Effects of topography and forest change on soil nutrient dynamics in wet tropical forests

Samantha Rose Weintraub
University of Colorado at Boulder, samanthaweintraub@gmail.com

Follow this and additional works at: https://scholar.colorado.edu/ebio_gradetds

Part of the Biogeochemistry Commons, Environmental Indicators and Impact Assessment Commons, Forest Biology Commons, and the Soil Science Commons

Recommended Citation
Weintraub, Samantha Rose, "Effects of topography and forest change on soil nutrient dynamics in wet tropical forests" (2014). Ecology & Evolutionary Biology Graduate Theses & Dissertations. 52.
https://scholar.colorado.edu/ebio_gradetds/52

This Dissertation is brought to you for free and open access by Ecology & Evolutionary Biology at CU Scholar. It has been accepted for inclusion in Ecology & Evolutionary Biology Graduate Theses & Dissertations by an authorized administrator of CU Scholar. For more information, please contact cuscholaradmin@colorado.edu.
EFFECTS OF TOPOGRAPHY AND FOREST CHANGE ON SOIL NUTRIENT DYNAMICS IN WET TROPICAL FORESTS

By

Samantha Rose Weintraub
B.S., University of California Berkeley, 2007

A thesis submitted to the
Faculty of the Graduate School of the
University of Colorado in partial fulfillment of the requirement for the degree of
Doctor of Philosophy
Department of Ecology and Evolutionary Biology
2014
This thesis entitled:
Effects of topography and forest change on soil nutrient dynamics in wet tropical forests
written by Samantha Rose Weintraub
has been approved for the Department of Ecology and Evolutionary Biology

(Alan R. Townsend)

(Jason C. Neff)

Date__________________

The final copy of this thesis has been examined by the signatories, and we find that both the content and the form meet acceptable presentation standards of scholarly work in the above mentioned discipline.
Weintraub, Samantha Rose (Ph.D., Ecology and Evolutionary Biology)

**Effects of topography and forest change on soil nutrient dynamics in wet tropical forests**
Thesis directed by Professor Alan R. Townsend

**ABSTRACT**

A widely accepted paradigm for lowland tropical forests is that phosphorus (P) commonly limits plant growth while nitrogen (N) cycles in relative excess. And yet, increasing evidence suggests substantial heterogeneity in the availability of these essential elements from local to regional scales, driven by high variability in both biotic and abiotic factors. In this thesis, I used a series of field experiments in Costa Rica alongside detailed laboratory analyses to explore how changes in organic matter inputs, tree species and topography can all affect tropical forest nutrient cycling. First, I showed that changes in organic matter inputs, such as those that could result from altered land-use or forest productivity under elevated CO$_2$, altered the availability of soil N and P and the production of enzymes that acquire them. Reduced leaf litter quantities exacerbated soil P limitation, yet leaf litter doubling shifted soils toward N constraints, similar to observations in temperate biomes exposed to elevated CO$_2$. Next, using a series of mono-dominant native tree plantations, I demonstrated that species promote up to eight-fold differences in soil emissions of nitrous oxide (N$_2$O), a potent greenhouse gas. Species differences in N$_2$O efflux were strongly and inversely related to rates of fine-root growth. This link between belowground C allocation and emission of N$_2$O may be important to forestry-related climate mitigation programs such as REDD+. Finally, I documented the importance of topography to N cycling in a diverse primary forest, where flat ridge-tops display much greater N-richness than do steep hillslopes. My data suggested that surface erosion might drive this landscape level pattern, thus for my last experiment I monitored fluxes of nitrogen in eroding soil, leaf litter, and runoff. I found that erosion of soil and organic N in steep regions is roughly equal to atmospheric
N inputs in the short and long term, implying that erosion may maintain N limitation across substantial portions of topographically complex tropical forests. Taken as a whole, my work underscores several multi-faceted aspects of heterogeneous tropical forest nutrient cycling. This complexity is necessary to unravel in order to tackle societally-relevant issues ranging from climate prediction to forest restoration.
ACKNOWLEDGEMENTS

I would like to thank the following people for their input and assistance with this thesis.

First, I am very grateful for the mentorship of my adviser, Alan Townsend. It has been rewarding and fun working with him to develop the ideas presented in this thesis, and I thank him for all his support and guidance over the last five years. Alan has inspired me to strive to address the environmental challenges of our time using creative, interdisciplinary approaches. His keen ability to communicate science and distill complex environmental problems into simple and compelling terms is something I aspire to.

I was fortunate to share the lab with several exceptionally smart, motivated and generous people. The research interests and perspectives of Philip Taylor, Rebecca Cole, John Mischler, Will Wieder, and Maggie Kriz have expanded my views on many topics in biogeochemistry and forest ecology. Moreover, their assistance with field and laboratory analyses was instrumental to the completion of the work presented here.

Many thanks are due to Robert Stallard, Ann Russell, Steven Porder, and Greg Asner – I have grown tremendously through collaborations with these scientists. I also wish to thank Cory Cleveland and the members of his lab, including Ben Sullivan, Megan Nasto and Adrienne Keller, for their input and assistance over the years.

I am grateful to all entities that assisted me in conducting fieldwork and accessing research sites – no small task when working in remote and rugged tropical forests. This includes the staff of Osa Conservation, the Organization for Tropical Studies (especially Francisco Campos-Rivera), the Drake Bay Wilderness Camp, and Sarah Torres. I would also like to acknowledge several excellent Costa Rican field technicians, most notably Marvin Lopez-Morales as well as Walkom Cambronero Castro, Ricardo Bedoya, Marlon Hernandez, Flor Cascante and Eduardo Paniagua.

Biogeochemistry is an inherently interdisciplinary field, and my research has benefited from the input of scientists with diverse perspectives, including John Moody, Deborah Martin, Sheila Murphy, Robert Anderson, Dick Smith and Deborah Repert. I would also like to thank my dissertation committee – Tim Seastedt, Jason Neff, Nicole Barger, and Steve Schmidt – for their valuable input on my research projects.

Finally, I wish to acknowledge the funding sources for this thesis, which include the National Science Foundation, the Organization for Tropical Studies, Osa Conservation, and the Ecology and Evolutionary Biology Department.
TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION

1.1 Role of nutrients in tropical carbon cycling and climate ................................ 1
1.2 Heterogeneity of tropical nutrient dynamics ................................................. 1
1.3 Environmental changes facing tropical forests ............................................. 3
1.4 Scope and aims of this thesis ......................................................................... 4

CHAPTER 2: ORGANIC MATTER INPUTS SHIFT SOIL ENZYME ACTIVITY AND ALLOCATION PATTERNS IN A WET TROPICAL FOREST

2.1 Abstract ............................................................................................................. 5
2.2 Introduction ......................................................................................................... 6
2.3 Methods ............................................................................................................. 9
   2.3.1 Site description ............................................................................................. 9
   2.3.2 Leaf litter and throughfall manipulations ................................................. 9
   2.3.3 Enzyme assays ............................................................................................ 10
   2.3.4 Soil biogeochemical analyses ................................................................. 12
   2.3.5 Statistical analyses .................................................................................... 14
2.4 Results .............................................................................................................. 15
2.5 Discussion ......................................................................................................... 21

CHAPTER 3: NATIVE TREE SPECIES REGULATE NITROUS OXIDE FLUXES IN TROPICAL PLANTATIONS

3.1 Abstract ............................................................................................................. 27
3.2 Introduction ....................................................................................................... 28
3.3 Methods ............................................................................................................. 31
### CHAPTER 4: TOPOGRAPHIC CONTROLS ON SOIL NITROGEN AVAILABILITY IN A LOWLAND TROPICAL FOREST

4.1 Abstract.................................................................45
4.2 Introduction..........................................................46
4.3 Methods....................................................................49
  4.3.1 Study site..........................................................49
  4.3.2 Field and laboratory methods.............................50
  4.3.3 Statistical analyses............................................54
4.4 Results......................................................................55
4.5 Discussion...............................................................60

### CHAPTER 5: HILLSLOPE EROSION AND NITROGEN LOSS FROM AN INCISED LOWLAND TROPICAL FOREST, OSA PENINSULA COSTA RICA

5.1 Abstract.....................................................................67
5.2 Introduction............................................................68
5.3 Methods...................................................................72
  5.3.1 Study site – geology, soils and climate...................72
  5.3.2 Erosion trough construction and monitoring............73
  5.3.3 Sample mass and nitrogen content.........................75
5.3.4 Trough contributing areas ......................................................76
5.3.5 Long-term watershed erosion .............................................78
5.3.6 Hillslope nitrogen fixation ..................................................78
5.3.7 Data treatment and statistical analyses .............................79
5.4 Results .................................................................................81
5.5 Discussion ............................................................................86

CHAPTER 6: CONCLUSIONS .................................................................93

REFERENCES ..................................................................................95

APPENDIX ..................................................................................114
TABLES

Table

2.1 Soil extracellular enzymes assayed in this study and their functions (EC: Enzyme Commission, MUB: methylumbelliferone).................................11

2.2 Mean values with one standard error in parentheses of soil biogeochemical properties in control and treatment plots (1× = control, 0× = litter removal, 2× = double litter, -50% = fifty percent throughfall reduction). Below are ANOVA results (F, P) from linear mixed effects models examining treatment effects on soil biogeochemical properties. Date effects are not shown. Letters indicate significant differences between treatments.................................19

2.3 Spearman’s product-moment correlation coefficients for soil biogeochemical variables and enzyme activities per gram soil. Only significant correlation coefficients are shown.................................................................20

3.1 Soil inorganic N concentrations and water filled pore space in the different vegetation types during wetter and drier season sampling period (mean values ± standard errors) ........................................................................38

4.1 Soil C and N values at the three landscape positions. Values are means ± SE, and letters indicate significant differences between positions at Bonferroni-adjusted α = 0.05 ........................................................................57

4.2. Mean values with standard deviations in parentheses of soil physico-chemical and mineralogical properties at the three landscape positions. Letters indicate significant differences between positions at α = 0.05. .................................................59

5.1 Slopewash, litterwash and runoff total amounts from the 4-month study period. Positions that do not share a letter had significantly different fluxes (P < 0.05) based on multiple comparisons (“kruskalmc”) following Kruskal-Wallis tests .........................................................................................83

5.2. Total wet-season biological N₂ fixation (BNF) inputs and measured overland N losses at the four landscape positions.........................................................86
FIGURES

2.1 Response ratios of enzymes activities (treatment/control) per gram soil (a), per gram soil carbon (b), and per milligram microbial biomass carbon (c). -50% = fifty percent throughfall reduction, 0x = litter removal, 2x = double litter. Horizontal dashed line indicates 1:1 value.

2.2 Ratios of soil enzyme activities (± standard error) in experimental plots. Letters indicate significant differences between treatments based on linear mixed effects models.

3.1 Soil fluxes of N₂O in the wetter season from the different vegetation types, including abandoned pasture, four species in mono-dominant plantations, and mature forest. Vegetation types that share a letter are not significantly different; hatched lines are standard errors.

3.2 Soil fluxes of N₂O in the drier season from the different vegetation types, including abandoned pasture, four species in mono-dominant plantations, and mature forest. Vegetation types that share a letter are not significantly different; hatched lines are standard errors.

3.3 Relationships between wetter-season N₂O fluxes and NO₃⁻ concentrations (a) and drier-season N₂O fluxes and soil WFPS (b) in the mono-dominant species plots. Pearson’s r values are shown, along with linear trend lines.

3.4 Relationships between fine-root growth and N₂O fluxes in the wetter (a) and drier (b) seasons in the mono-dominant species plots. Pearson’s r values are shown, along with linear trend lines.

4.1 Outline map of Costa Rica and neighboring countries with the Osa Peninsula highlighted, as well as a hillshade map of the Osa Peninsula with the study region indicated by a white star. Schematic representation of the study toposquence.

4.2 Principal components analysis (PCA) of soil N cycling at the three landscape positions (ridge = RDG, ridge-slope transition = RST, slope = SLP).

4.3 Relative values (means ± SE) centered and scaled by the range for eight soil N metrics (ridge = RDG, ridge-slope transition = RST, slope = SLP). Stars indicate a significant effect of landscape position.

4.4 Log-log relationships between soil C (a) and soil N (b) stocks and depth below the surface.
4.5 Digital elevation map of the study forest, with white crosses indicating sites of the larger landscape sampling and white circle indicating location of the study toposequence (a). Soil $\delta^{15}$N values versus slope angle from the larger landscape sampling, with error bars indicating two standard deviations of the isotope measurements (b). Gray shaded region is the 95% confidence interval for the linear regression: $\delta^{15}$N = -3.745*slope (sin$\theta$) + 6.553, $R^2 = 0.576$ ..................................................................................................................60

4.6 Soil $\delta^{15}$N values versus soil C:N ratios across the study forest, with error bars indicating two standard deviations of the isotope measurements (ridge = RDG, ridge-slope transition = RST, slope = SLP, hillslopes from larger landscape sampling = HS) .............................................................................................................................61

5.1 Erosion collector deployed on the steep slope post roof addition, July 2012 (a). Set of overflow buckets for an erosion collector on the shoulder slope (b). Topographic map of the study hillslope, with locations of the sixteen erosion collectors indicated (c) .........................................................................................................................74

5.2 Rainfall for the collection periods (a), and cumulative fluxes of water (b), soil (c) and fine litter (d) over the course of the study period. Points represent the mean of each collection date per slope position (n = 4) and hatched lines are standard errors ........................................................................................................................................82

5.3 Mean total overland nitrogen fluxes ((± standard error) during the 4-month study period .................................................................................................................................................................84

5.4 Erosion and runoff-associated N losses scaled by their relation to total biological nitrogen fixation (BNF) inputs at each slope position. Estimates of BNF include free-living and symbiotic components, sensu Table 5.2. Hatched lines are standard errors ..................................................................................................................................................................................87

5.5 Balance between total N inputs and total erosive losses estimated for the watershed incision zone.........................................................................................................................................................................................88
CHAPTER I: INTRODUCTION

1.1 Role of nutrients in tropical carbon cycling and climate

In many respects, tropical forest ecosystems are even more important than their land area suggests. While covering ~ 13% of Earth’s land surface, they store 25% of it’s terrestrial carbon (Bonan 2008), support tremendous levels of biological diversity (Condit et al. 2005), and play a crucial role in global climate regulation, both through biophysical processes and high rates of net primary production (Field 1998, Pan et al. 2011). Forest sinks for carbon dioxide are an increasingly significant factor in offsetting rising atmospheric carbon dioxide (CO₂), and highly productive humid tropical forests play a vital role (Bonan 2008, Pan et al. 2011). As is true for most forested ecosystems, nutrients are a key component regulating tropical productivity and decomposition (Cuevas and Medina 1988, Vitousek and Farrington 1997, Wieder et al. 2009). As those nutrients play key roles in C uptake and release, and thus are likely to affect carbon-climate feedbacks, it is essential to understand the controls on tropical nutrient availability and limitation (Townsend et al. 2011).

Beyond indirect links to climate via the carbon cycle, tropical nutrient dynamics can also have direct climate impacts. One noteworthy example is the emission of nitrous oxide (N₂O), a potent greenhouse gas with ~ 300 times the radiative forcing per molecule compared to CO₂ (Forster et al. 2007). While there is much spatial and temporal variability in patterns of N₂O emission (Werner et al. 2007), the pan-tropical N₂O flux is large (Vitousek and Matson 1992) – second only to agriculture at the global scale.

1.2 Heterogeneity of tropical nutrient dynamics
While there is clear impetus to understand nutrient cycling and availability in the humid tropical forest biome, the task is complicated by wide variation in abiotic and biotic state factors (sensu Jenny 1941), which cause heterogeneous patterns of essential element constraints. For instance, a widely-held notion for lowland tropical forests suggests that phosphorus (P) limits productivity while nitrogen (N) cycles in excess of demand. This holds true for some tropical forests, yet many exceptions have been found (Austin and Vitousek 1998, Kaspari et al. 2008, Nardoto et al. 2008, Wright et al. 2011, Alvarez-Clare et al. 2013), suggesting nutrient cycling patterns are actually quite complex. Factors such as high diversity of species, broad gradients in climate, as well as variations in topography and uplift make it difficult to establish universal rules for nutrient dynamics across all lowland forests (Townsend et al. 2008).

Topography is one such factor that can complicate patterns of nutrient availability. Since ecosystems tend to lose their rock-derived nutrients over time while gaining nitrogen from the atmosphere, many tropical forests (generally characterized as “old”) are thought to be P depleted and relatively rich in N. This belief has been influenced by age gradient and fertilization studies on geomorphically stable surfaces (Walker and Syers 1976, Vitousek 2004), and may indeed apply to areas of the tropics where land surface stability reigns. However, many tropical landscapes are not flat and static, but instead are dominated by hillslopes and channels where soil is mobile. Soil mobility (i.e. erosion) is a factor that confounds the dominant paradigm, as the age of the soil is decoupled from its parent rock (Porder and Hilley 2011). Recent studies have linked topography and soil erosion to variation in P fertility (Vitousek et al. 2003, Porder et al. 2005), yet shifts in N dynamics across complex terrain have received less attention to date.
1.3 Environmental changes facing tropical forests

Aside from natural variation in the factors that regulate nutrient availability, humid tropical forests are also experiencing myriad environmental perturbations from local to global scales. By the end of the 20th century, atmospheric CO$_2$ concentrations were 36% higher than their pre-industrial levels, driven both by changes in land use (much of that in the tropics) and rising anthropogenic CO$_2$ emissions (Forster et al. 2007). Land-use change, elevated CO$_2$, as well as changes in climate, will likely affect inputs of organic matter to tropical soils. A key question is how nutrient availability will change in response, especially since nutrients may mediate globally significant carbon-climate feedbacks (Thornton et al. 2007, Bonan and Levis 2010). In temperate forests, progressive nitrogen limitation commonly follows experimental CO$_2$ enrichment (Luo et al. 2004), yet parallel studies have not yet been conducted in tropical forests. There, leaf litter manipulations have been used to explore soil biogeochemical and nutrient responses to carbon-rich (or poor) conditions (Wood et al. 2009, Sayer et al. 2011), yet important aspects of this topic remain unresolved.

The land-use change footprint on the humid tropical biome is pervasive (Asner et al. 2009), and yet the biogeochemical consequences of forest change are not fully understood. By 2005, approximately half the tropical forest biome only had 50% tree cover; while deforestation rates remain high, rates of secondary forest regeneration are also on the rise (Asner et al. 2009). As secondary forests are an increasingly important feature of the tropical landscape (Chazdon 2008), including mono-dominant plantations (Mayaux et al. 2005), a better understanding of nutrient dynamics in such forests is necessary (Davidson et al. 2004). Previous work suggests aggrading forests cycle nutrients, especially nitrogen (N), differently than their primary forest counterparts (Keller and Reiners 1994, Davidson et al. 2007), but whether these dynamics result
from changes in species composition during succession or intrinsic properties of young forests is not well understood. This question is relevant to predicting forest successional trajectories and the diverse climate consequences of forest regeneration.

