriers to fish passage (Ramírez et al. 2009), suggesting that there is a food resource to support them in urban streams. The presence of decapods and fish in urban streams outside LEF indicated that enrichment with N and P did not exclude them, although lack of population measurements in urban streams prevented quantitative comparisons with reference stream populations. Greathouse et al. (2006) reported that grazing by atyid shrimp kept rock surfaces clean even in streams with high N levels (1.5 mg NO_3^--N/L). These findings suggest that decapods and native fish would not necessarily abandon other Puerto Rican streams when encountering N and P enrichment.

LEF streams have often been included in cross-site, regional, and global studies of stream nutrient flux because LEF has one of the most detailed stream chemistry records of any tropical/subtropical forest. Lewis et al. (1999) included data from Icacos, Sonadora, and Toronja in a multi-regional analysis of stream nitrogen exports from 31 undisturbed watersheds: 25 tropical streams from mostly American tropical forests and savannas, and six forested streams in

the Sierra Nevada of California. Fluxes of all forms of N were found to be correlated with total runoff. Despite its small watershed area, Icacos ranked highly in NO_3^- export among the studied watersheds due to its high runoff.

Argerich et al. (2013) analyzed trends over time in nitrate and ammonium concentrations in forested reference streams at seven EFRs across the USA, including four LEF catchments. The LEF streams all showed decreasing trends in NO_3^--N concentration, as did reference streams in Hubbard Brook EF in New Hampshire and H. J. Andrews EF in Oregon.

LEF has also been the site of two cross-site experimental studies of stream N cycling, the first and second Lotic Intersite Nitrogen eXperiments (LINX I and LINX II).) In LINX I stable isotope enrichment experiments using $^{15}NH_4^+$ were conducted at 10 North American stream sites from Arctic tundra to tropical forest (see Peterson et al. 2001 for overview). The LEF was the only tropical stream in the study (Merriam et al. 2002). Nitrification in the LEF stream (Bisley 1) accounted for the largest fraction of total NH_4^+ uptake of any of the streams studied in LINX I, although the absolute rate of nitrification was higher in a North Carolina stream (Webster et al. 2003). Forest streams in LEF plus North Carolina and Oregon experienced no N or N + P limitation on biofilm growth. All three of these sites had very low average light availability (Tank and Dodds 2003), suggesting that light may commonly limit biofilm production in streams under heavy canopy cover.

Potter et al. (2010) reported the effects of $^{15}NO_3^-$ enrichments of streams at eight sites across North America that were part of LINX II, which conducted $^{15}NO_3^-$ enrichments of streams at eight sites across North America. At each of the eight sites, streams were selected to represent reference, agricultural, and urban land use (see Mulholland et al. 2008 for overview). In Puerto

Rico, three LEF streams were used as reference sites, and compared with three agricultural and three urban streams. As expected, ambient NO_3^--N and NH_4^+-N concentrations were lowest in the LEF streams and highest in the urban streams. Across all land uses, the Puerto Rican streams showed NO_3^- uptake that was strongly correlated with background NO_3^--N concentration, indicating that stream organisms/communities have some capacity to adapt to higher NO_3^- loads than under reference conditions by processing more NO_3^-. In addition, NO_3^- uptake was also strongly correlated with GPP, suggesting that light exerts an indirect control on uptake where autotrophs are active. Denitrification in all Puerto Rican streams accounted for a third of total NO_3^- uptake on average, and denitrification rates were positively correlated with DOC concentrations.

Responses to management and natural disturbance

Most LEF research into the effects of forest management has focused not on streams but on forest biomass production. In the 1930s and 1940s over 2300 ha of mahogany, teak, and pine plantations were planted at lower elevations of LEF, and timber production was monitored. Experimental studies examined the effects of cutting and herbicides on regrowth of vegetation. Odum's Rain Forest Project (Odum & Pigeon 1970) examined effects of an experimental radioactive cesium irradiation, which, at highest exposures, was lethal to vegetation on a 50 ha forest plot. Potential impacts of management practices on groundwater or streams have not been studied, nor have LEF research findings been used to develop any best management practices (BMPs) for water quality protection.

Hurricanes and landslides are the major natural causes of disturbance. As noted above, hurricane-related disturbance caused large increases in NO_3^--N concentrations that took years to decades to return to pre-disturbance baseline (Schaefer et al. 2000, Shanley et al. 2011,

19

McDowell et al. 2013). No post-hurricane increases in P have been observed. Following Hurricane Hugo in 1989, Shanley et al. (2011) showed that NO_3^--N exports from Icacos in the 1990s were roughly double those of the 1980s. Groundwater N in disturbed riparian zones showed a similar pattern to stream water NO_3^-, increasing for years to decades following disturbance (McDowell et al. 1996, 2013).

Hurricane disturbance of forests has long been recognized as a major driver of N dynamics in tropical forest streams, due in part to research in LEF. Hurricanes affect stream N dynamics indirectly by damaging trees with wind, breaking boles and branches, causing tree mortality, and delivering fresh leaf and wood litter to the forest floor and stream channel in great excess of normal conditions. Following Hurricane Hugo in 1989, loss of vegetative uptake and litter decomposition caused post-hurricane increases in NO_3^--N concentrations and N export that took years to decades to return to pre-hurricane levels (Schaefer et al. 2000); the pattern was repeated following Hurricane Georges in 1998 (McDowell et al. 2013). Maximum post-hurricane stream nitrate concentrations were 0.37 mg NO_3^--N/L, several times higher than pre-hurricane conditions but below the federal drinking water standard of 10 mg NO_3^--N/L. P dynamics showed no response to hurricane disturbance, either because mineralized P did not reach the stream or because mineralization of biomass-bound P was insufficient to raise stream water concentrations above background conditions.

The Luquillo LTER Canopy Trimming Experiment (CTE) was an experimental simulation of the biophysical disturbances that accompany hurricanes (http://luq.lternet.edu/research/project/canopy-trimming-experiment-cte). Its purpose was to disentangle the effects of canopy opening from those of additions to the forest floor of wood and leaf debris on nutrient fluxes in soil solution. Neither canopy opening nor debris addition, on

their own, produced elevated NO_3^--N flux through soil, but the combination produced peak in NO_3^--N concentrations lasting 18 months that was similar to the post-hurricanes response of NO_3^- in streams (McDowell and Liptzin 2014). Levels in soil solution increased from 0.01-0.03 mg NO_3^--N/L to nearly 7.5 mg NO_3^--N/L over the first 10 months of the full hurricane simulation. Both reduced vegetative N demand and increased mineralization of organically bound N in litter appear to be necessary to produce a NO_3^- pulse in soil solution that was similar to the observed post-hurricane pulse in stream water. Evidence from the CTE has strengthened the argument that regrowth of trees causes stream N concentrations to eventually decline after a hurricane-induced pulse. Dissolved PO_4^{3-} in soil solution did not respond to canopy trimming treatments.

Although direct effects on stream biota of the hurricane-induced N pulse have not been studied, post-hurricane biological responses to increased channel inputs of plant litter have been reported. Shrimp populations temporarily increased in response to the abundance of litter inputs and debris dams from Hurricane Hugo, and then declined as pre-hurricane conditions returned over the following year (Covich et al. 1991). These temporary population booms may have altered nutrient cycling, considering the control shrimp exert on the benthic resource base, but any effects on N and P were not studied.

Reliability and limitations of findings

Research results and environmental monitoring datasets from LEF apply most directly to streams at other sites in Puerto Rico with relatively undisturbed forests, particularly where climate and geology are most similar to LEF. At sites where these factors are dissimilar to LEF, or where high accuracy is required, validation experiments or monitoring should be considered to test how well findings from LEF predict local conditions.

LEF streams have extremely flashy hydrology that makes study of storm flow conditions difficult and hazardous. As a result, most research has been conducted at base flow conditions which may miss the important processes that take place during storm flows. Deploying autosampler technology has made progress toward addressing this problem for water quality monitoring. USGS used autosamplers during the WEBB project to obtain grab samples at higher runoff rates than any other studies previously published (Stallard and Murphy 2012), and sensors have recently been deployed at LEF to obtain continuous records of dissolved oxygen and conductivity. However, field experiments remain difficult and often dangerous during high flows, and the scarcity of stream data collected during high flow events has limited parameterization of models of nutrient effects on LEF stream biota at high flows.

Research needs

The highest priority research question for the water quality regulatory community, related to N and P that could be filled at LEF is: How does flow reduction due to drought and/or water withdrawal affect stream biotic response to N and P?

Filling this need would be important for the regulatory community because regulators require information on the capacity of stream biota to cope with the interactive effects of flow reduction and nutrient increases, especially considering projections of increased drought frequency and severity. Compared to the impacts of hurricanes, relatively little is known about how drought conditions affect biological responses to N and P in rainforest streams. In periods of low flow, stream biotic processing of nutrients might mitigate the loss of dilution capacity by streams, or, alternatively, stream biota might be negatively affected by low flows and lose their natural capacity to process nutrients. If the latter is the case, stream nutrient levels would rise, perhaps making more costly wastewater treatment necessary in order to meet standards for

protected uses. LEF shrimp survey data have been used to determine instream flow, the minimum required flow for preserving a given set of ecological functions (Scatena and Johnson 2001), but no study has considered the effect of low flow on nutrients. As discussed above, low flows during the drought of 1994 caused both crowding of shrimp and accumulations of leaf litter, suggesting a limit on the ability of shrimp to break down leaf litter but not revealing the mechanism behind this limit (Covich et al. 2003). A better understanding of stream biotic response to drought might offer the potential to improve modeling for nutrient total maximum daily load models (TMDLs). If this research need were filled, water quality regulators might be better informed to decide when it might be necessary to modify nutrient endorsements during low flows.

The following work would need to be done at LEF to fill this research need: a) re-analyze existing LEF long-term rainfall, stream flow and stream nutrient datasets to develop a quantitative model of drought effects on stream nutrient concentrations, b) initiate focused monitoring of nutrient conditions and biotic surveys at low flows, c) conduct nutrient uptake experiments during low flows, and d) study biological responses to experimental flow reductions and drying of natural and/or artificially created streams.

Other relevant questions that might be addressed at LEF, but would be of lower priority for regulators, are:

- Can a response to global drivers such as climate change and atmospheric deposition of N and P be detected by monitoring LEF's forested, low-nutrient streams under reference conditions?
- How do N and P levels affect stream metabolism and BOD export downstream?

- In forested catchments, streams tend to export a higher proportion of the total N load as DON while urbanized catchments typically export a larger DIN fraction. Do these differences reflect differences in N sources or differences in aquatic N cycling?

- Are the organic forms of N and P in LEF watersheds more or less labile than the organic N and P in urban watersheds or in wastewater?

- Is the marine environment a significant source of nutrients to streams via migrating shrimp and fishes? What fraction of stream N and P is delivered to streams from marine environments, how much is taken up in freshwater food webs, and how much returns to the ocean as stream flux?

- Do large floods alter stream nutrient conditions on an enduring basis?

Potential utility to water quality regulatory agencies

Responses at LEF to disturbance such as hurricanes and droughts can be interpreted without the confounding effects of a complex land use history. In this context, watersheds at LEF might be useful to water quality regulatory agencies both as natural reference points for comparison with Puerto Rico's urban-impacted watersheds, and as sentinels of regional-to-global-scale drivers such as climate change and N deposition.

Long-term data on N and P concentrations and fluxes in LEF streams might be used to improve estimates of "best attainable" concentrations for the establishment of nutrient criteria for Puerto Rican streams. Nutrient criteria to protect biological and chemical integrity of inland waters in Puerto Rico are not in place, but are in development (Sotomayor et al. 2011). LEF stream chemistry datasets might augment the datasets currently being used for this purpose. Similarly, LEF stream data might also be used to represent background "natural" non-point source loads for determining nutrient TMDLs for streams elsewhere in Puerto Rico.

Stream biological parameters, such as typical assemblages of aquatic organisms, decapod and fish survey data, have been extensively studied at LEF. These data might be used to assist the development of indicators of stream biological integrity, because detailed representations of unimpacted biotic conditions in Puerto Rican streams with low nutrient inputs are rare.

Lastly, LEF research findings on the capacity of headwater streams and riparian zones to retain N might be used to establish BMPs for riparian buffer conservation. LEF findings demonstrate how important intact forested riparian zones and headwater streams are for protecting water quality and the biological integrity of streams in Puerto Rico and possibly elsewhere in the tropics.

Key Points

- LEF streams have characteristics typical of undisturbed tropical forests under natural conditions, such as relatively high N and low P concentrations.

- LEF stream biota have the ability to control N and P dynamics via microbial uptake of inorganic N and food web interactions such as detrital processing and grazing on algae by shrimp that prevents accumulation of autotrophic biomass.

- Although food web effects on N and P have been extensively studied, many important questions remain unanswered about how of N and P levels affect food webs.

- Low light conditions and scant DOC inputs to LEF streams limits energy available to biota and causes microbial nitrification of NH_4^+ to be important energy source.

- From the perspective of the regulatory community the highest priority research question that could be filled at LEF is: How does flow reduction due to drought and/or water withdrawal affect stream biotic response to N and P?

- Existing LEF datasets and research results might be used by regulators to establish nutrients criteria, to identify stream health bio-indicators, and to develop BMPs for conserving riparian buffers for Puerto Rico.

1.3 Biological responses to stream nutrients other than nitrogen and phosphorus

Issues of concern

Nutrients other than N and P do not currently present issues of concern in Puerto Rico's streams, but their export and delivery to rivers, estuaries and coastal waters may affect the functioning of those downstream ecosystems. For example, concentration and flux of SiO_2 are high in LEF watersheds relative to other forested streams, due to rapid weathering of primary minerals in bedrock. In downstream estuaries and offshore marine environments, SiO_2 limitation of primary productivity by diatoms is common, as diatoms require SiO_2 for the formation of their siliceous frustules (shells). Changes in SiO_2 flux from uplands might alter diatom production at the base of food webs in estuaries and coastal ecosystems.

In Puerto Rico, nutrients other than N and P are added to streams by urbanization and atmospheric deposition, the same vectors that add N and P. Urbanization can cause enrichment in chloride (Cl^-) of two to three times the concentrations in reference streams. Atmospheric deposition of SO_4^{2-} is of concern because, in excess, it may acidify streams. Low-level SO_4^{2-} loading may alleviate sulfur limitation of terrestrial vegetation or aquatic microorganisms.

While not a nutrient for the purposes of this synthesis, DOC merits discussion here because it is tightly coupled with nutrient cycling in aquatic ecosystems and can be an energy

source for the base of aquatic food webs. Dissolved organic carbon loads are low in LEF streams but are much higher in urban streams in Puerto Rico. Wastewater discharge can cause DOC enrichment and change the molecular composition of the DOC pool in streams (Figueroa-Nieves et al. 2014, Potter et al. 2014). Elevated DOC loads, especially more labile forms in wastewater, supports high stream bacterial populations and contribute to oxygen depletion.

Findings from studies

Overall conditions of nutrients other than N and P

Stream water Ca^{2+}, Mg^{2+}, Na^+, K^+, Cl^-, Br^-, SO_4^{2-} and SiO_2 have been monitored on a weekly basis in LEF reference watersheds since the 1980s, with Sonadora, Toronja, and Icacos beginning in 1983, Bisley 1-3 in 1988, and Mameyes-Puente Roto and Prieta in 1989 (McDowell and Asbury 1994, Schaefer et al. 2000). The dynamics of Ca^{2+}, Mg^{2+}, Na^+, K^+, Cl^-, Br^-, and SiO_2 are largely controlled by flow conditions, with strong dilution as flows increase. Additional controls also have been inferred from effects of hurricane-related disturbance on K^+, from spatial variability of dissolved SiO_2, and from the concentration-discharge relationships for SO_4^{2-}. Weekly monitoring for these constituents in wet deposition has occurred since 1985 at the El Verde NADP site. Wet deposition of SO_4^{2-} has increased in LEF precipitation, as has total acidity, since NADP monitoring began. Stallard (2012) attributed this increase to contamination from temperate zone sources.

Bedrock weathering and sea salt aerosol inputs supply base cations to LEF streams (McDowell et al. 1990, McDowell and Asbury 1994). High SiO_2 concentrations (≥ 25 mg SiO_2/L) are a ubiquitous feature of LEF streams, due to high rates of bedrock weathering from year-round abundant moisture and warm temperatures.

Responses of stream biota to nutrients other than N and P have not been studied extensively in LEF streams. It is likely that none of these other nutrients are biologically limiting, because their stream water concentrations, driven by inputs from weathering and sea salt aerosols, are thought to be sufficient to support stream biological processes (Stallard and Murphy 2012).

Ability of streams to regulate dynamics of nutrients other than N and P

Silica cycling by freshwater diatoms has not been studied at LEF. The role of terrestrial vegetation in the watershed SiO_2 cycle has been examined in the Icacos watershed. Using a novel geochemical technique the ratio of germanium to silicon (Ge/Si) in stream water, soil solution, and minerals in soil and bedrock, two studies assessed sources of SiO_2 to the stream (Derry et al. 2006, Lugolobi et al. 2010). Previously, Derry et al. (2005) had applied this technique to Hawaiian watersheds and shown that most of the SiO_2 in riverine flux had been cycled by terrestrial vegetation. However, at LEF Lugolobi et al. (2010) found that terrestrial plants actively cycle only a small fraction of SiO_2 close to the soil surface, retaining it from stream flow.

Sulfur cycling in streams also remains to be studied in more detail. The SO_4^{2-} concentration-discharge relationship is markedly different from Ca^{2+}, Mg^{2+}, Na^+, K^+, Cl^- and SiO_2. Instead of strong dilution at higher flows, SO_4^{2-} concentrations show little response to changing discharge (McDowell and Asbury 1994, Shanley et al. 2011). This difference in the behavior of SO_4^{2-} from other constituents suggests that biotic factors influence SO_4^{2-}, but the exact mechanism is unknown.

Studies of other factors

Numerous other factors that might influence biotic responses have been measured at LEF. These are the same factors that were mentioned as possible influence responses to N and P in Section A of this chapter: flow conditions, light and organic matter. Low light levels probably exert strong influence on SiO_2 cycling, as light limitation of diatom growth can suppress SiO_2 demand in streams.

Streams in LEF have low DOC concentrations during base flows, which has been attributed to high sorption capacity in the clayey soils limiting supply to streams (McDowell 1998) and to high stream respiration rates that may indicate DOC is rapidly mineralized (Ortiz-Zayas et al. 2005). A strong positive concentration-discharge relationship holds, with DOC increasing from 2 to 20 mg DOC-C/L from low to high flows, except at highest flows when the relationship changes to a negative slope (McDowell and Asbury 1994, Shanley et al. 2011). LEF streams and groundwater are often supersaturated with CO_2, indicating that high soil respiration and high weathering rates supply CO_2 that eventually is exported as bicarbonate or degasses from the stream surface. Riparian zones retained large amounts of DOC from groundwater before it reached streams. Chestnut and McDowell (2000) estimated that, without riparian processing, one headwater stream in the Icacos basin would have a fourfold higher DOC concentration. Some important aspects of stream carbon budgets have not yet been quantified at LEF, including the fraction of CO_2 due to stream respiration, the amount of CO_2 lost from streams by evasion, and the contribution of autochthonous production to the stream DOC pool.

