University of New Hampshire
University of New Hampshire Scholars' Repository

Master's Theses and Capstones

Student Scholarship

Winter 2007

Denitrification drives total nitrate uptake in small Puerto Rican streams

Jody D. Potter
University of New Hampshire, Durham

Recommended Citation

Potter, Jody D., "Denitrification drives total nitrate uptake in small Puerto Rican streams" (2007). *Master's Theses and Capstones*. 342.
https://scholars.unh.edu/thesis/342

DENITRIFICATION DRIVES TOTAL NITRATE UPTAKE IN SMALL PUERTO RICAN STREAMS

BY

JODY D. POTTER
B.S., University of New Hampshire, 2001

THESIS

Submitted to the University of New Hampshire
in Partial Fulfillment of
the requirements for the degree of

Master of Science
in
Natural Resources: Water Resources

December, 2007

UMI Number: 1449601

UMI Microform 1449601

ProQuest Information and Learning Company
300 North Zeeb Road
P.O. Box 1346
Ann Arbor, MI 48106-1346

This thesis has been examined and approved.

Thesis Director, William H. McDowell, Professor

Serita D Frey

Serita D. Frey, Associate Professor

Wilfred Wollheim

Wilfred M. Wollheim, Research Scientist III

10/22/07

Date

ACKNOWLEDGEMENTS

I would like to thank my thesis advisor, Bill McDowell, for his support and advice throughout my time as a graduate student and providing me the opportunity to work on such an interesting project. I would like to thank my committee members, Serita Frey and Wil Wollheim, for providing valuable advice on a draft of this thesis. Wil Wollheim also developed the models to estimate uptake rates and denitrification.

The project would not have been possible without assistance in the field, lab, and intellectually by Jeff Merriam, Suzanne Thomas, and Bruce Peterson. This was a collaborative project that required a team effort from all of these individuals. I would also like to thank Michelle Daley, Gretchen Gettel, Amy Burgin, and Daniel von Schiller for much field and intellectual assistance. Additional field assistance was provided by Jorge Ortiz-Zayas, Debora Figueroa-Nieves, Fred Scatena, and Joe Troester. Finally I would like to thank friends and family for their support.

This research was part of the Lotic Intersite Nitrogen eXperiment II (LINX II) funded from the Ecosystems Program, National Science Foundation (DEB-0111410). Additional support was provided by the National Science Foundation to the Terrestrial Ecology Division at the University of Puerto Rico and the Institute of Tropical Forestry (DEB-9411973) through the Luquillo Long Term Ecological Research (LUQ LTER) program.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES PAGE

LIST OF FIGURES

FIGURE TITLE PAGE

ABSTRACT

DENITRIFICATION DRIVES TOTAL NITRATE UPTAKE IN SMALL PUERTO RICAN STREAMS

by

Jody D. Potter
University of New Hampshire, December, 2007

An intensive study that was part of the Lotic Intersite Nitrogen eXperiment II (LINX II) project was conducted to determine nitrogen transformations in nine low-order streams with contrasting land use. Short term (24-hour) additions of $K^{15}NO_3$ and NaBr were performed on a gradient of anthropogenically impacted streams in Puerto Rico. Nitrate uptake was determined from longitudinal decline in $^{15}NO_3$ and denitrification rates were determined from the longitudinal pattern of $^{15}N_2$ and $^{15}N_2O$. Several physical, chemical, and biological variables were also measured to determine controlling factors. I performed these experiments to investigate: 1) the mechanisms for NO_3 uptake and denitrification in tropical streams, 2) how tropical streams differ from temperate streams in their rates and controls on NO_3 uptake and denitrification and 3) the functional responses of these streams as NO_3 concentrations increase due to anthropogenic impacts.

Background nitrate concentrations ranged from 105 to 997 μg N L^{-1} and stream nitrate uptake length varied from 315 to 8480 m (median of 1200 m).

Uptake length was mainly predicted by specific discharge (L s^{-1} m^{-1}) and ecosystem respiration rate (multiple regression analysis; $r^2 = 0.71$, $p < 0.05$). The other nitrate uptake parameters (V_f, cm/s and U, μg N m^{-2} s^{-1}) were primarily predicted by gross primary production and respiration, indicating strong biological control on nitrate uptake.

Denitrification rates ranged from 0.01 to 2.20 μg N m^{-2} s^{-1} (median = 0.25) and the strongest predictors were respiration and fine benthic organic matter ($r^2 = 0.89$, $p < 0.05$). Denitrification accounted for 1 to 97% of nitrate uptake with 5 of 9 streams having 35% or more of nitrate uptake via denitrification showing that denitrification is a substantial sink for nitrate in tropical streams.

In comparison to rates in other regions, nitrate uptake was low and denitrification was high. Whole stream nitrate uptake more closely followed Michealis-Menten kinetics than in other regions, indicating that high N streams are approaching nitrate saturation. The efficiency with which these streams assimilate and remove nitrate (through denitrification) generally declines with increasing nitrate concentrations and loading.

INTRODUCTION

Human activities have approximately doubled the rate of nitrogen (N) input into the terrestrial N cycle through fossil fuel burning, fertilizer production, and cultivation of N-fixing crops with large implications for ecosystem function (Galloway et al. 1995; Vitousek et al. 1997). Until recently, the majority of anthropogenic N inputs were concentrated in the industrialized, temperate regions of the world. This is rapidly changing with economic expansion in the tropics, as nearly 2/3 of Earth's energy-related N inputs will take place there by 2020 (Galloway et al. 1994). In general, tropical forests are not N limited and have high rates of N fixation, so increases in anthropogenic N could lead to decreases in primary productivity (due to acidification and cation leaching) and rapid increases in N flux with little or no lag time (Matson et al. 1999). The reduction in N retention could be exacerbated in the tropical forests of Puerto Rico where most of the N is stored in the soil (McDowell 2001) and there is little opportunity for uptake before it reaches lotic ecosystems.

Background N-export rates in tropical watersheds with minimal disturbance are about five-fold greater than in temperate regions with similar runoff (Downing et al. 1999). This export often has a high percentage of dissolved organic nitrogen (DON; ~ 35%), while dissolved inorganic N also makes up about 35% of that N export (Lewis et al. 1999; McDowell and Asbury 1994). Human activities induce a shift from organic to inorganic forms of exported N, with mobile nitrate (NO_3) dominating outputs (Cole et al. 1993). Nitrate loading from rivers is known to cause coastal eutrophication, so the understanding of ecosystem processing of NO_3 is critical in efforts to mitigate downstream effects. This is especially true in a rapidly urbanizing region such as Puerto Rico where N export in highly urbanized basins is higher than the most impacted zones in Northern Europe (Ortiz-Zayas et al. 2006). Small streams play a significant role in the retention and fate of NO_3. Alexander et al. (2000) demonstrated that the high width to depth ratio that allows more contact with the active stream bottom is important in the delivery of N in large river networks. Peterson et al. (2001) showed the shortest inorganic N uptake lengths occurred in the smallest streams.

Nutrients cycle in streams according to the spiraling concept (Webster & Patten 1979), where nutrients simultaneously cycle and are transported downstream. Nitrogen spiraling in streams is often described in terms of uptake length and can be measured by adding inorganic N (Stream Solute Workshop 1990). The use of stable isotope tracers is an effective way to measure N spiraling without causing the enrichment effects of adding inorganic solutes (Mulholland et al. 2002). The measurement of N spiraling is critical to our understanding of the fate of N in lotic ecosystems and this has received much recent attention. These studies have found both biological (Hall & Tank 2003; Webster et al. 2003; Mulholland et al. 2006) and hydrologic controls (Vallet et al. 1996; Wollheim et al. 2001) on inorganic N uptake. The hydrologic controls are likely indirect, as increases in transient storage and decreases in water velocity and depth primarily allow more contact with the primary producers. Primary producers are especially important for NO_3 uptake, since energy is required for its reduction before cellular use.

Studies on in-stream N dynamics are largely lacking in the tropics, with most of the published work that has been done focused on Costa Rica, Puerto Rico, and Amazonian Brazil. The few studies that have been published suggest that the high inorganic N concentrations typically found in tropical streams result in little or no limitation to primary production (Pringle et al. 1986; Neill et al. 2001). In the case of three heavily forested tropical streams in Costa Rica and Puerto Rico, NH_4 uptake was rapid, nitrification dominated stream N dynamics and there was little or no NO_3 uptake (Triska et al 1993; Duff et al. 1996; Merriam et al. 2002). Neill et al. (2006) found in a comparison of paired forest and pasture streams in the Amazon that forest streams again exhibited no NO_3 uptake, but uptake was measurable in a pasture stream. They attributed this to a shift to N limitation of algal production, an increase of denitrification in hypoxic conditions, or uptake by grasses in the stream channel. This pattern suggests that changes in land use can alter the structure and the function of small streams in the tropics and ultimately the delivery of N downstream.

To protect coastal water quality, the most desirable fate of bioavailable N to downstream ecosystems is denitrification, the microbial process of reducing NO_3 to unavailable gaseous N (N_2 and N_2O). In temperate streams denitrification can be a significant sink of total N inputs (20-35%) with higher rates occurring in systems that receive substantial anthropogenic N (Seitzinger 1988). In a study of Midwestern streams (Inwood et al. 2005), denitrification was primarily predicted by NO_3 concentration, although there was a reduction in the efficiency of denitrification at high NO_3. Other variables (i.e., dissolved organic carbon (DOC), dissolved oxygen, temperature, and sediment organic matter content) have also been found to predict denitrification. Denitrification in the near-stream zone of streams in Puerto Rico has been found to be important (Bowden et al. 1992; McDowell et al. 1992; McDowell et al. 1996; Chestnut and McDowell 2000), but in-stream denitrification has been largely ignored. In a study of a lowland tropical stream draining swamp forest in Costa Rica (Duff et al. 1996), there was significant potential for denitrification, but stream sediments appeared to be a source of NO_3 overall. Yet, there is reason to believe that denitrification rates might be relatively high in tropical compared to temperate streams because of high ambient nitrate concentrations, warm water temperatures, and large organic matter inputs due to high rates of terrestrial primary production. Recent advances in the use of ^{15}N tracers have allowed the quantification of whole stream denitrification rates without the physical and chemical perturbations of the acetylene block method (Mulholland et al. 2004). This is critical in our understanding of the role denitrification plays in the N cycle of aquatic ecosystems and could lead to a strategy for mitigating excessive N loading to coastal waters.

This study attempts to understand the controlling factors on NO_3 uptake and denitrification in streams of varying land use in the tropics. The questions I asked include: 1) what are the mechanisms for NO_3 uptake and denitrification in tropical streams?, 2) how are tropical streams different than their temperate counterparts in their rates and controls on NO_3 uptake and denitrification?, and 3) what are the functional responses of these streams as NO_3 concentrations increase due to anthropogenic impacts? My approach was to conduct field ^{15}N tracer experiments in small streams in Puerto Rico under ambient nitrate concentrations to determine ambient rates of denitrification in tropical streams.

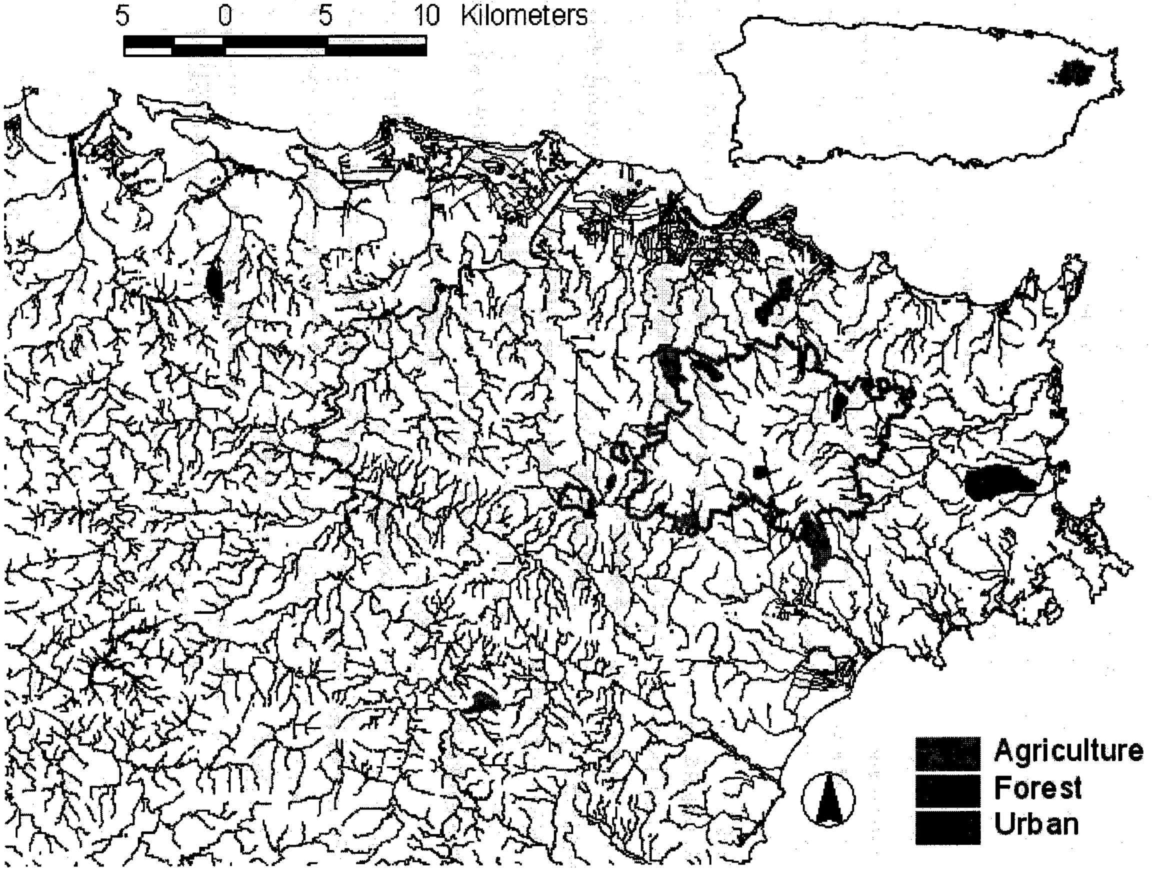
CHAPTER I

METHODS

Site Description

This study was conducted in and around the Luquillo Experimental Forest (LEF), on the Caribbean island of Puerto Rico (Figure 1). The area is characterized by steep slopes in forested areas at higher altitudes and moderate to steep slopes in the lower, human-impacted regions. The elevation of my study watersheds ranged from 10 to 675 m. Annual rainfall ranges from 150 cm in metropolitan San Juan to 250 cm in the lower altitudes of the forest and increases with elevation (Brown *et al.* 1983). The mean annual temperature ranges from 19°C to 26°C. Total agricultural land in Puerto Rico has decreased significantly in the last 50 years, while forested and urban land has increased due to socioeconomic factors (Grau *et al.* 2003). Much of the agricultural land is divided into small parcells and is interspersed with forest fragments. Urban land is developed intensively. Nine streams (1st to 3rd order) were selected in the study area in three different classes of land use: forest/reference, agriculture, and urban.

Figure 1: Study sites in northeastern Puerto Rico. Designated watershed land use classes are based on a stream's immediate surroundings. The pink outline is the Caribbean National a Forest, which is protected from development.



The forested streams were located in the mountainous region of the LEF and represent the reference nutrient conditions. The three streams, Quebrada Bisley, Rio Icacos Tributary (RIT), and Quebrada Pared, are steep, confined and very shaded (Table 1). Percent forested land was not below 99.7% in any of the 3 watersheds. These have higher slope than the other streams.

The agricultural streams were located at lower elevations in the coastal plains. Quebrada Grande is a sandy bottomed stream draining horse and cattle pasture. It has an incised stream channel that is heavily disturbed by frequent rain events. Quebrada Maizales is mostly cobble and boulder, draining banana plantation and horse pasture. Portions of its stream bank have been stabilized with concrete to maintain the stream course. Quebrada Vaca flows through cattle pasture and patchy forest. The streambed consists mostly of gravel and the riparian zone is mostly broad leaf trees. Grande and Maizales have very little vegetation in their riparian zone.

The three urban streams Quebrada Petunia, Rio Mameyes Tributary (MTrib), and Quebrada Ceiba are mostly gravel bottomed. Petunia and MTrib have thick, tree lined riparian cover, while Ceiba has a more open canopy. Petunia drains a residential area in metropolitan San Juan and contains the highest NO_3 concentrations of any of the study sites. MTrib and Ceiba also drain residential areas in the towns of Palmer and Ceiba, respectively. Evidence that sewage leaks directly into the streams was observed in all three of our urban streams.

Table 1: Stream location, watershed area, watershed % land use, stream substrate, experiment reach length, and stream slope.

Stream Name	Latitude (N)	Longitude (W)	Watershed Area (ha)	% Native	% Agriculture	% Urban	Dominant Substrate	Reach Length (m)	% Slope
Bisley	18.31633	65.74802	58.163	99.7	0	0.3	Boulder/bedrock	385	12.9
RIT	18.28048	65.78925	30.270	100	0	0	Sand/fine gravel	365	3.1
Pared	18.33409	65.82462	64.006	99.8	0.2	0	Gravel/cobble	525	14.2
Grande	18.16048	65.94535	95.169	28.3	69.3	2.4	Sand/fine gravel	690	1.4
Maizales	18.23354	65.75931	265.337	61.7	34.3	4	Gravel	450	2.5
Vaca	18.34340	65.84181	172.039	60.5	32.2	7.4	Gravel	490	2.5
Petunia	18.37925	66.08373	110.025	6.7	0	92.3	Gravel	350	4.0
MTrib	18.37040	65.77972	159.850	42	1.5	56.7	Gravel	375	2.4
Ceiba	18.27135	65.64887	505.302	44.2	2.7	53.2	Gravel	625	1.6

Materials and Methods

Experimental Procedures

$^{15}NO_3$ experiments were conducted in the nine streams, as part of the Lotic Intersite Nitrogen Experiment II (LINX II), in February and March, over 3 years (2004 – 2006). The ^{15}N addition experiment consisted of a 24-hour addition of ^{15}N (99% $K^{15}NO_3$), with a target enrichment of stream NO_3 to 20,000 ‰, together with a conservative tracer (NaBr). The injection solution was pumped at a constant rate (20 mL min^{-1}) for 24 hours with a peristaltic pump (Cole Palmer *Masterflex*). Injection sites were located in constricted, turbulent sections of the stream to ensure proper mixing.

The ^{15}N was sampled in several pools to quantify uptake rates and to understand the fate of N in streams. ^{15}N was sampled and analyzed in stream water (decline of $^{15}NO_3$ for total uptake; $^{15}NH_4$ and $DO^{15}N$ for N transformations), detrital organic matter (assimilation), and gas (denitrification). I also measured a variety of other stream characteristics (i.e. – ecosystem metabolism, transient storage, biological standing stocks) to gain insight into the controls on the fate of N.

Water samples for a variety of ^{15}N pools ($^{15}NO_3$, $^{15}NH_4$, and dissolved organic ^{15}N) and several other chemical species (Br, Cl, NO_3, NH_4, TDN, DOC and PO_4) were collected at six stations just prior to, during, and 24 hours, 72 hours, and 1 week after the ^{15}N addition ended. Hyporheic water (NO_3 and NH_4) was also collected before the experiment began using a groundwater "sipper", which consists of a hollow 3/8" metal tube with an opening cut in the lower 5 cm.

This tube is inserted into the streambed to bury the intake about 5-20 cm below the surface. Inside the tube is a stainless-steel fine-mesh fuel filter connected to 1/8" O.D. TFE tubing that leads through the tube to a 60-mL syringe equipped with a 4-way plastic stopcock. Sediment porewater is slowly extracted via the syringe. Samples were filtered through precombusted glass fiber filters (Whatman GF/F) and frozen within 4 hours of collection, except for $^{15}NH_4$ samples which were immediately processed for ^{15}N after filtration. Br, Cl, and NO_3 were analyzed by ion chromatography (Dionex), NH_4 and PO_4 were analyzed by robotic automated colorimetry (Westco Smartchem), and DOC & TDN were analyzed by high temperature catalytic oxidation (Shimadzu TOC-V) at the Water Quality Analysis Laboratory of the University of New Hampshire (UNH). Samples collected prior to the ^{15}N addition were used to determine background levels of each solute. The samples collected 24 hours, 72 hours, and 1 week after the end of the ^{15}N addition were used to measure total release of ^{15}N taken up during the experiment and the forms in which it was found. $^{15}NO_3$ samples were collected twice (1 am and 12 pm of day 2) during the ^{15}N addition to provide two estimates of nitrate uptake length and rates and to determine day/night differences.