1.4 Scope and aims of this thesis

Understanding tropical forest nutrient dynamics is critical, both in expanding our conceptual models to include more heterogeneous terrain and predicting what tropical forests of the future might look like. In this thesis, I use a series of field experiments in Costa Rica alongside parallel laboratory analyses to explore several of these topics. In Chapter 2, I detail the effects of organic matter inputs on nutrient availability and decomposition enzymes using soils exposed to *in-situ* leaf litter removal and addition. In Chapter 3, I document species-specific variations in the production of nitrous oxide in mono-dominant plantations and highlight linkages between species variations in root growth and N$_2$O efflux. I also discuss the significance of such findings for forestry-based climate mitigation schemes in tropical regions. In Chapter 4, I investigate how patterns of N richness vary across topographically dissected terrain and highlight key differences between flat and steep zones. And in Chapter 5, I build on this work to explore the role of erosion and particulate N loss in driving N availability trends in incised terrain.
2.1 Abstract

Soil extracellular enzymes mediate organic matter turnover and nutrient cycling yet remain little studied in one of Earth’s most rapidly changing, productive biomes: tropical forests. Using a long-term leaf litter and throughfall manipulation, we explored relationships between organic matter (OM) inputs, soil chemical properties and enzyme activities in a lowland tropical forest. We assayed six hydrolytic soil enzymes responsible for liberating carbon (C), nitrogen (N) and phosphorus (P), calculated enzyme activities and ratios in control plots versus treatments, and related these to soil biogeochemical variables. While leaf litter addition and removal tended to increase and decrease enzyme activities per gram soil, respectively, shifts in enzyme allocation patterns implied changes in relative nutrient constraints with altered OM inputs. Enzyme activity ratios in control plots suggested strong belowground P constraints; this was exacerbated when litter inputs were curtailed. Conversely, with double litter inputs, increased enzymatic investment in N acquisition indicated elevated N demand. Across all treatments, total soil C correlated more strongly with enzyme activities than soluble C fluxes, and enzyme ratios were sensitive to resource stoichiometry (soil C:N) and N availability (net N mineralization). Despite high annual precipitation in this site (MAP ~ 5 m), soil moisture was positively correlated with five of six enzymes. Our results suggest resource availability regulates tropical soil enzyme activities, soil moisture plays an additional role even in very wet forests, and relative investment in C, N and P degrading enzymes in tropical soils will often be distinct from higher latitude ecosystems yet is sensitive to OM inputs.
2.2 Introduction

Soil extracellular enzymes play a critical role in organic matter decomposition, regulating both carbon (C) storage and the supply of essential nutrients to below- and above-ground communities (e.g. Aber and Melillo 1980, Berg 2000, Burns and Dick 2002). A better understanding of the feedbacks between soil physico-chemical conditions and enzyme activities can help improve our understanding of steady-state biogeochemical dynamics as well as how soil biogeochemical cycles may respond to current and future global changes (Sinsabaugh et al. 2009, Allison et al. 2010, Cusack et al. 2011). Theoretical and empirical work to date suggests soil enzyme production is coupled to local resource availability. Economic, “optimal allocation” models of enzyme dynamics rest on the assumption that enzyme production is sensitive to resource stoichiometry, allowing decomposer organisms to target those resources most in demand (Sinsabaugh and Moorhead 1994). For example, in northern hardwood forests, nitrogen fertilization tends to shift enzyme allocation away from lignin oxidation (and the mining of N, Moorhead and Sinsabaugh 2006) toward hydrolysis of more labile carbon compounds via hydrolytic enzymes (Saiya-Cork et al. 2002, Sinsabaugh et al. 2002, Waldrop et al. 2004, Zak et al. 2008, Cusack et al. 2011). Elsewhere, N additions to N-poor Hawaiian forests caused an increase in phosphatase enzyme activity (Olander and Vitousek 2001).

While these studies support optimal allocation theory, evidence from both broad-scale syntheses and small-scale laboratory studies suggests links between resource availability and enzyme activities are complex. In a 2009 meta-analysis, Sinsabaugh et al. observed a convergence of C, N, and P-mineralizing enzyme activities on a ratio of 1:1:1 across broad ecosystem types encompassing diverse resource stoichiometries. This result may imply that the flexibility of decomposers to alter enzyme production in response to different resource
environments is limited by physiological or metabolic constraints. Other studies have observed that enzymes targeting specific bio-molecules display varied responses to resource levels, with production of some enzymes induced by supply of the decomposition product, while others are inhibited by the end product as optimal allocation models predict (Mcgill and Cole 1981, Sinsabaugh and Moorhead 1994, Geisseler and Horwath 2009, Hernandez and Hobbie 2010). These results highlight areas of uncertainty in our understanding of soil enzyme regulation in the context of resource availability.

As with a number of other components of ecosystem function (Townsend et al. 2008, Malhi et al. 2009, Randerson et al. 2009), our understanding of soil extracellular enzyme dynamics is poor in tropical forests. And yet, tropical forests are both a key driver of biosphere-atmosphere feedbacks to global change (Townsend et al. 1992, Field et al. 1998, Bonan 2008) and are experiencing rapid changes in climate, atmospheric conditions and direct human disturbance (Clark et al. 2003, Wright 2005, Lewis et al. 2009, Chai and Tanner 2010, Miettinen et al. 2012). All of these changes have the potential to substantially alter the amount of organic matter delivered to soils (Guariguata and Ostertag 2001, Murty et al. 2002, Nemani et al. 2003, Taylor 2012). In turn, the fate of such organic matter inputs will depend on soil enzyme activities.

There are reasons to suspect tropical soil enzymes might display different enzymatic patterns and linkages with soil resources than their temperate counterparts. For instance, at the global scale total soil C appears to be a robust predictor of hydrolytic enzyme activities (Sinsabaugh et al. 2008). However, current enzyme databases are dominated by temperate sites. In contrast to high-latitude forests, tropical C cycling is rapid (Parton and Silver 2007, Cusack et al. 2009), partly due to significant transfers of soluble C and nutrients from the litter layer to soil
during the early stages of litter decay (Allison and Vitousek 2004, Cleveland and Townsend 2006, Wieder et al. 2009). Such fluxes regulate short-term variations in soil respiration (Cleveland and Townsend 2006, Cleveland et al. 2010). As such, one might expect stronger links between enzyme activities and transfers of dissolved OM from litter to soil than with total soil C pools. Moreover, organic matter cycling in tropical forests is more likely to be constrained by P rather than N availability (Vitousek and Sanford 1986, Herbert and Fownes 1995, Cleveland et al. 2002, Reich and Oleskyn 2004, Cleveland et al. 2011). Heterotrophic microbes in P-limited environments with high N availability should be able to allocate more cellular resources to the procurement of P (Marklein and Houlton 2012), leading to different ratios of C, N and P mineralizing enzymes than those observed in temperate soils.

Here we used two forms of resource manipulation – a long-term leaf litter manipulation and a throughfall reduction experiment – to explore links between soil resources and soil enzyme activities in a wet lowland tropical forest. Previous work in these experimental plots has revealed links between litter-to-soil dissolved organic carbon (DOC) transfers and organic matter decomposition (Wieder et al. 2009, Cleveland et al. 2010), litter P content and rates of mass loss (Wieder et al. 2009) as well as changes in soil resource stoichiometry with changes in OM quantity (Nemergut et al. 2010, Leff et al. 2012). Specifically, the doubling of leaf litter inputs leads to an increase in dissolved and total soil organic matter C to N ratios. As such, we predicted that: 1) DOC concentrations would exhibit strong links with enzyme activities, 2) enzyme allocation patterns would generally support belowground P limitation, but 3) relative allocation to enzymes that mineralize C, N and P would change due to altered resource abundance and stoichiometry across the treatment plots. We tested these predictions by
measuring the potential enzyme activity of six hydrolytic enzymes as well as a suite of soil biogeochemical properties.

2.3 Methods

2.3.1 Site description

The study was conducted in a primary, lowland wet tropical forest of high species diversity located on the Osa Peninsula, southwestern Costa Rica, near the town of Progresso in the Drake River Valley (8° 43' N, 83° 37' W). This forest, part of the Golfo Dulce Forest Reserve, receives ~5000 mm rain per year and has a mean annual temperature of 26° C. Annual temperature fluctuations are small but precipitation is highly seasonal – a pronounced dry season occurs at this site from December through March. The soils are classified as Ultisols and are derived from basaltic parent material (Berrange and Thorpe 1988). Prior work at the site has demonstrated exceptionally high rates of litterfall and decomposition, with a strong influence of the seasonal rainfall pattern on carbon fluxes (Cleveland et al. 2006, Cleveland and Townsend 2006). For more detailed descriptions of this site, see Cleveland et al. (2006, 2002) and Bern et al. (2005).

2.3.2 Leaf litter and throughfall manipulations

In April 2007, we initiated a leaf litter manipulation experiment with three treatments: litter removal (0×), double litter addition (2×) and control (1×). At monthly intervals, all fine litterfall was raked and removed from the 0× plots, weighed, homogenized and redistributed onto the 2× plots. Control plots received no litter manipulation (n = 10 per treatment). Each treatment plot was 3 m x 3 m and plots were established in an area of the forest with minimal topographic
variation. For more details regarding this experiment, see Wieder et al. (2011) and Leff et al. (2012).

In September of 2007, a throughfall exclusion experiment was established in close proximity to the leaf litter manipulations. Throughfall exclusion shelters were constructed by cutting 5 cm diameter polyvinylchloride (PVC) pipes in half longitudinally and mounting them on 2.4 m x 2.4 m aluminum frames (Cleveland et al. 2010, Wieder et al. 2009). The PVC was mounted at 5 cm or 15 cm intervals in order to shield one-quarter or one-half of incoming throughfall, and plots were not trenched as the intent of the experiment was to focus on changes in litter-to-soil transfers of dissolved organic matter. In the present study, we focused on plots that received a 50% through-fall reduction (-50%; n = 10). The throughfall exclusions were dismantled in 2009, while the leaf litter manipulations are ongoing.

2.3.3 Enzyme assays

Sub-samples of frozen soil from litter manipulations and -50% throughfall plots were assayed for the activity of six hydrolytic extracellular enzymes. A previous test of sample storage methods for tropical soil enzyme activities indicated that soil freezing has only modest effects on overall activities, and no disproportionate effects on any given class of enzymes, thus making it a reasonable method for comparisons across enzyme types and treatments (Turner and Romero 2010). Assays were performed on soil samples collected at three time-points known from prior work to be especially important in understanding the biogeochemistry of this site: the early wet season (June 2008), the peak wet season (October 2008) and the dry season (March 2009). Soil samples were assayed using standard fluorometric microplate methods (Sinsabaugh et al 2002, Saiya-Cork et al. 2002). The activity of one P, two N, and three C-mineralizing enzymes were examined using fluorescently labeled substrates (Table 2.1). Since substrates were added in non-
Table 2.1 Soil extracellular enzymes assayed in this study and their functions (EC: Enzyme Commission, MUB: methylumbelliferone).

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Abbreviation</th>
<th>EC Number</th>
<th>Function</th>
<th>Substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid phosphatase</td>
<td>aP</td>
<td>3.1.3.2</td>
<td>Release of phosphate from ester-bonded P</td>
<td>4-MUB-phosphate</td>
</tr>
<tr>
<td>N-acetyl-β-D-glucosaminidase</td>
<td>NAG</td>
<td>3.2.1.14</td>
<td>Release of N-acetyl-β-D-glucos-aminide from chitin oligomers</td>
<td>4-MUB-N-acetyl-β-D-glucosaminide</td>
</tr>
<tr>
<td>Leucine aminopeptidase</td>
<td>LAP</td>
<td>3.4.11.1</td>
<td>Release of N-terminal hydrophobic amino acids, especially leucine, from polypeptides</td>
<td>L-Leucine-7-amin-4-methylcoumarin</td>
</tr>
<tr>
<td>β - glucosidase</td>
<td>BG</td>
<td>3.2.1.21</td>
<td>Release of glucose from glucosides, cellobiose</td>
<td>4-MUB-β-D-glucoside</td>
</tr>
<tr>
<td>1,4-β -cellobiosidase</td>
<td>CBH</td>
<td>3.2.1.91</td>
<td>Release of cellobiose from non-reducing end of cellulose chains</td>
<td>4-MUB-β-D-cellobiose</td>
</tr>
<tr>
<td>1,4-β -xylosidase</td>
<td>BX</td>
<td>3.2.1.37</td>
<td>Release of xylose from short xylan oligomers</td>
<td>4-MUB-β-D-xyloside</td>
</tr>
</tbody>
</table>

limiting quantities, the assays are a measure of the potential activity of the residual soil enzyme pool, not in-situ degradative activity (Wallenstein and Weintraub 2008).

Briefly, frozen soil was thawed for 30 minutes, then 1.0 g was combined with 125 ml of 50 uM sodium acetate buffer (pH 5.0) and homogenized with a Virtex 45 tissue homogenizer (Virtex Inc., Yonkers, NY, USA) for one minute. Soil slurries were continuously stirred on a magnetic stir plate while 200 ul aliquots were dispensed into black 96-well assay plates, with 16 analytical replicate wells per sample. After plating all suspensions, 50 ul of 200 uM substrate solutions (Sigma Aldrich, St. Louis, MO, USA) were added to the appropriate wells (Table 2.1). In addition to sample wells, negative control wells received 200 ul buffer plus 50 ul substrate solution, sample control wells received 200 ul sample plus 50 ul buffer, standard reference wells
received 200 ul buffer plus 50 ul standard (10 uM 4-methylumbelliferone or 7-amino-4-methylcoumarin), and quench wells received 200 ul sample plus 50 ul reference standard, all in analytical replicates of eight. Plates were incubated at room temperature for 3-6.5 hours, depending on the enzyme being assayed.

In order to optimize fluorescence, reactions were terminated by raising the pH in each well with 10 ul of 1M sodium hydroxide (NaOH). The time between NaOH additions and fluorescence readings was short (circa 8 minutes) and consistent for all samples and enzymes (German et al. 2011). Fluorescence was measured at 365 nm excitation and 460 nm emission using a Fluoroskan II microplate fluorometer (Thermo Labsystems, Franklin, MA, USA). Enzyme activity was determined after correcting for sample quenching and fluorescence in sample controls and negative controls.

2.3.4 Soil biogeochemical analyses

We monitored a host of soil biogeochemical parameters throughout the duration of the experiments in order to assess the impacts of changes in leaf litter quantity and throughfall on nutrient cycling (Cleveland et al. 2010, Wieder et al. 2011). For this study, we focused on total, extractable, and dissolved soil nutrient pools with hypothesized links to soil extracellular enzyme activities (EEA). For soil nutrient analyses, surface soils (0-10 cm) were collected fresh, transported on ice to the University of Colorado Boulder within 72 hours, sieved at 4 mm, and analyzed for gravimetric soil moisture.

Total soil C and N concentrations were determined using oven-dried soils that were ground and analyzed via combustion on a Carlo Erba EA 1110 elemental analyzer (CE Elantech, Lakewood, New Jersey, USA). Total soil P was measured using a hot sulfuric acid (H2SO4) and hydrogen peroxide (H2O2) digest of 0.5 g of soil on a block digester with a sequential heating
regime (maximum temperature = 360°C for 3 hours; Tiessen and Moir 1993). Reference soils were included to test for digest efficiency, and total P was quantified by colorimetric analysis of PO$_4^{3-}$ concentrations (ascorbic acid method) on an Alpkem autoanalyzer (OI Analytical, College Station, TX, USA).

Inorganic N was extracted from fresh surface soils using 2 M potassium chloride. Net rates of N mineralization were determined on a sub-sample of soil incubated at 25°C under field moisture conditions for 25 days. Net N mineralization was calculated as the difference between initial and day-25 total inorganic N concentrations (Hart et al. 1994). Ammonium (NH$_4^+$) and nitrate (NO$_3^-$) were measured colorimetrically using an Alpkem autoanalyzer (OI Analytical, College Station, TX, USA). Extractable P was determined on air-dried, ground soils using 0.5 M sodium bicarbonate (NaHCO$_3$), following a partial Hedley fractionation method (Tiessen and Moir 1993). After an 18-hour extraction, supernatants were decanted and digested with ammonium persulfate and sulfuric acid at 120°C for 1 hour. NaHCO$_3$-extractable P was quantified by colorimetric analysis of PO$_4^{3-}$ concentrations (ascorbic acid method) on an Alpkem autoanalyzer.

Dissolved organic matter fluxes from the litter layer to the soil were monitored using zero-tension surface lysimeters installed in experimental plots. For more detailed information on surface lysimeter construction, see Cleveland et al. (2010). Water from the lysimeters was collected bi-weekly and frozen. A sub-sample was sent to the University of Colorado for analysis of DOC and total dissolved nitrogen (TDN) concentrations on a total C-N analyzer (Shimadzu TOCvcpn, Kyoto, Japan). Microbial biomass (MB) C and N were determined on fresh soils extracted with 0.5 M K$_2$SO$_4$ with and without exposure to a 5-day chloroform fumigation (Brookes et al. 1985, Beck et al. 1997). Total C and N from extracts were measured via
combustion using a total C-N analyzer (Shimadzu TOCvpn, Kyoto, Japan). Microbial biomass was calculated as the difference between fumigated and unfumigated samples, without correction for extraction efficiency.

2.3.5 Statistical analyses

We calculated enzyme activities in three ways: nmol substrate converted per gram soil per hour (nmol g⁻¹ h⁻¹), per gram soil C per hour (nmol g soil C⁻¹ h⁻¹) and per milligram microbial biomass C per hour (nmol mg MB-C⁻¹ h⁻¹). We took this course because we knew that soil and microbial C could be important determinants of soil enzyme activities (Sinsabaugh et al. 2008) and that they changed in response to the treatments (Nemergut et al. 2010, Wieder et al. 2011, Leff et al. 2012).

Prior to statistical analyses, enzyme activities were natural log transformed (ln) to satisfy assumptions of normality and homoscedasticity. We used analysis of variance (ANOVA) of linear mixed effects models, with treatment and season as fixed factors and plot as a random effect, to determine the effects of litter or throughfall treatment on soil EEA. Since the interaction between the two fixed factors was insignificant in all cases, we removed the interaction term from enzyme statistical models and focused on the effects of the treatments rather than season. Quantile-quantile (qq) plots were used to check models for random distribution of residuals. In order to characterize enzyme allocation patterns, we calculated the ratios of enzymatic acquisition of P relative to C (BG:aP), N relative to C (BG:(NAG + LAP)) and P relative to N ((NAG + LAP):aP) and conducted ANOVA of mixed effects models to test for treatment effects on these ratios as above.

To determine the effect of the treatments on soil biogeochemical properties, we used ANOVA of mixed effects models with treatment and season as fixed factors and plot as a
random effect. In the cases where interactions between treatment and season were significant, the interaction was included in the model structure. We restricted our analyses to time-points when both biogeochemical properties/processes and enzyme activities were measured (i.e. June 2008, October 2008 and March 2009). For time-integrated biogeochemical measurements from these manipulations, see Cleveland et al. (2010), Wieder et al. (2011) and Leff et al. (2012). Soil environmental data were ln or square root transformed if distributions did not meet assumptions of normality prior to analysis. In order to explore links with soil variables and enzyme activities, we used Pearson’s product-moment correlations. All statistical analyses were conducted in R version 2.9.2 (R Core Development Team, Vienna Austria) and significant differences were determined at $P < 0.05$.

2.4 Results

Leaf litter manipulation caused substantial shifts in extracellular enzyme activities while throughfall reduction did not. Relative to controls, higher potential activity per gram soil was detected in $2\times$ plots for five of the six enzymes measured, with NAG displaying the largest response (Figure 2.1a, Table A1). Litter removal resulted in significantly lower activities per gram soil for NAG, BG, and CBH, with strongest declines in the two C-degrading enzymes. When normalized per gram soil C and microbial biomass C, EEA revealed a slightly different picture. NAG activity still exhibited a sizable increase in $2\times$ plots even when normalized by soil C and microbial biomass C. However, aP activity was not significantly higher (Figure 2.1b, c, Table A1). On the other hand, aP activity per gram soil C and per milligram microbial biomass C was significantly higher in $0\times$ plots relative to controls while NAG activity was unchanged. The
Figure 2.1 Response ratios of enzymes activities (treatment/control) per gram soil (a), per gram soil carbon (b), and per milligram microbial biomass carbon (c). -50% = fifty percent throughfall reduction, 0× = litter removal, 2× = double litter. Horizontal dashed line indicates 1:1 value.
Note: Stars indicate significant differences between treatment and control values based on results of linear mixed effects models (Table A1). One star means treatment values were lower than controls while two stars indicate treatment values were higher than controls.

Activity of the cellulose-degrading enzyme CBH increased significantly in 2× plots and decreased significantly in 0× plots even when normalized by soil C. The other two C-degrading enzymes showed more subtle responses: BG tended to increase and decrease with litter addition and removal (respectively) but BX displayed higher activity in both 2× and 0× plots per milligram MB-C (Figure 2.1c). We detected no significant changes in the activity of LAP in experimental plots and no differences in soil enzyme activities in response to the -50% treatment.