Cross-site and regional studies

Monitoring data collected at LEF for nutrients other than N and P are used in regular regional and national analyses and maps of atmospheric deposition produced by the NADP

Network (see NADP website). Stallard (2012) synthesized these data to analyze concentrations, loads, and trends in precipitation for the WEBB watersheds in northeastern Puerto Rico.

Stallard and Murphy (2012) reported stream chemistry findings from the USGS WEBB project for Icacos, Mameyes, and two other watersheds with both historical and current agricultural influences. Concentrations of Ca^{2+}, Mg^{2+}, Na^+, K^+, Cl^-, SO_4^{2-} and SiO_2 were higher in the developed watersheds, which the authors attributed to agricultural activities and domestic waste disposal.

Studies of urban watersheds near to LEF have elucidated the effects of urbanization on conditions of Na^+, Cl^- and DOC. Santos-Roman et al. (2003) showed that, among 15 watersheds across Puerto Rico, urban basins had higher Na^+ and Cl^- concentrations than forested watersheds, including two in LEF. Potter et al. (2014) found that Cl^- and DOC concentrations in sub-basins of the urban Rio Piedras watershed were positively correlated with spatial density of sewer pipes, suggesting that both constituents reach surface waters via leaky pipes or illicit discharges. Chloride concentrations averaged 34 mg Cl^-/L with a maximum of 52 mg Cl^-/L. These concentrations were below EPA recommendations for aquatic life criteria (200 mg Cl^-/L), but more than three times the highest concentrations in LEF reference watersheds. Concentrations of DOC were similarly enriched, with a maximum of 11 mg DOC-C/L observed.

Wastewater treatment facilities in Puerto Rico release effluent that significantly changes the DOC composition of streams, including some downstream of LEF (Figueroa et al. 2014). The compositional shift in urban streams is toward more labile DOC, which is more likely to be taken up by bacteria and respired, consuming dissolved oxygen in the process.

In a study of chemical weathering deduced from solute fluxes in stream water, White and Blum (1995) found that Rio Icacos had the highest known weathering rates in the world for a granitic watershed, likely due to high precipitation and a young pluton particularly susceptible to weathering (Stallard and Murphy 2012).

Response to management and natural disturbance

Concentrations and fluxes of nutrients other than N and P have been shown to be insensitive to hurricane disturbance in LEF, with the exception of K^+. In simulated hurricane treatments in the CTE, McDowell and Liptzin (2014) did not observe any response of DOC in soil solution.

The K^+ cycle is an important biogeochemical marker of tropical forest disturbance and recovery. Stream concentrations and export of K^+ were increased after hurricane disturbance of whole watersheds on a similar time scale to N (Schaefer et al. 2000, McDowell et al. 2013). This K^+ pulse was the result of mineralization of tree litter added to the soil by hurricane damage, as tree biomass was the largest K^+ pool in LEF systems (McDowell 1998). Schaefer et al. (2000) found that Hurricane Hugo had approximately doubled K^+ flux from LEF watersheds over the first year post-hurricane. However, in the CTE simulated hurricane disturbance, no K^+ pulse in soil solution was observed, although a NO_3^- pulse was (McDowell and Liptzin 2014). This lack of an expected K^+ response to the simulated hurricane disturbance was attributed to a lag in data collection that probably missed a rapid K^+ pulse in the first months after the treatment. Potassium pulses in streams are probably a typical feature of whole-watershed responses to hurricanes in the tropics.

Landslides occur frequently in LEF watersheds and often intersect with streams due to the dense drainage network. Landslides directly impact LEF streams by delivering sediment to stream channels (Larsen 2012). Bhatt and McDowell (2007) attributed the higher concentrations of SiO_2 observed in springs at higher elevations to the exposure and weathering of fresh bedrock by landslides. Even in heavily forested LEF watersheds, the presence of roads increases the likelihood of landslides; Larsen (2012) found that 43 to 63 percent of landslide-related erosion in Icacos and Mameyes was associated with roads.

Reliability and limitations of findings

Scientific findings and monitoring data sets from LEF related to nutrients other than N and P probably apply to other watersheds in Puerto Rico with climate and land use similar to LEF. Where environmental factors differ from LEF, or where greater accuracy is required, we suggest considering validation experiments or monitoring in order to test how well LEF results predict local conditions.

Research needs

The highest priority research question for the water quality regulatory community, related to nutrients other than N and P, that could be answered at LEF is: if tropical forested headwater streams actively process DOC, what will be the effect on aquatic food webs both in headwaters and in downstream second- and third-order streams?

Answering this question would be important for the regulatory community because DOC will either be used as an energy resource for stream food webs, as a source of mineralized nutrients or be passively transported to estuaries and coasts. The fate of DOC depends on its composition and on the environmental conditions and microbial communities it encounters in

transit. If DOC is mineralized in streams, it will contribute to dissolved oxygen depletion and release organically bound nutrients. If DOC is not mineralized in headwater streams, background levels of DOC loading are probably driven by riparian and in-stream DOC sources and by streamflow. Knowledge of DOC loading and active processing in headwater streams, such as those at LEF, might help water quality regulators better understand what background DOC would be in undisturbed streams for assessing effects of urban DOC enrichment from waste water discharges.

The following work would need to be done at LEF to answer this question: a) conduct assays of biodegradable dissolved organic carbon with incubations to determine DOC losses at different flow conditions, b) conduct field or mesocosm enrichment experiments using different compositions of DOC such as leaf litter leachate, glucose or acetate, and wastewater DOC, and c) develop a quantitative model of DOC mineralization that incorporates effects of source and molecular composition.

Other relevant questions/ that might be addressed at LEF, but of lower priority for regulators, are:

- Do tropical forested headwater streams exert any control on the SiO_2 cycle and downstream export from forested reference watersheds, or do they passively transport SiO_2 to downstream ecosystems?

- Do tropical forested headwater streams actively cycle SO_4^{2-} or do they passively transport SO_4^{2-} to downstream ecosystems?

Potential utility to water quality regulatory agencies

As noted above with N and P, the LEF provides an invaluable background of nutrient conditions against which urbanized or other heavily managed streams may be compared, and also as a baseline for assessing responses to regional or global scale drivers of change. As with riparian processing of groundwater N, LEF-based findings of DOC retention from groundwater passing through riparian zones might provide a scientific basis for riparian zone conservation BMPs to mitigate DOC delivery to streams. LEF also has extensive, long-term monitoring datasets on nutrients other than N and P that might be used by regulators should issues arise in the future concerning these other nutrients.

Key Points

- Potassium concentrations and fluxes increase greatly after hurricane-derived forest disturbance compared to pre-hurricane conditions.
- Silica can be a limiting nutrient in marine systems, and silica concentrations and exports are high in LEF streams owing to the high weathering rates of the underlying rocks.
- LEF streams carry low DOC loads, but nearby urban streams carry elevated DOC concentrations of labile forms from wastewater that may enhance dissolved oxygen depletion.
- The fate of DOC in headwater streams is a high priority research need of water quality regulators that might be addressed by future LEF research.
- Continuous monitoring datasets from LEF watersheds from the 1980s to present are readily available for use by water quality regulators.

1.4 Overview and Synthesis

The Luquillo Experimental Forest may be thought of as an island within an island, wherein largely intact montane forests are surrounded by a heavily urbanized landscape, all situated on an oceanic, tropical island. Free from the impacts of development, the green island of LEF has served for decades as an environmental laboratory for the study of important ecological functions, including processes that control the quality of water from intact forest watersheds. Below, on the densely populated coasts, the urban island of Puerto Rico is critically dependent on the green island in the interior to provide clean freshwater. These headwater streams also provide habitat that supports healthy communities of native aquatic species.

Research from LEF provides the most complete water quality baseline that exists for Puerto Rico and for tropical forest streams without confounding impacts from land uses. Past and future studies conducted at LEF may prove essential to disentangle the effects of global and regional drivers of change, such as drought and hurricanes, from the effects of local drivers such as urban development.

Many LEF studies discussed here have examined how streams process nutrients, showing that terrestrial inputs control fluxes of nutrients and organic matter in forest streams; that shrimp are the dominant organisms processing plant detritus in streams; and that natural disturbances, including hurricanes and landslides, strongly influence long-term stream nutrient dynamics. Fewer studies have addressed how stream biota respond to nutrients. Future research at LEF can broadens the scope to include more aspects of the responses of the biological community to stream nutrients. A better understanding of how LEF stream biota are affected by nutrients will undoubtedly be of use as regulatory agencies develop criteria to protect ecological function and maintain ecosystem services.

1.5 Works Cited

Argerich, A; Johnson, S.L.; Sebestyen, S.D. [et al.] 2013. Trends in stream nitrogen concentrations for forested reference catchments across the USA. Environmental Research Letters. 8(1): 1-8. doi:10.1088/1748-9326/8/1/014039.

Benstead, J. P.; Cross, W. F.; March, J. G. [et al.]. 2010. Biotic and abiotic controls on the ecosystem significance of consumer excretion in two contrasting tropical streams. Freshwater Biology. 55(10): 2047-2061.

Bhatt, M.P.; McDowell, W.H. 2007. Controls on major solutes within the drainage network of a rapidly weathering tropical watershed. Water Resources Research. 43(11): W11402. doi:10.1029/2007WR005915.

Boulton, A.J., Boyero, L.; Covich, A.P. [et al.]. 2008. Are tropical streams ecologically different from temperate streams? In: Dudgeon, D. ed. Tropical stream ecology. San Diego, CA: Academic Press. 257-284. Chapter 9.

Brown, S.; Lugo, A. E.; Silander, S. [et al.]. 1983. Research history and opportunities in the Luquillo Experimental Forest. Gen. Tech. Rep. SO-44. New Orleans, LA: U.S. Department of Agriculture, Forest Service, Southern Forest Experiment Station. 132 p.

Brookshire, E.N.J.; Gerber, S.; Menge, D.N.L. [et al.]. 2012. Large losses of inorganic nitrogen from tropical rainforests suggest a lack of nitrogen limitation. Ecology Letters. 15(1): 9-16.

Chestnut, T. J.; McDowell, W. H. 2000. C and N dynamics in the riparian and hyporheic zones of a tropical stream, Luquillo Mountains, Puerto Rico. Journal of the North American Benthological Society. 18(2): 53-75. doi:10.2307/1468065.

Comarazamy, D.E.; González, J.E.; Moshary, F. [et al.]. 2015. On the hydro-meteorological changes of a tropical water basin in the Caribbean and its sensitivity to midterm changes in regional climate. Journal of Hydrometeorology. 16(3): 997-1013.

Covich, A. P.; Crowl, T. A.; Johnson, S. L. [et al.]. 1991. Post-Hurricane Hugo increases in atyid shrimp abundances in a Puerto Rican montane stream. Biotropica. 23(4): 448-454.

Covich, A. P.; McDowell, W. H. 1996. The stream community. In: Reagan, D.P.; Waide, R.B. eds. 1996. The food web of a tropical rain forest. Chapter 13. 433-459.

Covich, A.P; Crowl, T.A.; Scatena, F.N. 2003. Effects of extreme low flows on freshwater shrimps in a perennial tropical stream. Freshwater Biology. 48(7): 1199-1206.

Covich, A.P.; Crowl, T.A.; Hein, C.L. [et al.]. 2009. Importance of geomorphic barriers to predator–prey interactions in river networks. Freshwater Biology. 54: 450-465.

Crook, K.E.; Scatena, F.N.; Pringle, C.M. 2007. Water withdrawn from the Luquillo Experimental Forest, 2004. Gen. Tech. Rep. IITF-GTR-34. San Juan, PR: U.S. Department of Agriculture, Forest Service, International Institute of Tropical Forestry. 26 p.

Crowl, T. A.; McDowell, W. H.; Covich, A. P. [et al.]. 2001. Freshwater shrimp effects on detrital processing and nutrients in a tropical headwater stream. Ecology. 82(3): 775-783.

Crowl, T. A., Brokaw, N., Waide, R. B. [et al.]. 2012. When and where biota matter: linking disturbance regimes, species characteristics, and dynamics of communities and ecosystems. In: Brokaw, N.; Crowl, T.A.; Lugo, A.E. [et al.]. eds. 2012. A Caribbean forest tapestry: the multidimensional nature of disturbance and response. New York: Oxford University Press. 272-304. Chapter 6.

Cuevas, E.; Clements, R. 1975. Changes in selected water quality parameters as influenced by land use patterns in the Espiritu Santo drainage basin. San Juan, PR: Center for Energy and Environment Research. Puerto Rico Nuclear Center 195.

de Jesús-Crespo, R.; Ramírez, A. 2011. Effects of urbanization on stream physicochemistry and macroinvertebrate assemblages in a tropical urban watershed in Puerto Rico. Journal of the North American Benthological Society. 30(3): 739-750.

Derry, L. A.; Kurtz, A. C.; Ziegler, K. [et al.]. 2005. Biological control of terrestrial silica cycling and export fluxes to watersheds. Nature. 433: 728-731.

Emanuel, K. 2013. Downscaling CMIP5 climate models shows increased tropical cyclone activity over the 21st century. Proceedings of the National Academy of Sciences. 110(30): 12219-12224.

FAO & JRC. 2012. Global forest land use change 1990–2005. In: E.J. Lindquist, R. D'Annunzio, A. Gerrand, K. [et al.]. 2012. FAO Forestry Paper No. 169. Rome: Food and Agriculture Organization of the United Nations and European Commission Joint Research Centre.

Figueroa-Nieves, D.; McDowell, W. H.; Potter, J. D. [et al.]. 2014. Effects of sewage effluents on water quality in tropical streams. Journal of Environmental Quality. 43(6): 2053-2063.

Gould, W. A.; Martinuzzi, S., Pares-Ramos, I. K.; Murphy, S. F.; Stallard, R. F. 2012. Land use, population dynamics, and land-cover change in eastern Puerto Rico. In: Stallard, R. F; Murphy S.F. eds. 2012. Water quality and landscape processes of four watersheds in eastern Puerto Rico. Reston, VA: U.S. Department of the Interior, U.S. Geological Survey: Professional Paper 1789. Chapter B.

Greathouse, E. A.; Pringle, C. M.; McDowell, W. H. [et al.]. 2006. Indirect upstream effects of dams: consequences of migratory consumer extirpation in Puerto Rico. Ecological Applications. 16(1): 339-352.

Heartsill Scalley, T.; Scatena, F.N.; Estrada, C. [et al.]. 2007. Disturbance and long-term patterns of rainfall and throughfall nutrient fluxes in a subtropical wet forest in Puerto Rico. Journal of Hydrology. 333(2): 472-485.

Heartsill Scalley, T.; Lopez-Marrero, T. 2014. Land-cover composition, water resources and land management in the watersheds of the Luquillo Mountains, northeastern Puerto Rico. Caribbean Geography, 14, 43-68.

Hedin, L.O.; Brookshire, E.N.J.; Menge, D.N.L. [et al.]. 2009. The nitrogen paradox in tropical forest ecosystems. Annual Review of Ecology, Evolution, and Systematics. 40: 613-635.

Hein, C.L.; Pike, A.S.; Blanco, J.F. [et al.]. 2011. Effects of coupled natural and anthropogenic factors on the community structure of diadromous fish and shrimp species in tropical island streams. Freshwater Biology. 56: 1002-1015.

Jacobsen, D.; Cressa, C.; Mathooko, J. M. [et al.]. 2008. Macroinvertebrates: composition, life histories and production. In: Dudgeon, D. ed. Tropical stream ecology. San Diego, CA: Academic Press. 66-96. Chapter 4.

Knutson, T. R., McBride, J. L., Chan, J. [et al.]. 2010. Tropical cyclones and climate change. Nature Geoscience. 3(3): 157-163.

Larsen, M.C. 2012. Landslides and sediment budgets in four watersheds in eastern Puerto Rico. In: Stallard, R. F; Murphy S.F. eds. 2012. Water quality and landscape processes of four watersheds in eastern Puerto Rico. Reston, VA: U.S. Department of the Interior, U.S. Geological Survey: Professional Paper 1789. Chapter F.

Lewis, Jr., W. M.; Melack, J. M.; McDowell, W. H. [et al.]. 1999. Nitrogen yields from undisturbed watersheds in the Americas. Biogeochemistry. 46(1-3): 149-162.

Liptzin, D.; Silver, W. L. 2009. Effects of carbon additions on iron reduction and phosphorus availability in a humid tropical forest soil. Soil Biology and Biochemistry. 41(8): 1696-1702.

Lugo, A. E.; Helmer, E. 2004. Emerging forests on abandoned land: Puerto Rico's new forests. Forest Ecology and Management. 190(2): 145-161.

Lugo, A.E.; Swanson, F.J.; González, O.R. [et al.]. 2006. Long-term research at the USDA Forest Service's experimental forests and ranges. BioScience, 56(1): 39-48.

Lugo, A.E.; Heartsill Scalley, T. 2014. Research in the Luquillo Experimental Forest has advanced understanding of tropical forests and resolved management issues. In: Hayes, D.C.; Stout, S.L.; Crawford, R.H. [et al.]. eds. USDA Forest Service Experimental Forests and Ranges: Research for the Long Term. New York: Springer. 435-461. Chapter 19.

Lugolobi, F.; Kurtz, A. C.; Derry, L. A. 2010. Germanium–silicon fractionation in a tropical, granitic weathering environment. Geochimica Et Cosmochimica Acta. 74(4): 1294-1308.

McDowell, W.H.; Sánchez, C.G.; Asbury, C.E. [et al.]. 1990. Influence of sea salt aerosols and long range transport on precipitation chemistry at El Verde, Puerto Rico. Atmospheric Environment. 24(11):.2813-2821.

McDowell, W.H.; Bowden, W.B.; Asbury, C.E. 1992. Riparian nitrogen dynamics in two geomorphologically distinct tropical rain forest watersheds: subsurface solute patterns. Biogeochemistry 18: 53-75

McDowell, W. H.; Asbury, C. E. 1994. Export of carbon, nitrogen, and major ions from three tropical montane watersheds. Limnology and Oceanography. 39(1): 111-125.

McDowell, W. H.; McSwiney, C. P.; Bowden, W. B. 1996. Effects of hurricane disturbance on groundwater chemistry and riparian function in a tropical rain forest. Biotropica. 28(4): 577-584. doi:10.2307/2389098.

McDowell, W.H. 1998. Internal nutrient fluxes in a tropical rain forest. Journal of Tropical Ecology. 14: 521-536.

McDowell, W. H.; Scatena, F.N.; Waide, R.B. [et al.]. 2012. Geographic and ecological setting of the Luquillo Mountains. In: Brokaw, N.; Crowl, T.A.; Lugo, A.E. [et al.] eds. A Caribbean forest tapestry: the multidimensional nature of disturbance and response. New York: Oxford University Press. 272-304. Chapter 3.

McDowell, W. H.; Brereton, R. L.; Scatena, F. N. [et al.]. 2013. Interactions between lithology and biology drive the long-term response of stream chemistry to major hurricanes in a tropical landscape. Biogeochemistry, 116, 175-186.

McDowell, W. H.; Liptzin, D. 2014. Linking soils and streams: response of soil solution chemistry to simulated hurricane disturbance mirrors stream chemistry following a severe hurricane. Forest Ecology and Management. 332:56-63.

Merriam, J.L.; McDowell, W.H.; Tank, J.L. [et al.]. 2002. Characterizing nitrogen dynamics, retention and transport in a tropical rainforest stream using an in situ ^{15}N addition. Freshwater Biology. 47(1): 143-160.