For $^{15}NO_3$ water samples, samples were processed using a modified Sigman et al. (1997) method based on an alkaline headspace diffusion following reduction of nitrate to ammonium. The headspace diffusion involves addition of MgO and NaCl to the water samples, and boiling to remove NH_4 and concentrate the samples to 100 mL. Samples were then transferred to a 250 mL bottle and

Devarda's alloy was added (to convert the NO_3 to NH_3) with an acidified filter packet, which consisted of a 1 cm glass fiber filter (Whatman GF/D) inside porous Teflon tape. Samples were heated to 60°C for 2 days and then shaken for 7 days to allow the NO_3-NH_4 and NH_4-NH_3 conversions and the absorption of the NH_3 on the filter. The filters were allowed to dry in a desiccator. All ^{15}N analyses of filters and ground organic matter samples were conducted at the Ecosystems Center Laboratory, Marine Biological Laboratory, Woods Hole, MA. The ^{15}N:^{14}N ratio was determined by mass spectrometry using a continuous-flow PDZ Europa "20-20" with an ANCA-SL elemental analyzer - gas chromatograph preparation system.

Samples for dissolved ^{15}N-gas (^{15}N in N_2 and N_2O) were collected in duplicate three times: prior to the ^{15}N release (morning of day 1) and during each of the plateau samplings (1 am and noon of day 2) at 10 stations over the stream reach. Samples were collected, processed, and analyzed according to Mulholland et al. (2004) and Hamilton (personal communication). Stream water was collected in 60-mL (2004) or 140-mL (2005 & 2006) syringes with 4-way male Luer stopcocks. Water was drawn in (40 mL or 120 mL) under the surface being careful not to introduce air bubbles into the syringe. Syringes were then closed and submerged in water and processed in a single location. Samples were injected with 20-mL of ultra-high purity helium in the syringe and equilibrated with the He headspace by shaking for 5 minutes. The headspace was injected into 12-ml Exetainer vials (evacuated Vial Type 3 screw-cap with septa, Labco). The Exetainers were stored and shipped in water filled centrifuge tubes to avoid air contamination. One replicate of the samples was shipped to the University of California Davis Stable Isotope Facility for ^{15}N:^{14}N ratio analysis by mass spectrometry using a Europa Hydra Model 20/20 continuous flow IRMS. The other replicates of samples were sent to the Biogeochemistry and Paleoproteomics Laboratory, Michigan State University for ^{15}N:^{14}N ratio analysis with a VG Prism Series II IRMS interfaced with a HP 5890 Series II Gas Chromatograph.

Samples of suspended particulate organic matter (SPOM) were collected 24 hours after the end of the ^{15}N addition. For concentration and flux, samples of stream water were collected from each station and a known volume was filtered through precombusted and pre-weighed glass fiber filters (Whatman GFF, 47 mm diameter), dried (60°C), weighed, combusted (500°C) and reweighed for determination of ash-free dry mass (AFDM) per unit volume. For ^{15}N, samples of stream water were collected for the Post 24 hours from the 6 downstream stations and one upstream station and from the most downstream station and the upstream station for Post 72 hours and Post 1 Week. Samples for ^{15}N were filtered through precombusted glass fiber filters (Whatman GF/F, 24 mm diameter), dried (60 C) and placed in labeled scintillation vial and capped tightly for later analysis.

Samples of detrital benthic organic matter from specific biomass compartments were collected at each station at Pre and Post 24 for ^{15}N and at 10 stations for standing stocks. A metal quadrat (0.28-m^2) was placed in the stream at random and all leaves, wood, macrophytes, filamentous algae, and roots associated with streamside vegetation that lay within the quadrat were collected. Standing stock samples were placed in dried and weighed paper bags and then dried (60 C) and weighed. A subsample was combusted (500°C) and reweighed for determination of AFDM. ^{15}N samples were dried and ground for subsequent ^{15}N analysis. A cylinder was used to sample surface and subsurface fine benthic organic matter (FBOM), in which all coarse material was removed and a sample was taken of the suspended sediments after surface agitation. The cylinder was then removed and the material was allowed to flush downstream and then the cylinder was placed in the same location and depth and the sediments were agitated as deep as possible with a hand. The FBOM samples were filtered through precombusted glass fiber filters (Whatman GFF, 24 mm diameter) and dried (60°C). ^{15}N samples were tightly capped and shipped for analysis. Standing stock samples were combusted (500°C) and reweighed for determination of AFDM. Epilithic biofilm samples were collected by scraping a known surface area of rock and were processed in the same manner as the FBOM samples. Chlorophyll *a* for FBOM and epilithon was collected as above and filtered through precombusted glass fiber filters (Whatman GFF, 24 mm diameter) and frozen until they were analyzed by a hot ethanol extraction method (Sartory and Grobbelaar, 1984).

Additional Experimental Procedures

Whole stream primary productivity and community respiration was measured using the 2-station dissolved oxygen (DO) method with data logging sondes (YSI 6920). DO was measured at 5 minute intervals for the duration of the ^{15}N experiments. Water travel time and air-water gas exchange were measured concurrently by addition of NaCl and a conservative gas, respectively. To measure air-water gas exchange, propane or sulfur hexafluoride (SF_6) was injected at a constant rate directly into the stream though a gas diffusing stone. 40 mL water samples were collected in 60 mL syringes and injected with air and shaken at the same location. The headspace was injected into evacuated glass vials (22 mL Wheaton with crimped septa), shipped to UNH, and analyzed on a gas chromatograph (Hewlett Packard 5890 Series II) equipped with a electron capture detector (SF_6) and flame ionization detector (propane). I calculated gas exchange rate in terms of the fractional decline in propane concentration (corrected for dilution due to groundwater input between stations) between two measurement stations. Gas exchange rates of O_2, N_2, & N_2O were calculated form the measured values of the gas exchange rate of propane using the relative values of their Schmidt numbers (Mulholland et al. 2004). The O_2 air-water exchange rate was then calculated as 1.396 times the calculated propane air-water exchange rate (1.345 for SF_6). The N_2 gas exchange rate was calculated as 1.335 times the propane rate (for correction of stream N_2 production) and the N_2O gas exchange rate was calculated as 1.308 times the propane rate (for correction of stream N_2O production). If SF_6 was used as the tracer gas, then the N_2 and N_2O conversion factors were 1.285 and 1.258, respectively.

Water travel time and transient storage zone size and exchange rates were determined from the NaCl injection. Conductivity and temperature were measured (YSI) at 5 minute intervals. The conductivity data were entered in the OTIS-P model, which was used to quantify the hydrologic parameters affecting solute transport. Photosynthetically active radiation (PAR) was measured at a representative location of the canopy cover along the ^{15}N experiment reach using a light data logger (HOBO, Onset corp). Canopy cover was also determined along 10 transects with a concave densiometer (Forestry Suppliers).

Calculations

Reported ^{15}N values expressed as $\delta^{15}N$ values (units of ‰) according to the following equation:

$$\delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 1000 \qquad (1)$$

where R = ^{15}N:^{14}N ratio and $R_{standard}$ is atmospheric N_2 ($R_{standard}$ = 0.0036765).

Tracer ^{15}N flux (μg ^{15}N sec^{-1}) was calculated from the measured ^{15}N values by first converting all $\delta^{15}N$ values to the isotopic mole fraction (MF) of ^{15}N or $^{15}N/(^{15}N + {}^{14}N)$ ratios using the following equation:

$$\frac{^{15}N}{^{15}N+{}^{14}N} = \frac{\left(\frac{\delta^{15}N}{1000}+1\right)*0.0036765}{1+\left(\left(\frac{\delta^{15}N}{1000}+1\right)*0.0036765\right)} \qquad (2)$$

The ^{15}N MF values were then corrected for the N content of Devarda's and ^{15}N flux was calculated at each station by multiplying the ^{15}N MF values by stream water NO_3 flux (F, calculated as discharge multiplied by stream water nitrate concentration) at each station and subtracting the total background $^{15}NO_3$ flux.

Uptake lengths (S_w) were calculated using regressions of the natural log of the ^{15}N flux value (corrected for background, and corrected for dilution) against distance below the ^{15}N injection. The slope of this regression is the distance-normalized NO_3 uptake rate and the inverse of the slope is the NO_3 uptake length. Whole stream nitrate uptake rate (U) was calculated by the following equation:

$$U\ (\mu g\ N\ m^{-2}\ s^{-1}) = F/(S_w \times w) \qquad (3)$$

where w is the average wetted width. The mass transfer coefficient (V_f) was calculated by dividing U by the stream water NO_3 concentration (Newbold *et al*, 1981; Stream Solute Workshop, 1990). Assimilatory ^{15}N uptake by biomass was calculated from the mass of ^{15}N tracer found in each biomass compartment.

Total nitrification rates were determined using a mass balance approach for NO_3. Inputs of nitrate are from the nitrate flux at the top of the reach and groundwater and the outputs are nitrate flux at the bottom of the stream and total nitrate uptake flux (calculated from S_w, nitrate flux, and reach length). Groundwater was sampled using a "groundwater sipper", which is a ½" aluminum hunting arrow with a whole cut near the tip of the shaft. PVC tubing is inserted into the aluminum arrow with micro-mesh at the bottom to prevent clogging of the sample. At the other end is a stop-cock and a syringe and a vacuum is created with the syringe to pull water up the tubing. The arrow is inserted into the ground in the riparian zone and water is sampled at several locations along a stream. Nitrification is the difference between these nitrate outputs and inputs.

Denitrification rates (production of N_2 and N_2O) were determined from the production of $^{15}N_2$ and $^{15}N_2O$ in the study reach using the approach described in Mulholland *et al.* (2004). First, tracer $^{15}N_2$ and $^{15}N_2O$ MF values were computed (equation 1) and $^{15}N_2$ and $^{15}N_2O$ flux were calculated with the MF values and the N_2 and N_2O mass values (statistically corrected for incomplete headspace mixing and air contamination). We solved for the $^{15}N_2$ and $^{15}N_2O$ production rates by fitting the following relationship to the longitudinal pattern in tracer $^{15}N_2$ and $^{15}N_2O$ flux (A) with distance x (in units of m) downstream from the ^{15}N addition point:

$$\mathbf{A} = \left(\frac{\mathbf{k}_{\mathbf{den}} \times \mathbf{N}_0}{\mathbf{k}_2 - \mathbf{k}_1}\right) \times \left(\mathbf{e}^{-\mathbf{k1x}} - \mathbf{e}^{-\mathbf{k2x}}\right) \qquad (4)$$

where k_{den} is the denitrification rate; N_0 is the flux ^{15}N-nitrate calculated at the point of the injection; k_1 is the measured rate of decline in streamwater ^{15}N-nitrate flux with distance due to all processes (in units of m^{-1}); and k_2 is the N_2 or N_2O gas exchange rate through the air-water interface per unit distance (units of m^{-1}). Values of k_2 are determined from the propane or SF6 injection experiment. I then used a least squares fitting procedure in Microsoft Excel (Solver Tool, Microsoft Excel 2003) to determine the values of k_{den} from fitting the model to A. k_{den} was then multiplied by stream NO_3 flux and divided by average stream width to give the areal denitrification rate.

Statistical Analysis

Regression analysis and multiple regression analysis were preformed to determine relationships between measured hydrological, physical, and chemical variables and N uptake parameters. Relationships were considered significant where $p < 0.05$. Non-normal data were log transformed. Regression analyses were performed with Systat 11.

The statistical models explaining the functional response of stream biota to increasing nutrient concentration were done with the following statistical tests. Linear regression analysis was used for 1st order response, in which the relationship between independent and dependent variables would be significant if near 1. The relationship would be considered saturated if there was a significant fit with the Michaelis-Menten model and calculated K_S was within the range of NO_3 concentrations in my study. The model with the better r-square value was considered the relationship that better explained the data in my study.

CHAPTER II

RESULTS

Physical and Chemical Parameters

Stream discharge varied among streams from 4.7 L/s (Q. Petunia) to 80.7 L/s (Q. Vaca) during the ^{15}N addition (Table 2; median = 20.3 L/s), but discharge was relatively constant throughout the experiments in each stream. The nine streams ranged in mean width from 1 m (Q. Grande) to 3.5 m (Q. Maizales) and in mean depth from 4 cm (Q. Pared) to 35 cm (Q. Vaca). Velocity was unrelated to discharge and was highest in a sandy bottom stream (Q. Grande, 20 cm s^{-1}) where there was little debris from riparian vegetation to impede its flow. PAR ranged from 0.02 (Q. Petunia) to 13.95 mol quanta m^{-2} d^{-1} (Q. Maizales) and was dependent on canopy cover.

Dispersion coefficients (D, Table 3) ranged from 0.024 (Q. Maizales) to 0.455 m^2 s^{-1} (Q. Grande) and were highly dependent on water velocity ($r^2 = 0.61$, $P = 0.01$). The area of the transient storage zone (A_S, Table 3) varied between 0 (Q. Maizales) and 0.13 m^2 (Q. Vaca), while the size of transient storage zone adjusted for stream size (A_S/A, Table 3) ranged from 0 (Q. Maizales) to 0.38 (Q. Bisley). Neither of the transient storage zone size parameters (A_S and A_S/A) were significantly related to any other physical parameter ($P > 0.05$). Storage zone exchange coefficient (α, Table 3) ranged from 0.00002 (Q. Pared) to 0.01875 s^{-1}

(Q. Grande), but most were below 0.001 s^{-1} and was not significantly related to any other parameter.

NO_3 concentrations varied from 105 μg N L^{-1} in Q. Pared to 997 μg N L^{-1} in Q. Petunia (Table 2) and were an indication of the severity of anthropogenic impacts on the streams. Concentrations of NH_4 were low (median = 7 μg N L^{-1}), except in Q. Ceiba (50 μg N L^{-1}) and MTrib (2204 μg N L^{-1}). NO_3 was the largest proportion of dissolved N, except in Q. Pared where DON was the dominant dissolved form. Nutrient concentrations during the ^{15}N experiment in MTrib were elevated from concentrations measured previously due to a pollution event, in which raw sewage was found leaking out of the top of a manhole and into the stream unbeknownst to the author prior to initiating the experiment. DIN:SRP was below 16 for Q. Bisley, Q. Pared, and MTrib.

Table 2: Stream chemical and physical characteristics during each of the ^{15}N addition experiments.

Stream Name	Experiment Date (Feb-March)	Q (L/s)	NO_3 (μg N/L)	NH_4 (μg N/L)	DON (μg N/L)	SRP (μg P/L)	Width (m)	Depth (m)	PAR (mol quanta m^{-2} d^{-1})
Bisley	2004	12.5	171	3	10	22	3.20	0.07	0.15
RIT	2005	20.0	131	7	23	0	1.18	0.27	0.50
Pared	2006	5.2	105	3	280	7	2.74	0.04	1.55
Grande	2004	12.3	276	11	70	13	1.02	0.06	6.39
Maizales	2005	25.0	206	7	78	12	3.53	0.35	13.95
Vaca	2006	80.7	440	3	167	9	1.96	0.16	1.52
Petunia	2004	4.7	997	15	53	26	1.80	0.11	0.02
MTrib	2005	23.2	174	2204	847	310	2.46	0.41	0.82
Ceiba	2006	49.5	512	50	11	22	2.73	0.07	1.98

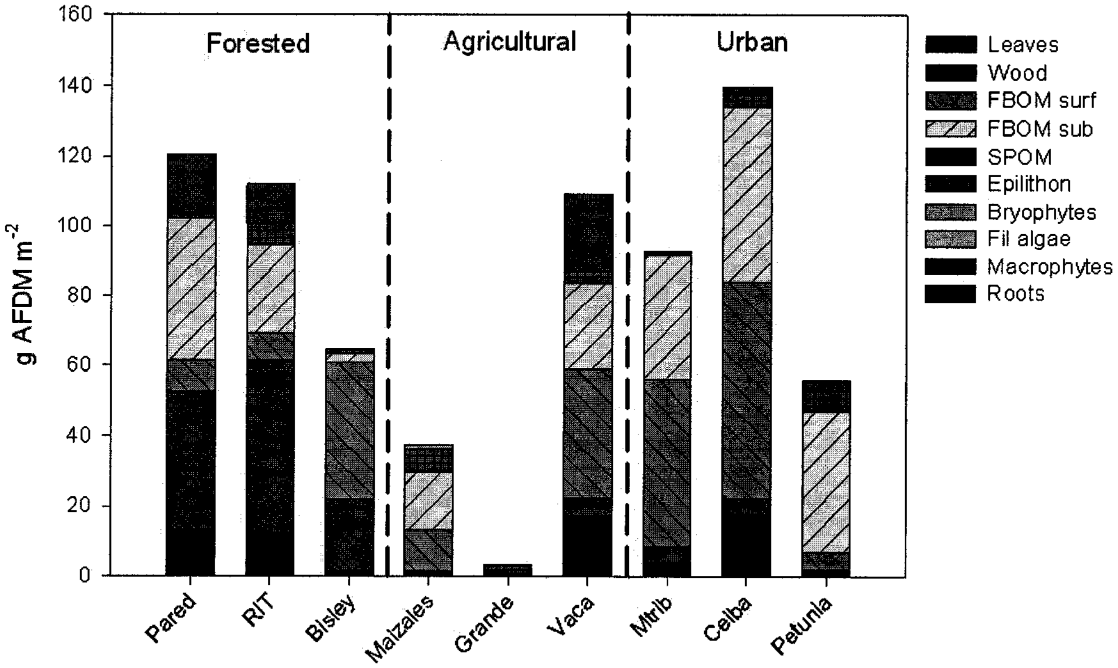
Table 3: Stream hydraulic characteristics determined from the OTIS-P simulations of the NaCl injections.

Stream Name	Velocity (v, cm s^{-1})	Dispersion coefficient (D, m^2 s^{-1})	Cross sectional area (A, m^2)	Area of storage zone (A_S, m^2)	A_S/A	Storage exchange coefficient (α, s^{-1})	Turnover time of storage (T_S, s)	Hydraulic retention factor (Rh, s m^{-1})	F_{med}^{200} (fraction)
Bisley	5.366	0.188	0.23	0.086	0.380	0.00013	2876	4.89	0.078
RIT	6.159	0.218	0.17	0.022	0.131	0.00005	2855	1.31	0.242
Pared	1.852	0.272	0.46	0.109	0.236	0.00002	11035	12.30	0.030
Grande	20.000	0.455	0.07	0.021	0.318	0.01875	17	1.58	0.010
Maizales	2.011	0.024	1.26	0.000	0.000	0.00576	0	0.01	0.000
Vaca	6.073	0.171	0.81	0.133	0.164	0.00004	3885	3.21	0.031
Petunia	2.315	0.052	0.30	0.038	0.128	0.00004	3320	5.13	0.038
MTrib	2.278	0.163	0.39	0.059	0.151	0.00005	3174	4.30	0.021
Ceiba	6.755	0.305	0.26	0.012	0.045	0.00005	902	0.65	0.006

Metabolism and Biomass

Fine Benthic Organic Matter (FBOM) was the dominant biomass compartment (mean = 57% of total AFDM, figure 2) in all streams except RIT, where CBOM was dominant (44% of total AFDM) and Q. Grande where epilithon was 35% of total AFDM. Q. Ceiba had the highest total standing stock of organic matter (140 g AFDM m^{-2}) and the mean was 82 g AFDM m^{-2} across all streams.

Figure 2: Habitat-corrected estimates of ash-free dry mass (AFDM) for compartments samples for N uptake.



Stream water temperature was fairly constant across sites (19 – 25.3 °C, Table 4). Light readings varied considerably from 0.02 mol quanta m^{-2} d^{-1} in an urbanized stream with very thick riparian cover (Q. Petunia) to 13.95 mol quanta m^{-2} d^{-1} in an agricultural stream with no riparian vegetation (Q. Maizales). All streams were net heterotrophic except Q. Maizales, which had a P/R above 1.

Gross primary production (GPP) varied considerably among streams, ranging 0.01 g O_2 m^{-2} d^{-1} (Q. Bisley) to 9.33 g O_2 m^{-2} d^{-1} (Q. Ceiba). GPP was positively correlated with PAR (log scale, $r^2 = 0.46$, $P = 0.046$) and watershed area ($r^2 = 0.67$, $P = 0.01$). Ecosystem respiration (R) was highly variable among streams, ranging from 0.42 (Q. Pared) to 15.69 g O_2 m^{-2} d^{-1} (Q. Vaca). R was negatively correlated with stream gradient ($r^2 = 0.47$, $P = 0.04$) and positively correlated with depth ($r^2 = 0.62$, $P = 0.01$) and discharge ($r^2 = 0.79$, $P = 0.01$). However, Q. Vaca had significant leverage on the R-discharge relationship and when that stream was removed from the regression the relationship was no longer significant. R dominated ecosystem metabolism in most streams and P/R was greater than one in only Q. Maizales.