Similar to the EEA trends above, enzyme stoichiometry was markedly impacted by the leaf litter treatments but not by reduction in throughfall. The mean BG:aP ratio of enzyme activity in control plots was 0.22. This ratio did not change significantly in double-litter plots but declined to 0.13 in litter removal plots ($F = 10.19, P < 0.001$; Figure 2.2). Enzyme N to P stoichiometry also showed robust response to the treatments. Control plot (NAG+LAP):aP ratios

![Figure 2.2](en)

**Figure 2.2** Ratios of soil enzyme activities (± standard error) in experimental plots (1× = control, 0× = litter removal, 2× = double litter, -50% = fifty percent throughfall reduction). Letters indicate significant differences between treatments based on linear mixed effects models.
averaged 0.151. Litter removal caused this ratio to fall to 0.117 and litter addition caused it to increase to 0.212 \((F = 12.88, P < 0.001; \text{Figure 2.2})\). Ratios of BG:(NAG+LAP) did not change significantly with treatment.

Litter manipulation altered many soil biogeochemical properties. Notably, litter addition resulted in elevated surface soil C concentrations (+30%) and a higher soil C:N ratio (13.8 vs. 11.7 in 1× plots; Table 2.2). Litter removal caused declines in soil C and N, but as they were of similar magnitude, soil C:N in 0× plots did not change. We did not detect changes in total soil P or extractable P in the treatment plots. Mean extractable \(\text{NH}_4^+\) concentration was significantly higher with litter addition, but net N mineralization rates were highest in control plots while tending to decline when litter was either added or removed (Table 2.2). This trend was only marginally significant but the largest decline was in double litter plots \((P = 0.081)\). None of the above soil properties were significantly altered by the throughfall reduction treatment.

The concentration of DOC in litter-to-soil fluxes was strongly affected by both litter and throughfall treatments, with a near tripling of [DOC] in -50% plots, a doubling of [DOC] in 2× plots and a 59% reduction in 0× plots compared to controls. However, the ratio of DOC to TDN was similar in -50% plots while it significantly increased and decreased in 2× and 0× plots respectively (Table 2.2). Both microbial biomass C and gravimetric soil moisture declined significantly with leaf litter removal but did not change in response to other treatments. The lack of a strong response in soil moisture to the throughfall reduction was intentional, as the plots were not trenched to focus the manipulation on rainfall-driven variations in litter-to-soil transfers of dissolved organic matter.
### Table 2.2

Mean values with one standard error in parentheses of soil biogeochemical properties in control and treatment plots (1× = control, 0× = litter removal, 2× = double litter, -50% = fifty percent throughfall reduction). Below are ANOVA results (F, P) from linear mixed effects models examining treatment effects on soil biogeochemical properties. Date effects are not shown. Letters indicate significant differences between treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Soil C (mg C/g)</th>
<th>Soil N (mg N/g)</th>
<th>Soil C: N</th>
<th>Soil P † (µg P/g)</th>
<th>NH₄⁺ (µg N/g)</th>
<th>PO₄³⁻ (µg P/g)</th>
<th>N min (µg g⁻¹ day⁻¹)</th>
<th>DOC♮ (mg/L)</th>
<th>TDN♮ (mg/L)</th>
<th>DOC: TDN</th>
<th>MB-C (mg C/g)</th>
<th>MB-N (mg N/g)</th>
<th>Soil Moisture* (%)</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1×</td>
<td>55.26⁺</td>
<td>4.64⁺</td>
<td>11.74⁺</td>
<td>667.93 (20.6)</td>
<td>9.14⁺</td>
<td>12.36 (0.51)</td>
<td>1.05 (0.20)</td>
<td>6.00⁺</td>
<td>0.83⁺</td>
<td>11.74⁺</td>
<td>667.93</td>
<td>0.11⁺</td>
<td>42.9⁺</td>
<td>16.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(2.59)</td>
<td>(0.16)</td>
<td>(0.27)</td>
<td>(0.88)</td>
<td>(0.51)</td>
<td>(0.20)</td>
<td>(0.69)</td>
<td>(0.15)</td>
<td>(0.9)</td>
<td>(0.03)</td>
<td>(0.007)</td>
<td>(0.44)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0×</td>
<td>38.02ᵇ</td>
<td>3.39ᵇ</td>
<td>11.16ᵇ</td>
<td>680.83 (26.93)</td>
<td>7.74ᵇ</td>
<td>11.58 (0.84)</td>
<td>0.70 (0.08)</td>
<td>2.47ᵇ</td>
<td>0.58ᵇ</td>
<td>6.36ᵇ</td>
<td>35.6ᵇ</td>
<td>0.08ᵇ</td>
<td>38.9ᵇ</td>
<td>3.39</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(1.94)</td>
<td>(0.16)</td>
<td>(0.1)</td>
<td>(0.84)</td>
<td>(0.42)</td>
<td>(0.08)</td>
<td>(0.32)</td>
<td>(0.12)</td>
<td>(0.73)</td>
<td>(0.02)</td>
<td>(0.005)</td>
<td>(0.37)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2×</td>
<td>72.41ᶜ</td>
<td>5.22ᶜ</td>
<td>13.83ᶜ</td>
<td>665.64 (18.67)</td>
<td>15.91ᶜ</td>
<td>13.70 (1.52)</td>
<td>0.65 (0.16)</td>
<td>11.31ᶜ</td>
<td>0.86ᶜ</td>
<td>15.22ᶜ</td>
<td>0.61ᶜ</td>
<td>0.13ᶜ</td>
<td>44.8ᶜ</td>
<td>16.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(5.42)</td>
<td>(0.41)</td>
<td>(0.26)</td>
<td>(0.97)</td>
<td>(0.97)</td>
<td>(0.16)</td>
<td>(0.86)</td>
<td>(0.11)</td>
<td>(1.38)</td>
<td>(0.05)</td>
<td>(0.011)</td>
<td>(0.77)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-50%</td>
<td>53.19ᵃ</td>
<td>4.21ᵃ</td>
<td>11.99ᵃ</td>
<td>614.44 (28.79)</td>
<td>9.07ᵃ</td>
<td>11.89 (1.03)</td>
<td>0.93 (0.13)</td>
<td>16.89ᵃ</td>
<td>2.14ᵃ</td>
<td>9.27ᵃ</td>
<td>0.56ᵃ</td>
<td>0.10ᵃ</td>
<td>43.2ᵃ</td>
<td>5.22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(3.09)</td>
<td>(0.20)</td>
<td>(0.39)</td>
<td>(0.31)</td>
<td>(0.31)</td>
<td>(0.13)</td>
<td>(1.74)</td>
<td>(0.18)</td>
<td>(1.27)</td>
<td>(0.03)</td>
<td>(0.007)</td>
<td>(0.54)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.65</td>
<td>0.09</td>
<td>0.65</td>
<td>0.13</td>
<td>0.09</td>
<td>0.13</td>
<td>0.03</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.65</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Only measured in June 2008, analyzed with a one-way ANOVA

*Does not include March 2009 due to lack of rain

*Soil Moisture, g H₂O/g wet soil *100
Many of these soil variables displayed some degree of positive correlation with enzyme activities. Concentrations of total soil C displayed stronger positive association with enzyme activities than concentrations of soluble C (Table 2.3). While soil C was more strongly correlated with individual enzymes, the soil C:N ratio was the strongest correlate of two of the three enzyme ratios. We did not detect any direct evidence from our soil chemical data of end-product inhibition, as inorganic N and P concentrations did not correlate negatively with N and P mineralizing enzyme activities or enzyme ratios. However, we did detect a positive correlation between rates of net N mineralization and BG:(NAG+LAP) activity and a negative correlation between rates of net N mineralization and (NAG+LAP):aP. Microbial biomass N was more strongly associated with enzyme activities than microbial biomass C, and soil moisture displayed significant positive correlations with five of the six enzymes assayed.

**Table 2.3** Spearman’s product-moment correlation coefficients for soil biogeochemical variables and enzyme activities per gram soil. Only significant correlation coefficients are shown.

<table>
<thead>
<tr>
<th></th>
<th>aP</th>
<th>BG</th>
<th>NAG</th>
<th>BX</th>
<th>CBH</th>
<th>LAP</th>
<th>BG: AP</th>
<th>BG: (NAG+LAP)</th>
<th>(NAG+LAP): aP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil C</td>
<td>0.579</td>
<td>0.673</td>
<td>0.792</td>
<td>0.637</td>
<td>0.730</td>
<td>0.283</td>
<td>0.347</td>
<td>--</td>
<td>0.496</td>
</tr>
<tr>
<td>Soil N</td>
<td>0.603</td>
<td>0.635</td>
<td>0.692</td>
<td>0.602</td>
<td>0.643</td>
<td>0.270</td>
<td>0.274</td>
<td>--</td>
<td>0.359</td>
</tr>
<tr>
<td>Soil P</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>-0.343</td>
<td>--</td>
</tr>
<tr>
<td>Soil C:N</td>
<td>0.275</td>
<td>0.499</td>
<td>0.718</td>
<td>0.415</td>
<td>0.613</td>
<td>0.233</td>
<td>0.407</td>
<td>-0.264</td>
<td>0.642</td>
</tr>
<tr>
<td>NH₄⁺</td>
<td>0.583</td>
<td>0.508</td>
<td>0.705</td>
<td>0.591</td>
<td>0.541</td>
<td>--</td>
<td>0.183</td>
<td>-0.192</td>
<td>0.381</td>
</tr>
<tr>
<td>PO₄³⁻</td>
<td>0.263</td>
<td>--</td>
<td>0.229</td>
<td>0.373</td>
<td>0.222</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>N-min</td>
<td>0.413</td>
<td>0.381</td>
<td>--</td>
<td>0.293</td>
<td>0.229</td>
<td>--</td>
<td>0.462</td>
<td>-0.266</td>
<td>--</td>
</tr>
<tr>
<td>DOC</td>
<td>0.264</td>
<td>0.414</td>
<td>0.377</td>
<td>--</td>
<td>0.465</td>
<td>--</td>
<td>0.305</td>
<td>--</td>
<td>0.268</td>
</tr>
<tr>
<td>TDN</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>0.273</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>DOC:TDN</td>
<td>0.338</td>
<td>0.386</td>
<td>0.509</td>
<td>0.287</td>
<td>0.376</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>0.422</td>
</tr>
<tr>
<td>MB-C</td>
<td>0.376</td>
<td>0.474</td>
<td>0.537</td>
<td>0.547</td>
<td>0.542</td>
<td>0.318</td>
<td>0.255</td>
<td>--</td>
<td>0.385</td>
</tr>
<tr>
<td>MB-N</td>
<td>0.641</td>
<td>0.640</td>
<td>0.737</td>
<td>0.651</td>
<td>0.660</td>
<td>--</td>
<td>0.255</td>
<td>--</td>
<td>0.324</td>
</tr>
<tr>
<td>Soil moisture</td>
<td>0.726</td>
<td>0.654</td>
<td>0.578</td>
<td>0.444</td>
<td>0.561</td>
<td>--</td>
<td>0.239</td>
<td>0.239</td>
<td>--</td>
</tr>
</tbody>
</table>
2.5 Discussion

Here we used two forms of resource manipulation to examine how tropical soil enzyme activities and ratios shift with OM inputs. We found leaf litter addition and removal had notable impacts on soil EEA while throughfall reduction did not. This was most likely due to robust links between total soil C and enzyme activities in the experimental plots but a lack of strong correlation between DOC and enzymes. As soil C changed markedly with litter manipulation but was not altered by throughfall reduction, the observed enzyme response per gram soil to the treatments was not surprising. Given the importance of DOC concentrations and fluxes for regulating soil respiration and litter decomposition rates in our study site (Cleveland et al. 2006, Wieder et al. 2009, Cleveland et al. 2010), we hypothesized that [DOC] would exhibit significant correlations with soil enzyme activities. This hypothesis was not supported; instead we found that five of the six soil enzymes measured showed strong positive correlations with soil C (Table 2.3), similar to what has been observed in other biomes (Sinsabaugh et al. 2008). The lack of correlation between [DOC] and enzyme activities (Table 2.3) and DOC fluxes and enzyme activities (data not shown) may be due to the high variability of DOC pools in space and time. Litter-to-soil DOC fluxes vary with litter composition, litter layer thickness, rainfall intensity, and other variables (Montano et al. 2007, Wieder et al. 2009, Fujii et al. 2011). As such, it may be a more fruitful strategy for soil decomposer microbes to attune enzymes, which can have long life-spans within the soil environment (Burns 1982, Allison 2006), with total soil OM, essentially integrating dissolved OM fluxes over time.

As the proximal agents of organic matter mineralization, soil enzymes can provide valuable information on belowground nutrient limitation (Sinsabaugh et al. 2008, Sinsabaugh and Follstad Shah 2012). As we predicted, enzyme stoichiometries suggest strong P constraints
in this tropical soil community. Our control plot BG:aP (0.22 ± 0.02) and (NAG+LAP):aP (0.15 ± 0.01) ratios are much lower than the global soil averages of 0.62 and 0.44, respectively (Sinsabaugh et al. 2009), with enzyme allocation favoring P acquisition. These allocation patterns indicate that soil organisms direct more effort toward acquiring and cycling P relative to C and N in our tropical site compared to extra-tropical ones. Though they diverge from global means, our enzyme ratios are quite comparable to the handful of tropical sites where similar measurements have been made (Sinsabaugh et al. 2008). While other factors may play a role, these allocation patterns are an indirect yet compelling line of evidence for elevated heterotrophic P demand in the dominant soil orders of lowland tropical forest soils (Ultisols and Oxisols; Sanchez et al. 1982), and complement studies that have observed microbial responses to the addition of P in such soils (Cleveland et al. 2002, Ilstedt and Singh 2005, Cleveland and Townsend 2006, Reed et al. 2010).

That said, the changes in soil enzyme allocation patterns we observed following leaf litter manipulation highlight the potential for OM inputs to impact not only nutrient supply, but relative nutrient limitation. The significant drop in the BG:aP activity ratio (Figure 2.2) and the increase in aP activity per unit soil C and microbial biomass C (Figure 2.1b, c, Table A1) upon litter removal suggest P constraints are further exacerbated when leaf litter inputs are curtailed. The importance of leaf litter as a source of P has been noted in other tropical litter manipulations (Wood et al. 2009) and is not surprising given the changes in P pools and P availability in highly weathered soils (Walker and Syers 1976, Crews et al. 1995, Turner and Engelbrecht 2011). Although we did not observe significant changes in chemically extractable or total P pools in treatment plots (similar to Sayer et al. 2012), the shifts in enzyme activities suggest demand for P was elevated upon leaf litter removal.
By contrast, leaf litter addition seemed to shift enzymatic investment toward the acquisition of nitrogen. The significant increase in the (NAG+LAP):aP ratio in 2× plots (Figure 2.2) indicates a change in enzyme allocation away from P and toward N-mineralization. The large increase in NAG activity per unit soil C and microbial biomass C (Figure 2.1b, c, Table A1) also supports this interpretation. This enzymatic reallocation points towards increasing N demand, which is probably linked to increases in the C:N ratio of both total and dissolved organic matter pools in double litter plots (Table 2.1). In extra-tropical ecosystems exposed to free-air CO₂ enrichment, C-rich conditions cause elevated production of detritus and higher tissue C:N ratios. Over time, this appears to trigger progressive nitrogen limitation (PNL; Luo et al. 2004, Reich et al. 2006). Many tropical forests have large N-stocks and a high capacity for biological N-fixation (Cleveland et al. 1999, Reed et al. 2007, Hedin et al. 2009), thus the nature and timing of increasing N constraints under C-rich conditions is not likely to mirror temperate systems. But while the common paradigm for lowland tropical forests suggests a relative abundance of N (Vitousek and Matson 1988, Martinelli et al. 1999, Hedin et al. 2009), the importance of N limitation seems to increase in wetter tropical forests (Austin and Vitousek 1998, Houlton et al. 2006, Nardoto et al. 2008, Posada and Schuur 2011). Our study site lies in the wetter end of the lowland tropical forest rainfall spectrum, and multiple lines of evidence suggest N may not cycle in excess even under baseline conditions (Cleveland and Townsend 2006, Wieder et al. 2012, Taylor 2012). Thus, the relative increase in N-specific enzyme activity under higher OM inputs suggests the potential for PNL to develop fairly quickly in at least some lowland forests. As well, the links we observed between net N mineralization and enzyme stoichiometry (Table 2.3) indicate that changes in N availability mediated by altered OM inputs may have cascading consequences for belowground nutrient cycling.
Enzymes that degrade OM to provide energy (C) versus nutrients (N and P) may be regulated by resource availability in different ways. While evidence suggests inverse relationships between N & P availability and N & P mineralizing enzyme activities (McGill and Cole 1981, Sinsabaugh and Moorhead 1994, Olander and Vitousek 2000), C-degrading enzymes may in fact be stimulated by elevated concentrations of their substrates and end-products (Hernandez and Hobbie 2010). Our results support the interpretation that C-degrading enzymes are up-regulated when their substrates are abundant, not scarce. In litter addition plots, the activity of the cellulose-degrading enzyme CBH was significantly higher than control plot levels, even when concentrations of soil C and microbial biomass C were accounted for. This result suggests shifts in the importance of cellulose as a substrate with increases in litter quantity. In 0× plots that received essentially no leaf litter for an extended period, C-mineralizing enzyme activities were largely suppressed. This trend was especially pronounced for CBH and is not surprising given the lack of plant-derived cellulose that would result from litter removal. These findings are consistent with laboratory incubations that showed an increase in cellulase activity following additions of cellulose (Geisseler and Horwath 2009).

Aside from direct regulation by soil resource availability, it is also possible that changes in the composition of the soil microbial community with litter addition and removal (Nemergut et al. 2010, Leff et al. 2012) may have mediated some of the observed changes in enzyme activities and allocation patterns. For example, litter-rich conditions in 2× plots may have promoted the proliferation of decomposer microbes that specialize on cellulose (Stursova et al. 2012), with consequent increases in cellulase production. The fact that we observed tighter links with EEA and microbial biomass N versus microbial biomass C suggests that functional aspects of the microbial community may be important to consider. Recent conceptual models have
highlighted the importance of fungi, both free living and mycorrhizal, in a C-rich world (Pendall et al. 2004, Carney et al. 2007, Johnson 2010). While it is possible that shifts in the relative abundance of bacteria and fungi impacted the enzyme (especially chitinase) patterns we observed in response to litter manipulation, both qPCR and fluorescent microscopy indicated a low abundance of soil fungi, regardless of treatment, within the study soils (D. Nemergut and S. Weintraub, unpublished data).