Morse, N. B.; Pellissier, P. A.; Cianciola, E. N. [et al]. 2014. Novel ecosystems in the Anthropocene: a revision of the novel ecosystem concept for pragmatic applications. Ecology and Society, 19(2): 1-12.

Mulholland, P. J.; Helton, A. M.; Poole, G. C. [et al.]. 2008. Stream denitrification across biomes and its response to anthropogenic nitrate loading. Nature 452: 202-205.

Murphy, S. F.; Stallard, R. F. 2012. Hydrology and climate of four watersheds in eastern Puerto Rico. In: Stallard, R. F; Murphy S.F. eds. 2012. Water quality and landscape processes of four watersheds in eastern Puerto Rico. Reston, VA: U.S. Department of the Interior, U.S. Geological Survey: Professional Paper 1789. Chapter C.

Murphy, S. F.; Stallard, R. F.; Larsen, M. C. [et al.]. 2012. Physiography, geology, and land cover of four watersheds in eastern Puerto Rico. In: Stallard, R. F; Murphy S.F. eds. 2012. Water quality and landscape processes of four watersheds in eastern Puerto Rico. Reston, VA: U.S. Department of the Interior, U.S. Geological Survey: Professional Paper 1789. Chapter A.

Odum, H.T. 1970. An emerging view of the ecological system at El Verde. In: Odum, H.T.; Pigeon, R.F. eds. 1970. A tropical rain forest: a study of irradiation and ecology at El Verde, Puerto Rico. Oak Ridge, TN: U.S. Atomic Energy Commission, Division of Technical Information. I-191-I-289. Chapter 1.

Odum, H.T.; Pigeon, R.F. eds. 1970. A tropical rain forest: a study of irradiation and ecology at El Verde, Puerto Rico. Oak Ridge, TN: U.S. Atomic Energy Commission, Division of Technical Information.

Ortiz-Zayas, J. R.; Lewis, Jr, W. M., Saunders III, J. F. [et al.]. 2005. Metabolism of a tropical rainforest stream. Journal of the North American Benthological Society. 24(4): 769-783.

Ortíz-Zayas, J. R.; Cuevas, E.; Mayol-Bracero, O. [et al.]. 2006. Urban influences on the nitrogen cycle in Puerto Rico. Biogeochemistry. 79: 109-133. doi:10.2307/20519823.

Pérez-Reyes, O.; Crowl, T.A.; Covich, A.P. 2015. Effects of elevation, food supplies, and water temperature on growth rates of freshwater topical shrimp in Luquillo Mountains, Puerto Rico. Freshwater Biology. 60: 1514-1524.

Pérez-Reyes, O.; Crowl, T.A.; Covich, A.P. 2016. Comparison of decapod communities across an urban-forest land use gradient in Puerto Rican streams. Urban Ecosystems. 19(1): 181-203.

Peterson, B. J.; Wollheim, W. M.; Mulholland, P. J. [et al.]. 2001. Control of nitrogen export from watersheds by headwater streams. Science. 292: 86-90.

Pett-Ridge, J. C. 2009. Contributions of dust to phosphorus cycling in tropical forests of the Luquillo Mountains, Puerto Rico. Biogeochemistry. 94(1): 63-80.

Potter, J. D.; McDowell, W. H.; Helton, A.M. [et al.]. 2014. Incorporating urban infrastructure into biogeochemical assessment of urban tropical streams in Puerto Rico. Biogeochemistry. 121(1): 271-286.

Potter, J.D.; McDowell, W.H.; Merriam, J. [et al.]. 2010. Denitrification and total nitrate uptake in streams of a tropical landscape. Ecological Applications. 20(8): 2104-2115.

Pringle, C.; Hemphill, N.; McDowell, W. [et al.]. 1999. Linking species and ecosystems: different biotic assemblages cause interstream differences in organic matter. Ecology. 80(6): 1860-1872.

Prudhomme, C.; Giuntoli, I.; Robinson, E. L. [et al.]. 2014. Hydrological droughts in the 21st century: hotspots and uncertainties from a global multimodel ensemble experiment. Proceedings of the National Academy of Sciences. 111(9): 3262-3267.

Pyron, M.; Covich, A. P. 2003. Migration patterns, densities, and growth of *Neritina punctulata* snails in Rio Espiritu Santo and Rio Mameyes, northeastern Puerto Rico. Caribbean Journal of Science. 39(3): 338-347.

Ramírez, A., de Jesús-Crespo, R., Martinó-Cardona, D. M., [et al.]. 2009. Urban streams in Puerto Rico: what can we learn from the tropics? Journal of the North American Benthological Society. 28(4): 1070-1079.

Ramírez, A., Rosas, K. G., Lugo, A. E. [et al.]. 2014. Spatio-temporal variation in stream water chemistry in a tropical urban watershed. Ecology and Society, 19(2): 45.

Rincón, J.; Covich, A.P. 2014. Effects of insect and decapod exclusion and leaf litter species identity on breakdown rates in a tropical headwater stream. International Journal of Tropical Biology and Conservation. 62: 143-154.

Rosemond, A.D.; Pringle, C.M.; Ramírez, A. [et al.]. 2002. Landscape variation in phosphorus concentration and effects on detritus-based tropical streams. Limnology and Oceanography. 47(1): 278-289.

Santos-Román, D.M.; Warner, G.S.; Scatena, F.N. 2003. Multivariate analysis of water quality and physical characteristics of selected watersheds in Puerto Rico. Journal of the American Water Resources Association. 39: 829–839. doi:10.1111/j.1752-1688.2003.tb04408.x

Scatena, F.N. 1989. An introduction to the physiography and history of the Bisley Experimental Watersheds in the Luquillo Mountains of Puerto Rico. Gen. Tech. Rep. SO-72. New Orleans, LA: U.S. Dept of Agriculture, Forest Service, Southern Forest Experiment Station. 22 p.

Scatena, F. N.; Johnson, S. L. 2001. Instream-flow analysis for the Luquillo Experimental Forest, Puerto Rico: methods and analysis. Gen. Tech. Rep. IITF-GTR-11. Rio Piedras, PR: US Department of Agriculture, Forest Service, International Institute of Tropical Forestry.

Schaefer, D. A., McDowell, W. H., Scatena, F. N. [et al.]. 2000. Effects of hurricane disturbance on stream water concentrations and fluxes in eight tropical forest watersheds of the Luquillo Experimental Forest, Puerto Rico. Journal of Tropical Ecology. 16(2): 189-207. doi:10.2307/3068756.

Shanley, J. B.; McDowell, W. H.; Stallard, R. F. 2011. Long-term patterns and short-term dynamics of stream solutes and suspended sediment in a rapidly weathering tropical watershed. Water Resources Research. 47(7): W07515.

Stallard, R. F. 2001. Possible environmental factors underlying amphibian decline in eastern Puerto Rico: Analysis of US Government data archives. Conservation Biology. 15(4): 943-953.

Stallard, R.F. 2012. Atmospheric inputs to watersheds of the Luquillo Mountains in eastern Puerto Rico. In: Stallard, R.F; Murphy S.F. eds. 2012. Water quality and landscape processes of four watersheds in eastern Puerto Rico. Reston, VA: U.S. Department of the Interior, U.S. Geological Survey: Professional Paper 1789. Chapter D.

Stallard, R.F.; Murphy, S.F. 2012. Water quality and mass transport in four watersheds in eastern Puerto Rico. In: Stallard, R.F; Murphy S.F. eds. 2012. Water quality and landscape processes of four watersheds in eastern Puerto Rico. Reston, VA: U.S. Department of the Interior, U.S. Geological Survey: Professional Paper 1789. Chapter E.

Sotomayor-Ramírez, D.; Martínez, G; Pérez-Alegría, L. 2011. Determination of numeric nutrient criteria in rivers and streams of Puerto Rico. Final Report submitted to Puerto Rico Environmental Quality Board. Mayaguez, PR: University of Puerto Rico Agricultural Experiment Station. 58 p.

Tank, J. L.; Dodds, W. K. 2003. Nutrient limitation of epilithic and epixylic biofilms in ten North American streams. Freshwater Biology. 48(6): 1031-1049.

Van Beusekom, A.E.; González, G.; Rivera, M.M. 2015. Short-term precipitation and temperature trends along an elevation gradient in Northeastern Puerto Rico. Earth Interactions. 19(3): 1-33.

Wadsworth, F. H. 1995. A forest research institution in the West Indies: The first 50 years. In: Lugo, A.E.; Lowe, C. eds., Tropical forests: management and ecology. New York: Springer-Verlag. 33-56. Chapter 3.

Webster, J. R.; Mulholland, P. J.; Tank, J. L. [et al.]. 2003. Factors affecting ammonium uptake in streams–an inter-biome perspective. Freshwater Biology. 48(8): 1329-1352.

White, A.F.; Blum, A.E.; Schulz, M.S. [et al.]. 1998. Chemical weathering in a tropical watershed, Luquillo Mountains, Puerto Rico: I. Long-term versus short-term weathering fluxes. Geochimica et Cosmochimica Acta. 62(2): 209-226.

Wright, M. S.; Covich, A. P. 2005. The effect of macroinvertebrate exclusion on leaf breakdown rates in a tropical headwater stream. Biotropica. 37(3): 403-408.

Zimmerman, J. K.; Covich, A. P. 2003. Distribution of juvenile crabs (*Epilobocera sinuatifrons*) in two Puerto Rican headwater streams: effects of pool morphology and past land-use legacies. Archiv für Hydrobiologie. 158(3): 343-357.

CHAPTER 2: HYDROLOGY MODULATES IN-STREAM AMMONIUM UPTAKE IN TROPICAL FOREST HEADWATER STREAMS

2.1 INTRODUCTION

Riverine exports of inorganic nitrogen from tropical forests are as much as an order of magnitude greater than those from temperate forests, tundra, grasslands, and other biomes (McDowell and Asbury 1994, Lewis et al. 1999, Brookshire et al. 2012). These consistently high N exports are the result of high rates of N transport from the landscape. Nitrogen limitation of net primary production occurs in many terrestrial environments around the globe (Vitousek and Howarth 1991, LeBauer and Treseder 2008), but tropical forests often are not N-limited (Vitousek and Farrington 1997, Menge et al. 2012). High N exports, frequent P limitation of primary production, high N:P ratios in plants and soils, and the continued proliferation of N-fixing plants have been described as the N paradox in tropical forests (Hedin et al. 2009).

Nitrogen dynamics in riparian zones and headwater streams remain curiously uncertain in N budgets for tropical watersheds, despite a growing body of research indicating that these landscape features are critically important N sinks. In temperate ecosystems, riparian zones have long been recognized as hot spots of inorganic N retention on the landscape (Peterjohn and Correll 1984, Hedin et al. 1998). It is also well established that headwater streams, including tropical forest streams, actively cycle and retain N and are critical to a river network's capacity for N retention and removal (Peterson et al. 2001, Mulholland et al. 2008). The fraction of N delivered to streams from the landscape that is subsequently lost as downstream flux depends on the rate of N uptake within streams (Newbold et al. 1981, Wollheim et al. 2006). Relatively few

studies have considered N retention in headwater streams and riparian zones within tropical forests, but those that have done so have demonstrated potentially large N sinks (McDowell et al. 1992, McClain et al. 1994, Chestnut and McDowell 2000, Merriam et al. 2002, Potter et al. 2010). Nitrogen retention in these environments is particularly important in tropical forests situated on small mountainous islands like Puerto Rico, where short distances from headwaters to estuaries mean that unprocessed N is quickly shunted to receiving waters where it may contribute to eutrophication.

Nitrogen uptake in streams is a function of biological demand and hydrologic transport (Newbold et al. 1981, Wollheim et al. 2006, 2008). As nutrients are transported downstream they are taken up by benthic microorganisms and cycled, causing an N spiral. Quantifying these spirals is essential for comparison between streams, land cover and land use types, and biomes, as well as for extrapolating from reach-scale studies to river networks and modeling delivery of nutrients downstream (Stream Solute Workshop 1990). Nitrogen uptake is commonly estimated by injecting nutrients and conservative tracer solutes into streams and measuring the disappearance of nutrients relative to tracers. Tracer experiments can also be carried out using isotopically enriched forms of N such as $^{15}N\text{-}NH_4^+$ (Peterson et al. 2001, Merriam et al. 2002, Webster et al. 2003) or $^{15}N\text{-}NO_3^-$ (Mulholland et al. 2008, Potter et al. 2010). Merriam et al. (2002) examined $^{15}N\text{-}NH_4^+$ uptake in a tropical forest stream in northeastern Puerto Rico and found high rates of uptake with nitrification as a dominant fate.

In this study, I asked whether the N paradox in tropical forests results from riparian and stream N sinks that are inadequate for retaining high hydrologic N fluxes from upland soils. My primary objectives were to characterize the variability of NH_4^+ uptake in streams of a tropical forest watershed, and of NH_4^+ delivery to those streams from groundwater, all in the context of

abundant N concentrations at our study site. I used whole-stream NH_4^+ pulse experiments and paired dilution gauging experiments to characterize NH_4^+ uptake and groundwater inputs in study reaches in the Rio Icacos watershed of the Luquillo Mountains in Puerto Rico. Nitrogen uptake experiments solely focused on streams may not capture processes that occur in the streambed or the near-stream riparian zone, particularly if the hydraulic characteristics of the stream do not force hyporheic flow through subsurface sediments (Wondzell and Swanson 1996, Chestnut and McDowell 2000). Thus, I chose a paired approach that allowed me to separately estimate in-stream and riparian control over solute concentrations. I further tested the hypothesis proposed by Merriam et al. (2002) that tropical forest headwater streams, with low light and organic carbon availability, rapidly take up NH_4^+ as an energy source. With this reasoning, we expected that uptake of added NH_4^+ would be rapid, and that nitrification would be a dominant fate of added NH_4^+, thus remaining consistent with the continued high NO_3^- exports observed in the tropical N paradox.

2.2 METHODS
Study site

The Rio Icacos basin (Fig. 1) is situated in the central Luquillo Mountains at 616-800 MASL spanning the palo colorado forest type, with palo colorado (*Cyrilla racemiflora*) dominant on hillslopes and ridges and sierra palm (*Prestoea montana*) in floodplains and riparian zones. The watershed is 100% forested with heavy shade cover above the stream channels of the Icacos and its tributaries. Mean annual rainfall is 4050 mm (Murphy et al. 2012b) and temperature is 21˚C (Shanley et al. 2011). The bedrock underlying the Icacos basin is quartz diorite of the Rio Blanco formation, and the soils are very deep clays, clay loams, and sandy clay loams of the Picacho and Utuado series, classified as Inceptisols (Murphy et al. 2012a)..
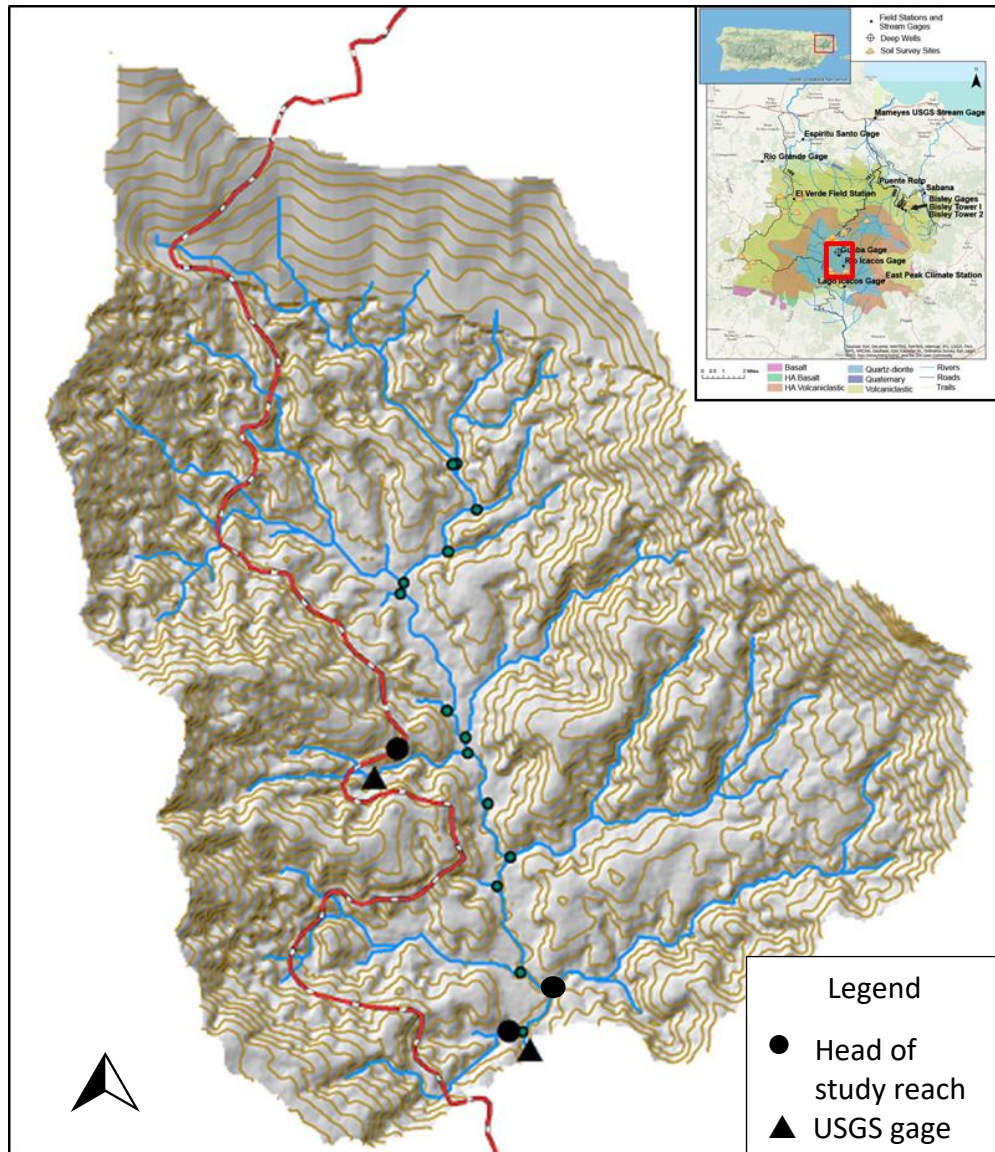
Figure 1. Map depicting the Rio Icacos basin in the Luquillo Experimental Forest of northeastern Puerto Rico (insets).

Three streams in the Rio Icacos watershed were selected to represent a range of stream size: the Icacos main stem (RI), Quebrada Guaba (QG, a 3rd order, larger tributary), and I-0 (a 2nd order, smaller tributary). For each stream we chose the longest study reaches without tributary inflows, for a range of reach lengths from 75 m (I-0) to 178 m (QG) (Table 1). Watershed area ranged from 13 in I-0 to 326 ha in RI (Table 1). Mean travel times were between 20 and 40 min for the reaches.

Table 1. Physiography of the three streams included in this study.