Table 4: Daily average water temperature, photosynthetically active radiation (PAR), gross primary production (GPP), ecosystem respiration (R), and P/R for each stream.

Stream Name	Water Temperature (°C)	Daily PAR (mol quanta m^{-2} d^{-1})	Daily GPP (g O_2 m^{-2} d^{-1})	Daily R (g O_2 m^{-2} d^{-1})	P/R
Bisley	21.3	0.15	0.01	2.42	0.00
RIT	19.0	0.50	0.47	4.49	0.10
Pared	22.1	1.55	0.39	0.42	0.92
Grande	23.0	6.39	5.18	7.64	0.68
Maizales	23.0	13.95	7.29	5.31	1.37
Vaca	23.0	1.52	3.08	15.69	0.20
Petunia	24.3	0.02	0.34	4.60	0.07
MTrib	20.9	0.82	7.13	7.41	0.96
Ceiba	25.3	1.98	9.33	11.74	0.79

Nitrogen Dynamics

The uptake kinetics of NO_3 varied considerably among streams, but there was no statistically significant pattern in the day to night variances in any of the

NO_3 uptake parameters. The distance specific uptake rate (*k*, Figure 3) for NO_3 was highest in Q. Pared (mean = 0.0032 m^{-1}; S_w mean =315 m), while *k* was lowest in Q. Vaca (mean = 0.0001; S_W = 8480 m). *k* was significantly related to specific discharge, which is stream discharge divided by width (Figure 4; log-log, r^2 = 0.67, P = 0.01, log kNO3 = -4.978 - (0.870 * log Q/W)). *k* was also related to stream gradient and R, but these relationships were not significant (r^2 = 0.38, P = 0.08 and r^2 = 0.41, P = 0.06 respectively). Distance specific uptake rate was not significantly related to any other chemical, physical, or biological variable.

Figure 3: NO_3 a) uptake lengths (S_W) and b) uptake rate coefficients (kNO_3) in the 9 streams for both day and night sampling periods. Error bars are the upper 95% confidence interval from the linear regression of the decline in $\ln^{15}NO_3$ over distance downstream. (Uptake length for Q. Vaca at night is infinity and therefore off scale.)

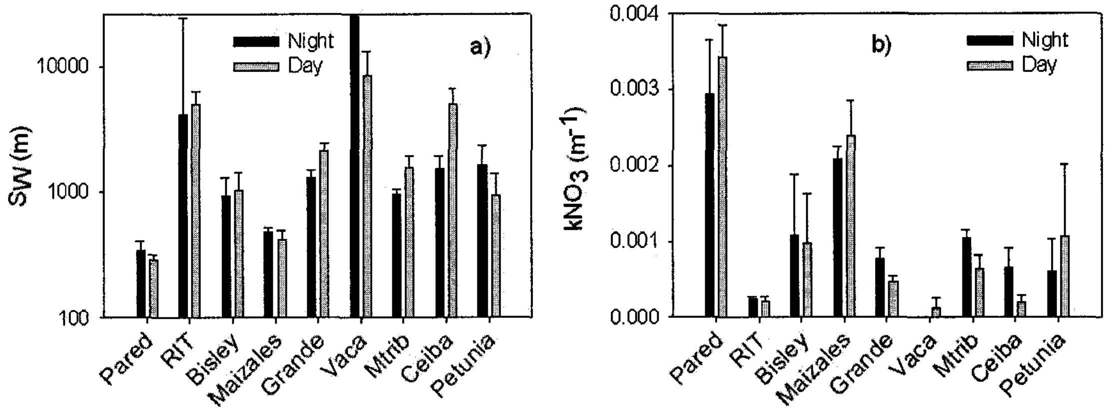
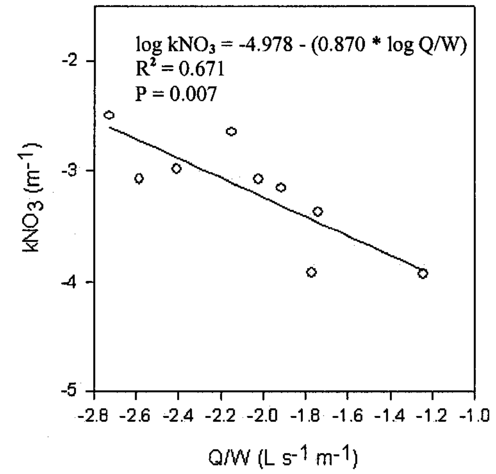


Figure 4: Nitrate uptake rate coefficient (k) as a function of specific discharge (Q/w). Significance found if P < 0.05.



The average mass transfer coefficient (V_f), or uptake velocity, ranged from 0.0002 cm s^{-1} (Q. Petunia; Figure 5) to 0.0016 cm s^{-1} (Q. Maizales). V_f had a significant positive relationship with PAR (Figure 6b; $r^2 = 0.77$, $P = 0.01$), GPP (Figure 6a; $r^2 = 0.61$, $P = 0.01$), and epilithon chlorophyll *a* ($r^2 = 0.54$, $P = 0.02$). Multiple regression analysis showed that GPP and PAR together explained 90% of the variation ($V_f\ NO_3 = 0.000350 + (0.0000502 * GPP) + (0.0000613 * PAR)$, GPP $P = 0.01$ and PAR $P = 0.00$), however GPP and PAR were also significantly related with each other (log-log, $r^2 = 0.46$, $P = 0.05$). V_f was not significantly related to NO_3 concentration (Figure 6c) or any other variable.

Figure 5: NO_3 mass transfer velocity (Vf, cm s^{-1}) in the 9 streams for both day and night sampling periods. Error bars are the upper 95% confidence interval from the linear regression of the decline in $\ln^{15}NO_3$ over distance downstream.

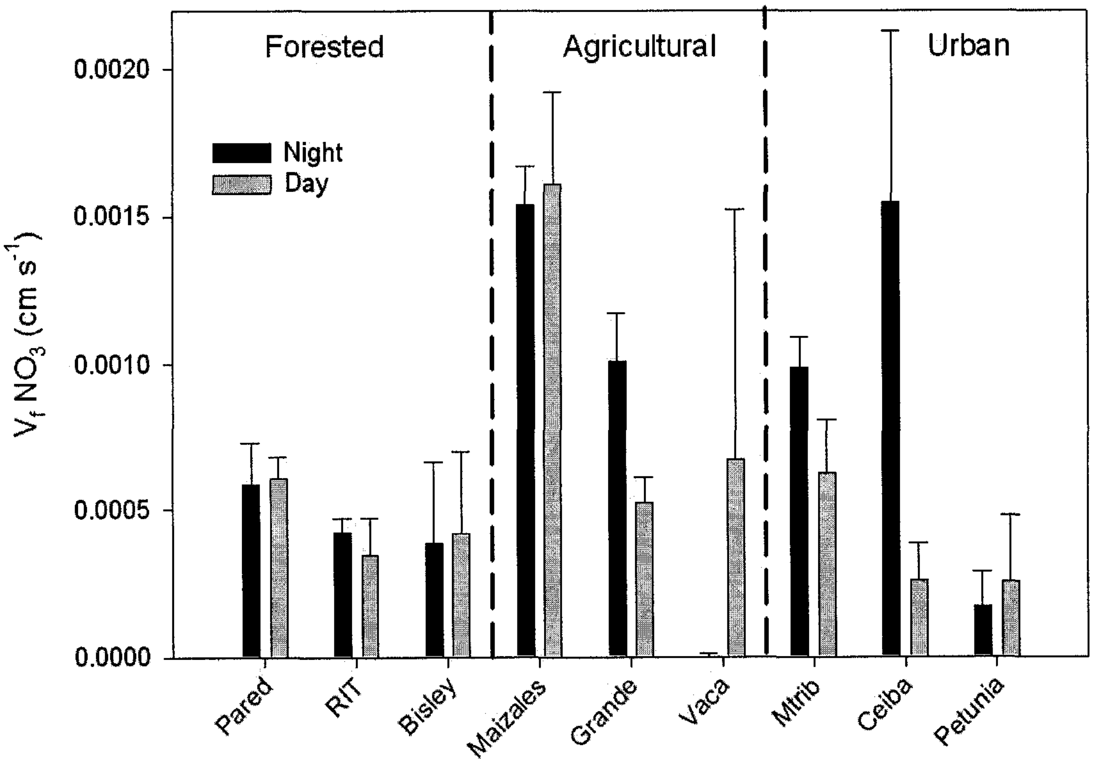
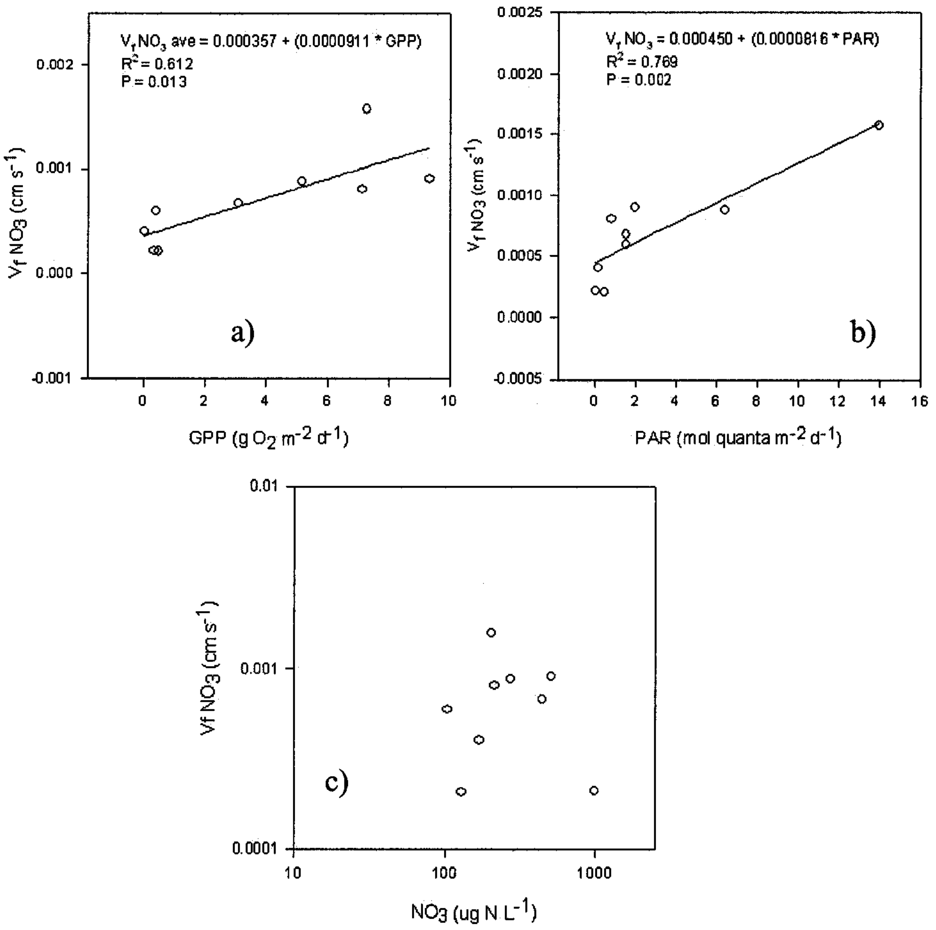


Figure 6: Nitrate mass transfer velocity (V_f) as a function of a) gross primary production (GPP), b) photosynthetically active radiation (PAR), and c) nitrate concentration (log scale). Significance found if P < 0.05.



Uptake per unit area (*U*, Figure 7) varied over an order of magnitude from 0.497 μg N m^{-2} s^{-1} (mean, RIT) to 4.185 μg N m^{-2} s^{-1} (Q. Ceiba). NO_3 uptake had a significant positive correlation with GPP (Figure 8a; r^2 = 0.61, P = 0.01), R (8b; r^2 = 0.53, P = 0.03), and stream temperature (8c; r^2 = 0.67, P = 0.01). GPP (P = 0.02), R (P = 0.05), and temperature (P = 0.01) explained 96% of the variation in *U* using multiple regression analysis (U NO_3 = -7.478 + (0.375 * Temp) + (0.147 * GPP) - (0.0852 * R)). *U* was correlated to NO_3 concentration (Figure 8d; r^2 =

0.71, P = 0.01) when Q. Petunia was removed, which had the highest NO_3 concentrations. *U* was not significantly related to any other variable.

Figure 7: NO_3 uptake (U) in the 9 streams for both day and night sampling periods. Error bars are the upper 95% confidence interval form the linear regression of the decline in $\ln^{15}NO_3$ over distance downstream.

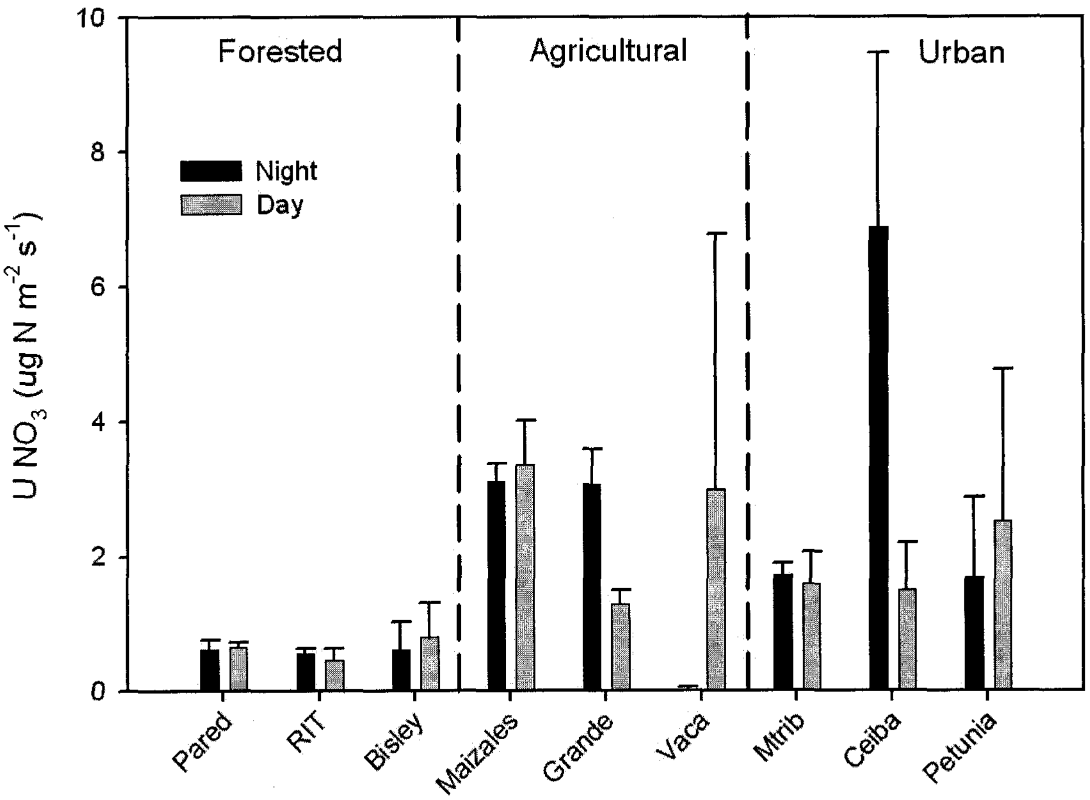
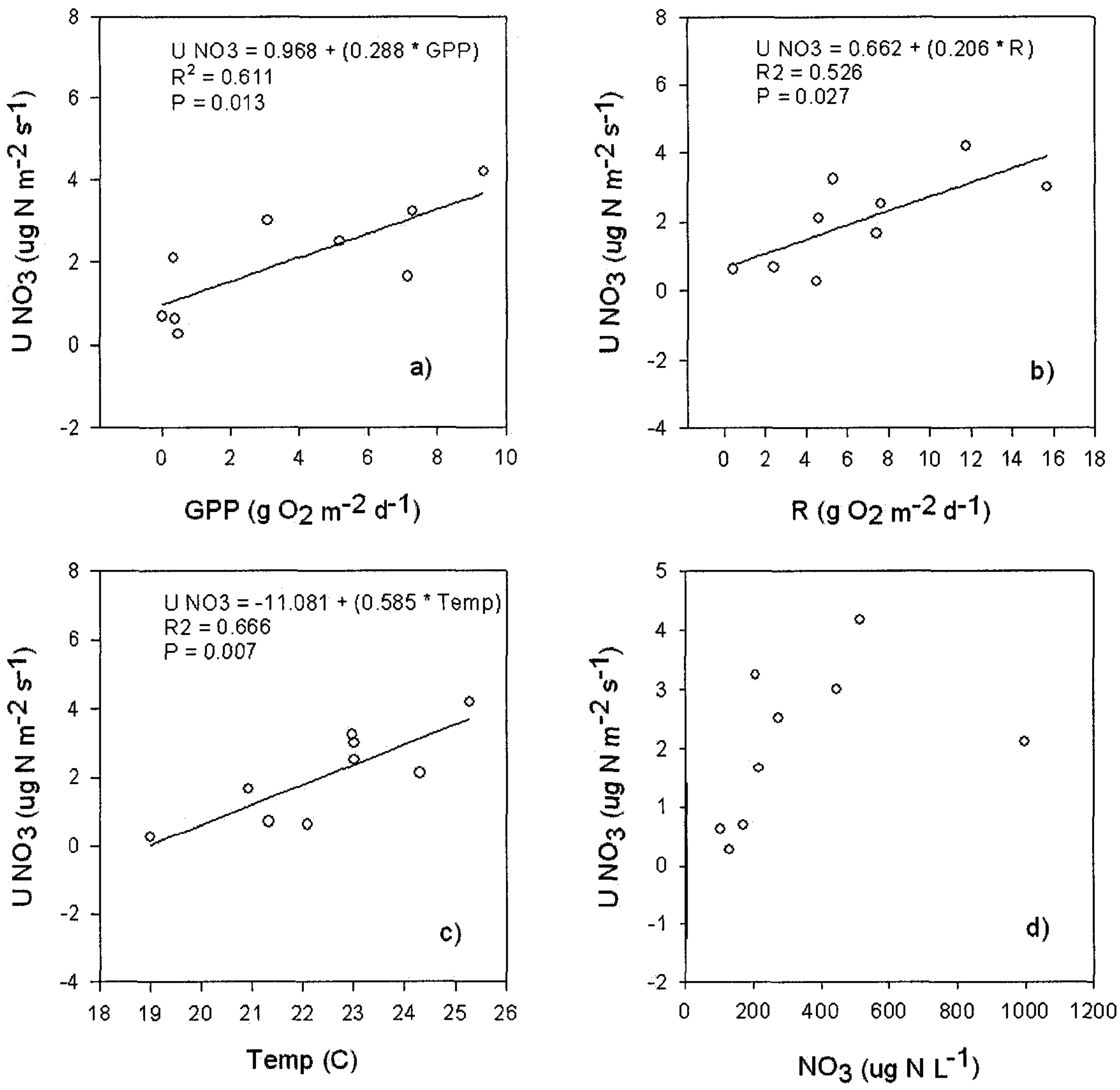
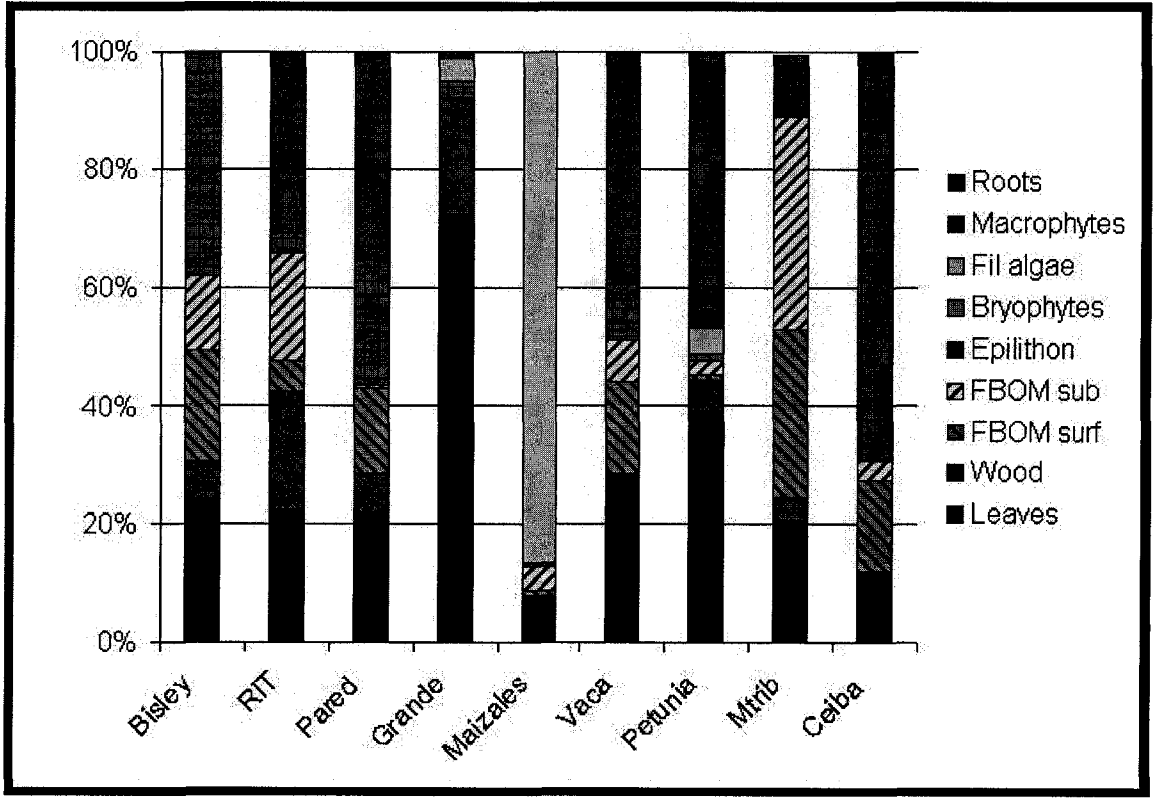


Figure 8: Nitrate uptake (U) as a function of a) gross primary production (GPP); b) ecosystem respiration (R); c) stream water temperature; and d) nitrate concentration (not significant). Significance found if $P < 0.05$.