Lastly, while soil resources and their ratios clearly exert significant control on enzyme activities, so too do climate variables (Freeman et al. 2001, Stursova et al. 2006, Wallenstein et al. 2009, Brzostek and Finzi 2011). In our study system, soil moisture content exhibited positive correlations with five of the six enzymes measured (Table 2.3). This result is notable in that overall moisture content in these high-clay but well-drained soils shows only minor temporal variation (Cleveland and Townsend 2006). Even so, we observed higher soil enzyme activities during the wet season when soils were slightly wetter than our dry season sampling date (data not shown). Differences in soil moisture between 0× and control plots likely also played a role. These results imply that variation in soil moisture conditions may be important for depolymerization rates, even in very wet tropical forests. Such variation can occur not only because of seasonal rainfall patterns, but via spatial variation in factors such as litter depth and topography. The mechanisms that underlie links with soil moisture and enzyme activities are not known but may include direct effects of water availability on microbial activity or indirect effects related to changes in soil oxygen levels (Silver et al. 1999) and/or the flux of labile C and nutrients (sensu Cleveland et al. 2006, 2010), although as noted above soluble C was not a significant predictor of EEA.
Taken as a whole, our study reinforces the growing conceptual understanding of enzyme regulation, in which soil C is an important predictor of enzyme activities (Sinsabaugh et al. 2008) yet soil nutrient availability and resource ratios regulate enzyme allocation patterns. Our results are broadly consistent with the theoretical framework underlying optimal allocation models (Sinsabaugh and Moorhead 1994, Treseder and Vitousek 2001), but also suggest that factors beyond resource-driven feedbacks – e.g. climate variability – may be important even at local scales. Our findings also support the idea that patterns in enzyme allocation to C vs. N vs. P acquisition will be distinct in tropical forests relative to higher latitude ecosystems and shifts in OM inputs can impact the balance between relative N and P limitation. Finally, the data presented here add to a body of literature (e.g. Waldrop et al. 2004, Carney et al. 2007, Allison et al. 2010, Cusack et al. 2011) that suggests soil enzyme activities are a useful tool for assessing changes in belowground nutrient limitation and OM cycling with environmental change, both within and beyond tropical ecosystems.
CHAPTER 3: NATIVE TREE SPECIES REGULATE NITROUS OXIDE FLUXES IN TROPICAL PLANTATIONS


3.1 Abstract

Secondary and managed plantation forests comprise a rapidly increasing portion of the humid tropical forest biome, a region that in turn is a major source of nitrous oxide (N$_2$O) emissions to the atmosphere. Previous work has demonstrated reduced N$_2$O emissions in regenerating secondary stands compared to mature forests, yet the importance of species composition in regulating N$_2$O production in young forests remains unclear. We measured N$_2$O fluxes beneath four native tree species planted in replicated, 21-yr-old mono-dominant stands in the Caribbean lowlands of Costa Rica, in comparison with nearby mature forest and abandoned pasture sites at two time points (wetter and drier seasons). We found that species differed eight-fold in their production of N$_2$O, with slower-growing, late-successional species (including one legume) promoting high N$_2$O fluxes similar to mature forest, and faster-growing, early successional species maintaining low N$_2$O fluxes similar to abandoned pasture. Across all species, N$_2$O flux was positively correlated with soil nitrate concentration in the wetter season and with soil water-filled pore space (WFPS) in the drier season. However, the strongest predictor of N$_2$O fluxes was fine-root growth rate, which was negatively correlated with N$_2$O emissions at both time-points. We suggest that tree-specific variation in growth habits creates differences in both N demand and soil water conditions that may exert significant control on N$_2$O fluxes from tropical forests. With the advent of REDD+ and related strategies for fostering climate mitigation via tropical forest regrowth and plantations, we note that species-specific traits as they relate to N$_2$O fluxes may be an important consideration in estimating overall climate benefits.
3.2 Introduction

The United Nations Framework Convention on Climate Change has identified reduced emissions from deforestation and degradation, alongside other forestry-related actions in tropical regions (REDD+), as a way to mitigate climate change (UNFCCC 2011). Not only are developing countries encouraged to slow deforestation through this program, but they may also receive financial compensation for enhancing national forest carbon (C) stocks. Some nations are currently pursuing the use of tropical tree plantations, as well as aggrading secondary forests, to accomplish these goals (Stickler et al. 2009). While carbon sequestration has been the primary focus, non-carbon environmental consequences (e.g. biodiversity effects and others) are enmeshed in this approach and are now being recognized and explicitly incorporated into REDD+ negotiations (Stickler et al. 2009, UNFCCC 2013).

One such non-carbon consideration is the production of nitrous oxide ($N_2O$), a potent greenhouse gas with nearly 300 times the radiative forcing per molecule compared to carbon dioxide and the capacity to destroy stratospheric ozone (Forster et al. 2007). Tropical forest soils are a globally important source of $N_2O$; estimates of annual $N_2O$ emissions from tropical forest soils range from 0.88 to 2.37 Tg N (Werner et al. 2007), second only to emissions from agriculture (Vitousek and Matson, 1992, Mosier et al. 2001, Davidson 2009). These considerable fluxes are attributed to high nitrogen (N) availability relative to demand in many humid tropical forests (Vitousek and Matson 1988, Hedin et al. 2009). In ecosystems that have accumulated more N than primary producers require (i.e. N is in excess, Martinelli et al. 1999), high rates of microbial N transformations coupled with warm temperatures and high water availability can cause substantial N losses via both gaseous and hydrologic pathways (e.g. Conrad 1996, Davidson et al. 2000, Houlton et al. 2006, Brookshire et al. 2012, Koehler et al. 2012).
In the case of N$_2$O losses, both nitrate availability and soil water-filled pore space (WFPS) regulate ultimate emissions from soils (Keller and Reiners 1994), the latter by influencing rates of gas diffusion and soil oxygen concentrations (Firestone and Davidson 1989, Perez et al. 2000). And while nitrous oxide production from the tropical biome is clearly significant in the global N$_2$O budget, the inherent spatial and temporal variation in controlling factors drives considerable variance in N$_2$O emission rates across tropical landscapes. For example, differences in topographic position (Reiners et al. 1998, McSwiney et al. 2001) and substrate age or fertility status (Hall and Matson 2003) can cause nitrous oxide production rates to vary by one or two orders of magnitude, due to differences in N availability and/or soil WFPS.

Variation is also driven by human disturbances. In general, deforestation followed by forest secondary succession reduces N$_2$O fluxes from tropical forests due to declines in N supply relative to the high demand of aggrading stands (Keller and Reiners 1994, Davidson et al. 2007). This makes aggrading forests both a carbon sink and a reduced N$_2$O source. Some evidence suggests post-disturbance recovery of high rates of N$_2$O efflux and a generally “open” N cycle with N in excess can take up to a century (Davidson et al. 2007), yet our understanding of the controls on N trace gas fluxes within aggrading stands remains limited and merits more attention as these forests continue to increase in extent and importance (Silver et al. 2000, Wright 2005, Chazdon 2008). The advent of policy metrics such as those discussed above, which promote tropical forest preservation, expansion and management as a key climate mitigation strategy (e.g. Stickler et al. 2009, Fearnside 2013, Lubowski and Rose 2013) heightens the need for this understanding, as N$_2$O emissions can represent a notable piece of the overall climate impact of a given tropical forest stand.
Both managed and natural reforestation result in shifts in tree species composition, yet links between species shifts and N\textsubscript{2}O emissions are poorly resolved. And while natural forest regeneration following pasture and cropland abandonment is still common, an increasing fraction of the globe’s tropical secondary forests are becoming actively managed, species-specific plantations (4% of global forest area; Lugo 1997, Mayaux et al. 2005, Chazdon 2008). Tropical tree species have been shown to affect ecosystem-level C and N cycling due to differences in species traits and ecological strategies that control growth, N acquisition, and litter decomposability (e.g. Vitousek et al. 1987, Binkley and Ryan 1998, Russell et al. 2010, Russell and Raich 2012). Results to date also suggest that individual tree species may affect rates of N\textsubscript{2}O emission in both humid tropical monocultures (Erickson et al. 2002) and mature mixed forests (van Haren et al. 2010). However, comparisons of N\textsubscript{2}O fluxes in multiple mono-dominant stands of tropical tree species in a controlled experiment, in combination with tree trait data that allows for analysis of the driving variables, are scarce.

We investigated the effects of tree species identity on soil N\textsubscript{2}O fluxes at La Selva Biological Station (hereafter referred to as La Selva) in northeastern Costa Rica. Mature forests growing on well-drained soils in this region are known to be large emitters of N\textsubscript{2}O, with fluxes averaging 5.86 kg N\textsubscript{2}O-N ha\textsuperscript{-1} yr\textsuperscript{-1} (Keller and Reiners 1994). Here, we used an experimental setting in which N\textsubscript{2}O fluxes from four 21-year-old mono-dominant plantations could be compared with adjacent abandoned pasture and mature forest. Previous work from this experimental site, wherein all planted species had similar climate, topography, parent material, and land-use history, has shown that key biogeochemical properties differ among the tree species (González and Fisher 1994, Raich et al. 2007, 2009, Russell et al. 2010). We hypothesized that differences in tree species traits would also cause inter-specific differences in the production of
nitrous oxide. While we assumed that all plantation plots would have lower N$_2$O fluxes than mature forest and higher fluxes than abandoned pasture (sensu Davidson et al. 2007, Keller and Reiners 1994), we predicted that fluxes of N$_2$O would be highest beneath species that promoted higher N availability in soil. We also predicted that species-driven differences in soil WFPS, which can emerge via traits that affect both soil bulk density and growth rates, would serve as an additional control over rates of N$_2$O emission.

3.3 Methods

3.3.1 Site description

For the last three decades, annual rainfall at La Selva has averaged 4,142 mm and annual temperature 25°C (Clark et al. 2010). Precipitation delivery is seasonal, with a wetter season from ~ May-December and a drier season from ~ January-April. However, long-term rainfall averages exceed 100 mm even in drier season months. The soil has been classified as Mixed Haplic Haploperox (Kleber et al. 2007) in the Matabuey consociation (Sollins et al. 1994). This soil is relatively high in organic matter and N stocks, acidic, highly leached, and low in base saturation (Russell et al. 2007).

The experimental site was situated in a 12-ha area of La Selva that had been deforested in the mid-1950’s, converted to pasture and grazed for 30 years before abandonment in 1987. In 1988 mono-dominant stands of 11 tree species were planted in 50 x 50 m plots in a randomized complete block design containing four blocks (Fisher 1995, Russell et al. 2007). By 2010, four of the 11 species originally planted had survived — all native species — and are the subject of this study. The species (and Family) include: Hieronyma alchorneoides Allemao (Phyllanthaceae); Pentaclethra macroloba (Willd.) Kunth. (Fabaceae); Virola koschnyi Warb. (Myristicaceae); and
*Vochysia guatemalensis* Donn. Sm. (Vochysiaceae). *Pentaclethra* is the only legume included in the experiment and is the most common overstory tree at La Selva. Whereas *Hieronyma* and *Vochysia* are typically found in the earlier successional stages, *Pentaclethra* and *Virola* become dominant as the forest matures. The understory in the plantations had been removed during the first 3-4 years of the experiment until canopy closure was reached. Thereafter, the understory was left unmanaged. By 2004, the understory contained 255 species, but their contribution to aboveground net primary production of 20-30% did not differ significantly among tree species (Russell et al. 2010).

To provide a reference of different land-cover types, four randomly selected 50 x 50 m plots within a single block (150 x 200 m) of mature forest were established in 2003 and four 14 x 6 m transects were established in an unplanted abandoned pasture in 2004. The northernmost corner of the mature forest block was situated 150 m from plantation block three, and the abandoned pasture transects, situated between plantation blocks one and four, were less than 50 m from the species plots. Both abandoned pasture and mature forest occurred on the same soil type and had the same climate as the plantations. Without true replication in these reference plots/transects, the full experimental design was classified as a randomized incomplete block.

### 3.3.2 Field and laboratory methods

Once during the wetter season (July 2010) and once during the drier season (January 2011), we measured N₂O fluxes from the 21-year-old plantations, and also in the abandoned pasture and mature forest to provide end-members for the study. Nitrous oxide fluxes were measured using static flux chambers (Keller and Reiners 1994, Wieder et al. 2011). On the day of sampling, 19.5 cm inner diameter PVC rings were installed in surface soils to equal depth at four randomly chosen locations within each plot, with one ring per quadrant. Surface leaf litter
was removed from the ring interior and volumetric soil moisture was recorded within 5 cm of the ring using a hand-held soil moisture probe inserted to 10 cm depth. We used this soil moisture measurement along with soil bulk density values to determine WFPS at the sampling time.

Closed chambers, constructed from 21.3 cm inner diameter PVC end-caps (headspace volume = 3.14 liters) fitted with brass bulkhead union fittings and 9.5 mm thermo-green septum, were placed on the rings using vacuum grease to ensure a gas-tight seal.

Gas samples (30 mL each) were collected from the chamber headspaces four times over the course of a thirty-minute incubation, including a time-zero sample immediately after the chamber was placed on the ring. Gas samples were stored in 20 mL serum vials that were sealed with thick butyl rubber stoppers (Bellco Glass, Vineland, NJ, USA). All vials were flushed with N₂ and evacuated prior to sample collection. Chamber incubations were conducted in the four species plots in each of the four blocks as well as in the mature forest plots and along the abandoned pasture transects. Gas vials were transported to the University of Colorado where they were analyzed using a Schimadzu GC-14A gas chromatograph via an electron capture device (ECD, Shimadzu Scientific Instruments, Columbia, MD, USA). Rates of N₂O efflux were calculated as the linear increase in N₂O concentration in the chamber headspace over time. The few sample vials that failed to hold their gas seal (i.e., were not over-pressurized) were excluded from our analyses.

In order to assess the size of inorganic N pools in soils with different plant-cover types, we conducted extractions of nitrate (NO₃⁻) and ammonium (NH₄⁺) with 2M potassium chloride (KCl). We sampled soils near the N₂O static chambers twice, once in the wetter and once in the drier period. Soil samples were taken from the surface (0-15 cm) using a 3.2-cm-diameter push-tube soil corer, placed in Ziploc bags, brought to the lab on ice, and processed immediately. Soil
solutions with a 1:5 dry soil/KCl ratio were shaken for 30 min, allowed to settle for 30 min, and then filtered through No. 42 Whatman paper. Two blanks were also extracted at every sample time to account for contaminant nitrate and ammonium in the filters and storage vials. Filtrates were kept frozen and transported on dry ice to Iowa State University where they were analyzed colorimetrically for NO$_3^-$–N and NH$_4^+$–N using an automated ion analyzer (QuickChem 4100, Lachat Instruments Division, Zellweger Analytics, Inc., Milwaukee, WI). Field-moist subsamples of 5 g were dried at 105°C for 48 hr to convert measurements to a dry-weight basis.

In January, potential net N mineralization was determined as the difference between ‘final’ and ‘initial’ quantities of inorganic N in fresh soil samples maintained at field moisture and incubated under aerobic conditions in the dark for seven days (Hart et al. 1994). Extractions for the ‘final’ samples were conducted as indicated above. We calculated net rates of N mineralization as the difference between total inorganic N (NH$_4^+$ + NO$_3^-$) on day 7 and day 1.

To determine whether the tree species continued to differ in fine root production (e.g. Russell et al. 2010), as this could affect belowground processes that regulate N$_2$O emissions, we measured fine-root growth in the 0-15 cm soil layer from May 2008 to May 2009 (Russell and Raich, unpublished data). We used the ingrowth core method, as described in Valverde-Barrantes et al. (2007). Measurements were conducted in all four plantation species. Fine-root growth was also measured using this technique in the mature forest in 2012-2013, thus providing a reference for that vegetation type.

3.3.3 Statistical analyses

Differences in N$_2$O emissions, inorganic N pools, and WFPS between treatments were analyzed using mixed effects models, with treatment as a fixed effect and block as a random effect. We used the Satterthwaite method for estimating the degrees of freedom for our analyses.
due to the incomplete nature of the block design (i.e. abandoned pasture and mature forest plots were not included in the original randomized complete block design) and restricted estimate maximum likelihood (REML) for parameter estimation within the SAS system. This statistical analysis took into account the pseudo-replication in the reference plots. All data except for WFPS values were natural log-transformed in order to meet assumptions for linear models (i.e. homoscedasticity and normal distribution of residuals). The differences of least squares means were used for pairwise comparisons between plant-cover types. We conducted separate analyses for July and January N\textsubscript{2}O fluxes rather than include sampling date as another variable in the model.

We conducted correlation analyses to determine if indices of N availability or soil WFPS were related to fluxes of N\textsubscript{2}O in either wetter or drier seasons. We also tested for relationships between fine-root growth and N\textsubscript{2}O production, hypothesizing that species with lower belowground productivity could allow for higher rates of exogenous soil N loss. For this analysis, we used mean fine root growth from Russell et al. (2010) and the 2008-2009 growth rates (Russell & Raich, unpublished data). Nitrous oxide fluxes were log-transformed for correlation analyses and only fluxes from the plantations were considered (n = 16, four species x four blocks). Correlation tests were conducted in R (R Core Development Team, 2011).

3.4 Results

Nitrous oxide fluxes differed significantly among the six vegetation types in both wetter (Figure 3.1; \( F = 18.31, P = 0.002 \)) and drier (Figure 3.2; \( F = 7.15, P = 0.024 \)) seasons. Emissions from \textit{Vochysia} were the lowest of all vegetation types (\(~ 0.5 \text{ ng N}_2\text{O-N cm}^{-2} \text{ hr}^{-1}\)) and were
Figure 3.1 Soil fluxes of N\textsubscript{2}O in the wetter season from the different vegetation types, including abandoned pasture, four species in mono-dominant plantations (full species names in text), and mature forest. Vegetation types that share a letter are not significantly different; hatched lines are standard errors.

significantly smaller than those in Pentaclethra, Virola and the mature forest during both sampling dates (pairwise comparisons, $P \leq 0.004$ in the wetter season and $P \leq 0.005$ in the drier season). Hieronyma was the next-lowest N\textsubscript{2}O-emitting species ($\sim 1.2$ ng N\textsubscript{2}O-N cm\textsuperscript{-2} hr\textsuperscript{-1}), exhibiting significantly smaller fluxes than Pentaclethra, Virola and the mature forest during the wetter (Figure 3.1) and drier (Figure 3.2) sampling periods. Emissions of N\textsubscript{2}O from the abandoned pasture ($\sim 1$ ng N\textsubscript{2}O-N cm\textsuperscript{-2} hr\textsuperscript{-1}) were most similar to Vochysia and Hieronyma, the two early successional species. Fluxes from Pentaclethra ($\sim 3.5$ and $1.5$ ng N\textsubscript{2}O-N cm\textsuperscript{-2} hr\textsuperscript{-1}, wetter and drier seasons respectively) were higher but not significantly different from the pasture in either season, yet emissions from Virola ($\sim 4$ and $2$ ng N\textsubscript{2}O-N cm\textsuperscript{-2} hr\textsuperscript{-1}, wetter and drier
seasons respectively) were significantly higher than pasture in both July (Figure 3.1; \( P = 0.038 \)) and January (Figure 3.2; \( P = 0.018 \)).

During the wetter season, mean N\(_2\)O efflux was one to two orders of magnitude higher in the mature forest (~11 ± 2.5 ng N\(_2\)O-N cm\(^{-2}\) hr\(^{-1}\)) compared to the lower-emitting plantation species and abandoned pasture. However, fluxes from *Virola* and *Pentaclethra* were not statistically different from the mature forest at \( P = 0.05 \) (Figure 3.1; \( P = 0.108 \) and 0.059, respectively). During the drier period, fluxes in the mature forest were greatly reduced (~1.5 ± 0.2 ng N\(_2\)O-N cm\(^{-2}\) hr\(^{-1}\)) yet were still significantly higher than *Vochysia* (\( P = 0.005 \)) and *Hieronyma* (\( P = 0.040 \)) and statistically similar to *Virola* (\( P = 0.218 \)) and *Pentaclethra* (\( P = 0.792 \)).

**Figure 3.2** Soil fluxes of N\(_2\)O in the drier season from the different vegetation types, including abandoned pasture, four species in mono-dominant plantations (full species names in text), and mature forest. Vegetation types that share a letter are not significantly different; hatched lines are standard errors.
Table 3.1 Soil inorganic N concentrations and water filled pore space (WFPS) in the different vegetation types during wetter and drier season sampling period (mean values ± standard errors).

<table>
<thead>
<tr>
<th>Vegetation Types</th>
<th>Wetter NO$_3^-$ (ug N/g)</th>
<th>Wetter NH$_4^+$ (ug N/g)</th>
<th>Wetter WFPS (%)</th>
<th>Drier NO$_3^-$ (ug N/g)</th>
<th>Drier NH$_4^+$ (ug N/g)</th>
<th>Net N-min (ug N/g)</th>
<th>WFPS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pasture</td>
<td>0.72$^b$ (0.22)</td>
<td>8.41$^b$ (0.77)</td>
<td>73.0$^b$ (1.3)</td>
<td>0.27$^b$ (0.10)</td>
<td>0.69$^{abc}$ (0.13)</td>
<td>12.76$^{bc}$ (9.08)</td>
<td>57.8</td>
</tr>
<tr>
<td>Vochysia</td>
<td>0.18$^a$ (0.09)</td>
<td>1.65$^a$ (0.40)</td>
<td>69.7$^b$ (2.2)</td>
<td>0.35$^b$ (0.15)</td>
<td>1.40$^c$ (0.33)</td>
<td>0.12$^a$ (0.39)</td>
<td>51.3</td>
</tr>
<tr>
<td>Hieronyma</td>
<td>0.33$^{ab}$ (0.12)</td>
<td>1.91$^a$ (0.37)</td>
<td>74.9$^{bc}$ (2.4)</td>
<td>0.01$^a$ (0.003)</td>
<td>1.29$^c$ (0.28)</td>
<td>3.17$^b$ (0.46)</td>
<td>57.2</td>
</tr>
<tr>
<td>Pentaclethra</td>
<td>2.29$^c$ (0.34)</td>
<td>1.17$^a$ (0.36)</td>
<td>63.3$^a$ (1.4)</td>
<td>0.45$^b$ (0.10)</td>
<td>0.82$^b$ (0.28)</td>
<td>7.54$^c$ (1.30)</td>
<td>48.7</td>
</tr>
<tr>
<td>Virola</td>
<td>0.20$^{ab}$ (0.07)</td>
<td>1.51$^a$ (0.35)</td>
<td>78.9$^{cd}$ (2.9)</td>
<td>0.02$^a$ (0.02)</td>
<td>0.24$^a$ (0.13)</td>
<td>2.61$^b$ (0.49)</td>
<td>63.6</td>
</tr>
<tr>
<td>Forest</td>
<td>2.98$^c$ (0.58)</td>
<td>3.80$^{ab}$ (1.03)</td>
<td>82.4$^d$ (2.0)</td>
<td>1.12$^c$ (0.16)</td>
<td>0.66$^{abc}$ (0.17)</td>
<td>7.33$^{bc}$ (2.53)</td>
<td>57.1</td>
</tr>
</tbody>
</table>

| F                | 31.19                     | 4.66                     | 10.44           | 17.41                    | 5.14                     | 7.49                 | 3.320    |
| P                | 0.001                     | 0.043                    | <0.001          | 0.036                    | <0.001                   | 0.087               |

Notes: Vegetation types include abandoned pasture, four species in mono-dominant plantations (full species names in text), and mature forest. Test statistics from mixed effects models ($F$, $P$) are shown; vegetation types that share a letter are not significantly different. Net N-min means net nitrogen mineralization over a 7-day period.