Study reach	Watershed area (ha)	Reach length (m)	Mean width (m)	Mean depth (cm)
I-0	13	75	0.66	24
QG	26	178	1.20	6.6
RI	326	147	4.6	24

Physical and chemical characteristics of study streams

On the day of each nutrient pulse experiment, stream discharge (Q) was measured with dilution gauging experiments at the bottom and top of the study reach. Groundwater discharge (Q_{GW}) was calculated as the difference between downstream and upstream Q. Wetted width (w) and depth were measured at 10-15 transects along each study reach. Mean transit time along study reaches was 10-35 minutes in all stream reaches. To characterize background stream chemistry, 2-5 grab samples were taken downstream before each nutrient pulse experiment, then 2-5 additional samples were taken immediately upstream of the pulse injection point during and after each experiment. The change in concentration along the reach (ΔNH_4^+) was calculated as $NH_4^+_{downstream}$ - $NH_4^+_{upstream}$.

Nutrient pulse experiments

In each of the three study streams, we carried out three nutrient pulse experiments (Covino et al. 2010, Gibson et al. 2015, Rodríguez-Cardona et al. 2016, Koenig et al. 2017) in March-June 2015, for a total of nine experiments. The target enrichment of each stream was 30 times background concentrations. A single slug addition of NH_4^+ (as NH_4Cl) and Cl^- (as $NaCl$) was added to a container of stream water and then poured into the stream at an upstream injection point. At a downstream sampling point, electrical conductivity (EC) during the breakthrough curve was monitored for specific conductance with a YSI 556 conductivity probe

logging at intervals of five seconds. Between 20 and 35 grab samples were taken over the full

range of the breakthrough curve as measured by EC.

Samples were filtered to 0.7 µm (Whatman GF/F) in the field or refrigerated for filtration

in the laboratory, and stored in 60 mL acid-washed HDPE bottles. Filtered samples were frozen

and shipped to the University of New Hampshire Water Quality Analysis Laboratory. Samples

were analyzed for NH_4^+ concentration using the phenol hypochlorite method (Solórzano 1969)

automated with a Westco Smartchem 200 discrete autoanalyzer. Concentrations of Cl^- and NO_3^-

were quantified on a Dionex ICS-1000 ion chromatograph. Samples were also analyzed for

dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) using the high temperature

catalytic oxidation method with a Shimadzu TOC-V CPH and TNM nitrogen unit. Dissolved

organic nitrogen (DON) was calculated by subtracting total dissolved inorganic nitrogen (NO_3^- +

NH_4^+) from measured TDN concentrations. For streams where the ambient NH_4^+ concentration

was lower than the laboratory method detection limit of 5 µg N L^{-1}, we assumed that ambient

NH_4^+ concentration was equal to half the detection limit (2.5 µg N L^{-1}).

Ammonium uptake estimates from pulse experiments

To quantify stream NH_4^+ uptake, we calculated three stream nutrient spiraling parameters

from each breakthrough curve of solute data from grab samples: the ambient NH_4^+ uptake length

(S_w, m), the stream distance a nitrogen atom travels before being removed from the water

column; the NH_4^+ areal uptake rate (U, µg m^{-2} s^{-1}); and the NH_4^+ uptake velocity (v_f, mm min^{-1}),

which represents NH_4^+ uptake normalized by NH_4^+ concentration (Newbold et al. 1981, Stream

Solute Workshop 1990). We based our calculations on the method introduced by Covino et al.

(2010).

For each grab sample across the breakthrough curve, the dynamic uptake length ($S_{w\text{-}add\text{-}dyn}$), dynamic areal uptake rate ($U_{add\text{-}dyn}$), and dynamic uptake velocity ($V_{f\text{-}add\text{-}dyn}$) were calculated. The difference in NH_4^+:Cl^- between the injection and sampling sites was assumed to result from exponential decay along the reach length, allowing calculation of the longitudinal decay rate $k_{w\text{-}add\text{-}dyn}$ (min^{-1}). To assess for hysteresis, samples were classified as rising limb or falling limb based on the field-recorded conductivity and the measured Cl^- concentration. The dynamic uptake length $S_{w\text{-}add\text{-}dyn}$ was taken as the negative inverse of $k_{w\text{-}add\text{-}dyn}$. The geometric mean of the grab sample concentration and the background upstream concentration ($NH_4^+{}_{upstream}$), called $[NH_4^+\text{-}N]_{add\text{-}dyn}$ (mg/L), was calculated for each grab sample. Dynamic areal uptake rate ($\mu g/m^2/min$) was calculated with Eqn. 1:

$$U_{add-dyn} = \frac{(Q \times [NH_4-N_{add-dyn}])}{(S_{w-add-dyn} \times w)} \qquad \text{Eqn. 1}$$

Dynamic uptake velocity (mm/min) was calculated with Eqn. 2:

$$V_{f-add-dyn} = \frac{U_{add-dyn}}{[NH_4-N_{add-dyn}]} \qquad \text{Eqn. 2}$$

We used the line of best fit between between $S_{w\text{-}add\text{-}dyn}$ and $N_{tot\text{-}dyn}$, the geometric mean of the background-corrected NH_4^+ concentration and the concentration that would result if NH_4^+ traveled conservatively like Cl^- (which represents the likely concentration experienced by stream biota [Covino et al. 2010]). The intercept of the line of best fit was taken as $S_{w\ amb}$ (m), which was subsequently used to calculate U_{amb} ($\mu g/m^2/min$) and $v_{f\ amb}$ (mm/min).

In addition to estimating metrics of NH_4^+ uptake, we also calculated the total net uptake of NH_4^+ during the pulse experiments. A notable feature of this site is that groundwater concentrations of NH_4^+ are as high as typical target concentrations for nutrient pulse experiments

of 20 to 30 times background concentrations in streamwater (Covino et al. 2010, Gibson et al. 2015, Rodríguez-Cardona et al. 2016). Therefore, the absolute magnitude of NH_4^+ removal during a pulse experiment is directly relevant to stream response to groundwater inputs. For each grab sample, NH_4^+ uptake was calculated as $[NH_4^+]_{expected} - [NH_4^+]_{observed}$ (background corrected). We calculated total net NH_4^+ uptake as the area under the NH_4^+ uptake curve vs. time, which we approximated using Riemann middle sums (Thomas, Jr. and Finney 1996) in Microsoft Excel 2013.

Statistical analyses

We focus on ambient NH_4^+ uptake velocity ($v_{f\,amb}$, mm min^{-1}) because $v_{f\,amb}$ facilitates comparison between streams of different depths and nutrient concentrations (Hall et al. 2002, Ensign and Doyle 2006). We used Pearson correlations to assess relationships between $v_{f\,amb}$ and five physical and chemical predictor variables: background NO_3^-, DIN, and DOC concentrations; groundwater specific discharge (q_{GW}); and stream specific discharge (Q/w). All statistical analyses were carried out in JMP (JMP Pro 13, SAS Institute, Cary, North Carolina)

Estimating riparian ammonium retention

We assessed whether or not the NH_4^+ removal observed from these experiments was sufficient to account for the potential groundwater NH_4^+ flux. We approached this question by taking the removal rate measured in each experiment and comparing it to the expected NH_4^+ from groundwater flux based on ambient concentrations upstream and downstream. Expected groundwater NH_4^+ flux was calculated as the fraction of stream discharge due to GW input, multiplied by the average NH_4^+ concentration from published values of streamside well chemistry (McDowell et al. 1992, Chapter 3 of this thesis).

We made a set of assumptions designed to give a conservative estimate of potential NH_4^+ flux from groundwater. First, we treated groundwater NH_4^+ flux as though it all enters the stream channel at the top of the reach and is therefore subject to the same removal rate as upstream NH_4^+ flux – that is, the maximum effect of NH_4^+ uptake. Second, we took the low end of the range of groundwater NH_4^+-N concentrations in the literature from this site, 500 µg L^{-1}, as the input concentration at all sites. This groundwater NH_4^+ concentration and the groundwater discharge we measured in each experiment were used to calculate a weighted average representing the combined stream and groundwater. We then applied the measured in-stream removal rate R for each experiment to decrease the potential groundwater NH_4^+ flux by the appropriate fraction (Wollheim et al. 2006). R is the fraction of nutrient flux removed (unitless), calculated with Eqn. 3:

$$R = 1 - \exp\left(-\frac{V_f LW}{Q}\right) \qquad\qquad \text{Eqn. 3}$$

Last, we calculated the difference between the potential NH_4^+ concentration (with R factored in) and the observed. This value is our inferred riparian removal, assumed to be the NH_4^+ retention which takes place in the aquifer as groundwater passes through the riparian zone, before discharging into the stream.

2.3 RESULTS

Physical and chemical characteristics of study streams

Stream discharge ranged from 4.6 to 229 L s^{-1} across the three streams and nine experiments (Table 2), and decreased in each stream by an average of 6-45% from March to June 2015 as a regional drought developed. On average, discharge in the main stem Icacos (168 L s^{-1})

was 19 times greater than that of the larger tributary Guaba (8.7 L s^{-1}), which was 1.7 times greater than that of the smaller tributary I-0 (5.0 L s^{-1}). Groundwater inputs ($Q_{downstream} - Q_{upstream}$ in Table 2) ranged from -4 to 37 L s^{-1}. When normalized to reach length, groundwater inputs ranged from -0.03 to 0.25 L m^{-1} s^{-1}. The lone negative value for Icacos on June 5 indicates that that experiment day was the only one in which discharge decreased along the study reach; all other experiment dates showed reaches gaining Q.

Table 2. Background physical and chemical parameters of study reaches during each of the nine ammonium pulse experiments in this study.

Reach	Date (2015)	$Q_{upstream}$ (L s^{-1})	$Q_{downstream}$ (L s^{-1})	NH$_4^+$$_{upstream}$ (μg N L^{-1})	NH$_4^+$$_{downstream}$ (μg N L^{-1})	NO$_3^-$ (μg N L^{-1})	DOC (μg C L^{-1})
I-0	March 17	5.2	5.3	11	19	107	546
	March 18	4.9	5.1	27	42	104	622
	June 4	4.6	4.8	13	9	66	590
Guaba	March 17	9.4	11.2	2.5	2.5	52	615
(QG)	March 18	9.5	11.2	4.5	2.5	48	548
	June 6	5.2	6.0	11	14	80	568
Icacos	March 16	209	229	10	18	82	568
(RI)	March 20	145	182	7	6	92	631
	June 5	124	120	5	4	114	549

In several instances, NH$_4^+$ concentrations showed sharp increases from the upstream to downstream sampling locations (Table 2). I-0 saw concentrations increase by 8 and 15 μg N L^{-1} during the two March 2015 experiments, while the Icacos mainstem showed an increase of 8 μg

N L^{-1}. We found no correlation between Δ[NH$_4^+$] (μg N L^{-1}), calculated as NH$_4^+$$_{downstream}$ −

NH$_4^+$$_{upstream}$, and Q$_{GW}$, calculated as Q$_{downstream}$ − Q$_{upstream}$ (statistics not shown).

Stream ammonium uptake

Three of the nine experiments showed negative dynamic uptake lengths for a majority of

the grab samples across the breakthrough curve (Fig. 2). These negative values resulted from

higher NH$_4^+$:Cl$^-$ ratios in grab samples than in the injectate. The three experiments with majority

negative S$_{w\ dyn}$ values (two at Icacos and one at I-0) were interpreted as below the limit of

detection of uptake via this method, or U_{amb} and $v_{f\ amb}$ equal to 0. Minimal hysteresis was

displayed during the nine experiments (Fig. 2).

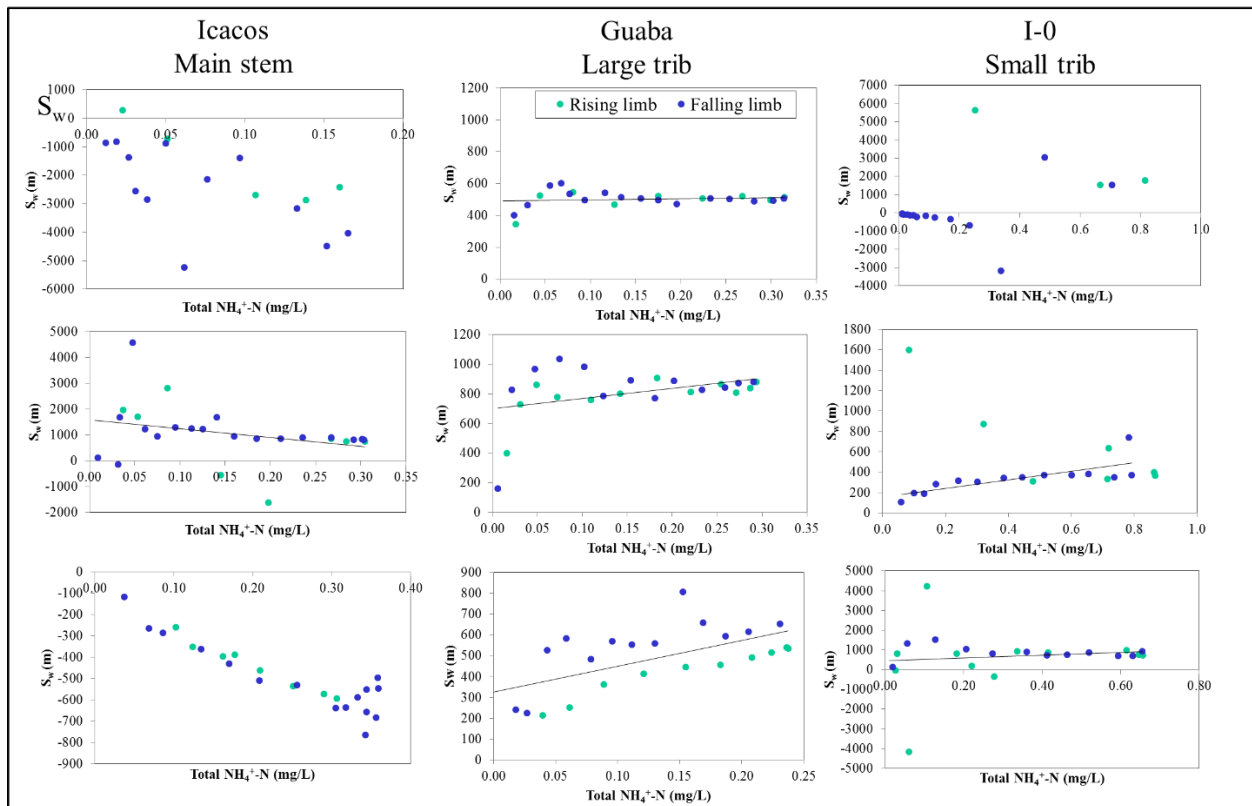Figure 2. Dynamic uptake length (S$_{w-add-dyn}$) vs. total NH$_4^+$-N concentration for
the nine ammonium pulse experiments in this study. Blue points represent rising
limb of breakthrough curve, turquoise points represent falling limb.

Ambient ammonium uptake metrics showed considerable variability (Table 3). $S_{w\,amb}$ was correlated with stream discharge (Table 4), with the shortest value (155 m, I-0) nearly an order of magnitude lower than the longest (1404 m, RI). U_{amb} was also highly variable. $v_{f\,amb}$ was slowest in the larger tributary and fastest in the smallest tributary.

Table 3. Ambient stream NH_4^+ uptake metrics (uptake length $S_{w\,amb}$, areal uptake rate U_{amb}, and uptake velocity $V_{f\,amb}$) and total NH_4^+ uptake for each pulse experiment.

Stream	Date (2015)	$S_{w\,amb}$ (m)	U_{amb} ($\mu g\ m^{-2}\ s^{-1}$)	$V_{f\,amb}$ ($mm\ min^{-1}$)	Total uptake (mg NH_4^+-N)	Tot. uptake / Tot. add. NH_4^+
I-0	March 17	-	0	0	-112	-0.09
	March 18	155	76	2.9	364	0.28
	June 4	441	11	1.0	161	0.12
Guaba	March 17	427	1	1.2	844	0.32
(QG)	March 18	766	2	0.7	543	0.21
	June 6	326	2.8	0.3	707	0.27
Icacos	March 16	-	0	0	-1539	-0.06
(RI)	March 20	1404	11	1.5	1648	0.04
	June 5	-	0	0	-779	-0.02

$S_{w\,amb}$ correlated with all hydrologic predictor variables considered: Q, Q/w, and q_{GW}. U_{amb} and $v_{f\,amb}$ were not correlated significantly with any of the physical/chemical predictor variables (Table 4).

Table 4. Pearson correlations for ambient uptake velocity of NH_4^+ ($v_{f\,amb}$) for six predictor variables. Relationships significant at p = 0.05 highlighted in **bold**.

Predictor variable	n	S_w		n	U_{amb}		$v_{f\,amb}$	
		P	r		P	r	P	r
NO_3^- (μg N L^{-1})	6	0.99	0.00	9	0.44	0.09	0.97	-0.02
DIN (μg N L^{-1})	6	0.76	0.03	9	0.20	0.22	0.75	0.47
DOC (μg C L^{-1})	6	0.79	0.02	9	0.43	0.09	0.23	0.45
DOC:DIN	6	0.92	0.00	9	0.40	0.10	0.92	0.04
Q (L s^{-1})	6	**0.01**	**0.82**	9	0.51	0.06	0.49	0.07
Q/w (m^2 min^{-1})	6	**0.01**	**0.83**	9	0.54	0.56	0.52	-0.25
q_{GW} (m^2 min^{-1})	6	**0.01**	**0.82**	9	0.81	0.01	0.77	0.11

In none of the nine experiments did we see any evidence of NO_3^- concentrations changing in response to NH_4^+ pulse experiments (Fig. 3). A lack of NO_3^- concentration increase during NH_4^+ releases indicates that no net NO_3^- production from nitrification occurred.
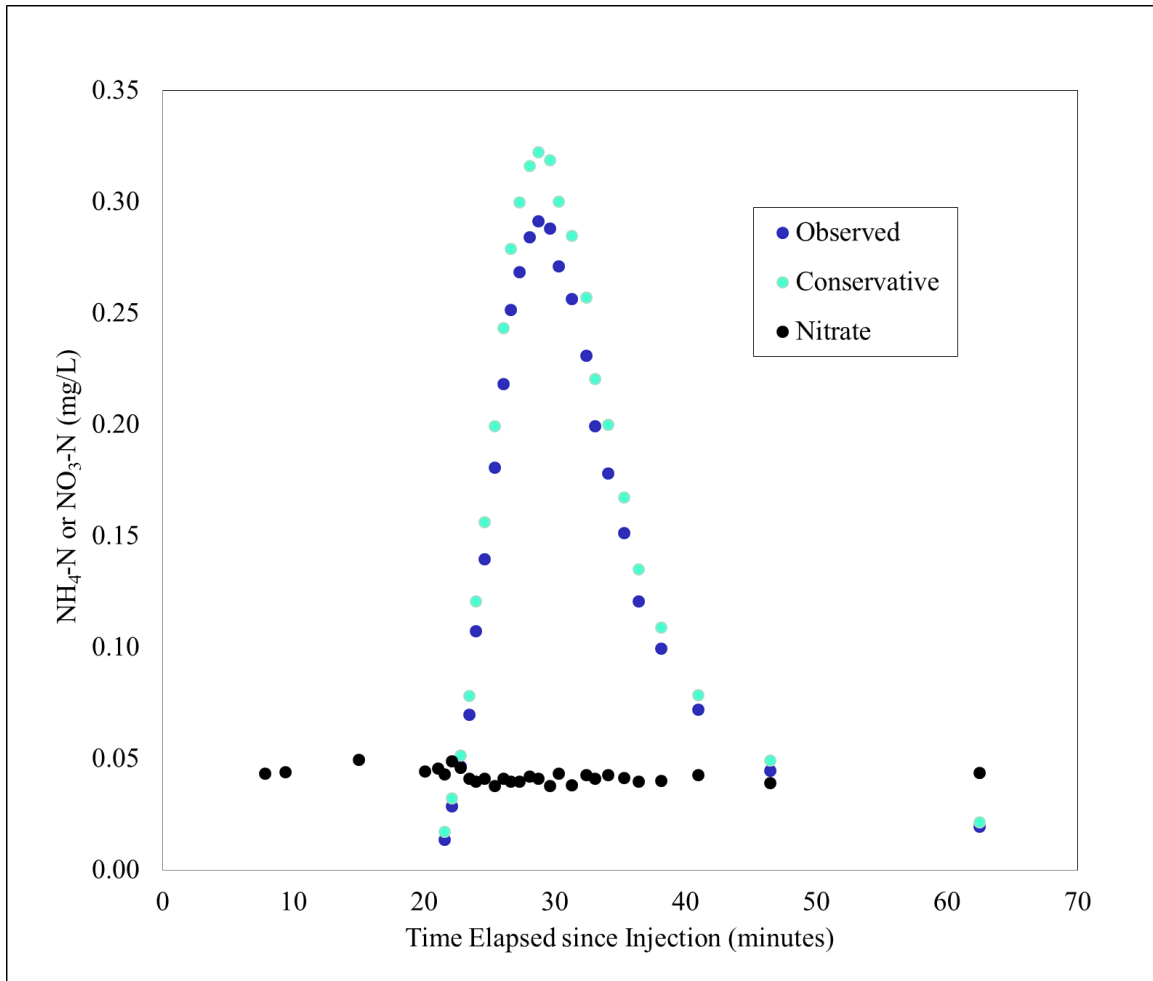
Figure 3. Time series demonstrating breakthrough curve of ammonium pulse experiment. Blue points are concentrations of observed NH_4^+; turquoise points are 'conservative' NH_4^+ concentrations - concentration expected if NH_4^+ behaved conservatively like the chloride tracer. Black points are nitrate concentrations which remain unchanged during experiment, which suggests that nitrate production was low to nonexistent.