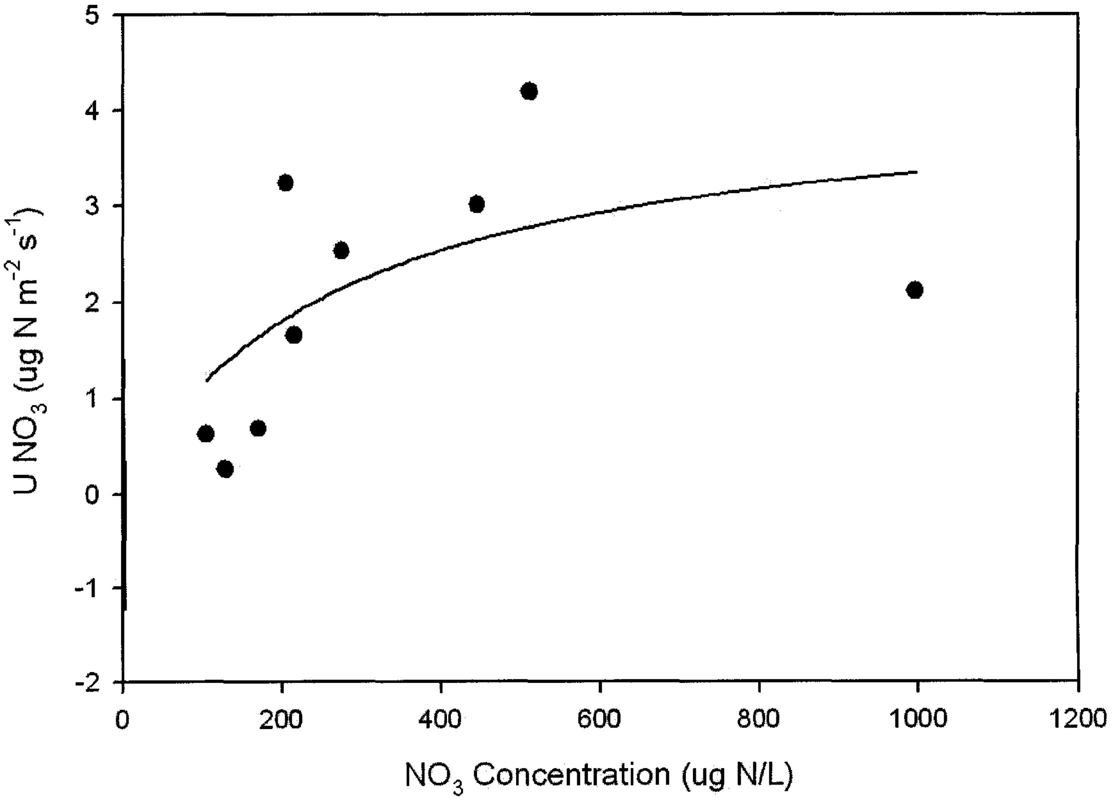
The proportion of the NO_3 that was assimilated for each of the major biomass compartments is presented in Figure 9. In several streams (Q. Pared, RIT, Q. Vaca, and Q. Petunia) the assimilation was highest in or on roots that were attached to riparian vegetation. In others, N uptake was the most rapid in epilithon (Q. Bisley and Q. Ceiba), CBOM as leaves (Q. Grande), filamentous algae (Q. Maizales) and FBOM (MTrib). Uptake by heterotrophic bacteria associated with leaves was also a significant fraction of uptake in most streams. Total autotrophic assimilation (mean = 15.62 mg N m^{-2} d^{-1}) was generally higher than heterotrophic assimilation (FBOM & CBOM; mean = 12.72 mg N m^{-2} d^{-1}), but that includes roots in autotrophic uptake. Assimilation by roots might not be autotrophic uptake in this case, since we observed significant biofilm on the roots at every stream but this biofilm was not separated from the bulk roots.

Figure 9: Proportion of nitrogen uptake for sampled biomass compartments. Streams on x-axis are arranged by land use.



There are two different models that can be used to explain the functional relationship between water column nutrient concentration and uptake by the benthos (Dodds et al. 2002). With first order kinetics, uptake depends entirely on mass transport, which would mean that total NO_3 *U* increases linearly with NO_3 concentration. In Michaelis-Menten kinetics, there is clear saturation of biotic uptake and there is a hyperbolic relationship between *U* and concentration. Michaelis-Menten relationship better explains total NO_3 uptake kinetics in the gradient of streams of my study (Figure 10; linear $r^2 = 0.19$, M-M $r^2 = 0.42$). The saturation kinetic calculations show that my streams approach saturation, as the half-saturation constant (K_S = 269 μg N/L) is below the mean NO_3 concentration in my streams.

Figure 10: Total areal nitrate uptake (U NO_3) as a function of nitrate concentration across the 9 streams of this study. The line represents the Michaelis-Menten model using non-linear regression.



Denitrification

Denitrification rates were high in several streams (Figure 11) and as was the case with total NO_3 uptake, denitrification varied unpredictably between night and day. Denitrification rates (*U den*) ranged from a stream day/night average of 0.008 μg N m^{-2} s^{-1} (Q. Pared) to 2.205 μg N m^{-2} s^{-1} (Q. Vaca). ^{15}N in N_2 and N_2O was detectable in all 9 streams and N_2 was the dominant end product of denitrification by two orders of magnitude. Denitrification had a significant positive relationship with NO_3 concentration (Figure 12a; log-log, $R^2 = 0.52$, $P = 0.03$), depth (12b; $R^2 = 0.58$, $P = 0.02$), DON (12c; $R^2 = 0.45$, $P = 0.05$), R (12d; $R^2 = 0.68$, $P = 0.01$), FBOM standing stock (12e; $R^2 = 0.57$, $P = 0.02$), and specific discharge (12f; $R^2 = 0.45$, $P = 0.05$). I also saw a significant negative relationship between denitrification rate and FBOM C:N (12g; $R^2 = 0.61$, $P = 0.02$). R was the most significant variable in predicting denitrification rate according to backward stepwise regression (log-log, $R^2 = 0.58$, $P = 0.02$). Denitrification efficiency (V_f *den*) was not significantly correlated with NO_3 concentration (Figure 13). Denitrification accounted for 1 to 97% of nitrate uptake with 5 of the 9 streams having denitrification accounting for greater than 35% of total nitrate uptake (Figure 14), indicating that denitrification has the potential to be a substantial sink for nitrate. The Michaelis-Menten relationship also better explains the relationship between denitrification rate (*U den*) and NO_3 concentration across streams (Figure 15; linear $r^2 = 0.15$, M-M $r^2 = 0.27$) on untransformed data compared to the 1st order response model. K_S is 452 μg N L^{-1}

for *U den*, so biotic uptake of NO_3 on the stream bottom saturates at lower NO_3 concentrations than denitrification.

Figure 11: Areal denitrification rates (*U den*) in the 9 streams for both day and night sampling periods.

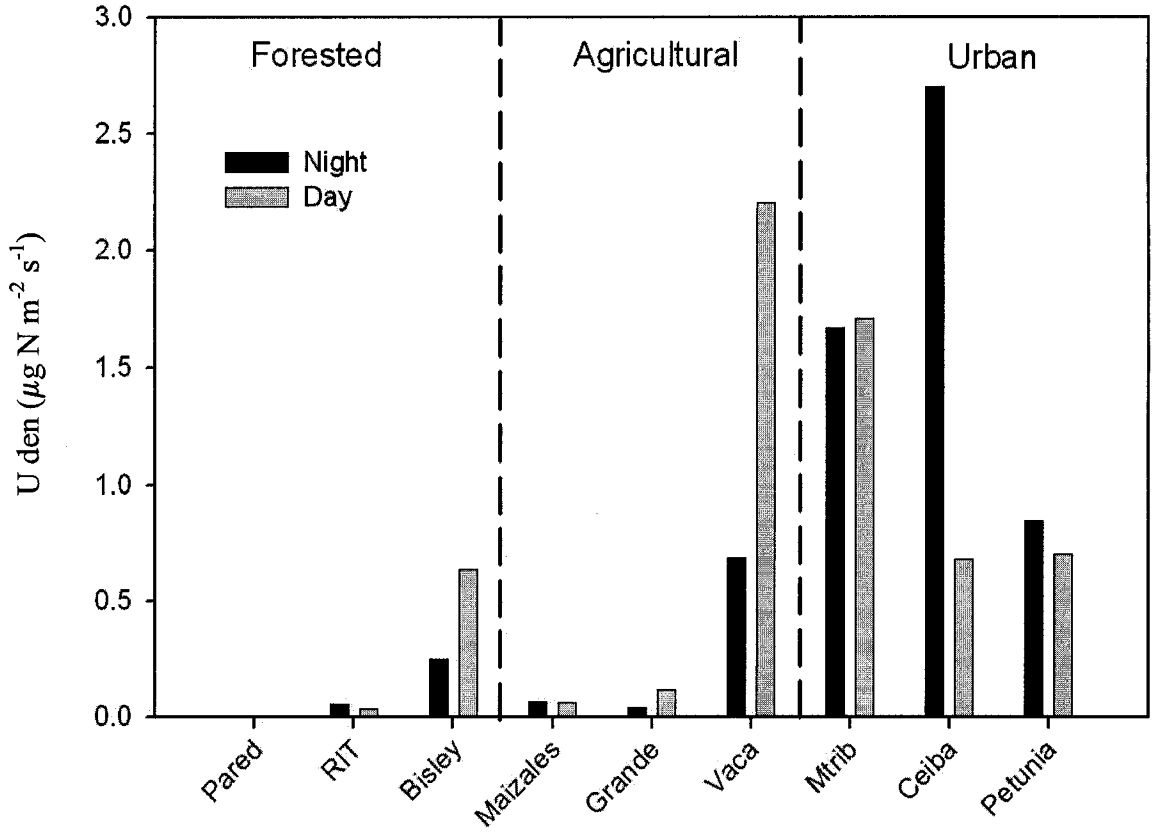


Figure 12: Areal denitrification rate as a function of a) NO_3 concentration, b) stream water depth, c) DON concentration, d) ecosystem respiration (R), e) fine benthic organic matter (FBOM) standing stocks, f) specific discharge (Q/w) and g) FBOM carbon to nitrogen ratio.

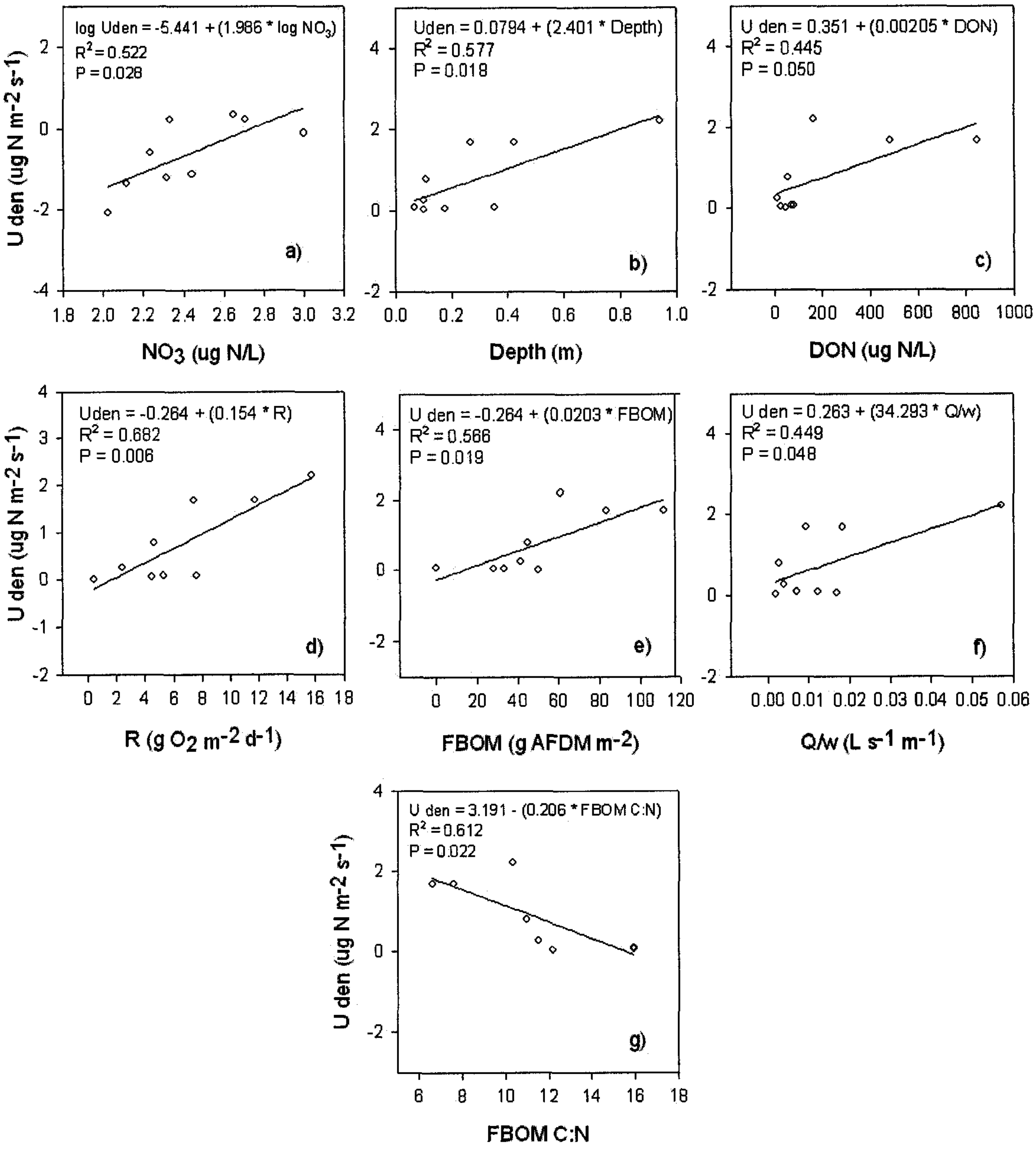


Figure 13: The relationship between denitrification mass transfer velocity (V_f den) and NO_3 concentration, which was not significant.

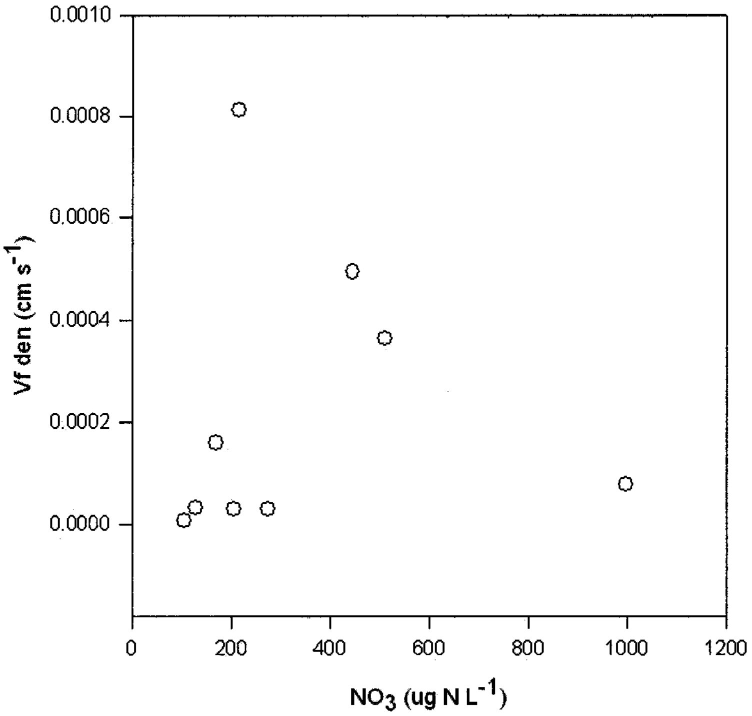


Figure 14: Denitrification rate (kden) as a fraction of NO_3 uptake rate (kNO_3) in the 9 streams.

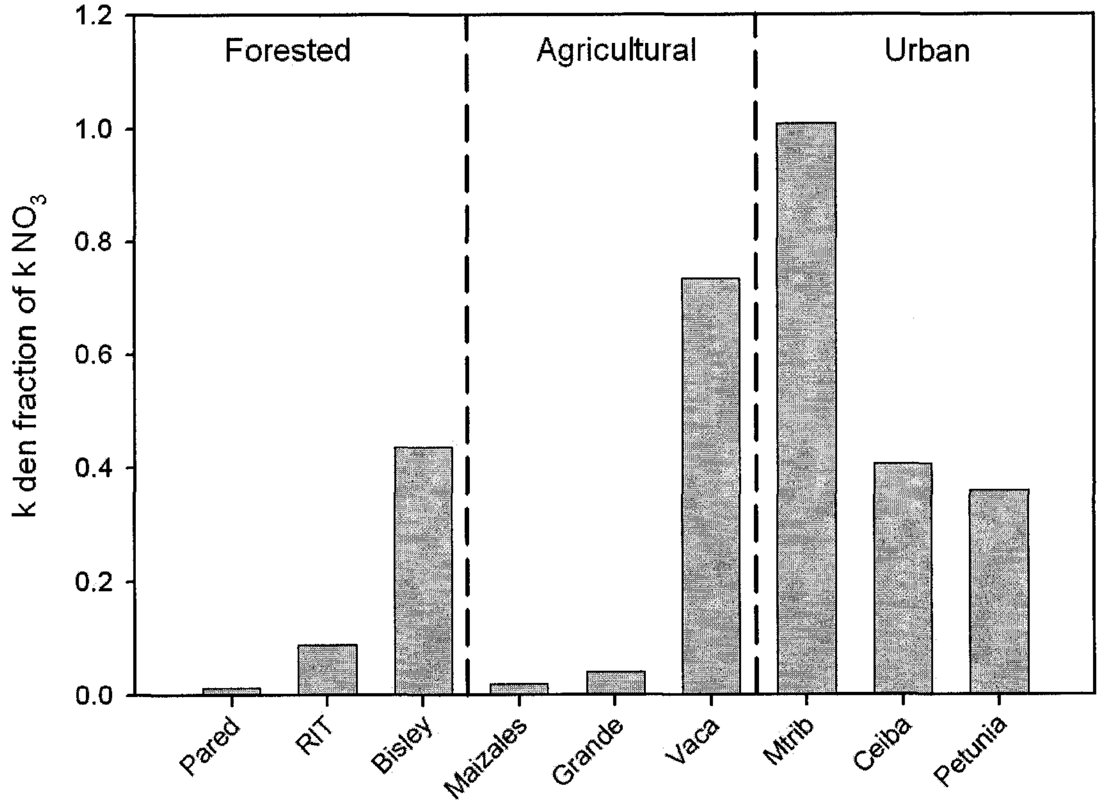
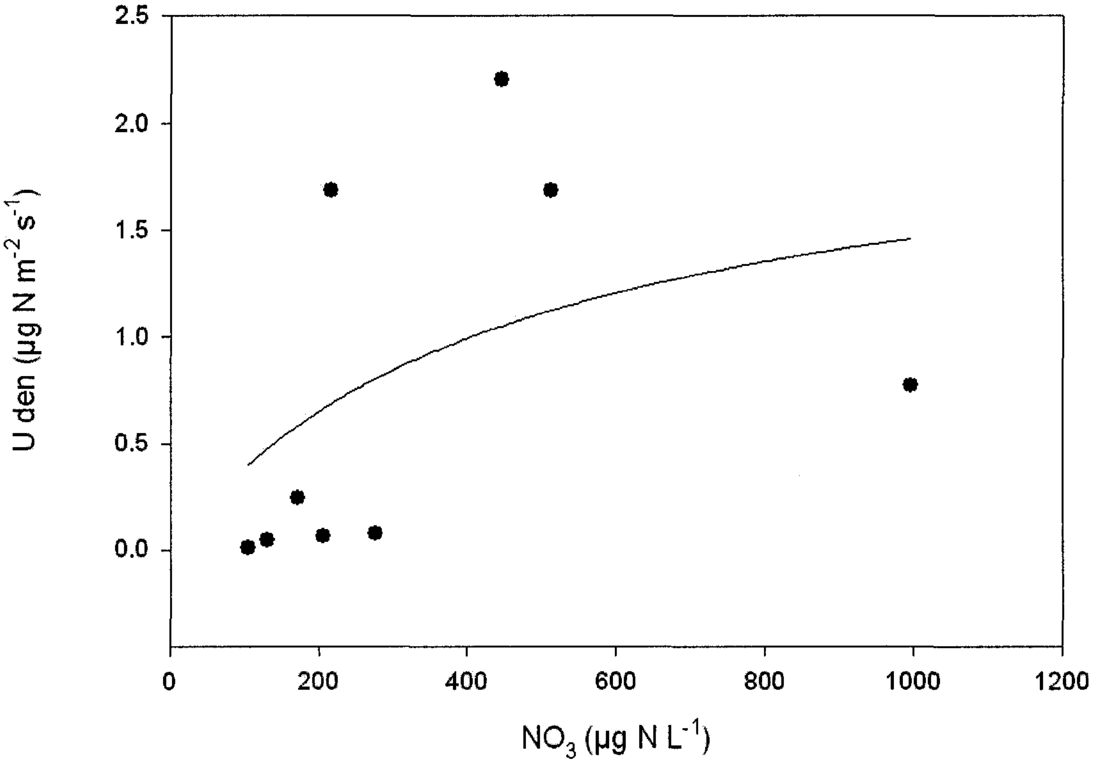