Soil nitrogen availability differed among the vegetation types. Extractable NO$_3^-$ concentrations were highest in the mature forest but Pentaclethra exhibited similarly high levels in the wet season and was the only species with a NO$_3^-$ to NH$_4^+$ ratio greater than 1 (Table 3.1). Concentrations of NH$_4^+$ were similar among species in the plantations during the wet season, but during the drier period Vochysia and Hieronyma had higher ammonium concentrations than the other two species. Net N mineralization was highest in Pentaclethra and mature forest and lowest in Vochysia soils. The species also differed in WFPS, with Pentaclethra being the driest (July = 63%, January = 49%) and Virola the wettest (July = 79%, January = 64%).
In the wetter season, N$_2$O fluxes and NO$_3^-$ concentrations were positively correlated (Figure 3.3a; Pearson’s $r = 0.49$, $t = 2.16$, $P = 0.048$). However, in the drier season, NO$_3^-$ did not correlate with N$_2$O emissions. Instead, WFPS was the only positively correlated soil predictor of N$_2$O fluxes in January (Figure 3.3b, Pearson’s $r = 0.54$, $t = 2.28$, $P = 0.039$). Neither net N mineralization nor NH$_4^+$ concentrations correlated significantly with N$_2$O fluxes at either time-point. However, fine-root growth rates from 2008-2009 were strongly and significantly negatively correlated with both wetter (Figure 3.4a; Pearson’s $r = -0.76$, $t = 4.33$, $P < 0.001$) and drier (Figure 3.4b; Pearson’s $r = -0.72$, $t = 3.91$, $P = 0.002$) season N$_2$O emissions.

**Figure 3.3.** Relationships between wetter-season N$_2$O fluxes and NO$_3^-$ concentrations (a) and drier-season N$_2$O fluxes and soil WFPS (b) in the mono-dominant species plots (full names in text). Pearson’s $r$ values are shown, along with linear trend lines.
3.5 Discussion

In an earlier study at La Selva, Keller and Reiners (1994) observed a distinct gradient of N$_2$O, with high fluxes from mature forest soils, low fluxes from grass-dominated pastures and intermediate fluxes from mixed, naturally regenerating secondary forest stands. While we also observed generally high fluxes in our mature forest plots at La Selva, not all aggrading forests were alike; the production of N$_2$O was dependent on the species composition of the overstory and not merely the successional stage. We propose that these significant species effects on N$_2$O emissions – eight-fold differences in wetter season fluxes and four-fold differences in drier-season fluxes – are linked to species traits and growth habits, such as fine-root production, which regulate both soil N availability and WFPS.

The two tree species in our study system that produced the most N$_2$O, Pentaclethra and Virola, are generally found in mature forests and appear to have traits that promote a more open, leaky N cycle. Pentaclethra, like many leguminous trees, has low litter and root C:N ratios (Russell et al. 2007, Raich et al. 2007). This species, with its N-rich tissues, low N use efficiency, and nodulated roots, sustains high fluxes of detrital N (Russell and Raich 2012). All of these traits point to high N fluxes through the plant-soil system, during the course of which more nitrogen can be lost. This is not an altogether surprising result, as others (such as Erickson et al. 2002) have also observed elevated N$_2$O fluxes from beneath leguminous trees in mono-dominant tropical stands. Furthermore, while Pentaclethra had the highest net N mineralization rates and largest NO$_3^-$ pools (Table 3.1), it may have had less capacity to take up mineralized N as a result of lower relative fine-root growth (Figure 3.4a,b, squares) and/or less need to take up soil N due to the capacity to fix atmospheric N$_2$. Thus, Pentaclethra promoted larger available N
pools but was potentially a poor competitor for its uptake, leaving N pools more vulnerable to loss via N$_2$O.

Figure 3.4 Relationships between fine-root growth and N$_2$O fluxes in the wetter (a) and drier (b) seasons in the mono-dominant species plots (full names in text). Pearson’s $r$ values are shown, along with linear trend lines.

Emissions from beneath *Virola* were on par with those from *Pentaclethra*, despite the fact that *Virola* did not have particularly N-rich tissues or elevated N flows within the plant-soil system (Russell and Raich 2012). However, *Virola* was among the least productive of the studied species and displayed notably low fine-root growth (Russell et al. 2010; Figure 3.4a,b, diamonds). This tendency toward slow growth and low belowground biomass likely reduced its potential for acquiring N and thus lowered N uptake, rendering soil N pools more prone to
transformations and losses via microbial and hydrologic pathways. Conversely, the early successional tree species that had the lowest N$_2$O fluxes in both wetter and drier seasons, *Vochysia*, was the most productive of the four species examined. This species tended to have higher fine-root growth (Figure 3.4a,b, triangles) and high above and below-ground biomass (González and Fisher 1994, Russell et al. 2010). As such, it had high N uptake (Russell and Raich 2012), leaving soil N less vulnerable to losses from the system. *Hieronyma*, the other plantation species with low N$_2$O fluxes, had the fastest average belowground growth rates (Russell et al. 2010; Figure 3.4a,b, circles), further supporting a link between tree growth patterns and N gas emissions.

Our findings of potential links between N$_2$O fluxes and plant traits that determine growth rates and N demand agree with the results of Van Haren et al. (2010), who observed variation in N$_2$O fluxes beneath individual tropical trees in a diverse stand of Amazonian humid mature forest. The highest emissions of N$_2$O detected in that study were beneath the crowns of species that had low productivity, none of which were legumes. The lowest emitters of N$_2$O tended to be the two most productive species (van Haren et al. 2010), similar to our results. Importantly, the coupling between productivity and the amount of N available for N$_2$O production was not completely apparent in our study by examination of inorganic N pools alone. For instance, high N$_2$O fluxes from slow-growing *Virola* were not well-predicted by its low NO$_3^-$ pools (Figure 3.3a, *Virola* plots above the line). In fact, a better predictor of N$_2$O fluxes in the plantations was fine-root growth rate, which scaled negatively with nitrous oxide emissions in both wet and dry periods. Consistent with correlations in the plantations, the mature forest reference plots had relatively high N$_2$O emissions and low fine-root growth, 1.46 ± 0.21 Mg C ha$^{-1}$ yr$^{-1}$ (Russell, unpublished data from 2012-2013), which was slightly less than rates in *Virola* and *Pentaclethra*
of 1.91 ± 0.25 and 1.84 ± 0.31 Mg C ha⁻¹ yr⁻¹, respectively. Thus, in order to tease out the mechanisms behind tree species effects, it seems essential to further examine processes that determine tree-specific variation in growth habits and how differences in tree N demand and N uptake affect N₂O emission.

Tree traits that affect soil water content may also lead to meaningful variability in N₂O emissions. For example, in the drier season soil WFPS was the only positive correlate with N₂O fluxes (Figure 3.3b), and we observed differences in soil WFPS across the three general vegetation types and between species in the plantations. Tree species traits that affect soil bulk density and water use, such as differences in organic matter inputs, root architecture and photosynthetic demand, may thus have important implications for soil N₂O fluxes via effects on soil WFPS, especially during drier periods. This may help to explain why Virola fluxes did not decline as strongly in January as in other vegetation types (e.g. Figure 3.2). Virola’s low belowground biomass contributed to a relatively higher soil bulk density (Russell et al. 2007, Russell et al. 2010), which, combined with lower growth rates, enabled WFPS in Virola plots to stay above 60% during the drier season (Table 3.1). These conditions may have continued to promote denitrification while soils beneath other species experienced hydrologic conditions unfavorable for nitrous oxide production (Davidson et al. 2000).

Our findings indicate that, through diverse mechanisms, tree species have the potential to alter the production of N₂O and the transition from conservative to leaky N cycling in aggrading tropical stands. Notably, those effects are not simply a product of soil N pools, but are also linked to plant traits that determine growth rates, soil water dynamics and N uptake. Links between tree species and biogeochemical processes in tropical forests recovering from human disturbance merit attention (Silver et al. 2000, Wright 2005, Chazdon 2008), since as of 2005,
half of the entire humid tropical biome had been deforested to the extent that it contained 50% or less tree cover (Asner et al. 2009). Tropical land managers and other environmental policy agents advocate the planting of trees and use of plantations to aid secondary succession and forest restoration (Lugo 1997, Rudel et al. 2005, Chazdon 2008), with new emphasis on C sequestration as well (Stickler et al. 2009, Lubowski and Rose 2013). Because species-specific variations in N\textsubscript{2}O emissions can be substantial, and given the potency of N\textsubscript{2}O as a greenhouse gas, these dynamics merit consideration in restoration efforts. We suggest that in meeting REDD+ goals, an improved understanding of the influence of individual tree species on N\textsubscript{2}O fluxes could: 1) guide species selection in restoration and C sequestration projects; 2) aid in the design of sustainable agro-ecosystems that include trees; and 3) promote land-use planning for mitigation of N\textsubscript{2}O emissions in a changing climate.
4.1 Abstract

Geomorphic position often correlates with nutrient cycling across landscapes. In tropical forests, topography is known to influence phosphorus (P) availability, but its effect on nitrogen (N) cycling has received less exploration, especially in lowland forests. Here, we report significant effects of topographic slope and landscape position on multiple aspects of the N cycle across a highly dissected wet tropical forest in southwest Costa Rica. A suite of N cycle metrics measured along a topographic sequence suggested a distinct gradient in N availability. δ\(^{15}\)N values, bioavailable N pools, net nitrification rates, and soil nitrification potentials, were all substantially lower on a flanking steep hillslope compared to a ridge top, indicating lower N availability and a less open N cycle. Slope soils also hosted smaller C and N stocks and less weathered soil minerals than did the ridge. These latter findings suggested that elevated N loss due to soil and particulate organic matter erosion could underpin the spatial variation in N cycling and availability. In order to explore links between N cycling and topography across the larger landscape, we analyzed soil δ\(^{15}\)N values in a set of spatially-distributed samples that varied only in their slope angle, and observed a strong negative correlation between soil δ\(^{15}\)N values and topographic slope. This trend can most easily be explained by an increase in nitrogen loss by erosive, non-fractioning pathways on steeper slopes, as other variables commonly assumed to affect soil δ\(^{15}\)N values (such as temperature, precipitation, and vegetation type) were held constant. Taken together, these results suggest that differences in openness of N cycling and relative N richness observed at the hillslope scale are likely widespread in our study region due
to increased erosive N loss from steep reaches. Our results illustrate the potential importance of
g geomorphology in driving nutrient availability, even for elements such as N that are not derived
from rock weathering.

4.2 Introduction

Tropical forests display notable heterogeneity in nutrient cycling (Cuevas and Medina
and nutrient constraints on ecosystem processes vary from local to regional scales even within
humid lowland forests (Kaspari et al. 2008, Townsend et al. 2011, Wright et al. 2011, Alvarez-
Clare et al. 2013). In large part, such variation is a product of unique and diverse combinations in
the state factors that regulate terrestrial ecosystem processes and soil development (i.e. climate,
organisms, topography, parent material, time; Jenny 1941, Amundson and Jenny 1997,
Townsend et al. 2008). This nutrient cycling heterogeneity presents a substantial challenge to
predicting ecosystem function across diverse tropical landscapes (Randerson et al. 2009,
Townsend et al. 2011, Cleveland et al. 2011). Resolving the drivers and resultant effects of this
variation is essential as tropical forests will play important roles in a host of global

Topography is one of the state factors that contributes to landscape-scale variation in
tropical nutrient cycling. On short timescales, high rainfall can cause significant hydrologic
transport of solutes, redistributing nutrients and carbon in dissolved phases along flowpaths
nutrient cycling via effects on soil formation and residence times (Jenny 1941, Walker and Syers
1976, Birkeland 1999). Slope gradient is among the central drivers of the rate of physical erosion
in soil-mantled landscapes (Heimsath et al. 1997, Roering et al. 1999), and differences in slope-mediated landscape stability create differences in soil development patterns across complex terrain. Humid, low-latitude regions are known for their deep, highly weathered soils that are depleted in primary minerals and nutrients derived from rock weathering (Johnsson and Stallard 1989, Thomas 1994, Buss et al. 2010). However, the extent of chemical weathering and associated rock-derived nutrient depletion is also strongly affected by topography. Particularly where landscapes are still adjusting to changes in erosion regime (Ahnert 1994), tropical hillslopes maintain thinner, less weathered soils than nearby ridges and tend to have a higher abundance of elements derived from primary mineral weathering, including phosphorus (P) (Scatena and Lugo 1995, Vitousek et al. 2003, Porder et al. 2007). The rejuvenation of rock-derived nutrients (particularly P) by erosion has broad ecological significance because P limitation is thought to be common in late-successional lowland tropical forests (Vitousek and Sanford 1986, Cleveland et al. 2011).

The physical removal of surface soils from slopes can rejuvenate the supply of rock-derived nutrients such as P, but it may also lead to substantial losses of nitrogen (N) (Hilton et al. 2013) as soil N concentrations are highest at the surface. Mature lowland tropical forests are commonly thought to have an open N cycle as a result of N accumulation in excess of demand (Vitousek and Matson 1988, Martinelli et al. 1999), a paradigm for which there is ample evidence (Vitousek and Sanford 1986, Vitousek and Farrington 1997, Hedin et al. 2009). However, much of this evidence is drawn from the study of stable, slowly eroding sites. For lowland tropical forests in actively eroding terrain, both nutrient inputs and losses due to erosion may regulate spatial patterns of soil fertility and associated ecosystem functions (Scatena and Lugo 1995, Porder and Hilley 2011) and should thus be considered. Where erosion rates vary
between ridges and slopes, the potential exists for significant effects on N as well as P cycling, but effects on N cycling have been less thoroughly explored, especially in the lowland tropics.

Unlike lithogenic nutrients, most N enters ecosystems via biological processes (i.e. N fixation) and tends to accumulate in surface soils over time (Jenny 1941, Olff et al. 1993, Chapin et al. 1994). Indeed, the broad paradigm of N-rich, P-poor tropical forest soils depends on sufficiently long soil residence times which allow for substantial N accumulation and concurrent P depletion via chemical weathering and leaching (Walker and Syers 1976, Crews et al. 1995). However, consistent loss of topsoil and detritus from eroding steep slopes may constrain N accumulation, preventing hillslopes from reaching the typical N-rich steady state found in many tropical flatlands. In a study site in California, Amundson et al. (2003) suggested that a decline in soil δ^{15}N values with an increase in hillslope gradient, an indicator of less open N cycling (i.e. less N loss from available pools), could be explained by soil N erosion. Hilton et al. (2013) observed a similar isotopic trend in montane tropical forests of Peru and Taiwan and modeled the changes as a function of increased non-fractionating, erosive N loss on steeper slopes. These studies suggest soil N dynamics may be under substantial geomorphic control, yet to date there have been few attempts to assess whether geomorphology causes biologically relevant variation in N availability across dissected lowland forests, where widespread N abundance is often assumed.

In this context, we explored links between topography and N cycling in a geomorphologically dynamic humid lowland tropical forest in southwest Costa Rica. The study region is characterized by a mixture of flat ridge tops, steep hillslopes, and bedrock streams. We hypothesized that the relatively flat, stable ridge tops would have higher N availability than steep
slopes, and that physical removal of surface soil and organic matter could be invoked to explain substantially lower stocks and flows of N in steep regions.

4.3 Methods

4.3.1 Study site

This study was conducted in a primary lowland tropical forest at the Rio Piro Research Station on the southern Osa Peninsula, southwest Costa Rica (8° 24' 42"N, 83° 20' 00"W). This carbon-dense forest has large-statured trees (mean canopy height = 45 m; Taylor et al. unpublished data), contains a diverse assemblage of canopy and understory plants (Weber 2001), and has no known history of deforestation or land-use change. The area receives ~ 3,400 mm of rain per year and has a mean annual temperature of 26°C. Rainfall is distinctly seasonal – from January to March (the dry season), rainfall averages less than 100 mm per month; thereafter, heavy rains are common with peak rains from September to November (Taylor et al. 2014). Soils in this region have been broadly classified as Ultisols (Berrange and Thorpe 1988). The parent material of all soils involved in this study is Miocene greywackes derived from continental and pelagic sediments deposited in a turbid marine environment. These sedimentary rocks overlay the Cretaceous basaltic rocks of the Osa Igneous Complex in a very thick (upto 800 m) layer (Buchs et al. 2009). The Osa Peninsula is undergoing uplift at a long-term average rate of 1-2 m/kyr due to the NE-directed subduction of the aseismic Cocos Ridge to its west (Gardner et al. 1992, Buchs et al. 2009). This high rate of uplift, combined with high rainfall, has created a highly dissected, geomorphologically dynamic landscape (Bern et al. 2007; Figure 4.1a). Prior data suggests suspended sediment fluxes from small Osa watersheds are substantial (Taylor et al. 2014).
4.3.2 Field and laboratory methods

To investigate links between topography and N cycling in this forest, we established a
topographic sequence in a headwater catchment of the Piro River (Figure 4.1b) where we
focused on sampling the three dominant geomorphic landscape units found in our study region:
1) broad ridge tops, 2) gentle shoulder slopes and 3) steep hillslopes bounded by streams. We

![Figure 4.1 Outline map of Costa Rica and neighboring countries, with the Osa Peninsula highlighted, as well as a hillshade map of the Osa Peninsula with the study region indicated by a white star (a). Schematic representation of the study toposequence (b).](image)

established 30-meter long transects along the contours of a single ridge (6°) – ridge-slope
transition (13°) – steep slope (28°) toposequence. Low angle toe-slopes and floodplains are
virtually absent in this region (Bern et al. 2007) and thus were intentionally excluded from the
study design. The distance between the upper and lowermost transect was ~ 90 meters and the
change in relative elevation was ~ 30 meters.

In order to characterize N-cycling and availability at these three landscape positions, a
suite of N pools and process rates were measured. Ten 0-20 cm soil samples per transect
(separated by ~ 3 meters each) were sampled for $\delta^{15}$N in July 2010. $\delta^{15}$N values have been
widely used as integrated metrics of ecosystem N status (Handley et al. 1999, Martinelli et al. 1999, Brenner et al. 2001, Amundson et al. 2003), as they are sensitive to rates and isotopic signatures of N inputs and losses. Soil samples (~ 100 g each) were homogenized and roots were removed. Then, they were transported to the University of Colorado where they were oven-dried, ground to a fine powder, and shipped to the Center for Stable Isotope Biogeochemistry at the University of California Berkeley for $\delta^{15}$N analysis. The natural abundance of soil $^{15}$N was calculated as: $\delta^{15}$N (‰) = [(R$_{\text{sample}}$/R$_{\text{standard}}$)-1] x 1000, where R = the $^{15}$N/$^{14}$N of the sample or standard (atmospheric N$_2$). The standard deviation of the isotope measurements, determined using laboratory standards, was 0.1 per mil (S. Mambelli, personal communication).