Estimates of groundwater ammonium retention

We calculated negative riparian NH_4^+ removal in two experiments in the small tributary I-0, owing to large $\Delta[NH_4^+]$ values on those two days, which indicates that the riparian zone was a NH_4^+ source during those experiments. We also had an experiment in which the study reach was losing Q to the subsurface, meaning that riparian removal was zero. The riparian zone was a

NH$_4^+$ sink of variable size during the remaining six experiments. Stream NH$_4^+$-N concentrations would have been between 16 and 117 µg L^{-1} greater without riparian processing (Table 4).

Table 4. Comparison of observed in-stream NH$_4^+$ concentrations ([NH$_4^+$]$_{stream}$) with the estimate of expected increases in NH$_4^+$ concentration with groundwater input.

Stream	Date (2015)	[NH$_4^+$]$_{GW + stream}$[†] (µg N L^{-1})	R (unitless)	[NH$_4^+$]$_{expected}$[‡] (µg N L^{-1})	Riparian removal (µg N L^{-1})
I-0	March 17	18	0	18	-5
	March 18	51	0.38	32	-10
	June 4	28	0.16	24	16
Guaba (QG)	March 17	97	0.34	64	61
	March 18	94	0.21	75	73
	June 6	91	0.42	53	37
Icacos (RI)	March 16	60	0	60	39
	March 20	136	0.10	123	117
	June 5	4	0	4	0

[†][NH$_4^+$]$_{GW+Stream}$ is calculated as the weighted average of [NH$_4^+$]$_{upstream}$ (Table 2) and groundwater [NH$_4^+$], weighted by hydrologic contribution of the two sources to total Q. The groundwater NH$_4^+$ concentration used in this calculation is 500 µg N L^{-1}, which represents the mean among the three streams. R is the removal factor (Eqn. 3).

[‡][NH$_4^+$]$_{expected}$ is calculated as [NH$_4^+$]$_{GW+Stream}$ * R. Riparian removal is calculated as [NH$_4^+$]$_{expected}$ − [NH$_4^+$]$_{downstream}$ (Table 2).

2.4 DISCUSSION

Factors affecting stream ammonium uptake

      Our study streams were shaded, carbon-poor, and bordered by NH_4^+-rich riparian groundwater. I expected to see evidence of high NH_4^+ demand consistent with energy limitation: relatively short ambient NH_4^+ S_w and relatively rapid U and v_f. Instead, I found that values of NH_4^+ $S_{w\ amb}$ were an order of magnitude longer in our study streams (Table 3, Fig. 4) than in the most comprehensive stream NH_4^+ uptake study to date, LINX I (Fig. 4) (Peterson et al. 2001, Webster et al. 2003). The fastest $v_{f\ amb}$ in the present study, 2.9 mm min^{-1}, was the lone value that fell into the range of 2.0-41 mm min^{-1} reported by Webster et al. (2003), which includes the Puerto Rico stream studied by Merriam et al. (2002) (v_f of 8.6 mm min^{-1}). The other most important comparison point is Koenig et al. (2017)'s study of seven forested Puerto Rico streams, in which a v_f. range of 0.3-8.5 was found. Two other recent studies employing nutrient pulse experiments to study stream NH_4^+ uptake dynamics in a boreal forest in Siberia and temperate forest in upstate New York similarly found NH_4^+ v_f values that dwarf those of the present study. The Siberian streams ranged from 0.2 to 19.5 mm min^{-1} (Diemer et al. 2015), while the New York streams ranged from 3.43 to 9.48 mm min^{-1} (Gibson et al. 2015). The present study uniformly found v_f. values below values in the literature.
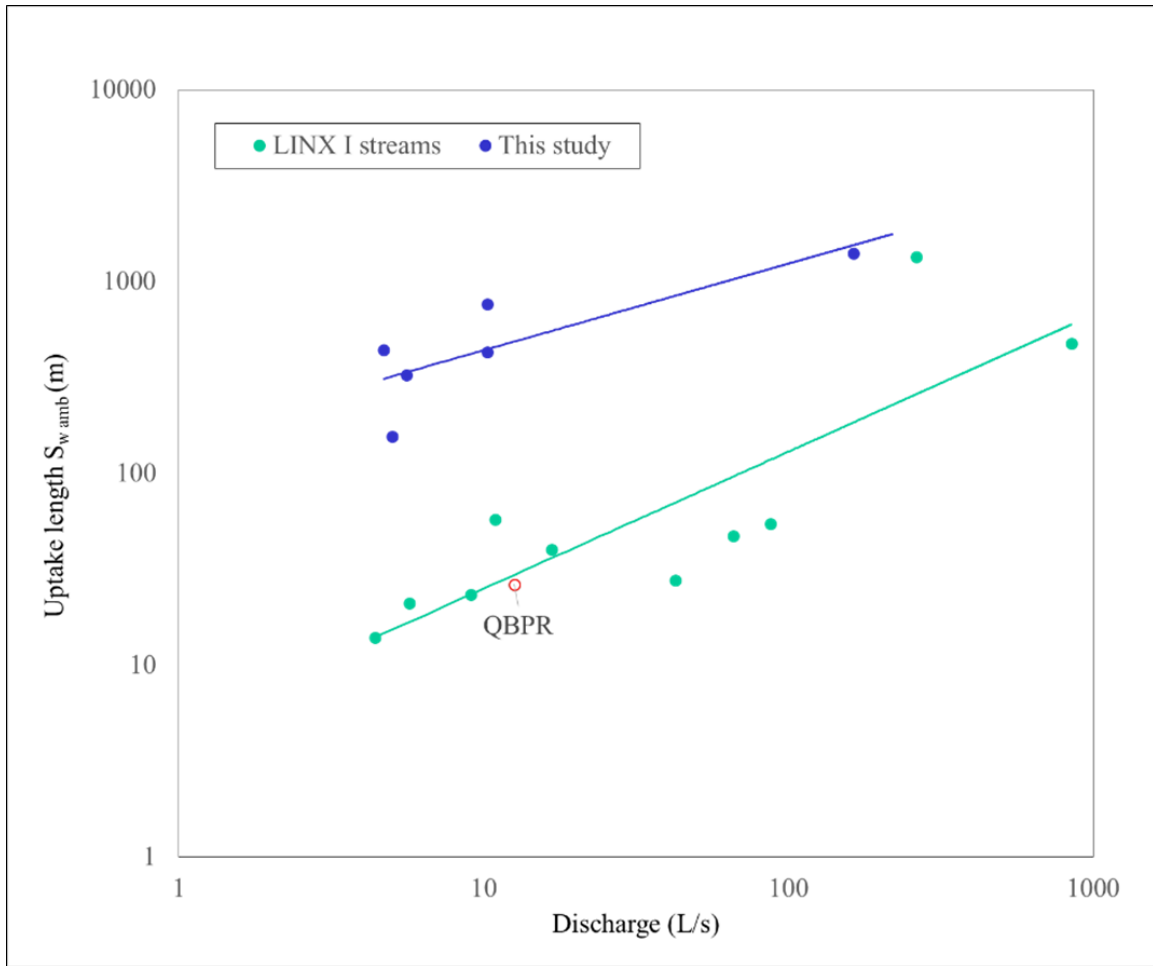
Figure 4. Log-log plot of ambient S_w as a function of stream discharge. Blue points and regression ($r^2 = 0.66$, p = 0.049) represent the experiments in this study. Turquoise points and regression ($r^2 = 0.71$, p = 0.001) represent LINX I study streams (Webster et al. 2003). QBPR is a Luquillo headwater stream with differing riparian geomorphology and N chemistry (low NH_4^+ concentrations).

In contrast to the $v_{f\ amb}$ results, U_{amb} in my study streams was relatively rapid – as high as 72 µg m^{-2} s^{-1}, compared with a range of 0.29-3.8 reported by Webster et al. (2003). The high background NH_4^+ concentrations in these study streams relative to those studied in Webster et al. (2203, particularly in I-0 (Table 2), account for the rapid U_{amb} values we found. Riparian zones in the Icacos watershed tend to be low-gradient, slow transport time, anoxic environments that

supply NH_4^+ to streams, as opposed to the stream studied in Merriam et al. (2002), which has little detectable NH_4^+ in near-stream wells.

Hydraulic variables were the only significant predictor variables for any uptake metric in this study. This finding agrees with Peterson et al. (2001) and Webster et al. (2003), who showed that S_w correlated strongly with Q. Peterson et al. (2001) conceptualize Sw as varying primarily with physical characteristics like depth and velocity, which correlate with discharge; thus, smaller, shallower streams at low flow with greater surface area to volume ratios will have shorter uptake lengths. They attribute the residual variation unexplained by transport processes to uptake processes.

Hyporheic exchange facilitates greater residence time within a given reach and allows oxygenated stream water to contact biologically active sediments that may be anoxic, leading to a wider range of redox processes that can take place (Duff and Triska 2000). Another possible explanation for the low uptake rates we observe in these streams is the relatively low slope of the channels, which may lead to less hydraulic forcing of hyporheic exchange and thus less contact with reduced sediments that could host NH_4^+-oxidizing microorganisms. Hyporheic zones may also be less oxygenated with reduced mixing of stream water. In some streams, debris dams can also facilitate hyporheic exchange and increase residence time, but in the study streams debris dams uncommon due to frequent spates that scour away leaves and coarse woody materials, leaving only scattered trunks of *P. montana* and *C. racemiflora*.

Frequent high flows might also be expected to affect biotic response to a pulse of NH_4^+. For example, scouring might limit periphyton biomass and thereby limit periphyton nutrient uptake, but one study observed no decrease in periphyton biomass after high flows in a stream in

an adjacent watershed (Ortiz-Zayas et al. 2005). Biofilms can be highly organized and stratified if they are given time to develop on durable substrates (Findlay and Sobczak 2000), and in their absence, demand for NH_4^+ may be strongly suppressed.

Although we did not measure light availability in this study, it is likely that photoautotrophic NH_4^+ demand was suppressed by a lack of light. Other studies have demonstrated low light conditions (Merriam et al. 2002, Potter et al. 2010) and highly heterotrophic metabolism (Ortiz-Zayas et al. 2005) in forested Puerto Rican streams. Thus, it is more likely that the high observed U values in this study were due to chemolithotrophic demand. The streams in this study had uniformly low DOC concentrations between 500 and 650 µg C L^{-1}, so C limitation likely played a role in suppressing assimilatory NH_4^+ demand by heterotrophs. Strauss and Lamberti (2000) showed that in sediment incubations, nitrification rates were highest when labile DOC was very scarce, suggesting that nitrifying bacteria were better able to compete with heterotrophs for nutrients in low-DOC conditions. In forested New Hampshire streams, NO_3^- $v_{f\,amb}$ was shown to vary with DOC: NO_3^- ratio, suggesting that higher relative availability of DOC influences NO_3^- demand (Rodriguez-Cardona et al. 2016). In this study, the lack of a relationship between NH_4^+ $v_{f\,amb}$ and DOC:DIN may simply reflect the lack of variance in DOC and NH_4^+ concentrations during these experiments, which were all conducted at baseflow. Higher flows bring increased DOC concentrations in streams of the Luquillo Mountains (McDowell and Asbury 1994, Shanley et al. 2011, Wymore et al. 2017), but uptake during storms is very difficult to assess with nutrient pulse experiments for reasons of logistics and researcher safety.

Phosphorus availability is typically very low in Puerto Rican forest streams (McDowell and Asbury 1994, McDowell et al. 2012) because soils with abundant iron oxide clay minerals

have a high capacity to bind phosphate (Liptzin and Silver 2009). Well-developed tropical forest soils, like those found at this study site, often show evidence of P limitation of primary productivity (Vitousek and Howarth 1991, Menge et al. 2012). This scarcity of P raises the possibility of P limitation of autotrophic as well as dissimilatory NH_4^+ demand; background soluble reactive P concentrations are less than half that in the extensive set of volcaniclastic streams in the Luquillo Mountains investigated by Koenig et al. (2017).

The lack of net NO_3^- production during NH_4^+ additions indicates that nitrification was not a dominant pathway in these study streams, despite what we expected based on the results of Merriam et al. (2002). Substrate type likely accounts for some of the difference between our study streams and the high-uptake and high-nitrification stream studied by Merriam et al. (2002). The substrates of streams in the Icacos basin (this study) are mostly sand, owing to the quartz diorite bedrock, whereas the stream studied by Merriam et al. (2002) was underlain by volcaniclastic bedrock and had cobble and boulder substrates. The high U values appear to persist despite the sandy substrate due to higher background NH_4^+ concentrations than in the volcaniclastic stream studied by Merriam et al. (2002). The possibility can be ruled out that gross NO_3^- production from nitrification occurred coupled with rapid uptake of the resulting NO_3^-. Potter et al. (2010) studied NO_3^- uptake in Puerto Rican streams, including a separate tributary in the Icacos watershed. They showed that NO_3^- uptake was slower in the Icacos tributary than in the stream studied by Merriam et al. (2002), which suggests it is unlikely that NO_3^- uptake was rapid enough to account for NO_3^- produced. In comparing NO_3^- uptake lengths to NH_4^+ uptake lengths, Diemer et al. (2015) and Gibson et al. (2015) both found that NO_3^- uptake lengths were longer, indicating a high likelihood that significant NO_3^- produced by nitrification would have appeared before being taken up in our study streams.

Inferring riparian ammonium uptake from in-stream experiments

We found that stream removal was not enough to account for the vanishing groundwater NH_4^+, and that groundwater NH_4^+ concentrations would range from 16 to 117 µg N L^{-1} greater than the background levels we observed in the streams if only stream removal were taking place (Table 4). We applied the maximum effect of in-stream NH_4^+ removal to the potential groundwater flux, a conservative assumption with respect to the estimate of riparian N retention. Chestnut and McDowell (2000) undertook a similar analysis on another tributary to the Rio Icacos. They characterized stream and riparian well N chemistry and quantified groundwater discharge to a stream reach, in order to estimate the potential groundwater N flux to the stream and infer the retention taking place along the entire flow path from riparian zone to open stream channel. Their findings were similar to the present study, as they found that groundwater flux would lead to far greater NH_4^+ flux in the stream than what is actually observed. The present study goes a step further by quantifying in-stream removal as a fraction of the groundwater N flux that is delivered into the stream bed. We found an even greater disparity between the potential and observed NH_4^+ concentrations than did Chestnut and McDowell (2000), suggesting that uptake of NH_4^+ from the thalweg is of minor importance in regulating stream N flux, and that stream bed processes occurring along groundwater flow paths prior to any interaction with flowing surface water drive stream NH_4^+ concentrations.

It should be noted that the ambient stream NH_4^+ uptake metrics and riparian NH_4^+ retention considered here hold only for base flow conditions. Following storm events that cause rapid increases in Q, uptake lengths are expected to increase as transport processes start to dominate over uptake processes. Likewise, quick flow through soil macropores, along with overland flow at higher rates of precipitation, are expected to overwhelm deeper groundwater flowpaths that are associated with riparian processing (Shanley et al. 2011). Concentration-

discharge relationships allow an examination of whole-system behavior in response to changing stream flow. If storm event flushing pushed NH_4^+-rich groundwater into streams bypassing riparian retention during high flows, we would expect NH_4^+ concentrations to increase with discharge. Instead, in the study streams, changing Q is a poor predictor of NH_4^+ concentration (Wymore et al. 2017), indicating that biological processes of uptake dominate over transport processes for determining watershed NH_4^+ response.

2.5 CONCLUSIONS

The N paradox in tropical forests results from an imbalance between N transport and N uptake at the watershed scale. Here we have shown that headwater streams display a similar imbalance. Stream Q is the only significant predictor variable for any NH_4^+ uptake metric, and transport processes become more dominant as Q increases. The finding of insufficient NH_4^+ removal in the stream channel to fit the observed pattern of high ambient groundwater NH_4^+ concentrations and low ambient stream NH_4^+ concentrations suggests that riparian N retention is a dominant component of watershed N retention, without which stream N export from these catchments would be much greater.

2.6 WORKS CITED

Brookshire, E.N.J., L.O. Hedin, J.D. Newbold [et al.]. 2012. Sustained losses of bioavailable nitrogen from montane tropical forests. Nature Geoscience 5: 123-126. doi:10.1038/ngeo1372

Chestnut, T. J., and W.H. McDowell. 2000. C and N dynamics in the riparian and hyporheic zones of a tropical stream, Luquillo Mountains, Puerto Rico. Journal of the North American Benthological Society. 18(2): 53-75. doi:10.2307/1468065.

Covino, T.P., B.L. McGlynn, and R.A. McNamara. 2010. Tracer additions for spiraling curve characterization (TASCC): quantifying stream nutrient uptake kinetics from ambient to

saturation. Limnology and Oceanography: Methods 8: 484-498. doi: 10.4319/lom.2010.8.484

Diemer, L.A., W.H. McDowell, A.S. Wymore [et al.]. 2015. Nutrient uptake along a fire gradient in boreal streams of central Siberia. Freshwater Science, 34(4), 1443-1456.

Duff, J.H., and F.J. Triska. 2000. Nitrogen biogeochemistry and surface-subsurface exchange in streams. In: Jones JB, Mulholland PJ (eds) Streams and Ground Waters. Elsevier, San Diego, CA. 425p.