Figure 15: Areal denitrification rate (*U den*) as a function of nitrate concentration across the 9 streams of this study. The line represents the Michaelis-Menten model using non-linear regression



CHAPTER III

DISCUSSION

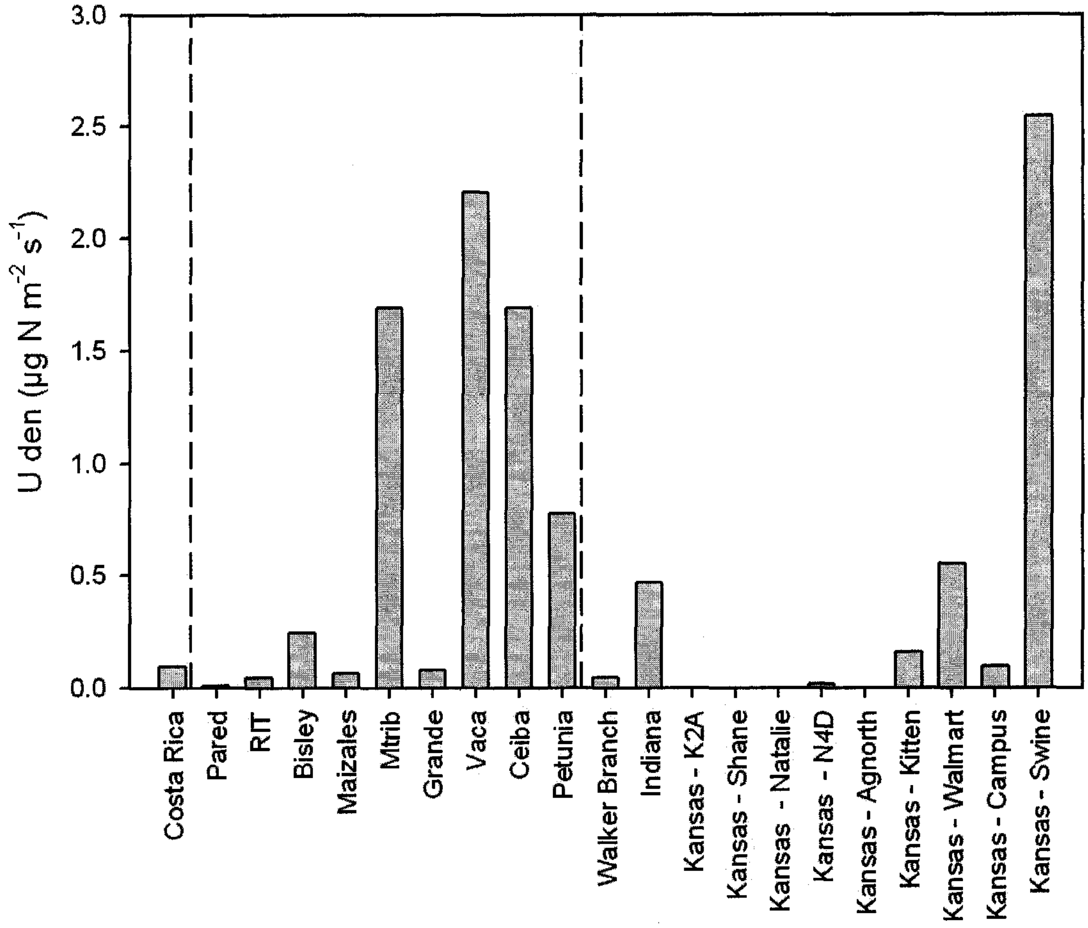
NO_3 Uptake in Tropical Streams

The data reported here provide some of the first empirical evidence that denitrification is a significant fraction of total N uptake in tropical streams. Denitrification was a significant fraction of NO_3 uptake in 5 of the 9 streams (Figure 14), comprising greater than 35% of uptake (mean = 33%). These results were slightly skewed by MTrib where the sewage input increased NH_4 concentrations from a background of 34 μg N L^{-1} to 5658 μg N L^{-1} at the start of the reach. This ammonium was taken up quickly, through both assimilation and nitrification, and benthic N demand was mostly met by NH_4. NO_3 assimilation was only 2% of uptake, so the only uptake of NO_3 was from denitrification. If MTrib is taken out, then denitrification still comprises 25% of NO_3 uptake on average. In their study on the importance of N removal in headwater streams in the Mississippi River basin, Alexander et al (2000) estimated an N mean annual loss rate (k_t) via denitrification of 45.5% d^{-1}. In headwater streams of Puerto Rico, the average k_t was 67% d^{-1} across streams. Another indicator of the impact of denitrification on stream N concentrations is the N:P ratio, where lower ratios can be a result of denitrification (Seitzinger et al 1988). The ratio of DIN to orthophosphate in my streams was low in comparison to streams that had high denitrification rates and similar orthophosphate concentrations in other regions in

LINX II (Mulholland, unpublished), apparently again showing the effectiveness of denitrification in Puerto Rican streams.

Denitrification rates in streams of Puerto Rico were highly variable within and across streams. In comparison to other published values (Figure 16), the rates found here at the high end of the range of values reported for streams across a variety of biomes and land uses (Seitzinger et al. 1988; Kemp & Dodds 2002; Inwood et al. 2005). Areal denitrification rates in another tropical study ($0.019 - 0.097$ μg N m^{-2} s^{-1}; Duff et al. 1996) were lower than most of the values I measured. The Duff et al. (1996) study, as in most studies of stream denitrification, measured rates by the C_2H_2 inhibition technique and by using benthic cores, approaches which are difficult to compare to the whole stream ^{15}N technique used here, as explained by Mulholland et al. (2004). There are three published studies to date that have used the reach-scale ^{15}N tracer technique to quantify ambient rates of denitrification. Areal denitrification rates were higher in all but two of my streams compared to Walker Branch (Mulholland et al. 2004), but this can be mostly explained by the higher NO_3 concentrations in my streams. My results are comparable to those found in Bohlke et al. (2004), but we had some substantially higher rates at similar NO_3 concentrations, discharge, and depth. This is also the case in comparison to several streams in Kansas that were part of the LINX II project (O'Brien et al. 2007), as rates in Puerto Rican streams were higher at similar NO_3 concentrations. The only stream that had a higher rate than in any of my streams was a Kansas stream that had NO_3 concentrations over 21 mg/L. This trend of higher denitrification rates in streams with similar NO_3 also appears to be the case when compared to streams using other techniques (Seitzinger et al. 1988), which supports my prediction that tropical streams have greater potential for denitrification than temperate streams.

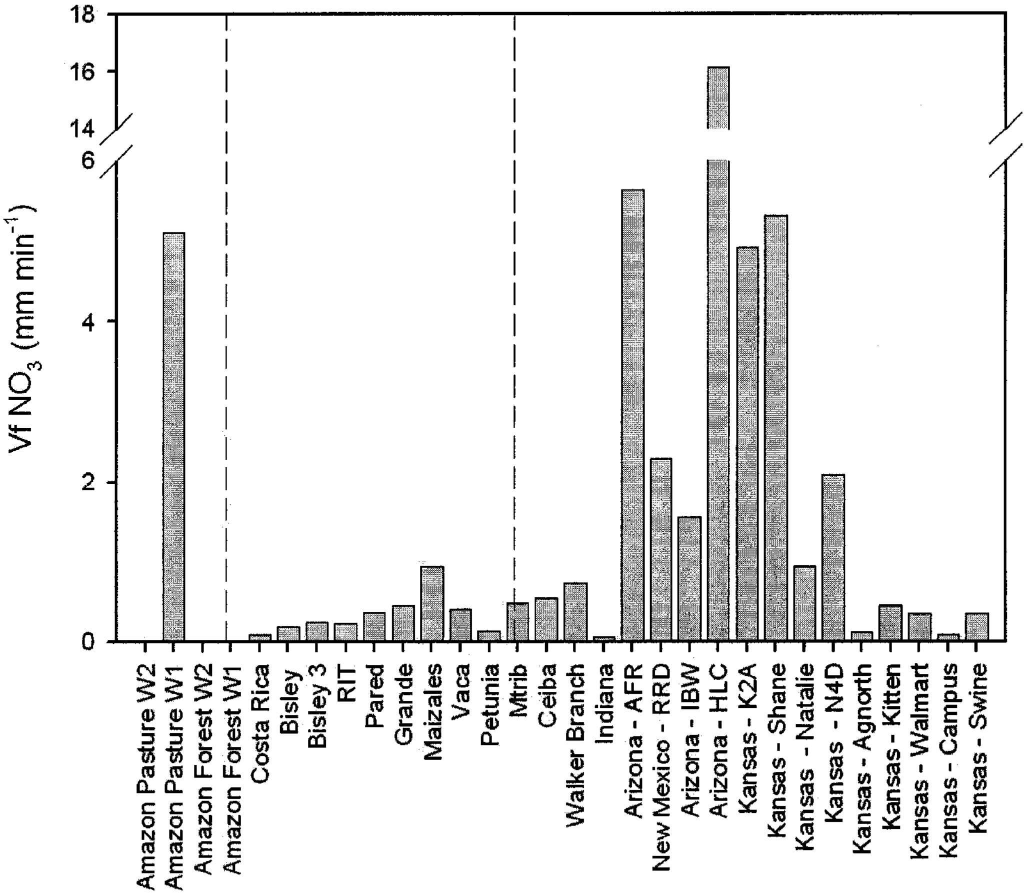
Figure 16: Denitrification rates (U den) in streams of this study (between dotted lines), in an acetylene block study in tropical Costa Rica (left of left dotted line; Duff et al. 1996), and in $^{15}NO_3$ tracer studies (right of right dotted line) in North America (Walker Branch, Mulholland et al. 2004; Indiana, Bohlke et al. 2004; Kansas, O'Brien et al 2007).



The importance of total NO_3 uptake in the delivery of N to the coastal ocean of Puerto Rico is evident in the fact that NO_3 is the dominant form of dissolved N in 7 of the 9 streams of this study. Nutrient uptake efficiency (V_f) is useful when comparing streams (Davis & Minshall 1999; Wollheim et al. 2001) because it reduces the variability associated with stream depth and velocity (S_W varies with depth and velocity) and because uptake rates (U) vary with concentration. V_f is therefore primarily a biological measure, describing the efficiency of removal for a given concentration of nutrients in the stream. Total uptake efficiency in the 9 streams was within the range of other tropical stream studies (Figure 17), although data from other tropical stream studies is limited and methodology varied across studies. In a fertilization study, uptake was undetectable in 3 of the 4 streams in the Amazon (Neill et al. 2001), but in one of the pasture streams V_f was much higher than V_f in all of my streams. Their value of V_f is likely an overestimate as uptake efficiency decreases with increasing nutrient concentration (Davis & Minshall 1999). Neill et al. (2001) attributed this high efficiency to lowered delivery of NO_3 from the pasture soils and because of high potential for denitrification. In a lowland stream in Costa Rica (Duff et al. 1996), uptake efficiency was below the V_f in all of my streams. They measured NO_3 uptake in stream sediments using benthic cores, so the lower uptake value is likely due to restricting uptake to the sediment. Merriam et al. (2002) measured NO_3 uptake from $^{15}NH_4$ injections in one of the stream reaches of this study (Q. Bisley) and the results were similar (0.192 and 0.241 mm min^{-1} respectively).

Comparisons to other $^{15}NO_3$ tracer studies are ideal because it is a more accurate method for determining ambient nutrient uptake rates (Mulholland et al. 2002). NO_3 uptake efficiency in Puerto Rico is in the low range of values reported from other regions (Figure 17). My streams have higher V_f than an agricultural stream in Indiana (Bohlke et al. 2004), which had NO_3 concentrations at the high end of the range in my stream nitrate concentrations. Uptake efficiency was higher in a heavily forested stream in Tennessee (Walker Branch, Mulholland et al. 2004) than in my forested streams. Uptake efficiency is high in N limited streams in the prairie (O'Brien et al. 2007), but uptake efficiency is similar to my streams when NO_3 concentrations are similar. On the other hand, N limited streams with potential for high primary productivity in the desert southwest (Grimm et al. 2005) have a much higher efficiency even at higher levels of NO_3 loading than what is found in my streams. These results suggest that N dynamics in my streams are similar to those in other regions that have either high NO_3 concentrations or are highly heterotrophic, but total N uptake are below those found in highly autotrophic, N-limited desert streams.

Figure 17: Comparison of total nitrate uptake velocity (V_f) between streams of this study and other published values. Values between the dotted lines are values from this study. Values to the left of the dotted line on the left side are from studies of tropical streams with different methodologies (Amazon, Neill et al. 2001, solute addition; Costa Rica, Duff et al. 1996, benthic cores; Bisley PR, Merriam et al. 2002, ${}^{15}NH_4$ tracer addition). Values to the right of right dotted line are values from other regions that use the ${}^{15}NO_3$ tracer approach (Walker Branch, Mulholland et al. 2004; Indiana, Bohlke et al. 2004; Arizona & New Mexico, Grimm et al. 2005; Kansas, O'Brien et al 2007). V_f values are arranged in increasing concentration from left to right for each study.



Controls on Total NO_3 Uptake

Uptake lengths in my streams were relatively long and this was expected with high NO_3 concentrations even in relatively pristine watersheds (105 – 171 μg N L^{-1}). Uptake lengths were the shortest in streams that had low specific discharge (Q. Pared) or had P/R above 1 (Q. Maizales). A low specific discharge primarily enhances contact time of stream water with the stream bottom, thus enhancing benthic uptake. In contrast to Q. Pared, Q. Vaca was especially deep relative to its flow (specific discharge was 30 times higher) and this translated into NO_3 uptake (S_W) that was over 27 times longer. Stream hydrology has been shown to control nitrate S_W in streams in New Mexico (Valett et al. 1996), but uptake length was only correlated with specific discharge here and not with discharge, velocity, and transient storage zone size as in the New Mexico study.

Transient storage describes the temporary retention of water separate from the main advection of the stream thalweg. Nutrient retention has been found to be correlated with the hydraulic storage that occurs in the hyporheic zone (Valett et al. 1996) and in the stream channel (Hall et al. 1998; Gucker & Boechat 2004; Ensign & Doyle 2005). NO_3 uptake in my Puerto Rican streams was unrelated to any of the transient storage parameters that I measured (Table 3). The lack of relationships is likely due to the relative lack of hydraulic variability compared to biotic variability in my streams, as was seen in the original LINX study (Webster et al. 2003). For instance, using what some consider the superior measure of transient storage for comparing between different streams (Runkel 2002; Ensign & Doyle 2005), values of F_{med}^{200} (median travel time attributable to transient storage) ranged from 0-24%. In tropical streams of South America where a relationship between NH_4 uptake and transient storage was found (Gucker & Boechat 2004), F_{med}^{200} ranged from 12-95%. The stream reaches of their study examined different morphotypes, including swamp, run, step-pool, and meandering. In my study morphological variability was often found within each stream rather than among streams and variability was not at the same scale (streams included only run and step-pool). The ratio of the hydraulic uptake length to the NO_3 uptake length (S_H/S_W) can be used to describe the potential role of transient storage in nutrient uptake (Ensign & Doyle 2006). Values above 1 indicate that uptake occurs before water reaches the transient storage zone. In my streams S_H/S_W averaged 0.7, which is an indication that there is potential for NO_3 uptake to occur in transient storage zones even though these relationships were not found.

The best predictors of spatial variability in NO_3 uptake were biological, which concurs with the majority of temperate studies that have shown that variability in U and V_f would be best predicted by biotic variables (Hall & Tank 2003; Webster et al. 2003; Mulholland et al. 2006). The high P/R and short S_W in Q. Maizales was due to a large standing crop of filamentous algae, where growth was made possible by an open canopy and an extended period of base flow. This relationship between short nitrate S_W and algal abundance has been demonstrated in streams of the desert southwest (Marti et al. 1997) where production of algae after floods results in an increase in N retention efficiency.

The heavily forested streams in Puerto Rico had very consistent total NO_3 V_f, while V_f in streams with anthropogenic impacts had significant variation among streams and even within streams (not due to day/night variation). This might be an indication of the in-stream variability caused by anthropogenic impacts, such as point source inputs. Total NO_3 V_f had a significant correlation with GPP, PAR, and epilithon chlorophyll a, indicating that biotic N demand in Puerto Rican streams is mostly autotrophic. In streams in the Grand Teton National Park with comparable V_f values and stream light variation to this study, Hall and Tank (2003) also concluded that autotrophic production (and not R) could account for most NO_3 uptake. The importance of autotrophy in the regulation of NO_3 concentration in even heavily forested headwater streams can also be seen diurnally in Walker Branch, Tennessee (Mulholland et al. 2006), but I did not see a discernable diurnal pattern in my streams. In the West Fork of Walker Branch in April they found that NO_3 uptake declined though the night with the depletion of photosynthate generated during the day and that there were no detectable differences in NO_3 uptake between midnight and midday (1 p.m.), although they did see differences between midnight and predawn (6 a.m.). The midnight-midday sampling coincides with the approximate times that I sampled uptake, so in order to see clear diurnal patterns in uptake I may need to sample in the predawn hours when uptake is lowest. At any rate, the importance of autotrophic N demand is apparent in Puerto Rico in comparisons among streams. Association of N demand with autotrophs suggests that light limitation ultimately drives biological N assimilation in streams across a variety of landscapes in Puerto Rico. Studies have also found energy limitation to be the driver of N dynamics in heavily forested streams in the LEF (Merriam et al. 2002) where there is little light reaching the stream. Across our gradient of streams inside and outside the LEF, it appears that energy and light limitation ultimately drive N assimilation even in streams where there are anthropogenic nutrient inputs. It is important to note that even where the potential exists for high autotrophic production in streams of Puerto Rico, the frequency and intensity of storms and the high rates of herbivory often suppress rates of GPP (Ortiz-Zayas et al. 2005) and therefore often influence N dynamics indirectly.

Controls on Denitrification

The factors that have been shown to control denitrification are the supply of NO_3 and organic carbon and the absence of oxygen. In their review of denitrification in aquatic ecosystems, Seitzinger et al. (1988) found that the highest denitrification rates were from streams and rivers that receive high anthropogenic nutrient inputs. This trend was also apparent in my streams, as stream water NO_3 concentration explained 52% of the variation in denitrification rate.