Four times over the course of the 2010 wet season (April, July, August, and October), soils were sampled for inorganic N by extracting ten 0-20 cm soil cores from along each transect. Within one day of soil collection for extractable N, *in-situ* soil nitrous oxide (N$_2$O) emissions were also measured using pre-installed sampling collars (n = 10 per transect) and following the field and laboratory methods described in detail in Wieder et al. (2011). For extractable N, ~8 g fresh soil was added to 30 ml of 2M potassium chloride (KCl), then after 3 hours of repeated shaking and one hour of settling, solutions were filtered through 0.7 µm Whatman glass fibre filters and frozen. Solutions were analyzed colorimetrically for ammonium (NH$_4^+$) using a Synergy HT microplate reader (Biotek, Highland Park, VT, USA) and nitrate (NO$_3^-$) using an Alpkem autoanalyzer (OI Analytical, College Station, TX, USA) at the University of Colorado. Soil subsamples were dried at 105°C for 48 hours and all measurements are reported on a dry-weight basis.

Rates of net N mineralization, net nitrification and nitrification potential were determined for each landscape position in July 2011. Ten 0-20 cm soil samples were collected from each
transect and split in two. One-half was sub-sampled for immediate KCl extraction; the other half was buried in a bag beneath the leaf litter for 5 days, then extracted, filtered and analyzed as described above. Net rates were calculated as the difference between initial and day-5 total inorganic N for N mineralization and NO$_3^-$ for net nitrification (Hart et al. 1994). Fresh subsamples from this collection were transported to the University of Colorado for analysis of nitrification potential. In the laboratory, ten grams of soil was added to 100 ml of a 0.5 mM NH$_4^+$–N buffer solution (Stark and Firestone 1996). A 10 ml subsample of this slurry was immediately removed, filtered and analyzed colorimetrically for NO$_3^-$. The remaining slurry was incubated aerobically in the dark on an orbital shaker for 24 hours, followed by filtration and colorimetric analysis. The nitrification potential was calculated as the difference in initial and final NO$_3^-$ concentrations.

In order to place these measurements of dynamic pools and fluxes in the context of long-term soil development trends, we measured a host of soil chemical, physical, and mineralogical properties along the toposequence. In July 2010 we sampled ten soil profiles per transect to a depth of one meter in 20-cm depth increments using a 3-cm diameter soil sampling tube. Soils from each depth interval were analyzed for % C and % N using a Carlo Erba EA 1110 elemental analyzer (CE Elantech, Lakewood, NJ, USA). C and N inventories for each soil depth were calculated by multiplying soil bulk density (g/cm$^3$) by C or N concentration (%) by the depth interval (cm). The former was estimated by digging pits down to one meter near each transect (n = 2). A 6 cm diameter bulk density corer (volume = 154 cm$^3$) was used to remove soil from the center of each depth interval. This soil was subsequently dried at 105°C for 48 hours to determine soil mass per unit volume.
Soil particle size distributions were measured on a sub-set of soils from upper and lower horizons \((n = 3\) per transect per depth interval) at the University of Montana using a Malvern Mastersizer 2000e (Malvern Instruments, Worcestershire, UK) and following the methods recommended by Sperazza et al. (2004). A sodium hexametaphosphate solution \((5.5 \text{ g/L})\) was used as a dispersant, the pump speed was set to \(2000 \text{ rpm}\), obscuration was mostly between 10-20 \% and the particle refractive index was set to 1.57 while absorption was set to one. The quantitative mineralogical composition was determined for a sub-set of surface soils \((n = 4\) per transect) using X-ray diffraction (XRD) with a Siemens D5000 X-ray Diffractometer in the University of Colorado Sediment Analysis Laboratory, following the protocol outlined in Dühnforth et al. (2012). XRD samples were treated with hydrogen peroxide to remove organics, dried, and ground to 20 microns in a McCrone mill with a zinc oxide internal standard. We prepared random mounts and XRD patterns were collected for 5–65 degrees 2\(\theta\) with a 0.02 degree step size. The RockJock software (Erbel 2003) was used to assign mineral identity and mass percentages. The pH of surface soils was measured using an Accumet AB15 pH meter (Fisher Scientific, Pittsburgh, PA, USA) with a 1:1 soil to deionized water ratio and a half-hour equilibration period.

Finally, in order to expand our exploration of links between topography and N cycling to the larger study landscape, we measured soil \(\delta^{15}\text{N}\) values at 26 distinct locations encompassing the range of slope angle variability found in the region \((\geq 5 \text{ to } \sim 45 \text{ degrees})\). This sampling was contained within a 100 hectare area and a 100 m range in elevation, thus the climate and forest type of all sampled locations did not vary. The lowest slope angles corresponded to ridge top and shoulder slope positions, as low-angle toe-slopes and floodplains (virtually absent in this region) were not part of the sampling. At each location, surface litter was removed and a shallow soil
sample (0-10 cm) was collected. The soil was homogenized and roots were removed. Global positioning system (GPS) coordinates were recorded in order to plot sampling locations and calculate surface slope angles using a digital elevation map (DEM) of the study site. This high-resolution DEM (1.12 x 1.12 m grid size) was generated from airborne light detection and ranging (LiDAR) data collected by the Carnegie Airborne Observatory (Asner et al. 2012) during an overflight of the region in 2012.

4.3.3 Statistical analyses

First, we employed a multivariate approach to examine whether N-cycle profiles differed significantly along the toposequence. Eight measurements of soil N pools and fluxes at the three landscape positions were used for this analysis: soil δ¹⁵N values; mean extractable NH₄⁺ and NO₃⁻ and mean N₂O fluxes; net nitrification and net mineralization rates; the ratio of net nitrification to net N mineralization; and nitrification potentials. These metrics were used to create a distance matrix and conduct a principle components analysis and PERMANOVA test to examine differences in N cycle profiles with landscape position.

The eight N-cycle metrics were also analyzed separately to examine which ones differed by landscape position. Mixed effects models were employed, with geomorphic position as a fixed effect and sampling location along each transect as a random effect, in order to account for non-independence of samples collected along the transects (Zuur et al. 2010). Date was also initially included as a fixed effect in linear models for [NH₄⁺], [NO₃⁻], and N₂O flux, as these metrics were measured multiple times over the wet season. But since there were no significant interactions between date and geomorphic position, and seasonal variation in these variables was not a central question of this study, those analyses were re-run using plot means and the date variable was dropped. N-cycle metrics were either natural-log or square-root transformed if
residuals were heteroskedastic. Although auto-correlation between measured variables was generally low (only three of 28 Spearman rank correlation tests returned $\rho$ values > 0.7), both unadjusted and Bonferroni-adjust $P$-values are reported in order to account for these multiple comparisons.

A mixed model approach similar to the one described above was used to test for differences in soil C and N concentrations and stocks as well as pH across the toposequence. A regression coefficient for the log of C or N pool as a function of the log of depth below the surface was calculated for each of the ten profiles per transect. Differences in these regression slopes by landscape position were then tested using mixed models. Means and standard deviations for soil particle size and mineralogical data were calculated, but statistical tests were not conducted due to small sample sizes.

Finally, linear regression was used to examine the relationship between soil $\delta^{15}$N and the sin of surface slope angle ($\theta$) in the larger landscape sampling. DEM-based slope angles at a 10-m length scale were used for this analysis. Pearson correlation was also used to examine the relationship between surface soil C:N and $\delta^{15}$N, including data from the toposequence as well as the wider landscape sampling. All statistical analyses were conducted using R (R Core Development Team, 2011).

4.4 Results

The principal components analysis and PERMANOVA test indicated that N-cycle profiles differed across the toposequence ($F = 10.47, P = 0.001, R^2 = 0.46$). Ridge and steep slope positions had the most dissimilar N-cycle profiles, while the ridge-slope transition zone was intermediate (Figure 4.2). Axis one of the N-metrics principle components analysis – which
explained 49.4% of the variance in N cycling profiles – was driven by metrics associated with production or accumulation of soil nitrate, including mean extractable NO$_3^-$ concentrations, net nitrification rate, and nitrification potential rate.

![Principal components analysis (PCA) of soil N cycling at the three landscape positions (ridge = RDG, ridge-slope transition = RST, slope = SLP).](image)

**Figure 4.2** Principal components analysis (PCA) of soil N cycling at the three landscape positions (ridge = RDG, ridge-slope transition = RST, slope = SLP).

When examined individually, six of the eight N metrics differed substantially between landscape positions (Figure 4.3, Table A2). Ridge soil NO$_3^-$ concentrations ($1.78 \pm 0.25$ µg N/g), net nitrification rates ($0.33 \pm 0.09$ µg N g$^{-1}$ d$^{-1}$), and nitrification potentials ($10.12 \pm 2.1$ µg N g$^{-1}$ d$^{-1}$) were all at least an order of magnitude higher ($P < 0.001$, unadjusted pairwise comparisons) than respective pools and fluxes on the slope. Net N mineralization rates did not vary with landscape position, but the ratio of net nitrification to net mineralization did ($F = 4.56$, unadjusted $P = 0.027$) and exceeded one only on the ridge (Table A2). For five of these six metrics that differed with topographic position, steep slope and ridge-slope transition zone were a statistical group. The only exception to this was δ$^{15}$N values, which were similar on the ridge
and ridge-slope transition (~ 6.3 ± 0.12 ‰) and significantly lower on the slope (5.0 ± 0.23 ‰). Soil N₂O fluxes (grand mean ~ 0.48 ± 0.06 ng N cm⁻² hr⁻¹) did not vary by landscape position.

**Figure 4.3** Relative values (means ± SE) centered and scaled by the range for eight soil N metrics (ridge = RDG, ridge-slope transition = RST, slope = SLP). Stars indicate a significant effect of landscape position.

Both concentrations and total stocks of soil C and N varied significantly with landscape position (Table 4.1). Ridge soils had higher surface C concentrations (4.0 %) compared to lower

**Table 4.1.** Soil C and N values at the three landscape positions. Values are means ± SE, and letters indicate significant differences between positions at Bonferroni-adjusted α = 0.05.

<table>
<thead>
<tr>
<th></th>
<th>Ridge</th>
<th>Ridge-slope transition</th>
<th>Slope</th>
<th>F²,18</th>
<th>P</th>
<th>P_Bon</th>
</tr>
</thead>
<tbody>
<tr>
<td>% C</td>
<td>4.00 (0.17)ᵃ</td>
<td>2.87 (0.12)ᵇ</td>
<td>2.86 (0.23)ᵇ</td>
<td>14.46</td>
<td>&lt;0.001</td>
<td>0.002</td>
</tr>
<tr>
<td>% N</td>
<td>0.39 (0.01)ᵃ</td>
<td>0.29 (0.01)ᵃᵇ</td>
<td>0.24 (0.01)ᵇ</td>
<td>39.75</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C:N</td>
<td>10.3 (0.17)ᵃ</td>
<td>10.0 (0.16)ᵃ</td>
<td>11.8 (0.32)ᵇ</td>
<td>17.33</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C Stocks (kg/m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20cm</td>
<td>5.33 (0.22)ᵃ</td>
<td>4.28 (0.18)ᵇ</td>
<td>4.30 (0.35)ᵇ</td>
<td>5.77</td>
<td>0.011</td>
<td>0.116</td>
</tr>
<tr>
<td>40cm</td>
<td>8.67 (0.28)ᵃ</td>
<td>6.70 (0.21)ᵇ</td>
<td>6.33 (0.49)ᵇ</td>
<td>13.25</td>
<td>&lt;0.001</td>
<td>0.002</td>
</tr>
<tr>
<td>100cm</td>
<td>14.47 (0.33)ᵃ</td>
<td>11.56 (0.42)ᵇ</td>
<td>8.85 (0.47)ᶜ</td>
<td>46.97</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>N Stocks (kg/m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20cm</td>
<td>0.51 (0.02)ᵃ</td>
<td>0.43 (0.01)ᵇ</td>
<td>0.36 (0.02)ᶜ</td>
<td>20.45</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>40cm</td>
<td>0.84 (0.03)ᵃ</td>
<td>0.67 (0.02)ᵇ</td>
<td>0.55 (0.04)ᶜ</td>
<td>24.82</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>100cm</td>
<td>1.42 (0.03)ᵃ</td>
<td>1.16 (0.04)ᵇ</td>
<td>0.76 (0.06)ᶜ</td>
<td>41.23</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
soil C in ridge-slope transition and slope soils (~ 2.9 %). Surface soil N concentrations were highest on the ridge, intermediate on the ridge-slope transition, and lowest on the slope. Surface soil C:N ratios (mass basis) were significantly higher on the steep slope (11.8) compared to other landscape positions (~10). Integrated to one-meter depth, ridge soils contained the most C and N, followed by the ridge-slope transition, with steep slopes containing roughly half of the C and N found on the ridge top (Table 4.1). Stocks declined with depth below the surface most strongly in the steep part of the landscape, displaying a more modest decline with depth on the ridge and shoulder slope (Figure 4.4). The regression coefficients of these relationships were significantly different for both C ($F = 7.0, P = 0.006$) and N ($F = 8.9, P = 0.002$).

![Figure 4.4 Log-log relationships between soil C (a) and soil N (b) stocks and depth below the surface.](image)

Soils displayed distinct physical and mineralogical characteristics along the toposequence. Fine silt particles ($2 – 30 \mu m$) dominated soil profiles across the site (55-70% by weight), while clays (< 2 \mu m) only accounted for 10-15% of soil particles at the surface. However, ridge profiles contained a zone of clay accumulation at one-meter depth where clay
Table 4.2. Mean values with standard deviations in parentheses of soil physico-chemical and mineralogical properties at the three landscape positions. Letters indicate significant differences between positions at $\alpha = 0.05$.

<table>
<thead>
<tr>
<th></th>
<th>Ridge</th>
<th>Ridge-slope transition</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle Size Distribution</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Sand / Silt / Clay)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-20 cm</td>
<td>15 / 74 / 11</td>
<td>14 / 70 / 16</td>
<td>13 / 74 / 13</td>
</tr>
<tr>
<td>80 - 100 cm</td>
<td>13 / 62 / 25</td>
<td>19 / 58 / 23</td>
<td>16 / 71 / 13</td>
</tr>
<tr>
<td>Clay enrichment (%)</td>
<td>127</td>
<td>44</td>
<td>0</td>
</tr>
<tr>
<td>Bulk Density (g/cm$^3$)</td>
<td>0.67</td>
<td>0.74</td>
<td>0.75</td>
</tr>
<tr>
<td>Gb / (Gb + Hal + Kao)</td>
<td>0.31 (0.04)</td>
<td>0.21 (0.04)</td>
<td>0.02 (0.02)</td>
</tr>
<tr>
<td>Smectite + Illite (weight %)</td>
<td>4.8 (1.4)</td>
<td>5.4 (1.3)</td>
<td>11.0 (3.5)</td>
</tr>
<tr>
<td>Quartz (weight %)</td>
<td>27.4 (2.5)</td>
<td>25.8 (3.6)</td>
<td>17.6 (4.0)</td>
</tr>
<tr>
<td>Soil pH</td>
<td>6.21 (0.06)$^a$</td>
<td>5.72 (0.07)$^b$</td>
<td>5.63 (0.06)$^b$</td>
</tr>
</tbody>
</table>

Notes: Sample sizes were as follows: Particle size distribution = 3, Bulk density = 2, Mineralogy = 4, pH = 10. Gb = Gibbsite, Hal = Halloysite, Kao = Kaolinite.

enrichment compared to the surface was 127% (Table 4.2). By contrast, we observed no change in the weight percentage of clay at depth on the slope (0%) and only moderate clay accumulation at depth on the ridge-slope transition (48%). Surface soils on the ridge contained ~ 13 % gibbsite, a completely desilicated aluminum hydroxide mineral, whereas slope soils contained only 0.05% gibbsite and were more abundant in siliceous aluminum-bearing secondary minerals such as halloysite and kaolinite. Thus, the ratio of gibbsite/gibbsite + halloysite + kaolinite, which is indicative of the degree of chemical weathering (Fisher and Ryan 2006), was an order of magnitude larger on the ridge than it was on the slope (Table 4.2). Ridge and ridge-slope transition soils were more enriched in quartz compared to the slope soils, while slopes had double the abundance of 2:1 clays (illite + smectite) in surface soils. Surface soil pH was lower on the two slope positions compared to the ridge (Table 4.2).
Finally, with a more widespread sampling of study landscape, a significant negative linear relationship between surface slope angle and soil $\delta^{15}N$ was observed ($F = 32.6, P < 0.001, R^2 = 0.58$; Figure 4.5). There was also a strong, negative linear association between soil C:N ratios and $\delta^{15}N$ values ($P < 0.001, r = -0.88$; Figure 4.6).

**Figure 4.5.** Digital elevation map of the study forest, with white crosses indicating sites of the larger landscape sampling and white circle indicating location of the study toposequence (a). Soil $\delta^{15}N$ values versus slope angle from the larger landscape sampling, with error bars indicating two standard deviations of the isotope measurements (b). Gray shaded region is the 95% confidence interval for the linear regression: $\delta^{15}N = -3.745 \times \text{slope (sin}\theta) + 6.553, R^2 = 0.576$.

### 4.5 Discussion

Variation in soil residence time and weathering status have been invoked to explain changes in relative nutrient content and constraints over large spatial gradients (i.e. high vs. low latitudes) and through time (i.e. chronosequences) (Walker and Syers 1976, Vitousek and Farrington 1997, Hedin et al. 2003, Reich and Oleksyn 2004, Porder and Hilley 2011). The results presented here concur with other studies suggesting tropical nutrient cycling dynamics.
and patterns of limitation may vary as much at local scales in dissected landscapes as they do across large spatial gradients and long time periods. This phenomenon and its drivers has been studied in the context of P availability (Scatena and Lugo 1995, Vitousek et al. 2003, Porder et al. 2005), but our results indicate topographic effects on N, which have received less attention to date, may be equally important to consider.

Evidence that N dynamics vary substantially with topography in this dissected forest comes both from detailed work along the topographic sequence as well as broader sampling of \( \delta^{15}N \) values across the landscape. Of eight N-cycle metrics measured along the toposequence, six firmly suggested N-poor conditions on the steep slope when compared with more open N cycling on the flat ridge top (Figure 4.3). Soil nitrate pools, net nitrate production rates, the ratio of net nitrate production to net N mineralization, and nitrification potential rates all hovered at detection levels on the slope but were substantially higher on the ridge. The gently sloping

\[
\text{Figure 4.6 Soil } \delta^{15}N \text{ values versus soil C:N ratios across the study forest, with error bars indicating two standard deviations of the isotope measurements (ridge = RDG, ridge-slope transition = RST, slope = SLP, hillslopes from larger landscape sampling = HS).}
\]
transition zone exhibited an N cycle profile that was intermediate between the steep slope and flat ridge end-members (Figure 4.2), sharing some characteristics with both. While N limitation on the steep slope was not assessed directly, the picture of N availability that emerged from the multiple indicators measured did not suggest that N cycles in excess of demand. Instead, the N cycle on steep slopes appeared similar to young or recently disturbed tropical forests (e.g. Hall and Matson 2003, Davidson et al. 2007) which tend to experience N constraints to ecosystem processes (Davidson et al. 2004).

Patterns observed at the hillslope scale were in line with observations across the larger landscape, specifically the negative correlation between surface slope angle and soil δ¹⁵N (Figure 4.5). High values of soil δ¹⁵N are associated with large losses of N from biologically available pools (Austin and Vitousek 1998) and are thus commonly interpreted as an indicator of N-rich conditions (Martinelli et al. 1999, McLaughlin et al. 2013). In the context of hillslopes, it has recently been postulated that lower soil δ¹⁵N values with increasing slope angle (similar to what we observed here) is indicative of a shift toward increased relative importance of N loss from non-bioavailable pools, such as particulate N erosion and organic N leaching (Amundson et al. 2003, Hilton et al. 2013). Models suggest that increased particulate nitrogen erosion on steep slopes can account for this trend because, as erosive losses do not cause isotopic fractionation, they lower the bulk fractionation factor of the ecosystem and cause δ¹⁵N values to approach isotopically light inputs (e.g. Figure 5, Hilton et al. 2013). We believe this explanation – a more important role for non-fractionating, erosive losses on steep slopes – is the most consistent explanation for observed patterns of δ¹⁵N with topography in our study site. It is possible that hydrologic losses of dissolved N and/or gaseous N fluxes also contribute to the δ¹⁵N pattern, but we believe these are not the dominant drivers of N isotopic trends. We did not find evidence for
elevated N\textsubscript{2}O production on slopes, and steep slope gradients do not promote optimal conditions for water-logging, high denitrification, and thus complete nitrate consumption leading to isotopic under-expression (\textit{sensu} Houlton et al. 2006). Moreover, soil nitrate concentrations in this wet forest are consistently low (Wieder et al. 2011), and regionally, both NO\textsubscript{3} and dissolved organic N losses are dwarfed by particulate N losses at the catchment scale (Taylor et al. 2014). When considered alongside results from the toposequence, our findings imply that steep hillslopes are not only more influenced by non-fractionating N loss pathways, but that total losses are likely greater than those in flat areas, causing large differences in relative N richness.