Ensign, S.H., and M.W. Doyle. 2006. Nutrient spiraling in streams and river networks. Journal of Geophysical Research 111: G04009.

Findlay S., and W.V. Sobczak. (2000) Microbial communities in hyporheic sediments. In: Jones JB, Mulholland PJ (eds) Streams and Ground Waters. Elsevier, San Diego, CA. 425p.

Gibson, C.A., C.M. O'Reilly, A.L. Conine [et al]. 2015. Nutrient uptake dynamics across a gradient of nutrient concentrations and ratios at the landscape scale. Journal of Geophysical Research Biogeosciences 120(2): 326-340.

Hall, R.O., Jr., E.S. Bernhardt, and G.E. Likens. 2002. Relating nutrient uptake with transient storage in forested mountain streams. Limnology and Oceanography 47(1): 255-265.

Hedin, L.O., E.N.J. Brookshire, D.N.L. Menge, and A.R. Barron. 2009. The nitrogen paradox in tropical forest ecosystems. Ann. Rev. Ecol. Evol. Syst. 40:613-635. DOI: 10.1146/annurev.ecolsys.37.091305.110246

JMP® Pro, Version 13. SAS Institute Inc., Cary, NC, 1989-2017.

Koenig, L.E., C. Song, W.M. Wollheim [et al.]. 2017. Nitrification increases nitrogen export from a tropical river network. Freshwater Science 36:699-712.

LeBauer, D.S., and K.K. Treseder. 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. Ecology, 89(2), 371-379. doi:10.1890/06-2057.1

Lewis, Jr., W. M., J.M. Melack, W.H. McDowell [et al.]. 1999. Nitrogen yields from undisturbed watersheds in the Americas. Biogeochemistry. 46(1-3): 149-162.

Liptzin, D, and W.L. Silver. 2009. Effects of carbon additions on iron reduction and phosphorus availability in a humid tropical forest soil. Soil Biology and Biochemistry 41(8):1696-1702.

McClain M.E., J.E. Richey, and T.P. Pimentel. 1994. Groundwater nitrogen dynamics at the terrestrial-lotic interface of a small catchment in the Central Amazon Basin. Biogeochemistry 27:113-127

McDowell, W.H., W.B. Bowden, and C.E. Asbury. 1992. Riparian nitrogen dynamics in two geomorphologically distinct tropical rain forest watersheds: subsurface solute patterns. Biogeochemistry 18: 53-75.

McDowell, W. H., and C.E. Asbury. 1994. Export of carbon, nitrogen, and major ions from three tropical montane watersheds. Limnology and Oceanography. 39(1): 111-125.

McDowell, W.H., F.N. Scatena, R.B. Waide [et al.]. 2012. Geographic and ecological setting. In: Brokaw, N., T.A. Crowl, A.E. Lugo [et al.] (eds.) A Caribbean Forest Tapestry: The Multidimensional Nature of Disturbance and Response. Oxford University Press, New York. 460p.

Menge, D. N. L., L.O. Hedin, and S.W. Pacala, 2012. Nitrogen and phosphorus limitation over long-term ecosystem development in terrestrial ecosystems. PLoS One, 7(8), e42045.

Merriam, J.L., W.H. McDowell, J.L. Tank [et al.]. 2002. Characterizing nitrogen dynamics, retention and transport in a tropical rainforest stream using an in situ ^{15}N addition. Freshwater Biology 47: 143-160.

Mulholland, P.J., A.M. Helton, G.C. Poole [et al.]. 2008. Stream denitrification across biomes and its response to anthropogenic nitrate loading. Nature 452: 202-205.

Murphy, S.F., R.F. Stallard, M.C. Larsen [et al.]. 2012. Physiography, geology, and land cover of four watersheds in eastern Puerto Rico: Chapter A in Water quality and landscape processes of four watersheds in eastern Puerto Rico. US Geological Survey Professional Paper 1789.

Murphy, S.F., and R.F. Stallard. 2012. Hydrology and climate of four watersheds in eastern Puerto Rico: Chapter C in Water quality and landscape processes of four watersheds in eastern Puerto Rico. US Geological Survey Professional Paper 1789.

Newbold, J.D., J.W. Elwood, R.V. O'Neill [et al.]. 1981. Measuring nutrient spiraling in streams. Canadian Journal of Fisheries and Aquatic Sciences 38(7): 860-863.

Ortiz-Zayas, J. R., W. M. Lewis Jr, J. F.Saunders III [et al.]. 2005. Metabolism of a tropical rainforest stream. *J N Am Benthol Soc, 24*(4), 769-783.

Peterjohn, W.T., and D.L. Correll. 1984. Nutrient dynamics in an agricultural watershed: observations on the role of a riparian forest. Ecology 65:1466-1475

Peterson, B.J., W.M. Wollheim, P.J. Mulholland [et al.]. 2001. Control of nitrogen export from watersheds by headwater streams. Science 292:86-90

Potter, J.D., W.H. McDowell, J.L. Merriam [et al.]. 2010. Denitrification and total nitrate uptake in streams of a tropical landscape. Ecological Applications 20:2104-2115

Rodríguez-Cardona, B., A.S. Wymore, and W.H. McDowell. 2016. DOC:NO_3^- ratios and NO_3^- uptake in forested headwater streams. Journal of Geophysical Research: Biogeosciences 121(1): 205-217.

Shanley, J.B., W.H. McDowell, and R.F. Stallard. 2011. Long-term patterns and short-term dynamics of stream solutes and suspended sediment in a rapidly weathering tropical watershed. Water Resour Res 47:W07515

Solórzano, L. 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. Limnology and Oceanography 14(5): 799-801.

Strauss, E.A., and G.A. Lamberti. 2000. Regulation of nitrification in aquatic sediments by organic carbon. Limnology and Oceanography 45: 1854-1859.

Stream Solute Workshop. 1990. Concepts and methods for assessing solute dynamics in stream ecosystems. J N Am Benthol Soc 95:95-119

Thomas Jr., G.B., and R. L. Finney. 1996. Calculus and Analytic Geometry (9th ed.). Addison Wesley, New York. 326p.

Vitousek, P. M., and R. W. Howarth. 1991. Nitrogen limitation on land and in the sea—How can it occur? Biogeochemistry 13:87–115.

Vitousek, P. M., and H. Farrington. 1997. Nutrient limitation and soil development: Experimental test of a biogeochemical theory. Biogeochemistry, 37(1), 63-75.

Webster, J.R., P.J. Mulholland, J.L. Tank [et al.]. 2003. Factors affecting ammonium uptake in streams – an inter-biome perspective. Freshwater Biology 48: 1329-1352. doi: 10.1046/j.1365-2427.2003.01094.x

Wollheim, W.M., C.J. Vörösmarty, B.J. Peterson [et al.]. 2006. Relationship between river size and nutrient removal. Geophysical Research Letters 33:L06410.

Wollheim, W.M., C.J. Vörösmarty, A.F. Bouwman [et al.]. 2008. Global N removal by freshwater aquatic systems using a spatially distributed, within-basin approach. Global Biogeochemical Cycles 22:GB2026.

Wondzell, S.M., and F.J. Swanson. 1996. Seasonal and storm dynamics of the hyporheic zone of a 4th-order mountain stream. I: Hydrologic processes. Journal of the North American Benthological Society, 15(1), 3-19.

Wymore, A.S., R.L. Brereton, D.E. Ibarra [et al.]. 2017. Critical zone structure controls concentration-discharge relationships and solute generation in forested tropical montane watersheds. Water Resources Research 53:6279–6295.

CHAPTER 3: BALANCE OF RIPARIAN ZONE RESIDENCE TIME AND REACTION RATE CONTROLS GROUNDWATER AMMONIUM FLUX IN A SMALL HEADWATER CATCHMENT

3.1 INTRODUCTION

The land-stream connection is a crucial component of elemental fluxes from watersheds. Riparian zones play a disproportionately large role in watershed nitrogen (N) cycling, and better understanding of riparian N cycling can improve our ability to accurately model N loads to aquatic ecosystems. Studies of the linkages between terrestrial and aquatic ecosystems often focus on the ability of riparian zones to retain dissolved N that would otherwise be transported by groundwater flow into headwater streams. Many researchers have demonstrated that riparian zones are biogeochemical hotspots that exert control on the flux of dissolved nitrogen, particularly nitrate carried by groundwater (e.g. Peterjohn and Correll 1984, Hedin et al. 1998, Clement et al. 2003, McClain et al. 2003, Vidon et al. 2010).

Mechanisms for riparian N retention remain curiously uncertain, particularly in N-rich tropical forests and their headwater stream networks. In the classic model of riparian N retention described mechanistically by Hedin et al. (1998), dissolved N as nitrate (NO_3^-) is produced by nitrification of NH_4^+ in oxygenated upland soils. Nitrate is then transported quickly along groundwater flowpaths because its negative charge allows it to bypass cation exchange processes in the soil. When NO_3^- in groundwater encounters the riparian zone, the redox conditions change; dissolved oxygen is depleted and NO_3^- becomes an energetically favorable electron acceptor and denitrification proceeds. Nitrate is reduced, forming a chain of compounds that ends in gaseous

N_2. In this scenario, because NH_4^+ is not as mobile in soil as NO_3^-, hillslopes are less likely to contribute significant fluxes of NH_4^+ to riparian zones through subsurface flowpaths.

Recently, the understanding of riparian zones as denitrification hotspots has expanded to include anaerobic NH_4^+ oxidation (Burgin et al. 2011, Zhu et al. 2013). In anoxic conditions that suppress nitrification, or in the presence of high NH_4^+ inputs, NH_4^+ can dominate the dissolved N load in riparian groundwater (e.g. Blackburn et al. 2017). In the absence of available oxygen, processes that couple NH_4^+ oxidation to reduction of alternative terminal electron acceptors such as NO_3^- (anaerobic ammonium oxidation, or anammox, Kuypers et al. 2003) and Fe(III) (Fe^{3+}-mediated ammonium oxidation or "Feammox," Yang et al. 2012) have been demonstrated in a wide range of anoxic environments, including wetland and upland soils, lake and marine sediments, and wastewater reactors (Burgin et al. 2011, Ding et al. 2014, Melton et al. 2014).

Tropical forests, in particular, are test cases for the importance of anaerobic forms of NH_4^+ oxidation. Watersheds draining tropical forests commonly have N export rates an order of magnitude higher than their counterparts in temperate and boreal zones (Lewis et al. 1999, Brookshire et al. 2012), a difficult-to-explain phenomenon that has been termed the N paradox in tropical forests (Hedin et al. 2009). The mechanisms for this sustained N richness are poorly understood, as is the effectiveness of riparian N retention. Nitrogen, C, and P cycling are coupled with iron oxidation-reduction in many tropical forest soils, aided by abundant rainfall and high soil respiration rates that encourage spatial variability of oxygen availability in soil (Liptzin and Silver 2009, Yang et al. 2012, Hall et al. 2016). Riparian zones of undisturbed tropical forests can have concentrations of NH_4^+ in riparian groundwater immediately adjacent to the stream that are much higher (1 mg N L^{-1}) than other undisturbed environments (McDowell et al. 1992, McClain et al 1994).

When considering riparian N retention, reaction rates are only partly responsible for controlling N flux. The balance of reaction rates and transport rates determines what fraction of N will be retained. This balance can be expressed as a Damköhler number, a dimensionless ratio of the reaction rate and the transport rate that has been used extensively in the engineering literature and is increasingly applied to reactive transport in hydrologic systems (Oldham et al. 2013):

$$\text{Da} = \frac{\tau_T}{\tau_R} \qquad\qquad \text{Eqn. 1}$$

where τ_T is the transport timescale (i.e. residence time, hr) and τ_R is the reaction timescale (hr). Damköhler numbers < 1 indicate that transport processes dominate over reaction processes, while the reverse is true for Da > 1. Ocampo et al. (2006) reviewed NO_3^- retention in riparian zones and demonstrated that 50% removal occurred when $D_a < 1$ and increased to nearly 100 % at $D_a = 2$–20.

Obtaining estimates of the rate of riparian N uptake, and thus the fraction of N retained from transport into the stream, has long posed a challenge to researchers. Complex interactions of hydrology and biogeochemistry control N cycling in these subsurface environments. Measurements of physical parameters in any aquifer are difficult to obtain, highly variable, and subject to high uncertainty (Trudell et al. 1986, Schroth and Istok 2006). Recently, methods that introduce solute tracers to the subsurface have increasingly been used to study in-situ degradation of pollutants or other biogeochemical processes including denitrification (e.g. Addy et al. 2002). Such methods allow a direct measurement of reaction rates without being subject to measurements of aquifer properties.

In this study, we examined rates and mechanisms of NH_4^+ retention in riparian zones of the Luquillo Mountains of northeastern Puerto Rico, the site of several previous studies of riparian N dynamics (McDowell et al. 1992, Chestnut and McDowell 2000, McSwiney et al. 2001). Our objectives were to 1) quantify NH_4^+ retention in the riparian zone using direct in-situ measurement techniques, 2) assess the balance between reaction rates and transport rates that determines how a riparian zone will respond to changing NH_4^+ inputs and hydrologic conditions, and 3) determine which mechanisms are responsible for riparian NH_4^+ retention in a tropical forest. I hypothesized that riparian zones would rapidly remove added NH_4^+ from solution based on observed high ambient NH_4^+ conditions in riparian groundwater and low ambient concentrations in stream water. I further expected that nitrification would be oxygen-limited in riparian groundwater. To test the possibility of Fe(III) as an alternate electron acceptor, I evaluated whether Fe(III) in soils would decline at depth as the environment becomes more reducing.

3.2 METHODS

Study Site
The Rio Icacos basin is situated in the central Luquillo Mountains at 616-800 MASL spanning the palo colorado forest type, with palo colorado (*Cyrilla racemiflora*) dominant on hillslopes and ridges and sierra palm (*Prestoea montana*) in floodplains and riparian zones. The watershed is 100% forested with heavy shade cover above the stream channels of the Icacos and its tributaries. Mean annual rainfall is 4050 mm (Murphy and Stallard 2012) and temperature is 21˚C (Shanley et al. 2011). Soils are very deep clays, clay loams, and sandy clay loams of the Picacho and Utuado series, classified as Inceptisols (Murphy et al. 2012).

Riparian hydrology and biogeochemistry were monitored in shallow (1.4-2.4 m depth) wells placed in the riparian zone and adjacent hillslopes of a 12.4 ha tributary to the Rio Icacos. The well field was installed beginning with 10 wells in 1988 and gradually expanded to 28 wells by 2011, on either side of the tributary. The well casings were 5.1 cm diameter PVC with 61 cm of slotted screen with 0.25 mm slots, placed in hand-augered holes 150 to 350 cm below the soil surface. Wells were positioned across the catena from the hillslope to 1 m from the stream channel. Water tables for all riparian wells persistently ranged from 0.4 to 1.4 m depth, meaning that the wells for this study never dried out, though nearby wells positioned on the hillslope did stop producing water during dry conditions in the watershed.

Background physical and chemical parameters of study wells

Hydraulic gradient $\frac{\delta H}{\delta L}$ was calculated from H, the measured difference in hydraulic head between the well and the stream, and L, the distance from the well to the edge of the stream along groundwater flow paths. Saturated hydraulic conductivity K_{sat} was taken as the average of measured K_{sat} at the study site from McDowell et al. (1992), 0.003 m hr^{-1}. For background chemical characterization, wells were bailed with a Teflon bailer and groundwater samples were collected upon refill (within 24 hours). Stream water samples were filtered to 0.7 µm (Whatman GF/F) in the field, while groundwater samples were refrigerated and fines allowed to settle before filtration in the laboratory. Filtered samples were frozen and shipped to the University of New Hampshire Water Quality Analysis Laboratory. Samples were analyzed for NH_4^+ concentration using the phenol hypochlorite method (Solórzano 1969) automated with a Westco Smartchem 200 discrete autoanalyzer. Concentrations of Cl^- and NO_3^- were quantified on a Dionex ICS-1000 ion chromatograph. Samples were also analyzed for dissolved organic carbon

(DOC) and total dissolved nitrogen (TDN) using the high temperature catalytic oxidation method

with a Shimadzu TOC-V CPH and TNM nitrogen unit. Dissolved organic nitrogen (DON) was

calculated by subtracting total dissolved inorganic nitrogen ($NO_3^- + NH_4^+$) from measured TDN

concentrations. For streams where the ambient NH_4^+ concentration was lower than the laboratory

method detection limit of 5 µg N L^{-1}, we assumed that ambient NH_4^+ concentration was equal to
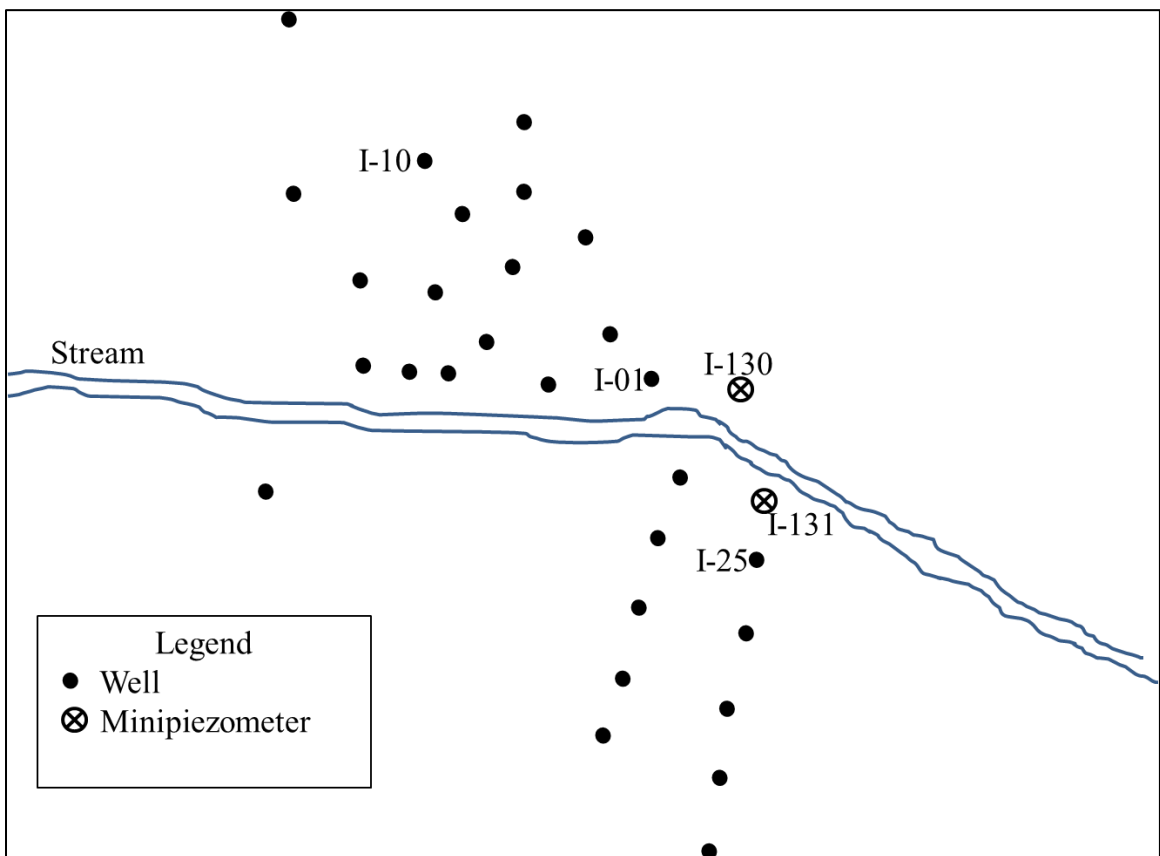
half the detection limit (2.5 µg N L^{-1}).