Variation in stream water dissolved oxygen concentrations did not explain variability in denitrification rates among my study sites, contrary to what has been found in other studies (Kemp & Dodds 2002; Inwood et al. 2005). However, stream water depth and specific discharge were found to be controlling variables and might be a surrogate for sediment oxygen levels, since oxygen should decrease with water depth. Also, stream water oxygen might not be indicative of the oxygen environment below the sediment-water interface. Christensen et al. (1990) reported that denitrification is inversely proportional to the thickness of the sediment oxic surface layer, which was found to extend no more than 7 mm into the sediments.

Another relationship that has significance here is the strong relationship between denitrification rates and FBOM standing stock. In their examination of denitrification on variety substrata, Kemp and Dodds (2002) found the highest denitrification rate was associated with FBOM. They attributed this to the fact that FBOM has high particle density, which creates abundant anoxic zones. I observed higher denitrification rates with lower FBOM C:N, suggesting that higher rates of denitrification are associated with a substrate that consisted of a more labile C source.

As with dissolved oxygen, variability in stream water column DOC did not explain variability in denitrification as was found in previous studies. Stream water concentrations of DOC in my streams were low (mean = 1.32 mg C/L) and there appears to be limited potential for water column DOC to serve as a significant C source even in anthropogenically disturbed systems. From these results and the trend of higher denitrification rates with lower FBOM C:N, it appears that particulate C is the C source for denitrification in streams of Puerto Rico.

The fact that R was a strong predictor of denitrification (68% of the variation) and GPP was a strong predictor of total NO_3 V_f rates bodes well for the use of metabolism as a predictor of total NO_3 uptake overall. Two of the highest rates of denitrification in my streams (Q. Vaca and MTrib) did not correspond to the highest concentrations of NO_3. In both of those cases R played a significant role in the high rates of denitrification. In the case of Q. Vaca, the stream was deep and incised with a thick riparian zone, which was more conducive to accumulation of organic matter and higher R. In the case of MTrib, the sewage input created a system that had low saturation of oxygen (61%) and a substantial supply of FBOM. It appears that in my streams where there is an abundant supply of NO_3 even in pristine watersheds, the physical variables that cause an increase in R (low gradient, greater depth and discharge, greater abundance of FBOM, warmer water temperatures) are the factors that drive the highest rates of denitrification. The majority of streams where these physical attributes are present in Puerto Rico are in watersheds with anthropogenic impacts and elevated NO_3 inputs, since forested areas are typically limited to the mountainous interior. This has significant implications on ecosystem dynamics of the whole river network.

As stated earlier in the discussion, the data reported here provide some of the first empirical evidence that denitrification is a significant fraction of total N uptake in tropical streams. These high rates of denitrification in tropical streams are likely due to denitrifying organisms evolving in an environment where there are climatic factors that lead to high rates of R (year round warm water temperatures, large inputs of organic matter, high rainfall) and high ambient NO_3. While my study was conducted under idealized conditions of base flow and my values represent rates at only one time of year, it does help to illustrate the importance of denitrification in the removal of N in Puerto Rican streams. This is especially true if you consider that these denitrification rates are the minimum, as they only includes denitrification of water column NO_3 and not the coupled nitrification-denitrification pathway and future denitrification of assimilated NO_3

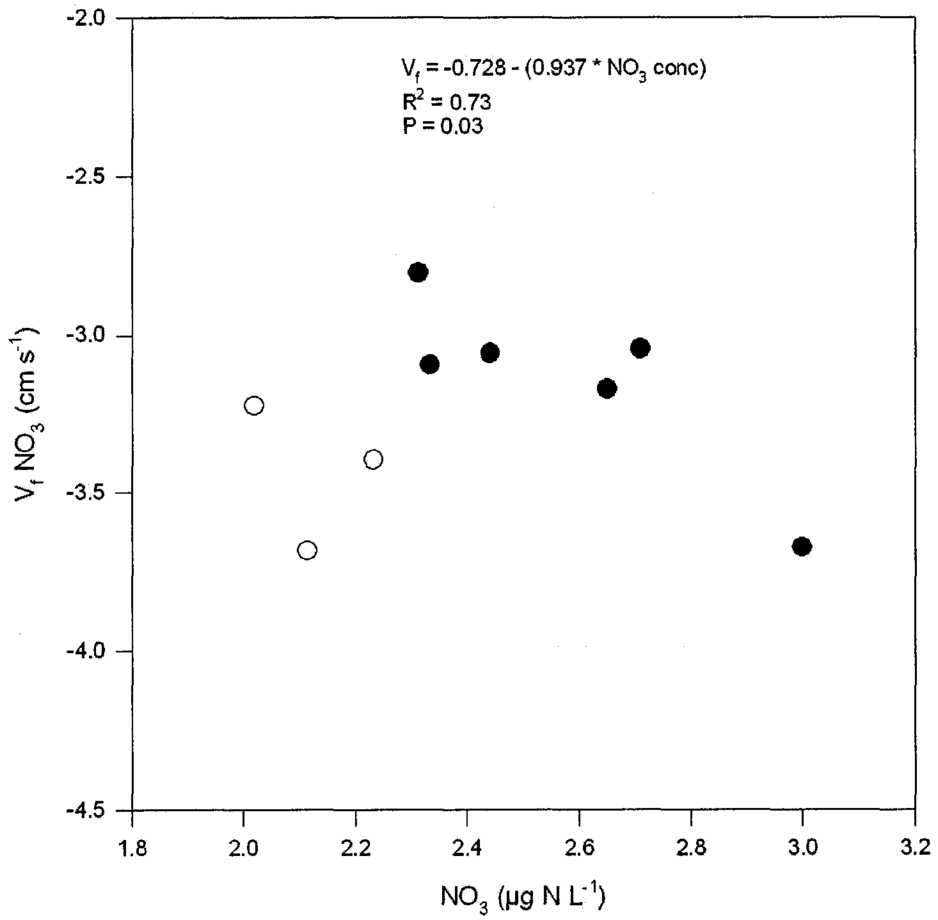
(Seitzinger et al. 2006), nor the denitrification associated with riparian N retention, which can be substantial (Chestnut and McDowell 2000).

NO_3 Saturation & Retention Efficiency

The Michaelis-Menten model better explains the biotic response to increasing NO_3 concentration for both total NO_3 uptake and denitrification. The NO_3 concentrations in my streams only span a wide enough range of concentrations to approach saturation in one stream (Q Petunia) however, even though N loading in Puerto Rican streams is pervasive. In a survey of 42 streams over 3 years in an urbanizing watershed in Puerto Rico (Rio Piedras), mean NO_3 concentrations were 736 μg N L^{-1} and several streams had concentrations between 1000 and 2500 μg N L^{-1} (Potter et al. unpublished). This level of N loading likely would help fill in the details about N saturation in the tropical streams.

It was surprising that there was not a significant relationship between NO_3 uptake efficiency (V_f) or denitrification efficiency (V_f *den*) and NO_3 concentration. I expected that as NO_3 concentrations increased with anthropogenic impacts, that there would be a reduction in the ability of these streams to retain N (Bernot & Dodds 2005). There was a relationship between uptake efficiency and NO_3 concentration that was significant in the urban and agricultural streams alone, however (Figure 18). This reduction in efficiency along the NO_3 gradient illustrates the need to reduce loading of N to surface waters to maintain their ability to reduce N loading to N-limited coastal waters. The impacted streams behaved differently than the reference streams. This offset has been seen in desert streams (Grimm et al. 2005), although the relationship there was the mirror image of our streams. The reference streams were higher than the urban streams in the desert, whereas my reference streams have a lower V_f vs NO_3 concentration relationship than the human impacted streams. Also, V_f increased with NO_3 concentration in their urban streams, but declined in my urban and agricultural streams. This functional difference is likely due to the fact that streams in the desert southwest are highly N-limited, which is not the case in Puerto Rico. At any rate, it appears that human impacted streams in Puerto Rico behave differently possibly because they have higher rates of biological activity, in terms of stream metabolism (Table 4). The impacts associated with human land use changes, such as the removal of stream side vegetation and the loading of organic wastes that leads to higher rates of GPP and R, is what likely causes these streams to function differently in their processing of inorganic nutrients.

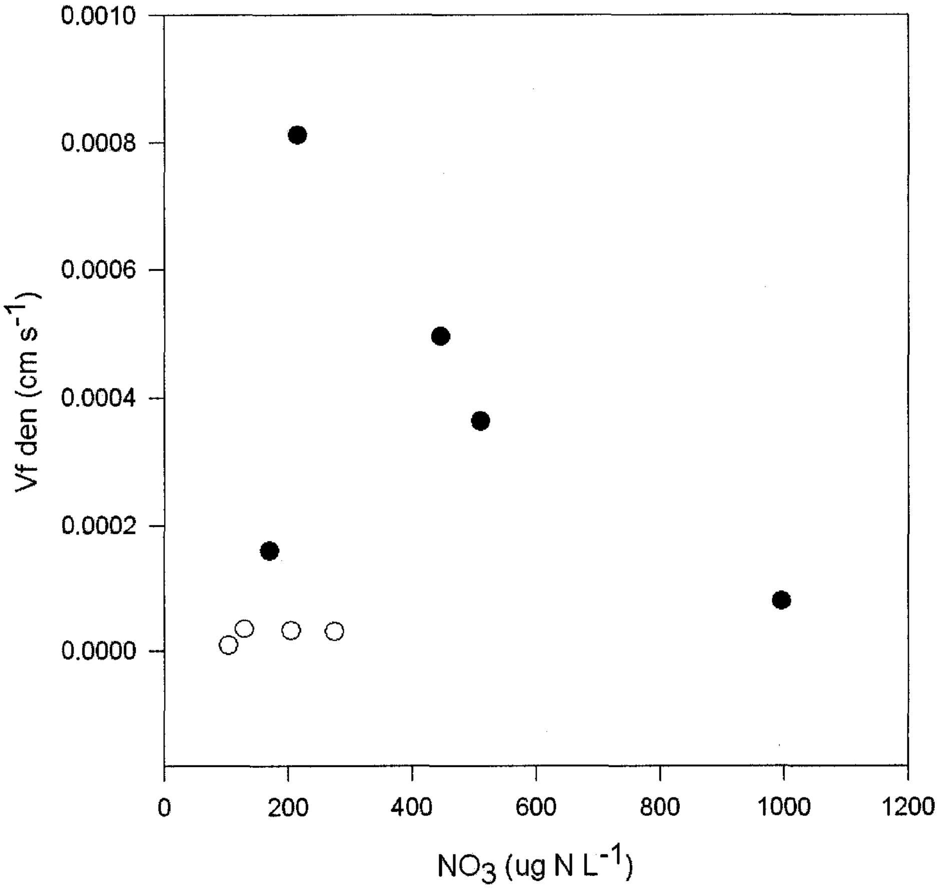
Figure 18: NO_3 mass transfer coefficient (V_f) as a function of NO_3 concentration in the 9 streams of this study (log-log scale). Open circles are forested streams; filled circles are urban and agricultural streams. Regression equation is for the relationship with urban and agricultural streams only.



To further investigate the importance of NO_3 levels in the removal of NO_3, we can look at the relationship between denitrification efficiency and NO_3 concentration (Figure 19). A clear relationship was not found between all sites, but there appears to be a pattern of decreasing NO_3 removal with higher NO_3 loads at two different scales; in streams with denitrification rates below 0.1 μg N m^{-2} s^{-1} and in streams above 0.1 μg N m^{-2} s^{-1}. This pattern of high NO_3 removal at lower NO_3 loads has been found in streams in temperate North America (Kemp &

Dodds 2002; Inwood et al. 2005) and corresponds well with the loss of total uptake efficiency with N loading. These results show that when anthropogenic impacts increase N loading to surface waters these small streams lose their ability to mitigate N loading downstream, which will increase the likelihood of eutrophication in coastal waters.

Figure 19: NO_3 loss as denitrification as a function of NO_3 concentration. Open circles are streams with denitrification rates below 0.1 $\mu g\ N\ m^{-2}\ s^{-1}$; filled circles are streams with denitrification rates above 0.1 $\mu g\ N\ m^{-2}\ s^{-1}$.



Conclusion

Rates of total in-stream uptake of N in streams of Puerto Rico are low, but denitrification rates are high and lead to significant removal of NO_3. As has been seen in the temperate zone, it is the factors that control autotrophic and heterotrophic production that drive inorganic N dynamics in these streams. The relationships found here need to be studied further to find the causes for what controls nitrate uptake and retention.

Headwater streams in the tropics are often under intense pressure from development and their conservation is critical in efforts to mitigate coastal eutrophication. Management efforts should aim to maintain stream and riparian structure to maximize NO_3 removal via denitrification. While these streams have shown the capacity to remove NO_3 at high N loads, their ability to remove N is impaired as NO_3 loads increase. There are several questions that arise from these findings. What fraction of benthic NO_3 assimilation is a sink through denitrification and burial? What role does the benthic community play in the NO_3 cycle in streams (for instance the dominant benthic species, freshwater shrimp *Xiphocaris elongata*, process CBOM into FBOM and might have an effect on denitrification)? How much NO_3 is lost via denitrification from terrestrial to riparian to stream ecosystems in Puerto Rico? Is the NO_3 actually lost in this terrestrial environment with high rates of N-fixation? Insight into these questions will allow us to predict some of the effects of climate change and land use change on the N cycle in the tropics.

LIST OF REFERENCES

Alexander R.B., Smith R.A., & Schwarz G.E. (2000) Effect of stream channel size on the delivery of nitrogen to the Gulf of Mexico. *Nature*, **403**: 758-761.

Bohlke J.K., Harvey J.W., & Voytek M.A. (2004) Reach-scale isotope tracer experiment to quantify denitrification and related processes in a nitrate-rich stream, mid-continent United States. *Limnol. Ocenogr.*, **49**(3): 821-838.

Bowden W.B., McDowell W.H., Asbury C.E., & Finley A.M. (1992) Riparian nitrogen dynamics in two geomorphologically distinct tropical rainforest watersheds: nitrous oxide fluxes. *Biogeochemistry*, **18**: 77-99.

Brandes J.A., McClain M.E., & Pimentel T.P. (1996) ^{15}N evidence for the origin and cycling of inorganic nitrogen in a small Amazonian catchment. *Biogeochemistry*, **34**: 45-56.

Brown S., Lugo A.E., Silander S., & Liegal L. (1983) Research history and opportunities in the Luquillo Experimental Forest. General Techinical Report SO-44, U.S. Department of Agriculture, Forest Service, Southern Forest Experiment Station. 128 pp.

Chestnut T.J. and McDowell W.H. (2000) C and N dynamics in the riparian and hyporheic zones of a tropical stream, Luquillo Mountains, Puerto Rico. *J. North Am. Benthol. Society*, **19**: 199-214.

Christensen P.B., Nielsen L.P., Sorensen J., & Revsbech N.P. (1990) Denitrification in nitrate-rich streams: diurnal and seasonal variation related to benthic oxygen metabolism. *Limnol. Oceanogr.* **35**: 640-651.

Cole J.J., Peirls B.L., Caraco N.F., & Pace M.L. (1993) Nitrogen loading of rivers as a human driven process. In: McDonnell M.J. & Prickett S.T.A. (Eds) Humans as Components of Ecosystems: The Ecology of Subtle Human Effects and Populated Areas (pp 141-157). Springer-Verlag, New York, NY, USA.

Dodds W.K., Lopez A.J., Bowden, W.B., Gregory S., Grimm N.B., Hamilton S.K., Hershey A.E., Marti E., McDowell W.H., Meyer J.L., Morrall D., Mulholland P.J., Peterson B.J., Tank J.L., Valett H.M., Webster J.R., & Wollheim W. (2002) N uptake as a function of concentration in streams. *J. N. Am. Benthol. Soc.*, **22**(2): 206-220.

Downing J.A., McClain M., Twilley R., Melack J.M., Elser J., Rabalais N.N., Lewis, JR. W.M., Turner R.E., Corredor J., Soto D., Yanez-Arancibia A., Kopaska J.A., & Howarth R.W. (1999) The impact of accelerating land-use change on the

N-Cycle of tropical aquatic ecosystems: Current conditions and projected changes. *Biogeochem.*, **46**: 104-148.

Duff J.H., Pringle C.M., & Triska F.J. (1996) Nitrate reduction in sediments of lowland tropical streams draining swamp forest in Costa Rica: An ecosystem perspective. *Biogeochemistry*, **33**: 179-196.

Ensign S.H. & Doyle M.W. (2005) In-channel transient storage and associated nutrient retention: Evidence from experimental manipulations. *Limnol. Oceanogr.*, **50**(6): 1740-1751.

Ensign S.H. & Doyle M.W. (2006) Nutrient spiraling in streams and river networks. *J. Geophys. Res,* **111**, GO4009.

Galloway J.N., Schlesinger W.H., Levy II H., Michaels A., & Schnoor J.L. (1995) Nitrogen fixation: Anthropogenic enhancement-environmental response. *Global Biogeochemical Cycles*, **9**: 235-252.

Galloway J.N., Levy H. II, & Kasibhatla P.S. (1994) Year 2020: Consequences of population growth and development on the deposition of oxidized nitrogen. *Ambio* **23**: 120-123.

Grau H.R., Aide T.M., Zimmerman J.K., Thomlinson J.R., Helmer E., & Zou X. (2003) The ecological consequences of socioeconomic and land-use changes in postagricultural Puerto Rico. *BioScience*, **53**: 1159-1168.

Grimm N.B., Sheibley R.W., Crenshaw C.L., Dahm C.N., Roach W.J., & Zeglin L.H. (2005) N retention and transformation in urban streams. *J. N. Am. Benthol. Soc.*, **24**(3): 626-642.

Gucker, B. & Boechat I.G. (2004) Stream morphology controls ammonium retention in tropical headwaters. *Ecology*, **85**(10): 2818-2827.

Hall R.O., Peterson B.J., & Meyer J.L. (1998) Testing a nitrogen cycling model of a forest stream by using a nitrogen-15 tracer addition. *Ecosystems*, **1**: 283-298.

Hall R.O. & Tank J.L. (2003) Ecosystem metabolism controls nitrogen uptake in streams in Grand Teton National Park, Wyoming. *Limnol. Oceanogr*, **48**(3): 1120-1128.

Holmes R.M., McClelland J.W., Sigman D.M., Fry B., & Peterson B.J. (1998) Measuring ^{15}N-NH$_4^+$ in marine, estuarine, and fresh waters: An adaptation of the ammonia diffusion method for samples with low ammonium concentrations. *Marine Chemistry*, **60**: 235-243.

Inwood S.E., Tank J.L., & Bernot M.J. (2005) Patterns of denitrification associated with land use in 9 midwestern headwater streams. *J. N. Am. Benthol. Soc.*, **24**(2): 227-245.

Lewis JR. W.M., Melack J.M., McDowell W.H., McClain M., & Richey J.E. (1999) Nitrogen yields from undisturbed watersheds in the Americas. *Biogeochem.*, **46**: 149-162.

Marti E., Grimm N.B., & Fisher S.G. (1997) Pre- and post-flood retention efficiency of nitrogen in a Sonoran Desert stream. *J. N. Am. Benthol. Soc.*, **16**(4): 805-819.

Matson P.A., McDowell W.H., Townsend A.R., & Vitousek P.M. (1999) The globalization of N deposition: ecosystem consequences in tropical environments. *Biogeochemistry*, **46**: 67-83.

McClain M.E., Boyer E. W, Dent C. L., Gergel S. E., Grimm N. B., Groffman P. M., Hart S. C., Harvey J. W., Johnston C. A., Mayorga E., McDowell W. H., & Pinay G. (2003) Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems. *Ecosystems*, **6**: 301-312.

McDowell W.H., Bowden W.B., & Asbury C.E (1992) Riparian nitrogen dynamics in two geomorphologically distinct tropical rainforest watersheds: subsurface solute patterns. *Biogeochemistry*, **18**: 53-75.

McDowell W.H. & Asbury C.E. (1994) Export of carbon, nitrogen, and major ions form three tropical montane watersheds. *Limnol. Oceanogr*, **39**(1): 111-125.

McDowell W.H., Lugo A.E., & James A. (1995) Export of nutrients and major ions from Catibbean catchments. *J. North Am. Benthol*, **14**(1): 12-20.

McDowell W.H., McSwiney C.P., & Bowden W.B. (1996) Effects of hurricane disturbance on groundwater chemistry and riparian function in a tropical rainforest. *Biotropica*, **28**: 577-584.

McDowell W.H. (2001) Hurricanes, people, and riparian zones: controls on nutrient losses from forested Caribbean watersheds. *For. Ecol. Manage.*, **154**: 443-451.