Demand-independent, erosive N losses may thus have a significant role in regulating spatial patterns of N availability in dissected tropical forests. The importance of demand-independent losses for nutrient budgets of undisturbed ecosystems is widely recognized (Hedin et al. 1995, Perakis and Hedin 2002, Neff et al. 2003), yet discussion of such losses to date has mostly concerned elements in dissolved phases. However, soil and organic matter-associated particulate nutrient losses can be of greater importance than dissolved losses in erosion-prone ecosystems (Shanley et al. 2011, Michaelides et al. 2012). This is the case in our study region, where particulate organic N (PON) losses are the dominant form of N export from undisturbed watersheds (Taylor et al. 2014). Our data complement those of Taylor et al. (2014) by highlighting some of the upland biogeochemical and ecological consequences of potentially large, demand-independent PON export from high-angle hillslopes – roughly 28% of the lowland forests in the immediate region of our study (i.e. areas where surface slope angle \( \geq 25^\circ \)).

Distributions of soil organic matter and particle sizes with depth across the topographic sequence further support a link between particulate erosion and soil N availability. On the ridge, larger soil C and N stocks, more organic matter at depth, and a zone of clay accumulation at one
meter suggest soils are relatively stable and water translocates material vertically through soil profiles. On the slope, a sharp reduction in soil C and N stocks, less organic matter at depth, and lack of deep-soil clay accumulation suggests that surface soil material is continuously removed. Taken together, these pedogenic traits point toward a mechanism for PON mobilization and transport on slopes. A combination of steep terrain and high-intensity storms, during which rainfall can exceed infiltration rates at depth in weathered tropical soils (Elsenbeer 2001, Lohse and Dietrich 2005, Stallard and Murphy 2012) may lead to overland flow and even landslipping/sliding, moving near-surface soil material downslope (Larson et al. 1999). Higher C:N ratios on the slope also point toward less processing of organic matter inputs, and the negative correlation between soil C:N and δ¹⁵N further supports links between soil organic matter residence times, demand-independent losses, and N availability.

Differences in soil weathering extent across the toposequence also correlate with N availability, suggesting a common geomorphic link. The mineralogical composition of the more N-poor slope soils suggests shorter residence times than the N-rich ridge: the former harbor negligible amounts of gibbsite, the desilated end-product of alumino-silicate mineral dissolution, compared to ridge soils and also contain a higher weight percentage of 2:1 clays, namely illite and smectite (Table 4.2). Both of these traits indicate a less advanced state of soil weathering (Fisher and Ryan 2006, Kleber et al. 2007). Slopes are also less enriched in quartz, a recalcitrant mineral that accumulates as other minerals weather away over time (Birkeland 1999), compared to the ridge In a study along the Pacific Coast of Costa Rica ~ 200 km north of the study site, Fisher and Ryan (2006) measured the kinetics of mineral transformations during pedogenesis using terraces of known ages to link residence time with soil mineralogy. According to their findings, the composition of the Piro ridge soils is more similar to old (> 37ka) terraces
that have reached an advanced, steady condition of pedogenesis, whereas the slope soils are more similar to young terraces that are still in a transitory phase of soil development. That N availability also follows patterns suggestive of differences in soil age (Vitousek 2004) is a notable finding.

While these results are compelling, the specific regional geomorphologic and hydrologic settings are important to consider in exploring links between N availability and topography across the tropics. Variation in surface slope angle may drive differences in demand-independent erosive N losses, thereby creating heterogeneity in N constraints, across some dissected landscapes but not others. For instance, ridges and slopes did not exhibit differences in inorganic or total soil N concentrations along a toposequence in Manaus, Amazonia (Luizao et al. 2004). This lack of difference in N availability may be a product of reduced erodability of hillslopes in that region compared to our site (and others), resulting from differences in precipitation patterns (2,200 vs. 3,400 MAP), steepness of slope angles (mean 6° vs. 20°), and subsurface infiltration rates (Elsenbeer 2001). In general, tropical areas that posses high rainfall rates, steep terrain, and high rates of physical denudation, have the greatest potential for erosion to mediate variation in nutrient cycling and availability with topography. This could include (but is not limited to): forested areas in Central America (Morell et al. 2012), the southern Central Range of Taiwan (Stolar et al. 2007), the Andean foothills (Dosseto et al. 2006) and Southeast Asia (Hovius et al. 1998). In low-gradient tropical regions where denudation rates are slower and dominated by chemical weathering (Stallard 1985), differences in nutrient availability along topographic sequences are more likely to be driven by transport and transformation of solutes (Lohse and Matson 2005, Chaves et al. 2009) or differences in soil type and redox status that affect soil nutrient retention capacity (Tiessen et al. 1994).
Accordingly, we do not assert that ridges or hillcrests will be N-rich everywhere compared to hillslopes, but that differences in erosion regimes, where they exist, may drive considerable variation in tropical N cycling. As discussed above, the specific geomorphic and hydrologic context matters, yet the relationships described in this study may be relevant to other highly productive rainforests. Moreover, these findings imply that N and P availability are likely to vary inversely with topography in steep landscapes. This dynamic could be added to the growing list of reasons why it is challenging to identify a single, specific limiting nutrient in lowland tropical forests (Townsend at el. 2011, Cleveland et al. 2011). A better understanding of how natural spatial variation in N richness is linked to topographic position and erosion will help us predict and model how certain tropical forests will respond to widespread environmental changes, such as N deposition and precipitation change.
CHAPTER 5: HILLSLOPE EROSION AND NITROGEN LOSS FROM AN INCISED LOWLAND TROPICAL FOREST, OSA PENINSULA COSTA RICA

5.1 Abstract

Topographically dissected landscapes can exhibit notable spatial variation in nitrogen availability – this includes lowland tropical forests, which are often characterized as N rich. Dissolved N transport along subsurface flowpaths has received attention as an important driver of this heterogeneity, yet the movement of N via erosion and overland transport, and the relation of such losses to spatial patterns of N richness, has not been examined in detail. We quantified spatial and temporal patterns of overland N loss by deploying erosion collectors for one wet season across an incised lowland tropical forest in southwest Costa Rica. We hypothesized that soil erosion by water (slopewash) as well as downslope movement of fine litter (litterwash) would be increasingly important in steep regions, and that N losses associated with these transport processes would approach the size of biological N\textsubscript{2} fixation inputs in steep terrain.

Across the landscape, surface runoff and slopewash rates increased from the early to the late wet season, coincident with increasing storm sizes, while litterwash dynamics showed the opposite trend. Summed over the whole wet season, soil, litter and water moving overland carried notable amounts of nitrogen. Cumulative fluxes of N per unit area were nearly an order of magnitude larger in steeper downslope zones (100 ± 43 mg N m\textsuperscript{-2}), where losses were dominated by litterwash, compared to the gently sloping ridge top (13 ± 5 mg N m\textsuperscript{-2}), where losses were mostly in dissolved form. Overland losses from the steeper downslope zones were on par with wet-season biological N fixation inputs, suggesting they help constrain N accumulation and promote conservative N cycling. Further analyses of N inputs and losses to include modeled atmospheric deposition and all erosive processes (of which slopewash is only a small fraction) also point toward erosive losses as important in determining spatial N dynamics in this geomorphologically
active region. These results suggest that hillslope erosion of particulate nitrogen can be an important pathway of N loss in steep tropical forests. Intriguingly, internal drainage patterns along the hillslope indicate that organisms play a role in shaping surface micro-topography, reducing relative erosional susceptibility on slopes and thus promoting some nutrient retention.

5.2 Introduction

Nitrogen (N) is an essential biological element that often limits ecosystem processes (Vitousek and Howarth 1991, LeBauer and Treseder 2008). In the tropics, previous research has painted a picture of lowland forests as often being nitrogen rich, with high N availability and N supply exceeding demand (Vitousek and Sanford 1986, Martinelli et al. 1999, Hedin et al. 2009). Reasons given for this pattern include: relatively long periods of time for N accumulation (Vitousek 2004), a high abundance of N\textsubscript{2} fixers and favorable climates for their activities (Cleveland et al. 1999), and depletion of phosphorus (Walker and Syers 1976, Crews et al. 1995), making N abundant in comparison. Increasingly, however, experimental results suggest this view is an oversimplification. In lowland forests of Central America, N has been shown to constrain ecosystem functions including root growth and respiration (Cleveland and Townsend 2006, Wright et al. 2011), tree investment in reproduction (Kaspari et al. 2008), and growth rates of several species (Alvarez-Clare et al. 2013). As evidence mounts that N limitation of growth or other ecosystem processes may be more widespread in lowland tropical forests than previously thought, this heightens the need to determine the spatially variable controls over nutrient limitation, given the links between such limitation and several aspects of global change (Bonan 2008, Hedin et al. 2009, Townsend et al. 2011). This is especially relevant in the context of a rapidly changing N cycle in low-latitude regions (Hietz et al. 2011, Austin et al. 2013).
Tropical forests experience a notably wide range in the biotic and abiotic state factors (sensu Jenny 1941) that determine major variation in ecosystem function, which may help explain why patterns of nutrient availability are more complex than previously thought (e.g. El-Swaify et al. 1982, Townsend et al. 2008, Hedin et al. 2009). For instance, relative N richness has been shown to vary with climate (Austin and Vitousek 1998, Nardoto et al. 2008) and soil type (Silver et al. 2000, Nardoto et al. 2008). In landscapes subject to erosion and uplift, previous work has also documented substantial effects of topography on spatial patterns of nutrient availability. The focus thus far has been on erosion’s role in promoting higher P availability on steep slopes compared to ridges due to soil rejuvenation (Vitousek et al. 2003, Porder et al. 2005), yet the same processes that rejuvenate rock-derived nutrients can also remove nutrients that are most abundant in topsoil, such as nitrogen (see Chapter 4). This phenomenon could help explain the observed N constraints to varied biotic processes in the relatively more fertile, topographically dissected Central American forests mentioned above.

Indeed, in regions dominated by hillslopes and channels, landscape morphology sets the stage for zones of N accumulation and hotspots for N loss (Tiessen et al. 1994, Reiners et al. 1998, McSwiney et al. 2001). To date, much of the work investigating topographic controls on N cycling in tropical forests has focused on documenting solution-phase N transport along subsurface hydrologic flowpaths and monitoring rates of denitrification in topographic lows (Reiners et al. 1998, McSwiney et al. 2001, Chaves et al. 2009). In the Amazon basin, contrasts between upland forest soils, comparatively rich in N in relation to nearby quartz sand soils of seasonally inundated bottomlands, have also received attention (Tiessen et al. 1994, Luizao et al. 2004, Nardoto et al. 2008). This work, and the conceptual models built upon it, has illuminated important links between hydrology, topography, and N cycling. And yet, as it has concerned
mostly low-gradient hillslopes and “classic” catenas terminating in concave toe slopes or flooded riparian zones, it may not capture all of the important N transport processes in landscapes where slope gradients are steep and stream incision is rapid.

In steep landscapes, patterns of relative N availability and zones of N accumulation and loss may still track topography, but in a different manner. For instance, Hilton et al. (2013) found that the enrichment of soil and plant $^{15}\text{N}$ declines as slope angle increases in montane tropical forests. While this trend can be interpreted in several ways, the most plausible explanation is increased relative importance of demand-independent nitrogen losses (which include particulate N erosion and dissolved organic N leaching) in steep regions compared to gentler sloping terrain (Townsend-Small et al. 2005, Hilton et al. 2013). More particulate N erosion is consistent with theoretical predictions that erosion rates should increase on steep slopes (Heimsath et al. 1997, Roering et al. 1999), and brings up an interesting question – do these patterns persist in steep tropical lowlands as well? In a dissected lowland forest with high-gradient bedrock streams and no toe slopes, the relative importance of demand-independent N losses does appear to increase with slope angle (see Chapter 4), similar to patterns observed in montane forests (Hilton et al. 2013). Moreover, the most N-rich zones of this landscape seem to be the flat ridges, while steep slopes are N poor. Together, these findings indicate that demand-independent losses, including erosion of particulate N, may have the power to influence N availability in steep lowland catchments, and point toward a possible common geomorphic control on N losses in erosion-prone landscapes.

That physical erosion could be an important pathway for nutrient loss from forested tropical hillslopes may not be intuitive – indeed, erosion potential below dense tropical forest is substantially lower than in degraded or agricultural landscapes experiencing otherwise similar
conditions (Lal 1984, Millward and Mersey 1999, Sidle et al. 2006). And yet, erosive losses from forests can still be substantial. Lal (1984) estimated that erosion from very wet, undisturbed tropical forests can be up to 100 tons km^{-2} yr^{-1}, dependent upon interactions between rainfall erosivity, topography, and vegetation cover. Rubin and Hyman (2000) estimated average erosion rates of 260 tons km^{-2} yr^{-1} for Costa Rican tropical forests. Over time, the N lost with eroding soil could affect forest N status, especially if losses are large in relation to inputs. On the input side of this equation, recent analyses suggest that early estimates of biological N\textsubscript{2} fixation rates in primary tropical forests (Cleveland et al. 1999) may have greatly overestimated annual N inputs (Sullivan et al. 2014). On the output side, hillslopes erode by the action of diverse processes (Anderson and Anderson 2010) – an important one for removing nutrients in steep, humid forests could be entrainment of topsoil and organic matter by surface runoff during large storms. These processes may be especially prevalent in Ultisols, the soil type that commonly underlies tropical forests in relatively younger, more fertile areas. In these soils, declines in saturated hydraulic conductivity with depth (Elsenbeer 2001) have been linked to the activation of near-surface and overland flowpaths during the large storm events that many humid tropical regions experience (Godsey et al. 2004, Zimmermann et al. 2012). Quantifying the amount of N mobilized by such overland processes would help fill a gap in our understanding of how topography and hydrology affect N cycling in steep tropical forests.

The goal of this study was to quantify overland N transport along a wet, forested tropical hillslope where slope angle increases with distance from the divide. We hypothesized that overland N losses would increase with slope angle, and that erosive N losses would be large enough to constrain N accumulation in the steepest regions. For one wet season, we measured overland transport and associated nitrogen fluxes in solid and aqueous phases. The magnitudes of
overland N fluxes were then related to N\textsubscript{2} fixation inputs in order to assess whether erosion and overland transport could affect spatial patterns of N availability.

5.3 Methods

5.3.1 Study site – geology, soils and climate

This research was conducted in the humid lowland tropical forest surrounding the Rio Piro Research Station on the southern Osa Peninsula, southwest Costa Rica (8° 24’ 42”N, 83° 20’ 00”W). The region is undergoing uplift at an average rate of ~ 2 m/kyr due to the NE-directed subduction of the aseismic Cocos Ridge beneath the Caribbean Plate directly seaward of the peninsula (Gardner et al. 1992, Buchs et al. 2009). As such, the Osa has a young and mountainous core, with a maximum elevation of ~ 780 masl, and consists of highly dissected landforms, even at low elevations. Soils in the region are broadly classified as Ultisols (Berrange and Thorpe 1988), and previous work in the area has revealed an advanced state of soil weathering, with quartz, aluminum and iron sesquioxides and 1:1 clays dominating the soil mineral makeup (see Chapter 4). Soils are derived from Miocene greywackes that originate from continental and pelagic sediments, which overlay the Cretaceous basaltic rocks of the Osa Igneous Complex (Buchs et al. 2009). Atop these soils grow high-biomass, large statured forests (Taylor et al. unpublished data) with a diverse assemblage of canopy and understory plants (Weber 2001).

The climate of the region is wet and warm. The study site receives an average of ~ 3,400 mm of rain per year and has a mean annual temperature of 26°C. While temperatures are largely invariant, precipitation inputs show distinct seasonality. From January to March (the dry season), rainfall rates average less than 100 mm per month; thereafter, heavy rains are common, with
peak rains from September to November. In order to monitor rainfall and storms during the study period, two HOBO data logging rain gauges (Onset Computer Corporation, Bourne, MA, USA) were installed in a clearing next to the study forest. One gauge was attached to a HOBO Pendant event data logger; these data were used to identify and characterize storms, which we defined as any rain event lasting longer than 2 hours or delivering at least 3 mm of precipitation and separated by at least 3 hours from subsequent rainy periods. The other rain gauge was hooked up to a HOBO Micro Station data logger that recorded rain every 5 minutes; these data were used to calculate total rainfall for the year and monitoring periods.

5.3.2 Erosion trough construction and monitoring

In order to intercept surface runoff, soil particles, and leaf litter traveling downslope, modified Gerlach troughs (Gerlach 1967) were constructed and deployed. These troughs have been used successfully in a wide variety of ecosystems (Moody and Martin 2001, Gellis et al. 2004) including tropical forests (Larsen et al. 1999, 2012) and have the advantage of collecting both overland water and any solid particles mobilized by it. Two 60-cm long sections of 11-cm diameter polyvinylchloride (PVC) tubing connected by a 90° PVC elbow joint formed the main sections of the trough, and a 50 cm x 5 cm opening was cut into one of the tubing sections to allow material to enter (Figure 5.1a). A rectangular section of aluminum flashing was attached to this opening, which was gently embedded into the soil directly upslope of the trough to facilitate entry of water, soil and litter. Troughs were installed so that openings were flush with the ground surface. Each trough was attached to a series of storage buckets, connected to each other by 2.5-cm diameter vinyl tubing so that excess water and suspended sediment could be contained until collection (Figure 5.1b). All connections between trough components were sealed with silicon to prevent leaks.
Troughs were constructed and installed in early May 2012 along the same 90-m convex hillslope that has been the subject of prior study (see Chapter 4 for a more detailed description of this topographic sequence). Sixteen troughs were installed in total along four slope position transects, on the ridge, shoulder slope, upper slope, and lower slope. Land surface slope angles in the 180 m² rectangular zone directly upslope of the transects were 7°, 12°, 22°, and 28° respectively, as determined by analysis of a high-resolution digital elevation model (DEM). The four troughs per slope position were staggered and separated by approximately eight meters, and
the distance between transects ranged from 16 to 30 m (Figure 5.1c). Contrary to other studies (Zimmermann et al. 2013), we sought to examine overland transport processes in non-channelized sections of the hillslope. Accordingly, trough placement was shifted to avoid obvious zones of concentrated flow and incipient channels. Troughs were sampled approximately every 10 days beginning on May 15 and continuing through November 22. However, collections prior to July 18 were excluded from our analyses because roofs covering the aluminum flange and opening of the troughs, required to exclude rainwater (Figure A1), were not installed until July 18.

On each monitoring date (n = 13 from July 18 – November 22), water volumes in the buckets attached to each erosion trough were measured, and litter and soil trapped in the trough and buckets were collected. A volume-weighted, homogenized water sample was taken from any trough bucket that had water and the remaining water was removed using a hand pump. If saturated sediment was present in the bottom of the buckets, it was also collected. At the field station laboratory, water samples were filtered through pre-weighed Whatman glass fiber filters (GE Healthcare Bio-Sciences, Pittsburgh, PA, USA) and both the filter and a 60 mL filtered water sub-sample were saved. Soil, litter, filters and water samples from each collection data were frozen and subsequently transported to the University of Colorado. Standing forest floor fine litter densities (g/m²) in the zones of the collectors were also monitored throughout the 2012 wet season. This was accomplished using a 0.25 m² quadrat to randomly sample litter mass at five locations per transect roughly every two weeks. Litter subsamples were saved so that weights could be corrected for moisture content.