Figure 1. Map of the well field showing the stream (I-0, a tributary to Río Icacos) and the locations of the wells and minipiezometers used in this study (as well as other wells not used).

Push-pull tests and determination of ammonium retention rates

Push-pull tests (PPTs) are a family of methods used to determine groundwater reaction and transport rates and allow researchers to experimentally determine in-situ hydrological and biogeochemical processes in aquifers (Addy et al. 2002). Briefly, groundwater is extracted from piezometers or wells and amended with reactive solute and conservative tracer solute, and gently pumped back into the aquifer during the "push" phase. The resulting plume of amended water is allowed to incubate for a period of several hours to days, and samples are extracted during the "pull" phase.

Five PPTs were conducted from March 2012-July 2013 using minipiezometers at 75-100 cm depth below the soil surface. Runoff conditions varied from low to high flow across the dates, but this variation is assumed to be of little consequence for perennial groundwater 0.4-1.4 m below the surface. Well positions were all in the riparian zone 0.4-8.5 m from the stream. These five separate minipiezometers were used to extract water, prepare injectate, and carry out the "push" and "pull" phases. To prepare the injectate, ten liters of groundwater were withdrawn from each target well using a peristaltic pump or Teflon bailer. Groundwater was amended with reactive dissolved NH_4^+ (as NH_4Cl) and additional Cl^- (as $NaCl$) as a conservative tracer, to approximately 30 times background concentration of the well. The solution was also bubbled with CO_2 (g) for approximately 20 min to return the solution to background DO concentration in case of any incidental DO introduction. The solution was then pushed into the well with a peristaltic pump at a moderate rate (10 L hr^{-1}) to minimize change in hydraulic head. The solution was allowed to incubate in the aquifer for a variable range of time, from 5-180 hr. During the pull phase, an equal rate of pumping was used to extract the remaining injectate, and samples were collected at regular intervals of volume extracted. Breakthrough curves were sampled 4-8 times.

Calculation of ammonium retention rates

Under the assumption that the aquifer behaves as a well-mixed reactor, the value of k can be modeled as an exponential decay rate beginning at the end of the "push" phase (Haggerty et al. 1998). The first order reaction for NH_4^+ retention (i.e. the disappearance of NH_4^+ from solution over the course of the PPT) is described as:

$$C_r^*(t) = C_{tr}^*(t)e^{-kt}$$

Eqn. 1

where $C_r^*(t)$ is the concentration of the reactive added solute NH_4^+ at time t divided by the injectate concentration; $C_{tr}^*(t)$ is the concentration of the tracer at time t divided by the injectate concentration, and k is the exponential decay rate. With the assumption that the reaction begins as soon as the first parcel of injected solution enters the aquifer, Eqn. 1 can be rewritten as the ratio of NH_4^{+*} to Cl^{-*}, the reactive solute and tracer for these experiments:

$$\ln\left(\frac{NH_4^{+*}}{Cl^{-*}}\right) = \ln\left[\frac{1-exp(-kT_{inj})}{kT_{inj}}\right] - kt^*$$

Eqn. 2

where NH_4^{+*} and Cl^{-*} are concentrations (mg L^{-1}) equal to $C_r^*(t)$ and $C_{tr}^*(t)$, respectively, from Eqn. 1, and T_{inj} is the incubation time (hr) from the end of the injection phase to the time of the beginning of the pull phase. Eqn. 2 yields a line with slope $-k$ and an intercept $\ln[(1-e^{-kT_{inj}})/kT_{inj}]$. The above equation was fit to PPT breakthrough curves with a nonlinear least squares routine using the Microsoft Excel Solver tool to solve for k in Microsoft Excel 2013. This approach assumes that the injectate enters the subsurface where it behaves as a well-mixed reactor, following Schroth and Istok (2006).

Rate constants were used to calculate ambient reaction rates for riparian groundwater based on most recent grab samples. Ammonium removal potential was calculated by scaling to

the linear distance along the groundwater flowpath from the well to the stream, obtained from field surveys.

Calculation of Damköhler Numbers

The Damköhler number is a dimensionless ratio of the rate of a reaction over the rate of fluid transport, allowing a direct comparison of the importance of reaction vs. transport processes. It is calculated as

$$Da = \frac{reaction\ rate}{transport\ rate} = \frac{transport\ time}{reaction\ time} = \frac{d\ k}{v}$$

where d is the distance traveled through the riparian zone (here defined by the distance from a well to the wetted perimeter of the stream along the groundwater flowpath), k is the rate constant of NH_4^+ retention, and v is the Darcy velocity (hydraulic conductivity K_{sat} * hydraulic gradient $\frac{\partial H}{\partial L}$). A Damköhler number less than one indicates that transport is more important than reaction for determining how much of the reactive solute is retained, while a value greater than one indicates that reaction dominates transport. For the purpose of illustrating the sources of variability in Damköhler numbers, I estimate k for each individual well and then apply the reaction rate to the transport time scale, making the assumption that riparian processing along the flowpath will remain the same from the well to the stream. A spatially heterogeneous model that passes reactants from one sub-zone to the next would be appropriate for some riparian zones in which sub-zones are functionally different due to k, K_{sat}, or some other environmental variable.

Determination of soil exchangeable iron

To assess the availability of Fe(II) and Fe(III) in the exchangeable pool in soil, I constructed depth profiles for Fe(II) and Fe(III) in riparian soils adjacent to the study wells and minipiezometers and within 2 m of the bankfull width of the stream. Two boreholes were dug to

the depth at which they filled with water, below which we were unable to obtain competent soil samples. In the field, soil samples were immediately immersed in hydrochloric acid in pre-weighed 50 mL PP centrifuge tubes after extraction to prevent Fe(II) from oxidizing with exposure to air. Upon returning to the laboratory, soil samples were centrifuged. Fe(II) concentrations were determined using the ferrozine colorimetric method (Lovley and Phillips, 1986). After reduction by hydroxylamine, total free Fe (Fe(II) + Fe(III)) was determined and Fe(III) was calculated as total free Fe – Fe(II). Fe(II) concentration was corrected for interference from Fe(III)-ferrozine complexes following Viollier et al. (2000).

3.3 RESULTS

Physical and chemical characterization of study wells

Background NH_4^+ concentrations were high in the wells immediately adjacent (0.4-2.1 m) to the stream, while NH_4^+ in the well farthest away (8.5 m) from the stream was an order of magnitude lower (Table 1). The same pattern held for DOC; concentrations were nearly an order of magnitude lower in the well 8.5 m from the stream (I-10) than in the other four wells within 2.1 m of the stream. NO_3^- showed two orders of magnitude of variation and did not correspond to distance from the stream. Well water concentrations of dissolved SiO2 (data not shown) were consistently below 8 mg L^{-1} for all wells, while the stream SiO2 concentration was consistently above 12 mg L^{-1}, indicating that stream water mixing in the near-stream wells was minimal to nonexistent. The hydraulic gradient was steep, ranging from 0.17 to 0.44 m vertical distance for every meter of horizontal distance (Table 1).

Table 1. Background physical and chemical characteristics of the three wells (I-10, I-25, and I-1) and two minipiezometers (I-130 and I-131) used during push-pull tests in this study. DO is the mean dissolved oxygen concentration as measured with a field meter in-situ during the

78

experiment. (Available field meters were submersible and not flow-through cell designs, so no DO data are available for the minipiezometers 1-130 and I-131 [though DO readings on extracted aliquots is problematic due to introduced air bubbles, the readings on these aliquots were consistent with the in-situ DO values from the wells, between 1 and 3 mg L^{-1}].)

Study well	Experiment date	Distance from stream (m)	Background concentration (mg L^{-1})					$\dfrac{\delta H}{\delta L}$ (m/m)
			DO	NH_4^+-N	NO_3^--N	DOC-C	Cl^-	
I-10	March 12, 2012	8.5	1.65	0.08	0.02	0.58	7.3	0.44
I-25	March 9, 2012	2.1	3.27	0.79	0.07	3.1	6.0	0.38
I-1	March 11, 2012	1.8	1.88	1.22	0.01	1.97	7.7	0.22
I-130	July 12, 2013	0.6	nd	0.60	0.18	3.7	6.5	0.17
I-131	July 14, 2013	0.4	nd	0.44	0.00	2.5	6.4	0.20

During the five PPTs, breakthrough curves progressed rapidly, yielding decreases in concentrations of Cl^- and NH_4^+ within the first 3 h (Fig. 2). Ammonium concentrations decreased with each successive grab sample, continuing to decline even 50-75 hr after the end of the injection phase when testing ceased (Fig. 2). Across the five experiments, rates of decline did not appear to slow even as concentrations approached ambient levels, allowing for a good modeling fit using first-order reaction kinetics. The five values of the rate constant k ranged from 0.13 to 0.68 hr^{-1}, with a mean of 0.30 (Table 2).
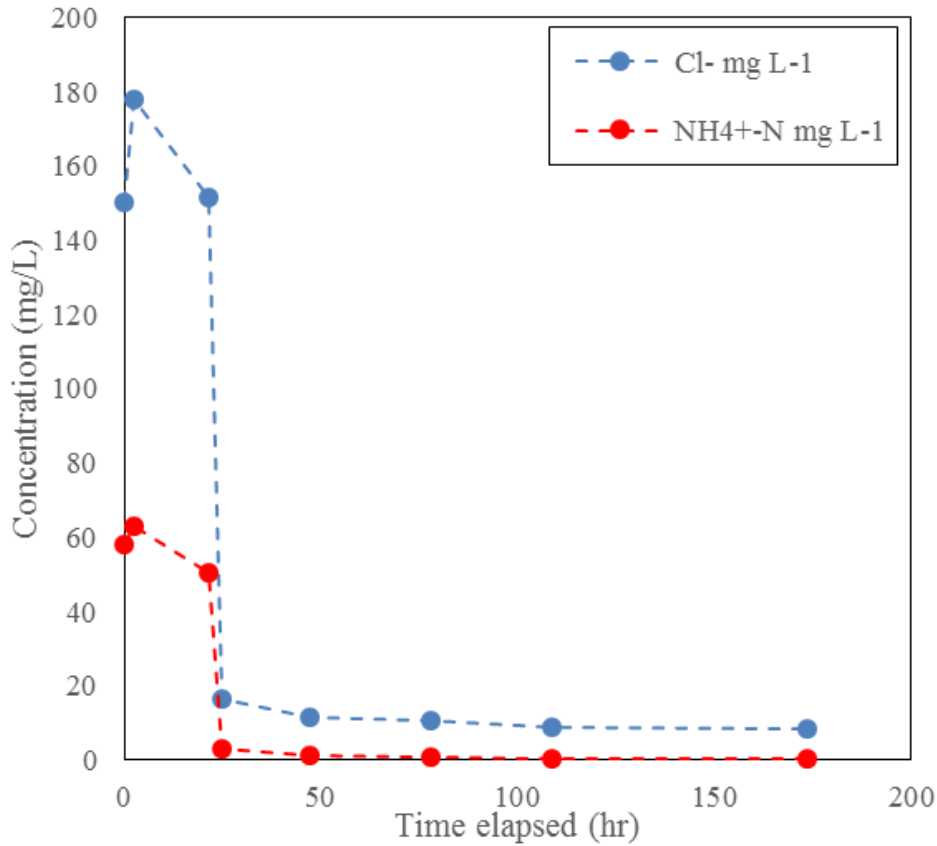
Figure 2. Breakthrough curve for a single push-pull test in a riparian well (I-1). Points represent background-corrected concentrations of Cl^- (blue) and NH_4^+ (red) vs. time during push-pull test. NO_3^- is not shown but did not show marked increases above background concentrations in any PPT.

The breakthrough curves typically showed a low rate of change in concentration for the first hours of the pull phase followed by a rapid decline over a short time period (Fig. 2). This rapid rate of change in absolute concentration was frequently accompanied by a simultaneous rapid decline in the $NH_4^+*:Cl^-*$ ratio (i.e., rapid NH_4^+ removal) (Fig. 3). Concentrations of NO3- were monitored in grab samples and did not increase markedly above background concentrations reported in Table 1 in any of the five PPTs.
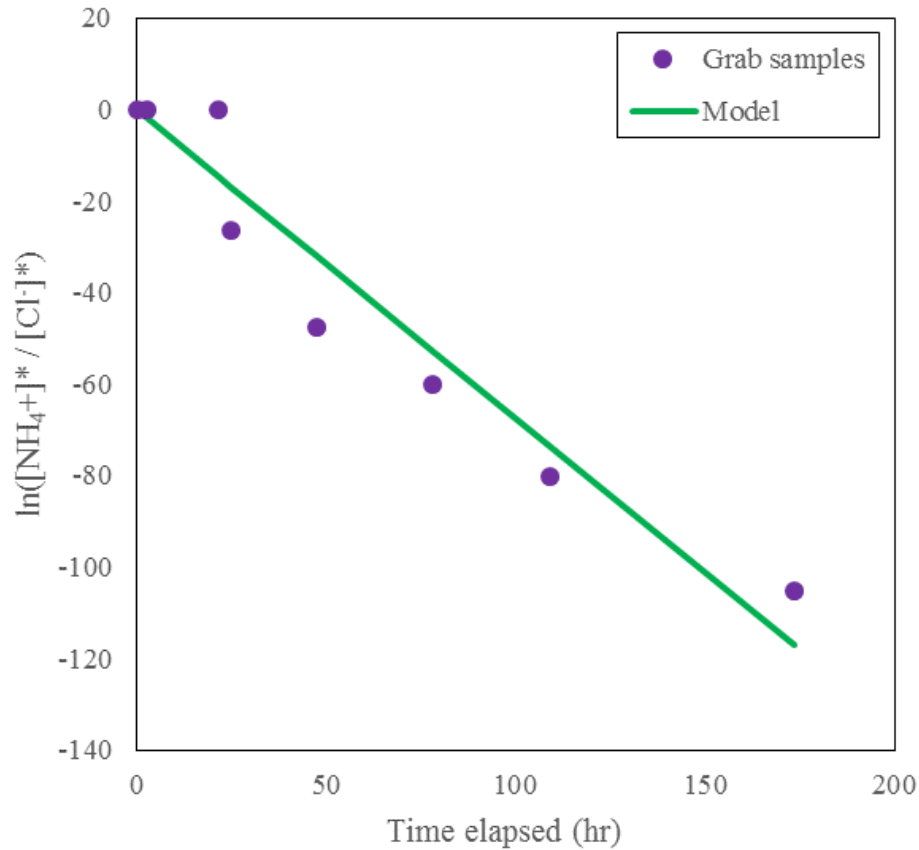
Figure. 3. Log-transformed $NH_4^+*/Cl-*$ ration vs. time for a single push-pull experiment in a riparian well (I-1). Exponential decay equation (green line) fit to experimental data (purple points) with a nonlinear least squares routine, following assumptions of a well-mixed reactor (Haggerty et al. 1998, Schroth and Istok 2006). Parameterization of rate constant k for NH_4^+ uptake obtained from line of best fit.

Results of Damköhler number calculations

The Damköhler numbers calculated for the five PPTs ranged from 72 to 11620

with a mean of 2584. (Table 2). Reaction rate constants for NH_4^+ ranged from 0.13 to

0.68 hr^{-1}. Residence times in this study ranged from 27 to 512 d (Table 2).

Table 2. Input variables required to calculate reaction time, transport time, and Damköhler numbers to describe riparian ammonium uptake in the Icacos tributary well field. Five PPTs are described; results are averaged to reflect overall

81

conditions in the riparian zone. Background NH_4^+ is the concentration of grab sample(s) taken immediately before PPTs. K (hr^{-1}) is the rate constant for ammonium uptake determined by the exponential decay fit. v is the Darcy velocity as the product of Ksat and dH/dL. τ transport is the transport time as L/v where L is the horizontal distance along the groundwater flowpath from the well to the edge of the stream. D_a is the Dahmköhler number.

Study well	Experiment date	k (hr^{-1})	v ($mm\ hr^{-1}$)	$\tau_{transport}$ (hr)	L (m)	D_a
I-1	March 12, 2012	0.68	1.6	1308	8.5	759
I-25	March 9, 2012	0.17	1.4	1781	2.1	260
I-10	March 11, 2012	0.33	0.2	12282	1.8	11620
I-130	July 12, 2013	0.21	0.6	1163	0.6	210
I-131	July 14, 2013	0.13	0.7	646	0.4	72

Availability of Fe(III) as an alternate electron acceptor in riparian soils

Iron in both redox states was present in the soil exchangeable pool from surface soils down to the water table. In both soil cores, Fe(II) concentrations generally increased with depth, while Fe(III) concentrations generally decreased (Fig. 4). Surface soils (0-10 cm depth) contained the lowest concentrations of Fe(II) of any depth (>0.1 mg Fe(II)/g dry soil), while lower B horizon soils (80-100 cm and 120-150 cm depth) contained higher and more variable concentrations (5-15 mg Fe(II)/g dry soil). Conversely, Fe(III) was most abundant in surface soils (mean 5 mg Fe(III)/g dry soil) and upper B horizon soils (40-60 cm, mean 4.8 mg Fe(III)/g dry soil). Mean Fe(III) concentrations were much lower and more variable at 80-100 cm depth (range of 0.2-6.5 mg Fe(III)/g dry soil, mean of 3.5 mg Fe(III)/g dry soil) (Fig. 4).
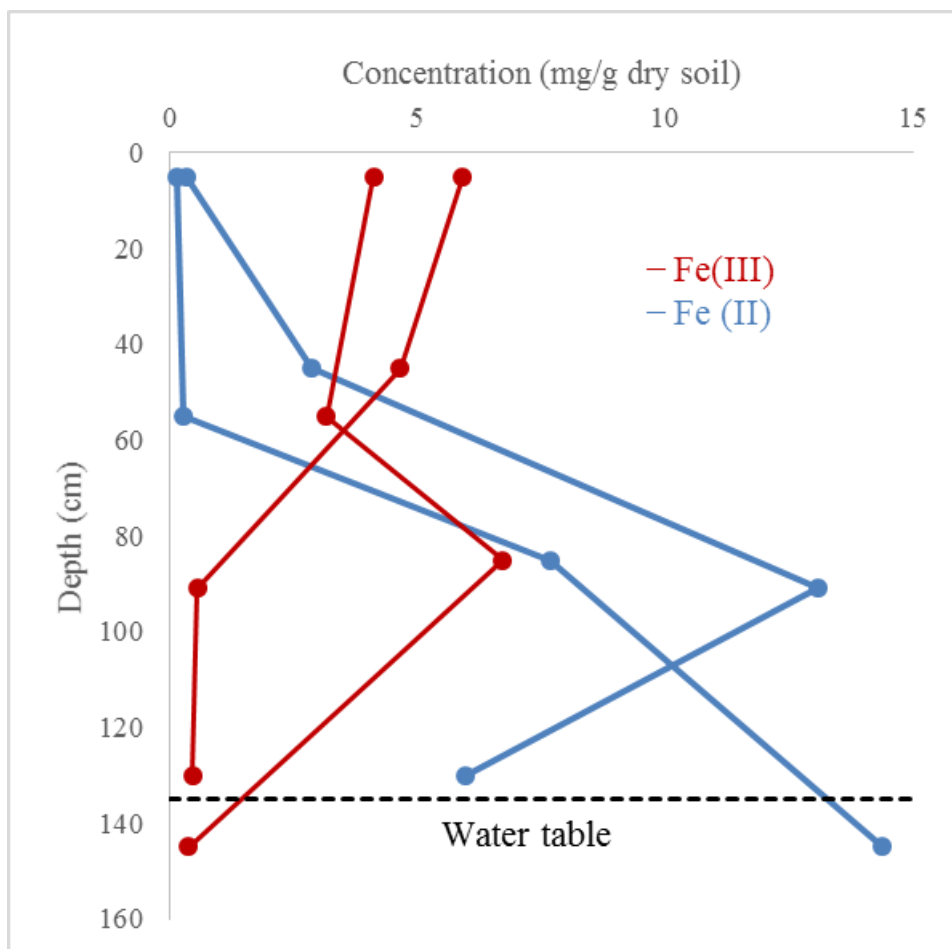
Figure 4. Soil depth profiles of Fe(II) (blue) and Fe(III) (red) concentrations for two soil cores taken in riparian zone. Water table at time of sampling indicated by black dashed line.