Meybeck, M. (1982) Carbon, nitrogen, and phosphorous transport by world rivers. *Am. J. Sci.*, **282**: 401-450.

Merriam J.L., McDowell W.H., Tank J.L., Wollheim W.M., Crenshaw C.L., & Johnson S.L. (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., Tank J.L., Webster J.R., Bowden W.B., Dodds W.K., Gregory S.V., Grimm N.B., Hamilton S.K., Johnson S.L., Marti E., McDowell W.H., Merriam J.L., Meyer J.L., Peterson B.J., Valett H.M., & Wollheim W.M. (2002) Can uptake length in streams be determined by nutrient addition experiments? Results from an interbiome comparison study. *J. N. Am. Benthol. Soc.*, **21**(**4**): 544-560.

Mulholland P.J., Valett H.M., Webster J.R., Thomas S.A., Cooper L.W., Hamilton S.K., & Peterson B.J. (2004) Stream denitrification and total nitrate uptake rates measured using a field ^{15}N tracer addition approach. *Limnol. Oceanogr*, **49(3)**: 809-820.

Mulholland P.J., Thomas S.A., Valett H.M., Webster J.R., & Beaulieu J. (2006) Effects of light on NO_3^- uptake in small forested streams: diurnal and day-to-day variations. *J. N. Am. Benthol. Soc.*, **25**(3): 583-595.

Neill C., Deegan L.A., Thomas S.M., Haupert C.L., Krusche A.V., Ballester V.M., & Victoria C.L. (2006) Deforestation alters the hydraulic and biogeochemical characteristics of small lowland Amazonian streams. *Hydrol. Process.* **20**: 2563-2580.

Newbold J.D., Elwood J.W., O'Neill R.V., & Van Winkle W. (1981) Measuring nutrient spiraling in streams. *Canadian Journal of Fisheries and Aquatic Sciences*, **38**: 860-863.

O'Brien J. M., W. K. Dodds, K. C. Wilson, J. N. Murdock, & J. Eichmiller. (2007) The saturation of N cycling in Central Plains streams: 15N experiments across a broad gradient of nitrate concentrations. Biogeochemistry DIO 10.1007/s10533-007-9073-7.

Ortiz-Zayas J.R., Cuevas E., Mayo-Bracero O.L., Donoso L., Trebs I., Figueroa-Nieves D., & McDowell W.H. (2006) Urban influences on the nitrogen cycle in Puerto Rico. *Biogeochemistry*,

Ortiz-Zayas J.R., Lewis Jr. W.M., Saunders III J.F., & McCutchan Jr. J.H. Metabolism of a tropical rainforest stream. *J. N. Am. Benthol. Soc.*, **24**(4): 769-783.

Peterson B.J., Wollheim W.M., Mulholland P.J., Webster J.R., Meyer J.L., Tank J.L., Marti E., Bowden W.B., Valett H.M., Hershey A.E., McDowell W.H., Dodds W.K., Hamilton S.K., Gregory S., & Morrall D.D. (2001) Control of Nitrogen Export from Watersheds by Headwater Streams. *Science*, **292**: 6 April 2001, 86-90.

Pringle C.M., Paaby-Hansen P., Vaux P.D., & Goldman C.R. (1986) *In situ* nutrient assays of periphyton growth in a lowland Costa Rican stream. *Hydrobiologia*, **134**: 207-213.

Runkel R.L. (2002) A new metric for determining the importance of transient storage. *J. N. Am. Benthol. Soc.*, **21**: 529-543.

Sartory, D. P. & J. E. Grobbelaar. (1984) Extraction of chlorophyll *a* from freshwater phytoplankton for spectrophotometric analysis. *Hydrobiologia*, **114**:177-187.

Seitzinger S.P. (1988) Denitrification in freshwater and coastal marine ecosystems: Ecological and geochemical significance. *Limnol. Oceanogr.* **33**(**4**): 702-724.

Seitzinger S.P., Harrison J.A., Bohlke J.K., Bouwman A.F., Lowrance R., Peterson B., Tobias C., & Van Drecht G. (2006) Denitrification across landscapes and waterscapes: A synthesis. *Ecol. Appl.* **16**(6): 2064-2090.

Sigman D.M., Altabet M.A., Michener R., McCorkle D.C., Fry B., Holmes R.M. (1997) Natural abundance-level measurement of the nitrogen isotopic composition of oceanic nitrate: an adaptation of the ammonia diffusion method. *Marine Chemistry*: **57**: 227-242.

Stream Solute Workshop (1990) Concepts and methods for assessing solute dynamics in stream ecosystems. *J. N. Am. Benthol. Soc.*, **9**(2): 95-119.

Valderrama J.C. (1981) The simultaneous analysis of total nitrogen and phosphorus in natural waters. *Mar. Chem*, **10**:109-122.

Valett H.M, Morrice J.A., Dahm C.N., & Campana M.E. (1996) Parent lithology, surface-groundwater exchange, and nitrate retention in headwater streams. *Limnol. Oceanogr.*, **41**(2): 333-345.

Vitousek P.M., Aber J.D., Howarth R.W., Likens G.E., Matson P.A., Shindler D.W., Schlensinger W.H., & Tilman D.G. (1997) Human alteration of the global nitrogen cycle: sources and consequences. *Ecological Applications*, **7**(3): 737-750.

Webster J.R. & Patten B.C. (1979) Effects of watershed pertubations on stream potassium and calcium dynamics. *Ecol. Monog*, **49**: 51-72.

Wollheim W.M., Peterson B.J., Deegan, L.A., Hobbie J.E., Hooker B., Bowden W.B., Edwardson K.J., Arscott D.B., Hershey A.E., & Finlay J. (2001) Influence of stream size on ammonium and suspended particulate nitrogen processing. *Limnol. Oceanogr.*, **46**: 1-13.

APPENDICES

APPENDIX A
Stream nitrate uptake rates

Site	Stream name	LU type	k NO_3 m^{-1}	V_f NO_3 (cm/s)	U NO_3 $ugN/m^2/s$
Plat 1	Bisley	REF	1.09E-03	3.83E-04	0.5937
Plat 2	Bisley	REF	9.78E-04	4.19E-04	0.7816
Plat 1	RIT	REF	2.42E-04	4.24E-04	0.5464
Plat 2	RIT	REF	2.02E-04	3.42E-04	0.4472
Plat 1	Pared	REF	2.93E-03	5.85E-04	0.6056
Plat 2	Pared	REF	3.43E-03	6.06E-04	0.6417
Plat 1	Grande	AGR	7.72E-04	1.01E-03	3.0737
Plat 2	Grande	AGR	4.64E-04	5.22E-04	1.2849
Plat 1	Maizales	AGR	2.08E-03	1.54E-03	3.1080
Plat 2	Maizales	AGR	2.39E-03	1.61E-03	3.3594
Plat 1	Vaca	AGR	2.11E-05	5.32E-05	0.2314
Plat 2	Vaca	AGR	1.18E-04	6.73E-04	3.0009
Plat 1	Petunia	URB	6.01E-04	1.69E-04	1.6868
Plat 2	Petunia	URB	1.07E-03	2.54E-04	2.5284
Plat 1	Mtrib	URB	1.04E-03	9.87E-04	1.7199
Plat 2	Mtrib	URB	6.39E-04	6.21E-04	1.5934
Plat 1	Ceiba	URB	6.60E-04	1.55E-03	6.8841
Plat 2	Ceiba	URB	2.01E-04	2.57E-04	1.4865

Site	Stream name	LU type	kden N_2 m^{-1}	kden N_2O m^{-1}	$V_f$den cm/s	Udentot $ugN/m^2/s$
Plat 1	Bisley	REF	4.51E-04	6.15E-07	1.59E-04	2.47E-01
Plat 2	Bisley	REF	7.97E-04	6.51E-07	3.42E-04	6.37E-01
Plat 1	RIT	REF	2.37E-05	4.98E-07	4.24E-05	5.47E-02
Plat 2	RIT	REF	1.46E-05	4.48E-07	2.55E-05	3.33E-02
Plat 1	Pared	REF	3.84E-05	3.51E-07	7.75E-06	8.02E-03
Plat 2	Pared	REF	4.51E-05	3.19E-07	8.03E-06	8.50E-03
Plat 1	Grande	AGR	9.74E-06	4.09E-07	1.32E-05	4.04E-02
Plat 2	Grande	AGR	4.05E-05	4.43E-07	4.61E-05	1.13E-01
Plat 1	Maizales	AGR	4.28E-05	4.13E-07	3.20E-05	6.47E-02
Plat 2	Maizales	AGR	4.42E-05	2.54E-07	2.99E-05	6.25E-02
Plat 1	Vaca	AGR	6.20E-05	4.53E-07	1.58E-04	6.87E-01
Plat 2	Vaca	AGR	8.63E-05	3.11E-07	4.95E-04	2.20E+00
Plat 1	Petunia	URB	3.00E-04	1.87E-06	8.48E-05	8.47E-01
Plat 2	Petunia	URB	2.95E-04	2.54E-06	7.07E-05	7.04E-01
Plat 1	Mtrib	URB	1.01E-03	5.91E-06	9.58E-04	1.67E+00
Plat 2	Mtrib	URB	6.80E-04	4.12E-06	6.65E-04	1.71E+00
Plat 1	Ceiba	URB	2.56E-04	3.25E-06	6.07E-04	2.70E+00
Plat 2	Ceiba	URB	9.08E-05	8.31E-07	1.17E-04	6.79E-01

APPENDIX B
Raw ^{15}N Data

$\delta^{15}N\text{-}NO_{3\ measured}$

Station	Distance (m)	Pre	Plateau 1	Plateau 2	Post 24	Post 72	Post 1 Week
Q. Bisley							
up	-10				9	4.63	4.65
1	40	3.06	18104	14859	48		
1	40		19786	13136			
2	75	4.55	12742	8574	73		
2	75		12549	10270			
3	130	4.31	12908	8230	72		
3	130		13108	7955			
4	200	4.16	12326	7429	82		
4	200		12104	7480			
5	350	3.67	8347	8469	125		
5	350		8464	9612			
6	425	3.5	10167	8851	122	36.67	14.02
6	425		9711	8964			
RIT							
up	-10				14.1	14.1	14.1
1	60	9.1	2940.3	3341.7	9		
1	60		3155.5	3306.7			
2	115	33.7	2916.7	2939.5	21.1		
2	115			2896.1			
3	190	20.8	2614.9	2902.1	95.8		
3	190		2831.4	2830.4			
4	255	13.8	2584.6	2593.7	19.1		
4	255		2645.3	2603.9			
5	345	10.8	2515.1	2490.6	22.5		
5	345		2410.1	2546.5			
6	425	36.7	2501.9	2313.1	206.6	418.2	1112.7
6	425		2537.8	2414.2			
Q. Pared							
up	-10				400.6	219.8	62.3
1	75	91.7	12602.6	11674.4	213.4		
1	75		12505.6	11466.8			
2	150	477	7176.6	7697.4	62.4		
2	150		9065.3	7574.5			
3	250	549	6293.7	5631.8	226.5		
3	250		6153.7	5096.3			
4	350	577	2439.6	2459.9	249.5		
4	350		2507.2	2123.1			
5	500	71.6	1569.5	1258.9	222.2		
5	500		1518.6	1489.6			
6	600	108.2	2241.8	1155.5	186.6	78.6	651.7
6	600		2313.7	1162.5			

Station	Distance (m)	Pre	Plateau 1	Plateau 2	Post 24	Post 72	Post 1 Week
Q Grande							
up	-10				10	8.88	34.13
1	50	39.1	19178	23167	19		
1	50		11537	23005			
2	100	11.68	19080	21227	121		
2	100		12516	21221			
3	200	35.46	18731	20859	115		
3	200		19180	20906			
4	400	18.42	18310	19674	179		
4	400		17995	19878			
5	600	7.79	16359	17854	131		
5	600		16115	17948			
6	740	7.75	15796	16969	98	95.29	51.98
6	740		15661	17715			
Q. Maizales							
up	-10				17.4	12.4	43.9
1	60	220.6	3780.4	5902.7	31.2		
1	60		3689.9	5875.7			
2	125	294.7	3561.4	6727	52.8		
2	125		3539.8	6793.1			
3	235	231.6	3013.9	3044.6	117.2		
3	235		2974.6	2989.7			
4	325	131.2		2039.4	210.3		
4	325		2357.9	2028.4			
5	450	16.4	1718.1	1377.5	340.1		
5	450		1755	1339.1			
6	510	20.9	1540.9	1280.5	389.7	158.1	85.6
6	510		1519.7	1263.1			
Q. Vaca							
up	-10				66.2	61.9	70.5
1	75	63.5	2345.2	1604.5	23.8		
1	75	DATA	2385.7	1576.2	DATA		
2	145	98.1	2391.8	1567.1	33.3		
2	145	DATA	2381.3	1587.4	DATA		
3	225	71.3	2322.9	1499.3	29.2		
3	225	DATA	2337.2	1483.6	DATA		
4	330	28.8	2345.2	1443.2	26.5		
4	330	DATA	2331.6	1417.6	DATA		
5	450	48.8	2394.6	1354.8	45.6		
5	450	DATA	2372.4	1375.8	DATA		
6	565	70.5	2322.6	1290.2	67.3	44.4	35
6	565	DATA	2325	1257.4	DATA		

Station	Distance (m)	Pre	Plateau 1	Plateau 2	Post 24	Post 72	Post 1 Week
Q. Petunia							
up	-10				17	11.74	36.22
1	25	12.01	37042	32635	28		
1	25		36557	32111			
2	50	11.32	36346	31407	29		
2	50		35883	31502			
3	100	11.13	32855	31360	28		
3	100		32413	31714			
4	150	10.94	30587	29725	45		
4	150		30153	30343			
5	225	10.96	29957	30587	61		
5	225		29226	30632			
6	375	14.18	17052	13602	61	62.99	53.09
6	375		16682	13214			
Mtrib							
up	-10					14.1	16.1
1	35	68.2	3831.2	3451	10.5		
1	35		3749.7	3367.7			
2	70	71	3545.6	3220.9	3.4		
2	70		3590.1	3242.2			
3	130	16	3270.3	2886.7	-0.2		
3	130		3226.5	2830.2			
4	200	28.2	2946.2	2743.3	0.7		
4	200		2918.4	2818			
5	300	4.4	2618	2457.8	4.4		
5	300		2513.3	2441.2			
6	410	2.6	2180.5	2050.4	4.3	8.8	17.3
6	410		2179.3	2048.5			
Q. Ceiba							
up	-10				27.7	22.5	15.3
1	75	21.5	1426	2027.2	14.9		
1	75		1425	2045.4			
2	200	19.8	1459.6	1930.5	32.1		
2	200		1501.7	2025.1			
3	300	21.3	1534.7	1883	14.7		
3	300		1518.9	1886.2			
4	425	23.9	1466.3	1804	21.3		
4	425		1438.7	1780.3			
5	575	19.2	1719.4	1621.1	24.3		
5	575		1658.8	1547.3			
6	700	22.2	1741.7	1515	25.8	27.9	32.6
6	700		1694.3	1510			

Gas ^{15}N and N mass Data

		del $^{15}N_2$ (per mil)			N_2 Mass (mmoles)		
Station	**Distance (m)**	**Pre**	**Plateau 1**	**Plateau 2**	**Pre**	**Plateau 1**	**Plateau 2**
Q. Bisley data from UC Davis							
1	40	1.07	1.34		0.0122	0.0134	
1	40	0.60	1.35	1.52	0.0134	0.0107	0.0119
1B	55	1.03	1.01		0.0165	0.0095	
1B	55	0.90	0.38	1.36	0.0128	0.0113	0.0118
2	75	0.78	0.79		0.0134	0.0135	
2	75	0.81	1.32	0.54	0.0137	0.0097	0.0118
2B	85	0.94	1.36		0.0142	0.0099	
2B	85	0.75	1.08	0.78	0.0154	0.0137	0.0116
3	130	-0.56	1.97		0.0100	0.0088	
3	130	-0.45	1.12	0.39	0.0136	0.0100	0.0124
3B	165	-0.76	1.30		0.0153	0.0097	
3B	165	-0.26	1.36	0.26	0.0105	0.0103	0.0117
4	200	-0.31	0.86		0.0122	0.0088	
4	200	-0.46	0.28	1.46	0.0111	0.0093	0.0107
4B	275	-0.62	0.26		0.0121	0.0094	
4B	275	-0.76	0.53	1.20	0.0120	0.0085	0.0104
5	350	-0.22	-0.49		0.0113	0.0200	
5	350	-0.35	0.06	1.78	0.0115	0.0088	0.0111
6	425	-0.44	-0.12		0.0110	0.0100	
6	425	-0.37	0.49	1.47	0.0110	0.0073	0.0109
RIT data from UC Davis							
1	60	3.75	2.44	2.84	0.0296	0.0303	0.0306
1B	85		2.92	2.77		0.0304	0.0333
2	115	3.46	2.27	3.32	0.0259	0.0331	0.0234
2B	150		2.93	3.93		0.0297	0.0291
3	190	3.00	2.97	2.38	0.0224	0.0334	0.0325
3B	210		2.27	2.90		0.0326	0.0316
4	255	3.51	2.42	3.42	0.0273	0.0356	0.0260
4B	295		3.03	3.51		0.0311	0.0270
5	345	3.45	2.69	3.51	0.0268	0.0322	0.0263
6	425	3.42	3.34	3.21	0.0274	0.0311	0.0247
RIT data from Michigan State							
1	60	0.14	0.44	0.17			
1B	85		0.62	0.59			
2	115	-0.01	0.38	0.58			
2B	150		0.54	0.29			
3	190	0.21	0.67	0.43			
3B	210		0.59	0.52			
4	255	0.26	0.46	0.37			
4B	295		0.60	0.48			
5	345	0.29	0.68	0.44			
6	425	0.34	0.56	0.44			

		del $^{15}N_2$ (per mil)			N_2 Mass (mmoles)		
Station	**Distance (m)**	**Pre**	**Plateau 1**	**Plateau 2**	**Pre**	**Plateau 1**	**Plateau 2**
Q. Pared data from UC Davis							
1	75	-0.22	0.02	0.16	0.0296	0.0281	0.0269
1B	100	-0.26	0.07	0.58	0.0351	0.0286	0.0220
2	150	-0.17	0.37	0.29	0.0330	0.0335	0.0273
2B	200	-0.29	0.01	0.29	0.0341	0.0317	0.0221
3	250	-0.15	0.09	0.59	0.0297	0.0327	0.0172
3B	300	-0.10	-0.10	-0.29	0.0309	0.0305	0.0242
4	350	-0.14	-0.07	-0.08	0.0328	0.0263	0.0253
4B	400	-0.02	-0.17	-0.30	0.0346	0.0312	0.0242
5	500	0.05	-0.20	-0.36	0.0289	0.0292	0.0194
6	600	-0.09	-0.37	-0.20	0.0327	0.0250	0.0190
Q. Pared data from Michigan							
1	75	0.12	0.62	0.63			
1B	100	0.22	0.83	0.91			
2	150	0.32	0.62	0.55			
2B	200	0.32	0.59	0.37			
3	250	0.33	0.54	0.60			
3B	300	0.41	0.30	0.10			
4	350	0.21	0.21	0.11			
4B	400	0.38	0.35	0.28			
5	500	0.41	0.07	0.36			
6	600	0.42	0.16	0.34			
Q Grande							
0B	25	2.39	1.23		0.0119	0.0128	
0B	25		1.21	2.02		0.0124	0.0165
1	50	2.44	1.68		0.0111	0.0122	
1	50		1.61	2.10		0.0118	0.0163
1B	75	2.29	1.96		0.0121	0.0114	
1B	75		2.19	1.97		0.0108	0.0163
2	100	2.04	2.63		0.0125	0.0120	
2	100		2.70	0.23		0.0112	0.0224
2B	150	2.24	2.71		0.0117	0.0107	
2B	150		2.56	2.78		0.0107	0.0146
3	200	2.63	3.13		0.0118	0.0117	
3	200		3.34	4.06		0.0114	0.0146
3B	300	2.63	3.13		0.0116	0.0125	
3B	300		3.62	3.67		0.0107	0.0156
4	400	2.78	2.74		0.0109	0.0101	
4	400		2.63	3.37		0.0117	0.0150
4B	500	1.83	2.40		0.0117	0.0080	
4B	500		2.85	3.36		0.0110	0.0141
5	600	1.98	1.69		0.0111	0.0084	
5	600		2.11	0.34		0.0072	0.0270
Q. Maizales data from UC Davis							
1	60	0.83	0.51	0.16	0.0289	0.0295	0.0294
1B	75		0.57	0.39		0.0331	0.0283
2	125	0.47	1.17	0.46	0.0314	0.0299	0.0311
2B	175		1.81	0.96		0.0295	0.0325
3	235	0.66	1.91	1.71	0.0337	0.0316	0.0276
3B	275		1.80	2.06		0.0346	0.0331
4	325	0.70	2.02	2.34	0.0287	0.0272	0.0313
4B	375		1.60	2.64		0.0333	0.0304
5	450	1.21	1.32	1.91	0.0231	0.0299	0.0284
6	510	0.71	1.08	1.61	0.0322	0.0307	0.0312