There was extensive gleying of clay-rich soils below 30cm soil depth, indicative of strong reducing conditions. When the water table was reached in both cores, a sulfide odor confirmed the presence of reducing conditions. While exchangeable Fe(III) concentrations declined precipitously in the reduced soils at depth, concentrations were still not equal to zero, averaging 0.45 mg Fe mg dry soil^{-1}.

3.4 DISCUSSION

Factors affecting riparian ammonium uptake

        This study is the first that I am aware of at this time to quantify NH_4^+ uptake in riparian

zones using in-situ experimental data. Further, though the Dahmköhler framework has been

applied to riparian NO3 uptake and transport, this study is the first to Damköhler numbers to

riparian NH_4^+ uptake and transport. For points of comparison, we must turn to estimates of

removal of other dissolved N forms from riparian groundwater. A study by Ocampo et al. (2006)

collected observational data and used numerical modeling to estimate NO_3^- removal rates in an

agriculturally influenced watershed. They also did a meta-analysis in which they calculated

Damköhler numbers for other published studies. Damköhler numbers for NO_3^- uptake in those

studies ranged from 0.21 to 8.33, making the values reported in my study far larger. To break the

comparison down into the constituent variables that go into the Damköhler numbers, the reaction

rate constants in this study (Table 2) were 1 to 4 orders of magnitude higher than the studies of

NO_3^- reviewed by Ocampo et al. (2006). The range of residence times in Ocampo et al. (2006)

was 0.5 to 105 d with a mean of 17 d. Thus, the riparian residence time in this study is relatively

long due to the fact that hydraulic conductivity is very low (as is typical in sandy clay soils). The

k values for riparian NH_4^+ retention found herein remain to be compared with future studies.

        It was not surprising to find that rate constants in this study exceeded those reviewed by

Ocampo et al. (2006). Ammonium is often preferentially taken up in a diverse array of

environments because it is a reduced form of N that does not require production of a reductase

enzyme, making it more energetically favorable (Findlay and Sobczak 2000). The fact that our

Damköhler numbers exceed those found in Ocampo et al. (2006) by many orders of magnitude

can be explained by the short reaction times and especially the long transport times in the

riparian zones studied. Schroth and Istok (2006) note that the well-mixed reactor assumption that is essential to calculating first-order reaction constants from push-pull data tends to overestimate values of k in computer simulations, but only slightly. Groundwater velocity is slow wherever K_{sat} or $\frac{\delta H}{\delta L}$ are very small, and in steep catchments with clay-rich soils such as those in the study site, a very small K_{sat} dwarfs the influence of a high $\frac{\delta H}{\delta L}$ and produces a very slow transport process.

Damköhler numbers are also highly influenced by the distance L over which reactive transport occurs (e.g., the distance from a well to the stream channel), making distance to the stream a scaling factor that changes the residence time within the riparian zone. It is entirely possible to find a wide range of Damköhler numbers for riparian NH_4^+ processing in a small spatial extent, as the residence timescale can shift radically with only a few meters of additional distance to be travelled within the riparian zone. Residence time distribution is a familiar concept in watershed hydrology, widely used in models of reactive transport and contaminant transport (Kirchner et al. 2000, Oldham et al. 2013). There is a different, but related, distribution of Damköhler numbers in watersheds because transport processes are inherently scaled to the distribution of travel times. With respect to riparian N retention, the key determinant is the areal extent of riparian hotspots, which are discrete locations within riparian zones. In order to be truly representative of the spatial heterogeneity of riparian N processes, a reactive transport model should have fine spatial resolution (m or finer) and should pass reactants through each zone in the riparian (with its corresponding Dahmköhler number) like a series of stream reaches. For solutes that readily sorb to and release from soil or sediment exchange sites, the Damköhler number should be considered accordingly – precisely what reaction or group of reactions is

measured by k must be carefully considered when modeling the balance between transport and reaction and making inferences.

**Fate of ammonium taken up in riparian zones**

Ammonium uptake rates as measured by push-pull tests were sufficiently rapid compared to transport times to account for the complete removal of NH_4^+ entering the riparian zone, but the observation of high ambient NH_4^+ concentrations appears to contradict this finding. The riparian zones investigated herein appeared to be very active in removing NH_4^+ from the groundwater flowpath leading to the stream, at the same time that high ambient NH_4^+ concentrations were maintained. Under ambient conditions, ammonium removal mechanisms are either limited by environmental factors (e.g. oxygen limitation of aerobic NH_4^+ oxidation), or rapid NH_4^+ production occurs to offset the measured rates of NH_4^+ removal. It is likely that the key difference between NH_4^+ in this study and NO_3^- in Ocampo et al. (2006) is the possibility of a significant source of NH_4^+ within the riparian zone, rather than only subsurface transport to the riparian zone.

Despite small but notable concentrations of DO in the wells, the lack of net NO_3^- production observed in the PPTs indicates that nitrification was not a dominant process of NH_4^+ removal. Sorption to mineral exchange sites is a potentially important temporary, abiotic NH_4^+ sink. Triska et al. (1994) found NH_4^+ sorption to sediments in riparian groundwater to be highest further from the stream where hyporheic mixing was minimal to nonexistent and nitrification rates were lowest. Their study also found that the total amount of NH_4^+ sorbed was highest during low-flow conditions, suggesting that lack of oxygen was the limiting factor for all perennially-submerged sediments (as are the deep riparian soils in this study). In our study site, sorption and desorption could account for the maintenance of high equilibrium NH_4^+

concentrations despite rapid measured uptake, but it is likely that this sorption-desorption-mediated equilibrium would saturate at some point as soil exchange sites become occupied. A nutrient pulse experiment allows for observation of the system in response to nutrient loads above and beyond the range of ambient conditions, but even at 30-60 times ambient NH_4^+, I cannot rule out abiotic retention/storage as a mechanism for the observed removal on the basis of these results.

The pattern of increasing Fe(II) concentrations at greater depth (Fig. 4) is expected as more reducing, anoxic conditions are encountered deeper in saturated riparian soils. This study did not consider depth profiles of NH_4^+ alongside the depth profiles of Fe(II), but McSwiney et al. (2001) measured NH_4^+ in soil solution with lysimeter nests, finding that NH_4^+ concentrations increased from $>0/1$ mg L^{-1} to 3.5 mg L^{-1} at 150 cm. Recent research in the Luquillo Mountains has indicated the importance of coupled N and Fe cycling (Yang et al. 2012). In this study, the presence of large quantities of Fe and NH_4^+ in groundwater raises the possibility of less well understood N removal pathways such as anammox and Feammox that produce N_2 directly from NH_4^+ without the intermediate step of nitrification.

The balanced reaction for anammox is:

$$NH_4^+ + NO_2 \rightarrow N_2 + 2H_2O \qquad \qquad \text{Eqn. 3}$$
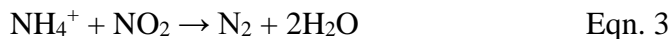
Nitrite is a necessary substrate for this reaction. In anoxic riparian groundwater, NO_2 would likely be the limiting reagent to anammox-based removal of NH_4^+ because oxidized forms of nitrogen are scarce. The more likely reaction is Feammox (Yang et al. 2012):

$$3Fe(OH)_3 + 10H_+ + NH_4^+ \rightarrow 3\ Fe(II) + 9H_2O + 0.5N_2 \qquad \text{Eqn. 4}$$
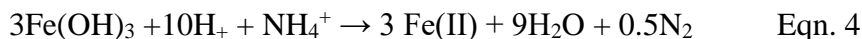
This NH_4^+ oxidation reaction uses ferrihydrite as the terminal electron acceptor instead of NO_2, which yields -245 kJ/mol reaction, making it energetically favorable for microbes. Ferrihydrite can exist as a poorly crystalline solid or as dissolved $Fe(III)$ and $3OH^-$ bound on soil cation exchange sites (Liptzin and Silver 2009 Appendix A contains example calculations using the NH_4^+ and pH data collected in this study, along with a range of realistic $Fe(II)$ concentrations. These calculations show that this reaction is thermodynamically favorable at field conditions in the Icacos aquifer, providing evidence that microorganisms can gain energy by consuming NH_4^+ and ferrihydrite, and releasing $Fe(II)$ and N_2.

Figure 4. Accumulation of iron flocs in a backwater alongside the Rio Icacos. These flocs develop during intervals of low flow when they can accumulate without being washed downstream.

In a study of tropical upland soils, Hall et al. (2016) found that $Fe(II)$ concentrations declined with depth, which the authors attributed to a lack of biologically-driven Fe cycling at greater depths. This study was conducted in riparian soils, which typically display a stronger

redox zonation than upland soils, analogous to aquatic sediments. Despite the strong reducing conditions in these riparian soils, the amount of Fe(III) present was enough to suggest that it could be an important electron acceptor. In a sandy clay with bulk density 1.45 g cm^{-1} and porosity 0.30, 1 L of water will occupy 4.83 kg of soil, which contains 1.9-2.4 mg exchangeable Fe(III), enough to react with 0.63-0.8 mg NH_4^+-N at a 3:1 molar ratio. Thus, there is enough Fe(III) in the exchangeable pool alone, without considering mineral phases, to have a quantitatively important effect by reacting with NH_4^+). The presence of flocculated Fe in surface water indicates that the Icacos watershed supplies abundant dissolved Fe(II) to stream water, routed through the subsurface. Fe(III) would have to be continuously produced to keep up with NH_4^+ oxidation via Feammox, so a more thorough evaluation of this pathway would estimate Fe(II) oxidation rates via possible pathways (i.e. abiotic vs. biotic, aerobic vs. anaerobic, N-mediated vs. organic carbon-mediated [see Melton et al. 2014 and references therein]).

3.5 CONCLUSIONS

Riparian NH_4^+ retention in this tropical forested headwater stream is rapid and can account for complete removal of ambient concentrations of NH_4^+, but evidence for complete removal is not seen. Transport processes are slow due to the clay-rich soil. The comparison of transport to reaction processes demonstrates a range of Damköhler numbers that reflect the physical reality of many different flowpaths and residence timescales within the riparian zone. The high availability of Fe(III), an energetically favorable terminal electron acceptor for dissimilatory NH_4^+ oxidation, shows that Fe(III)-rich soils can potentially be large NH_4^+ sinks and warrants further investigation.

Hedin et al. (2009) proposed a hypothesis to resolve the N paradox in tropical forests, the "leaky nitrostat" model, wherein N availability is strongly spatially segregated into zones of N limitation (canopy, upper soil) and zones of N richness (lower soil). Nitrogen limitation can thus continue to drive N fixation, while N richness in the lower soil perpetuates high hydrologic N flux. Riparian zones in this study appear to both "plug the leak" in the nitrostat in the sense that they retain N lost from the lower soil, but to also allow the leak to continue via high ambient NH_4^+ concentrations immediately adjacent to the stream. The balance depends on their areal extent, the residence time of groundwater, their reactivity, and the ultimate fate of the retained N, which remains for future studies to fully ascertain.

3.6 WORKS CITED

Addy K, Kellogg DQ, Gold AJ, Groffman PM, Ferendo G, Sawyer C (2002) In-situ push-pull method to determine ground water denitrification in riparian zones. J. Env. Qual. doi:31:1017-1024

Blackburn M, Ledesma JLJ, Näsholm T, Laudon H, Sponseller RA (2017) Evaluating hillslope and riparian contributions to dissolved nitrogen (N) export from a boreal forest catchment. Journal of Geophysical Research: Biogeosciences 122 324-39

Brookshire ENJ, Hedin LO, Newbold JD, Sigman DM, Jackson SK (2012) Sustained losses of bioavailable nitrogen from montane tropical forests. Nature Geoscience 5: 123-126. doi:10.1038/ngeo1372

Burgin AJ, Yang WH, Hamilton SK, Silver WL (2011) Beyond carbon and nitrogen: how the microbial energy economy couples elemental cycles in diverse ecosystems. Frontiers in Ecology and the Environment 9:44-52

Chestnut TJ, McDowell WH (2000) C and N dynamics in the riparian and hyporheic zones of a tropical stream, Luquillo Mountains, Puerto Rico. J. N. Am. Benthol. Soc. 19:199-214

Clément J, Holmes RM, Peterson BJ, Pinay G (2003) Isotopic investigation of denitrification in a riparian ecosystem in western France. J Appl Ecol 40 1035:1035-1048

Ding L, An X, Li S, Zhang G, Zhu Y (2014) Nitrogen Loss through Anaerobic Ammonium
Oxidation Coupled to Iron Reduction from Paddy Soils in a Chronosequence. Environ
Sci Technol 48 10641-7

Findlay S, Sobczak WV (2000) Microbial communities in hyporheic sediments. In: Jones JB,
Mulholland PJ (eds) Streams and Ground Waters. Elsevier, San Diego, CA. 425p.

Hall SJ, Liptzin D, Buss HL, DeAngelis K, Silver WL (2016) Drivers and patterns of iron redox
cycling from surface to bedrock in a deep tropical forest soil: a new conceptual model.
Biogeochemistry 130:177-90

Hedin LO, von Fischer JC, Ostrom NE, Kennedy BP, Brown MG, Robertson GP (1998)
Thermodynamic constraints on nitrogen transformations and other biogeochemical
processes at soil-stream interfaces. Ecology 79:684-703

Kirchner JW, Feng X, Neal C (2000) Fractal stream chemistry and its implications for
contaminant transport in catchments. Nature 403:524-7

Kuypers MMM, Sliekers AO, Lavik G, Schmid M, Jorgensen BB, Kuenen JG, Sinninghe
Damste JS, Strous M, Jetten MSM (2003) Anaerobic ammonium oxidation by anammox
bacteria in the Black Sea. Nature 422:608-11

Liptzin D, Silver WL (2009). Effects of carbon additions on iron reduction and phosphorus
availability in a humid tropical forest soil. Soil Biology and Biochemistry 41(8):1696-
1702.

Lovley D, Phillips E (1986) Organic matter mineralization with reduction of ferric iron in
anaerobic sediments. Applied and Environmental Microbiology 51 683–689.

McClain ME, Richey JE, Pimentel TP (1994) Groundwater nitrogen dynamics at the terrestrial-
lotic interface of a small catchment in the Central Amazon Basin. Biogeochemistry
27:113-127

McClain ME, Boyer EW, Dent CL, Gergel SE, Grimm NB, Groffman PM, Hart SC, Harvey JW,
Johnston CA, Mayorga E, McDowell WH, Pinay G (2003) Biogeochemical hot spots
and hot moments at the interface of terrestrial and aquatic ecosystems. Ecosystems
6:301–312

McDowell WH, Bowden WB, Asbury CE (1992) Riparian nitrogen dynamics in two
geomorphologically distinct tropical rain forest watersheds: subsurface solute patterns.
Biogeochemistry 18: 53-75

McSwiney CP, McDowell WH, Keller M (2001) Distribution of nitrous oxide and regulators of
its production across a tropical rainforest catena in the Luquillo Experimental Forest,
Puerto Rico. Biogeochemistry 56: 265-286.

Melton ED, Swanner ED, Behrens S, Schmidt C, Kappler A (2014) The interplay of microbially mediated and abiotic reactions in the biogeochemical Fe cycle. Nature Reviews Microbiology 12 797-808

Murphy SF, Stallard RF (2012) Hydrology and climate of four watersheds in eastern Puerto Rico: Chapter C in Water quality and landscape processes of four watersheds in eastern Puerto Rico. US Geological Survey Professional Paper 1789

Murphy SF, Stallard RF, Larsen MC, Gould WA (2012) Physiography, geology, and land cover of four watersheds in eastern Puerto Rico: Chapter A in Water quality and landscape processes of four watersheds in eastern Puerto Rico. US Geological Survey Professional Paper 1789

Ocampo CJ, Oldham CE, Sivalapan M (2006) Nitrate attenuation in agricultural catchments: shifting balance between transport and reaction. Water Resour. Res. 42 W01408. doi:10.1029/2004WR003773

Oldham CE, Farrow DE, Peiffer S (2013) A generalized Damköhler number for classifying material processing in hydrological systems. Hydrology & Earth System Sciences 17 1133-48

Peterjohn WT, Correll DL (1984) Nutrient dynamics in an agricultural watershed: observations on the role of a riparian forest. Ecology 65:1466-1475

Schroth MH, Istok JD (2006) Methods to determine first-order rate coefficients from single-well push-pull tests. Ground Water 44(2):275-283

Shanley JB, McDowell WH, Stallard RF (2011) Long-term patterns and short-term dynamics of stream solutes and suspended sediment in a rapidly weathering tropical watershed. Water Resour Res 47 W07515

Solórzano L (1969) Determination of ammonia in natural waters by the phenolhypochlorite method. Limnology and Oceanography 14(5):799-801

Triska FJ, Jackman AP, Duff JH, Avanzino RJ (1994) Ammonium Sorption to Channel and Riparian Sediments: A Transient Storage Pool for Dissolved Inorganic Nitrogen. Biogeochemistry 26 67-83

Trudell MR, Gillham RW, Cherry JA (1986) An in-situ study of the occurrence and rate of denitrification in a shallow unconfined sand aquifer. J. Hydrology 83:251-268

Vidon P, Allan C, Burns D, Duval TP, Gurwick N, Inamdar S, Lowrance R, Okay J, Scott D, Sebestyen S (2010) Hot spots and hot moments in riparian zones: Potential for improved water quality management. JAWRA Journal of the American Water Resources Association 46:278-298

Viollier E, Inglett P, Hunter K, Roychoudhury A, van Cappellen P (2000) The ferrozine method revisited: Fe(II)/Fe(III) determination in natural waters. Applied Geochemistry 15:785–790

Yang WH, Weber KA, Silver WL (2012) Nitrogen loss from soil through anaerobic ammonium oxidation coupled to iron reduction. Nature Geosci. 5:538-541

Zhu G, Wang S, Wang W, Wang Y, Zhou L, Jiang B, Op dC, Risgaard-Petersen N, Schwark L, Peng Y, Hefting MM, Jetten MSM, Yin C (2013) Hotspots of anaerobic ammonium oxidation at land-freshwater interfaces. Nature Geosci 6 103-7. doi:10.1038/ngeo1683