		del $^{15}N_2$ (per mil)			N_2 Mass (mmoles)		
Station	**Distance (m)**	**Pre**	**Plateau 1**	**Plateau 2**	**Pre**	**Plateau 1**	**Plateau 2**
Q. Maizales data from Michigan State							
1	60	0.34	0.69	0.10			
1B	75		0.87	0.13			
2	125	0.35	0.98	0.32			
2B	175		1.68	0.60			
3	235	0.28	1.87	1.31			
3B	275		1.78	1.82			
4	325	0.35	2.11	1.90			
4B	375		1.76	2.29			
5	450	0.35	1.25	1.51			
6	510	0.49	1.00	1.50			
Q. Vaca data from UC Davis							
1	75	-0.70		0.27	0.0227		0.0285
1B	115	-0.42	-0.37	-0.11	0.0246	0.0255	0.0293
2	145	-0.38	-0.61	-0.07	0.0180	0.0258	0.0275
2B	175	-0.11	-0.42	0.21	0.0178	0.0242	0.0286
3	225	-0.13	-0.22	0.24	0.0166	0.0264	0.0293
3B	275	-0.20	0.15	0.89	0.0207	0.0244	0.0299
4	330	-0.16	0.06	0.48	0.0179	0.0217	0.0266
4B	375	-0.21	0.53	0.61	0.0192	0.0235	0.0263
5	450	-0.89	0.48	0.34	0.0340	0.0241	0.0286
6	575	-0.36	0.18	0.18	0.0169	0.0230	0.0260
Q. Vaca data from Michigan State							
1	75	-0.12	0.17	0.55			
1B	115	-0.13	0.68	0.40			
2	145	0.22	0.33	0.44			
2B	175	0.14	0.70	0.30			
3	225	-0.34	0.59	0.47			
3B	275	0.42	1.09	1.21			
4	330	-0.21	1.10	0.32			
4B	375	-0.30	1.55	1.13			
5	450	0.85	1.10	0.90			
6	575	-0.33	1.06	0.91			

		del $^{15}N_2$ (per mil)			N_2 Mass (mmoles)		
Station	Distance (m)	Pre	Plateau 1	Plateau 2	Pre	Plateau 1	Plateau 2
Q. Petunia							
1	25	0.92	16.85	31.37	0.0135	0.0145	0.0137
1B	40	1.25	18.20	19.38	0.0109	0.0168	0.0144
2	50	1.26	29.36	25.04	0.0113	0.0132	0.0139
2B	75	1.18	32.48	32.54	0.0115	0.0134	0.0148
3	100	1.41	81.02	40.76	0.0108	0.0124	0.0126
3B	125	1.44	37.99	36.76	0.0103	0.0127	0.0148
4	150	1.28	37.64	36.55	0.0108	0.0132	0.0131
4B	190	1.23	39.53	39.67	0.0112	0.0123	0.0140
5	225	1.78	56.76	47.77	0.0111	0.0155	0.0145
6	375	1.14	23.03	30.12	0.0138	0.0124	0.0115
MTrib data from UC Davis							
1	35	0.63	17.21	11.90	0.0330	0.0336	0.0295
1B	50		16.45	12.51		0.0369	0.0317
2	70	0.51	21.28	17.41	0.0305	0.0346	0.0348
2B	100		32.69	32.70		0.0292	0.0268
3	130	0.34	39.64	28.95	0.0381	0.0313	0.0301
3B	175		44.87	30.35		0.0284	0.0274
4	200	0.46	49.50	34.06	0.0336	0.0262	0.0302
4B	250		52.56	36.44		0.0296	0.0284
5	300	0.35	60.37	37.05	0.0299	0.0320	0.0203
6	410	0.24	66.55	40.92	0.0305	0.0287	0.0320
MTrib data from Michigan State							
1	35	0.47	20.83	15.54			
1B	50		17.09	12.72			
2	70	0.26	22.83	17.60			
2B	100		40.82	37.18			
3	130	0.17	39.52	28.70			
3B	175		44.20	30.50			
4	200	0.22	48.74	33.96			
4B	250		54.71	36.33			
5	300	0.12	66.18	36.83			
6	410	0.06	66.16	40.85			
Q. Ceiba data from UC Davis							
1	75	0.37		4.60	0.0259		0.0237
1B	150		0.04	3.09		0.0244	0.0252
2	200	0.72	0.26	2.22	0.0290	0.0272	0.0261
2B	250	1.03	0.84	2.34	0.0275	0.0284	0.0283
3	300	0.49	1.03	1.78	0.0249	0.0274	0.0269
3B	370	0.37	1.76	1.31	0.0246	0.0275	0.0232
4	425	0.43	0.85	1.13	0.0250	0.0267	0.0234
4B	510	0.21	1.12	1.12	0.0252	0.0268	0.0250
5	575	0.27	1.42	0.52	0.0251	0.0262	0.0254
6	700	0.17	1.27	0.16	0.0250	0.0279	0.0250

		del $^{15}N_2$ (per mil)			N_2 Mass (mmoles)		
Station	Distance (m)	Pre	Plateau 1	Plateau 2	Pre	Plateau 1	Plateau 2
Q. Ceiba data from Michigan State							
1	75	0.90	1.48	4.06			
1B	150	1.01	1.78	3.05			
2	200	0.96	2.79	1.96			
2B	250	0.99	2.22	2.91			
3	300	0.96	3.19	2.41			
3B	370	0.86	3.99	2.60			
4	425	0.95	2.92	2.89			
4B	510	0.85	3.63	2.05			
5	575	1.21	3.80	1.97			
6	700	1.47	3.34	1.85			

		del $^{15}N_2O$ (per mil)			N_2O Mass (nmoles)		
Station	Distance (m)	Pre	Plateau 1	Plateau 2	Pre	Plateau 1	Plateau 2
Q. Bisley							
1	40	28.72	151.17		0.1055	0.1176	
1	40	20.18	138.75	135.19	0.0945	0.1095	0.1020
1B	55	25.66	117.19		0.1130	0.1040	
1B	55	2.80	128.66	102.47	0.0859	0.0935	0.1055
2	75	50.01	97.50		0.0960	0.1065	
2	75	32.37	78.15	91.64	0.1326	0.1070	0.1115
2B	85	14.24	104.01		0.1156	0.0990	
2B	85	31.46	77.96	90.27	0.1266	0.0965	0.1120
3	130	55.70	53.36		0.1261	0.0965	
3	130	16.49	73.08	106.95	0.1316	0.1221	0.1000
3B	165	52.28	88.20		0.1306	0.1216	
3B	165	51.29	88.67	73.90	0.1266	0.1221	0.0935
4	200	58.91	76.08		0.1276	0.1201	
4	200	37.34	70.18	84.70	0.1311	0.1276	0.0980
4B	275	6.45	110.04		0.1286	0.1065	
4B	275	50.10	95.43	77.20	0.1236	0.1211	0.0874
5	350	32.02	90.14		0.1161	0.1176	
5	350	37.18	69.03	30.21	0.1286	0.1176	0.1020
6	425	141.42	63.38		0.1010	0.1130	
6	425	47.93	82.35	66.83	0.1125	0.1141	0.1035
RIT							
1	60	17.85	200.64	179.85	0.2611	0.2380	0.2479
1B	85		230.77	227.07		0.2347	0.2479
2	115	17.80	256.05	254.66	0.2396	0.2330	0.1735
2B	150		269.56	271.88		0.2429	0.2479
3	190	29.97	285.02	299.32	0.1785	0.2545	0.2495
3B	210		309.50	304.07		0.2479	0.2297
4	255	15.04	311.73	315.97	0.2528	0.2363	0.2380
4B	295		314.06	343.35		0.2495	0.2479
5	345	16.33	386.93	407.94	0.2545	0.2363	0.2347
6	425	21.24	415.89	493.52	0.2314	0.2132	0.2396

		del $^{15}N_2O$ (per mil)			N_2O Mass (nmoles)		
Station	Distance (m)	Pre	Plateau 1	Plateau 2	Pre	Plateau 1	Plateau 2
Q. Pared							
1	75	48.20	175.22	112.09	0.1738	0.1916	0.1869
1B	100	54.39	275.01	294.67	0.1785	0.1728	0.1394
2	150	31.08	305.75	250.25	0.1879	0.1838	0.1942
2B	200	47.82	189.60	183.94	0.1765	0.1707	0.1561
3	250	54.18	197.79	187.36	0.1979	0.1686	0.1143
3B	300	37.60	87.61	61.92	0.1853	0.1869	0.1832
4	350	38.08	89.64	64.04	0.2046	0.2057	0.2125
4B	400	61.54	68.53	45.76	0.1827	0.1738	0.2062
5	500	40.76	48.94	53.38	0.1712	0.1796	0.1765
6	600	48.66	49.75	86.40	0.1801	0.1838	0.1498
Q. Grande							
0B	25	29.94	163.42		0.1841	0.1900	
0B	25		204.00	250.40		0.1867	0.1720
1	50	17.98	318.44		0.1923	0.2005	
1	50		328.20	340.27		0.1948	0.1767
1B	75	14.05	362.36		0.1971	0.1848	
1B	75		361.34	397.18		0.1801	0.1915
2	100	18.40	707.72		0.1875	0.1615	
2	100		652.97	547.38		0.1701	0.1178
2B	150	22.26	832.40		0.1779	0.1611	
2B	150		805.48	744.12		0.1601	0.1625
3	200	4.01	1345.85		0.2081	0.1535	
3	200		1331.00	1471.44		0.1582	0.1696
3B	300	23.70	1528.07		0.1841	0.1953	
3B	300		1488.23	1251.96		0.1810	0.1539
4	400	8.73	850.74		0.1515	0.1402	
4	400		819.11	956.04		0.1568	0.1535
4B	500	24.09	814.74		0.1458	0.1260	
4B	500		807.19	843.49		0.1354	0.1283
5	600	11.58	746.13		0.1405	0.1227	
5	600		838.49	905.51		0.1097	0.1216
Q. Maizales							
1	60	3.07	126.08	89.87	0.2503	0.2666	0.2599
1B	75		146.34	81.85		0.2674	0.2678
2	125	0.33	256.08	151.26	0.2344	0.2611	0.2571
2B	175		606.63	238.61		0.2396	0.2157
3	235	1.34	598.08	328.30	0.2045	0.2185	0.2232
3B	275		636.64	410.34		0.2368	0.2252
4	325	22.71	648.03	401.01	0.2638	0.2833	0.2571
4B	375		503.61	439.93		0.2268	0.2312
5	450	11.17	451.62	369.40	0.1966	0.2304	0.2328
6	510	23.45	459.40	318.44	0.2109	0.2475	0.2300

		del $^{15}N_2O$ (per mil)			N_2O Mass (nmoles)		
Station	**Distance (m)**	**Pre**	**Plateau 1**	**Plateau 2**	**Pre**	**Plateau 1**	**Plateau 2**
Q. Vaca							
1	75	33.28		73.42	0.2398		0.2393
1B	115	28.55	145.48	39.05	0.2269	0.2258	0.2300
2	145	35.93	212.51	125.20	0.2160	0.2310	0.2611
2B	175	56.36	217.24	125.16	0.2238	0.2885	0.2808
3	225	31.82	249.94	136.76	0.2538	0.2403	0.2590
3B	275	33.51	343.86	168.98	0.2523	0.2290	0.2445
4	330	38.05	317.83	196.41	0.2165	0.1896	0.2517
4B	375	29.46	411.85	229.01	0.2274	0.1932	0.1782
5	450	55.48	413.35	207.61	0.1772	0.2046	0.2144
6	575	23.53	337.29	182.08	0.2321	0.2196	0.1580
Q. Petunia							
1	25	3.77	1767.34	1835.25	0.3457	0.3131	0.4528
1B	40	9.09	2131.93	2359.81	0.2987	0.3045	0.1763
2	50	8.32	3761.43	2837.72	0.2791	0.2893	0.3107
2B	75	9.61	4455.80	4015.86	0.2887	0.2855	0.3625
3	100	11.16	11308.88	5293.02	0.3357	0.3544	0.3126
3B	125	8.04	5412.77	4899.88	0.3074	0.2893	0.3345
4	150	24.71	5803.80	5299.61	0.2556	0.2570	0.2637
4B	190	19.59	6955.71	6423.56	0.2522	0.2195	0.2437
5	225	7.58	10223.69	5733.45	0.2383	0.2855	0.3530
6	375	16.27	4404.69	4404.76	0.2412	0.2485	0.2475
MTrib							
1	35	-2.52	2100.56	1319.79	0.6168	0.4178	0.6009
1B	50		2127.67	1373.78		0.4497	0.7879
2	70	-3.85	2322.84	1467.12	0.7481	0.5611	0.9272
2B	100		2726.73	1729.64		0.6208	1.3530
3	130	-17.29	3070.08	1534.70	1.0347	0.6646	1.1899
3B	175		3440.26	1580.45		0.7203	1.1262
4	200	-21.03	3493.37	1593.34	1.0546	0.6924	1.2416
4B	250		3173.94	1517.46		1.0108	1.1819
5	300	-17.63	3630.99	1544.13	1.1978	1.0426	1.0506
6	410	-15.06	3149.49	1429.32	1.0744	1.2456	1.1501
Q. Ceiba							
1	75	36.73	555.53		0.2864		0.3657
1B	150		347.94	310.97		0.3787	0.2813
2	200	24.87	377.75	435.38	0.3165	0.3538	0.2533
2B	250	32.84	573.76	610.91	0.2823	0.3175	0.2683
3	300	58.18	489.99	986.29	0.2398	0.3175	0.2419
3B	370	53.46	481.96	858.69	0.2937	0.3336	0.2150
4	425	17.54	511.51	847.71	0.2455	0.2978	0.2326
4B	510	19.36	563.40	895.65	0.2357	0.2745	0.2269
5	575	28.42	508.05	1093.12	0.2321	0.2606	0.2191
6	700	26.02	506.76	939.51	0.2248	0.2290	0.2129

^{15}N in Biomass

Q. Bisley				**d^{15}N (per mil)**			
Compartment	**-10m**	**40m**	**75m**	**130m**	**200m**	**350m**	**425m**
Leaves	1.0	9.8	23.6	101.8	118.9	43.3	38.3
Wood	1.1	113.9	24.7	56.2	70.4	29.7	98.5
FBOM Surface	3.8	15.5	16.4	14.4	22.8	9.2	45.0
FBOM Subsurface	2.9	9.2	14.2	7.5	5.3	7.0	2.7
Epilithon	4.1	137.3	400.5	69.9	137.7	57.4	57.4
Bryophytes							
Filamentous Algae							
Macorphytes							
Roots							

RIT				**d^{15}N (per mil)**			
Compartment	**-10m**	**40m**	**75m**	**130m**	**200m**	**350m**	**425m**
Leaves	0.1	56.8	14.9	7.4	70.3	15.4	4.3
Wood	2.0	16.6	15.8	9.4	20.8	19.7	22.6
FBOM Surface	2.0	19.9	26.1	14.5	6.5	15.0	32.0
FBOM Subsurface	3.7	20.8	8.7	1.0	6.3	35.4	44.9
Epilithon	3.0	20.4	179.5	19.6	60.5	102.6	138.3
Bryophytes							
Filamentous Algae							
Macorphytes							
Roots	2.7	85.2	48.0	36.9	51.5	18.8	40.2

Q. Pared				**d^{15}N (per mil)**			
Compartment	**-10m**	**40m**	**75m**	**130m**	**200m**	**350m**	**425m**
Leaves	0.6	148.4	248.4	316.8	64.9	47.9	98.9
Wood	-0.1	77.3	30.4	55.3	23.4	10.7	5.7
FBOM Surface	3.3	54.1	101.3	68.5	7.2	7.6	6.5
FBOM Subsurface	3.2	5.5		14.0	5.9	3.7	1.1
Epilithon	6.9	545.5	780.7	1106.7	311.0	101.0	118.7
Bryophytes							
Filamentous Algae							
Macorphytes	0.7					8.9	398.1
Roots	0.3		422.4	296.7	9.5	71.3	14.8

Q. Grande				**d^{15}N (per mil)**			
Compartment	**-10m**	**40m**	**75m**	**130m**	**200m**	**350m**	**425m**
Leaves		221.4	338.0	49.5	30.2	43.4	19.2
Wood		307.9	37.2	14.4	42.3	100.3	41.2
FBOM Surface							
FBOM Subsurface							
Epilithon	7.1	272.4	256.8	162.7	271.6	81.3	32.2
Bryophytes							
Filamentous Algae		1365.3	817.6	137.3	408.1	277.1	175.5
Macorphytes		695.1	859.6	320.2	510.1	432.2	213.1
Roots							

Q. Maizales				**d^{15}N (per mil)**			
Compartment	**-10m**	**40m**	**75m**	**130m**	**200m**	**350m**	**425m**
Leaves	4.7	255.9	184.9	317.3	174.2	229.0	915.3
Wood							
FBOM Surface	2.0	19.9	26.1	14.5	6.5	15.0	32.0
FBOM Subsurface	3.7	20.8	8.7	1.0	6.3	35.4	44.9
Epilithon	3.0	20.4	179.5	19.6	60.5	102.6	138.3
Bryophytes							
Filamentous Algae	5.2	2421.3	3329.3	3913.8	3603.7	2802.6	2611.6
Macorphytes							
Roots							

Q. Vaca				**d^{15}N (per mil)**			
Compartment	**-10m**	**40m**	**75m**	**130m**	**200m**	**350m**	**425m**
Leaves	2.1	188.4	65.6	151.7	368.9	31.9	295.8
Wood	4.4	143.1	7.6	35.5	10.4	16.8	
FBOM Surface	4.8	33.2	89.6	33.6	38.3	47.0	39.1
FBOM Subsurface	3.0	29.6	63.9	29.9	28.9	30.7	17.4
Epilithon	10.0	31.3	381.6	53.3	65.4	50.5	171.9
Bryophytes	8.0	92.1			163.4		73.2
Filamentous Algae							
Macorphytes							
Roots	3.7	74.2	52.5	84.7		353.2	249.3

Q. Petunia				**d^{15}N (per mil)**			
Compartment	**-10m**	**40m**	**75m**	**130m**	**200m**	**350m**	**425m**
Leaves		382.1	434.1	456.3	339.0	570.2	427.5
Wood			125.0	236.7	62.9	109.0	38.0
FBOM Surface	8.1	14.9	48.7	161.8	62.4	47.4	40.2
FBOM Subsurface	8.2	22.5	36.1	48.2	29.4	24.5	27.1
Epilithon	11.6	32.6	32.5	70.0	148.0	76.1	61.9
Bryophytes							
Filamentous Algae						1401.9	
Macorphytes							
Roots		1460.8	1450.9	1255.3	910.5	2919.5	1057.6

Mtrib				**d^{15}N (per mil)**			
Compartment	**-10m**	**40m**	**75m**	**130m**	**200m**	**350m**	**425m**
Leaves	1.6	6.8	8.3	8.4	10.9	15.1	25.6
Wood	5.3	7.9	5.3	11.1	6.4	14.5	13.1
FBOM Surface	2.5	3.9	3.1	2.3	3.6	6.4	3.7
FBOM Subsurface	3.3	3.5	6.2	3.3	5.4	5.2	7.1
Epilithon	1.5	1.5	3.1	4.0	2.8	3.5	6.1
Bryophytes							
Filamentous Algae	7.5	7.6	4.4	5.6			
Macorphytes							
Roots	2.1	31.7	15.0	15.4	5.0	8.0	53.2

Q. Ceiba				**d^{15}N (per mil)**			
Compartment	**-10m**	**40m**	**75m**	**130m**	**200m**	**350m**	**425m**
Leaves	5.3	43.6	54.9	31.9	163.2	165.3	74.2
Wood	4.0	11.8	42.8	13.9	26.4	1.9	
FBOM Surface	1.0	31.5	21.5	13.3	12.5	24.4	25.816329
FBOM Subsurface	7.3	50.0	33.5	31.4	27.9	30.5	42.4
Epilithon	10.8	358.8	510.0	368.4	476.6	363.5	446.4
Bryophytes							
Filamentous Algae							
Macorphytes	9.5	123.1		19.5			
Roots	9.1	320.5	127.9	240.0	193.4	440.4	