5.3.3 Sample mass and nitrogen content
Soil and litter samples collected from the troughs were oven-dried at 105°C and 65°C, respectively, and weighed to determine dry masses. Glass-fiber filters were also oven-dried at 105°C and weighed; dry soil mass on each filter was calculated by subtracting the initial filter weight from the filter plus soil weight. Soil and litter samples were ground with a mortar and pestle, packed in aluminum capsules, and analyzed for % N using a Carlo Erba EA 1110 elemental analyzer (CE Elantech, Lakewood, NJ, USA). Soils were ground until they became a homogeneous fine powder, but fine litter samples appeared heterogeneous even after thorough grinding. As such, one-quarter of leaf litter samples were analyzed with replication to test the variability associated with this sample preparation method. The CV of these replicates averaged 5.2%, similar to the range of variation for check standards on the elemental analyzer, and as such, the remaining litter samples were analyzed without replication. Filtered trough-water samples were analyzed for total dissolved nitrogen using a Total C-N Analyzer (Shimadzu TOCvcpn, Kyoto, Japan).

5.3.4 Trough contributing areas

Artificial contributing areas were not imposed on the Gerlach troughs, akin to Larsen et al. (1999) and unlike Larsen et al. (2012). Instead the contributing areas were estimated using observations of rainfall and overland flow during two exceptionally stormy periods that occurred late in the wet season. Only the biggest storms are appropriate for this technique since large water volumes are needed to induce maximum hillslope hydrologic connectivity (McDonnell 2013), thus approaching a condition equivalent to the maximum possible contributing area for each collector. The two exceptionally stormy periods were between October 13 – 19 and 22 – 25. During these periods, 349.8 and 314.5 mm of rain fell over the course of 5.4 and 2.8 days, respectively. According to the criteria specified above, the latter period was composed of one
storm but the earlier period was composed of two large storms (each delivering > 100 mm of rainfall) separated by three smaller storms falling over the course of two days. As the erosion troughs could not be monitored in the midst of this interval, all five storms were aggregated for the analysis. Water volumes collected by each trough were measured at the end of the two rainy periods.

Rainfall data from these stormy intervals, expressed in mm d\(^{-1}\), were used to estimate runoff generation (in the same units), using a regression equation from Larsen et al. (2012). This regression is an appropriate way to estimate runoff from rainfall using our data since it was derived using collectors nearly identical to the ones in this study (except those of Larsen et al. (2012) were bounded) in a tropical forest with similar soils, topography and rainfall. Runoff estimates from this regression were then used to calculate the areas needed to generate observed water volumes for each erosion trough and each storm. The two contributing area estimates per trough, one from each storm, were on average very similar, with a mean coefficient of variation of 20%. Moreover, there was notable qualitative consistency in which troughs collected small and large water volumes. Thus, the average value from the two storms was assigned as the contributing area for each trough.

This method was employed for the troughs located on the shoulder, upper and lower slope (n = 12), but was not applied to the collectors located on the ridge. This is because of the difficulty in estimating contributing areas from rainfall-runoff patterns in irregular, nearly flat terrain (Renard et al. 1997). Therefore, the contributing areas for the ridge troughs (n = 4) were determined using erosion estimates from the slopes and the predictions of the Revised Universal Soil Loss Equation (RUSLE; Renard et al. 1997) as applied to surfaces with varied slope angles. The average wet-season erosion rate (in g/m\(^2\)) observed for the three slope positions was used as
a baseline erosion value, then a coefficient was applied to that average to model the reduced erosion potential of the flat ridge (average “length-slope” or LS factor in RUSLE = 4.5 for the slope, 0.54 for the ridge). Contributing areas for the ridge were extrapolated from the observed soil masses and this modeled erosion rate.

5.3.5 Long-term watershed erosion

In order to put slopewash erosion in the context of total erosive processes, the minimum eroded volume (Menéndez et al. 2008, Giaconia et al. 2012, Cooley 2013) of the incised part of the study catchment was calculated and used to estimate a long-term watershed erosion rate. To calculate the minimum eroded volume, a 1.12 x 1.12 m bare-earth DEM of the research site, derived from airborne LiDAR during a site fly-over by the Carnegie Airborne Observatory (Asner et al. 2012) was utilized. Visual inspection of this DEM suggested that watersheds in the Piro forest are forming from the incision of a planar surface, most likely an uplifted marine terrace (e.g. Figure 4.5). Using ArcGIS, the original planar surface that capped the study watershed was modeled by connecting the lateral divides using triangular irregular networks. Then, a raster ‘DEM of difference’ was created based on the difference in height between the original modeled surface and the modern topography. From the change in height and area of the eroding zone, a minimum eroded volume of rock was calculated. Eroded volume was converted to mass using rock bulk density (2.65 g/cm$^3$), and the current height of the ridge (80 masl) and long-term average uplift rate (2 m/kyr) were used to estimate when incision began (i.e. approximately 40,000 years ago). The eroded mass was divided by area and time to derive a long-term average erosion rate of ~ 240 g m$^{-2}$ yr$^{-1}$.

5.3.6 Hillslope nitrogen fixation
In order to put erosive N losses in the context of biological N inputs, rates of free-living biological N\textsubscript{2} fixation (BNF) in soil and litter were measured using the acetylene reduction assay (Hardy et al. 1968) following methods detailed in Reed et al. (2007, 2013). Rates were measured three times, once each during the early (May), mid (July) and late (October) wet season, along the four hillslope position transects. Samples of surface leaf litter (n = 12) and the soil beneath to 4 cm depth (n = 10) were collected and then incubated at ambient moisture and temperature with a 10% acetylene headspace in 55 mL vessels for 18 hours. Sample blanks as well as acetylene blanks for both soil and litter were included. At the end of the incubation period, vessel headspaces were mixed, subsampled and injected into pre-evacuated Exetainer vials (Labco Limited, Lampeter, Wales, UK).

Gas samples were transported to the University of Colorado and analyzed for ethylene concentration on a Shimadzu 14-A Gas Chromatograph equipped with a flame ionization detector (Shimadzu Scientific Instruments, Columbia, MD, USA) within two weeks of collection. Rates of ethylene production per unit time were corrected for background ethylene concentrations using the blanks, then converted to nitrogen-fixation rates assuming the common 3-to-1 ratio of ethylene to N\textsubscript{2} fixation (Hardy et al. 1968). The area of the collection vessel (2.54 cm\textsuperscript{2}) was used to estimate areal rates of soil BNF to 4 cm depth. In order to estimate leaf litter N fixation on an areal basis, assayed material was oven-dried at 65°C, then the mass was converted to area using observed mean litter densities in the month of sampling at each position.

5.3.7 Data treatment and statistical analyses

Water, soil and litter collected by each trough were normalized by that trough’s contributing area to calculate rates of slopewash (g/m\textsuperscript{2}), litterwash (g/m\textsuperscript{2}) and surficial runoff (mm), respectively. Fluxes of N in these components were calculated by multiplying sample N
concentrations by sample mass or volume. Glass fiber filter soil masses were assumed to have the same %N as soils. Trough collections of water, soil, litter and their associated nitrogen loads were summed over the monitoring period to estimate cumulative fluxes. Cumulative fluxes of material and nitrogen were analyzed with non-paramteric Kruskal-Wallis tests, followed by multiple comparison tests ("kruskalmc"; Giraudoux 2013). Kruskal-Wallis was used instead of ANOVA of linear models because the data did not satisfy assumptions for parametric statistics (i.e. sample sizes were small and the data was strongly overdispersed). For slopewash-related metrics (both total slopewash mass and slopewash N content), ridge troughs were excluded from statistical tests because ridge erosion rates were modeled from the data. Hence, only slopes were compared.

Measured stocks of standing leaf litter and rates of free-living biological nitrogen fixation were tested using linear mixed models, with position and sampling date as fixed effects and repeatedly sampled plots as a random effect. Leaf litter masses were natural-log transformed and soil and litter BNF rates were square-root transformed to ensure homoscedasticity and normal distribution of linear model residuals. In order to estimate cumulative wet-season BNF inputs to compare to overland N losses, BNF rates measured during each phase of the wet season were multiplied by the number of days in that phase (90, 75 and 76 days for the early, middle, and late wet season, respectively), then the three phases were summed for a wet season total. This applies to the soil; for leaf litter, the grand mean at each hillslope position was multiplied by the length of the whole wet season since fluxes were time-independent (see 5.4 Results). As a final step, a projected contribution from symbiotic N2 fixation was added to those of free-living inputs to estimate total BNF. This was accomplished by applying the average free-living-to-symbiotic BNF ratio (symbiotic inputs = 35% and free-living inputs = 65% of total BNF) determined by
Sullivan et al. (2014) in an extensive empirical study of primary forest plots within 1 km of the study hillslope. Since N₂ fixation rates in this region are much higher in wet months than during the short dry season (Reed et al. 2007), and since our intent here was to compare them to measured erosion losses, projected inputs are wet season values only.

5.4 Results

Annual precipitation for 2012 was 3,594 mm, a slightly wetter than average year for the study site. During the erosion trough monitoring period from July 18 – November 22 (127 days or 34% of the year), 60.1% of the annual rainfall, or 2,161 mm, fell in 89 discrete storms. Four of the year’s five largest storms, which delivered > 100 mm of precipitation each, occurred between September 21 and October 25. Storm precipitation amounts increased from the early to late wet season, as did storm durations. Accordingly, storm intensities, or total storm rainfall divided by storm length, did not change systematically with time. The mean storm intensity was 6.97 mm hr⁻¹, with a minimum of 0.31 mm hr⁻¹ and a maximum of 32.36 mm hr⁻¹.

Overland flow and surface soil erosion generally tracked rainfall patterns while surface litter transport displayed a different trend (Figure 5.2). Water and soil flow into the troughs began to increase in the end of September and continued to increase through the heart of the wet season along with storm lengths and volumes. On the other hand, litter transport rates were highest early in the monitoring period and declined in the late wet season. Overland water and soil flow exhibited similar spatial as well as temporal dynamics – water and soil collections were lower on the ridge compared to the slopes and largest quantities were captured by troughs on the shoulder slope. For litter, lower slope troughs received the most material followed by the upper slope, with minimal litter mobility on the shoulder and ridge. Standing forest floor leaf litter
Figure 5.2 Rainfall for the collection periods (a), and cumulative fluxes of water (b), soil (c) and fine litter (d) over the course of the study. Points represent the mean of each collection date per slope position (n = 4) and hatched lines are the standard error of the mean. Lack of hatched lines indicates standard errors are smaller than the point size.
densities varied significantly with slope position ($F_{3,211} = 13.01, P < 0.001$) and time, ($F_{12,211} = 12.02, P < 0.001$). The shoulder slope had the lowest forest floor litter densities, but densities converged on shoulder slope values in the late wet season (Figure A2).

Cumulative slopewash rates for the three slope positions ranged from $2.67 \pm 0.57 \text{ g/m}^2$ to $8.65 \pm 2.36 \text{ g/m}^2$ and the difference between slope positions was marginally significant (Table 5.1). These slopewash totals for the monitoring period were $\sim 1 – 4\%$ of long-term average erosive losses calculated from the minimum eroded volume. Low values are to be expected because we did not sample any truly huge storms and avoided sampling channelized flow.

**Table 5.1** Slopewash, litterwash and runoff total amounts from the 4-month study period. Positions that do not share a letter had significantly different fluxes ($P < 0.05$) based on multiple comparisons (“kruskalme”) following Kruskal-Wallis tests.

<table>
<thead>
<tr>
<th></th>
<th>Ridge</th>
<th>Shoulder</th>
<th>Upper slope</th>
<th>Lower slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slopewash* (g soil m$^{-2}$)</td>
<td>mean</td>
<td>S.E.</td>
<td>mean</td>
<td>S.E.</td>
</tr>
<tr>
<td>0.49</td>
<td>0.1</td>
<td></td>
<td>8.65$a$</td>
<td>2.36</td>
</tr>
<tr>
<td>Litterwash (g litter m$^{-2}$)</td>
<td>0.04$a$</td>
<td>0.02</td>
<td>0.21$ab$</td>
<td>0.16</td>
</tr>
<tr>
<td>Runoff (mm)</td>
<td>2.67$a$</td>
<td>0.38</td>
<td>20.62$b$</td>
<td>3.75</td>
</tr>
</tbody>
</table>

*Kruskal-Wallis and post-hoc tests were run excluding the Ridge site for slopewash since this response variable was modeled from the data using RUSLE (see 5.3.4 Trough contributing areas)

Cumulative litterwash rates were highest on the lower slope at $5.39 \text{ g/m}^2$, with a significant effect of position (Kruskal-Wallis chi-squared = 10.76, $P = 0.013$). Cumulative runoff also varied significantly across the hillslope (Kruskal-Wallis chi-squared = 11.21 $P = 0.011$), with the shoulder slope generating the most runoff at $20.62 \text{ mm} (~ 1\%$ of rainfall) and the ridge generating the least at $2.67 \text{ mm} (0.1\%$ rainfall).
Figure 5.3 Mean total overland nitrogen fluxes during the 4-month study period. Slopewash N fluxes (excluding the ridge) were marginally different by position \( (P = 0.05) \) and litterwash N fluxes differed significantly \( (P = 0.01) \). TDN fluxes did not vary significantly by position. Hatched lines are standard errors of the mean.

The amount and partitioning of surface nitrogen transport varied along the hillslope (Figure 5.3). Fluxes of soil N from the shoulder were 43.6 ± 11.4 mg N/m\(^2\), which was more than twice the quantity of slopewash N mobilized from the other slope positions (~13 and 19 mg N/m\(^2\) on the upper and lower slope, respectively) and this difference was just significant (Kruskal-Wallis chi-squared = 6.00, \( P = 0.049 \)). Litterwash N loss on the lower slope was 56.9 ± 27.4 mg N/m\(^2\), which was nearly four times larger than those from the upper slope (14.9 ± 8.7 mg N/m\(^2\)) and an order of magnitude greater than the ridge and shoulder (Kruskal-Wallis chi-squared = 11.29, \( P = 0.010 \)). Dissolved N fluxes were highest on the lower slope (24.5 ± 10.5 mg N/m\(^2\)) but the effect of position was not significant (grand mean DON flux = 13.9 mg N/m\(^2\)). Different forms of N thus dominated surface N transport at the different slope positions. Total
overland N fluxes were predominantly dissolved nitrogen on the ridge, soil nitrogen on the shoulder, roughly equal contributions from all three components on the upper slope, and litter nitrogen on the lower slope.

Free-living soil nitrogen fixation rates differed significantly with slope position ($F_{3.36} = 3.79, P = 0.018$) and sampling date ($F_{2,73} = 11.10, P = 0.001$), with no interaction between these two fixed factors. Averaged across the year, highest rates were observed in lower slope soils ($0.11 \pm 0.02 \text{ mg N m}^{-2} \text{ d}^{-1}$), which were statistically similar to those in shoulder and upper slope soils and roughly twice as large as those on the ridge ($0.05 \pm 0.01 \text{ mg N m}^{-2} \text{ d}^{-1}$). Litter free-living $N_2$ fixation rates differed significantly by slope position ($F_{3,44} = 7.43, P < 0.001$) but not by season ($P = 0.880$), and highest fluxes were observed on the upper slope ($0.51 \pm 0.08 \text{ mg N m}^{-2} \text{ d}^{-1}$) with 2-3 times lower fluxes at the other hillslope positions. Litter densities were not significantly higher on the upper slope (Figure A2), thus differences were due to higher rates of $N_2$ fixation per unit of litter. When extrapolated to the 2012 wet season, projected total BNF inputs were highest on the upper slope due to these large litter BNF contributions. The next-highest inputs were on the shoulder slope, with similar (and lower) inputs on the ridge and lower slope (Table 5.2).

Total overland N transport summed to variable but substantial proportions of wet-season BNF inputs (Figure 5.4, Table 5.2). On the lower slope, mean total surficial N losses were roughly equal to estimated biological N inputs – in fact, they exceeded inputs by $\sim 4 \text{ mg N/m}^2$. On the shoulder, overland N losses were approximately 40% of estimated inputs. On the ridge and upper slope, small overland N losses and large BNF inputs, respectively, meant that overland losses represented a smaller fraction (13 and 18% respectively) of wet-season biological N fixation inputs.
Table 5.2  Total wet-season biological N\(_2\) fixation (BNF) inputs and measured overland N losses at the four landscape positions. \(P\)-values refer to the effect of landscape position on BNF fluxes (g m\(^{-2}\) d\(^{-1}\)) based on results of linear mixed models, except for overland N loss where \(P\) refers to the result of a Kruskal-Wallis test. BNF standard errors were compounded to account for extrapolating daily rates to the whole wet season; positions that do not share a letter differ significantly according to mixed models or multiple comparison Kruskal test.

<table>
<thead>
<tr>
<th>Position</th>
<th>Ridge</th>
<th>Shoulder</th>
<th>Upper slope</th>
<th>Lower slope</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>S.E.</td>
<td>mean</td>
<td>S.E.</td>
</tr>
<tr>
<td>Soil N-fix (mg N/m(^2))</td>
<td>10.14(\text{a})</td>
<td>2.05</td>
<td>23.11(\text{b})</td>
<td>6.95</td>
</tr>
<tr>
<td>Litter N-fix (mg N/m(^2))</td>
<td>51.48(\text{a})</td>
<td>9.14</td>
<td>69.10(\text{a})</td>
<td>13.05</td>
</tr>
<tr>
<td>Total BNF* (mg N/m(^2))</td>
<td>94.80</td>
<td>17.21</td>
<td>141.85</td>
<td>30.76</td>
</tr>
<tr>
<td>Total overland N loss (mg N/m(^2))</td>
<td>12.65(\text{a})</td>
<td>5.64</td>
<td>54.52(\text{ab})</td>
<td>14.88</td>
</tr>
</tbody>
</table>

\*A 35\% scalar was used to estimate the added contribution of symbiotic inputs to total wet season BNF fluxes. Based on data from Sullivan et al. (2014) (see 5.3.6 Hillslope nitrogen fixation).

5.5 Discussion

Intact rainforests are not typically thought of as highly susceptible to erosion by rainfall. Accordingly, patterns of overland N loss have not been examined as potential contributors to spatial variation of N richness. However, in wet climates where high rainfall erosivity intersects with steep slopes, erosion can be an important process, redistributing water, litter and sediment across the landscape (Lal 1984, Larsen et al. 1999, Zimmermann et al. 2012). Here, we have shown that sufficient nitrogen is lost with overland material to play a role in constraining N accumulation in steep zones of the landscape. Unlike nitrate leaching and denitrification, these losses occur largely independent of biological N demand. Tropical forests are known for their efficiency in biotic recycling of nutrients, especially those that are limiting (Cuevas and Medina 1988); this may include N in more fertile forests (Kaspari et al. 2008, Wright et al. 2011, Alvarez-Clare et al. 2013). And yet, some forms of N loss, such as those documented here,
Figure 5.4 Erosion and runoff-associated N losses scaled by their relation to total biological nitrogen fixation (BNF) inputs at each slope position. Estimates of BNF include free-living and symbiotic components, *sensu* Table 5.2. Hatched lines are standard errors of the mean.

are driven more by hydrologic and geomorphologic processes, with less direct biotic (or demand-dependent) control. Previous studies have explored the importance of demand-independent losses on forest nitrogen cycling (Hedin et al. 1995, Neff et al. 2003), yet the focus has mostly been on losses of dissolved organic N. We extend this body of work to include myriad forms of nitrogen moving overland (and beyond the reach of organisms) in wet, steep locations.

On the steep slope, rates of overland N loss rival biological inputs (Figure 5.4), which probably contributes to the development of a more conservative N cycle in this part of the landscape (see *Chapter 4*). However, slopewash is only a small fraction (~ 1-4%) of total erosion, and abiotic atmospheric nitrogen deposition occurs alongside biological N$_2$ fixation. To explore the longer-term constraints to ecosystem N cycling in actively eroding terrain, we estimated rates of N inputs and outputs due to these processes. We assumed that one-half of the total eroding-zone mass loss (~ 240 g m$^{-2}$ yr$^{-1}$) was soil erosion (vs. chemical weathering), and
applied the average soil N content of the three hillslope positions (0.45 %) to get an annual N loss estimate due to all erosion processes from the watershed incision zone – approximately 533 mg N m$^{-2}$ yr$^{-1}$. This assignment of mass loss to physical versus chemical weathering processes may be conservative; Stallard and Murphy (2012) observed closer to 75% of total watershed mass loss by sediment in the Mameyes watershed in Puerto Rico, and the fraction of erosion that is physical versus chemical at the continental scale for Central & South America is estimated to be $\sim$ 65% (El-Swaify et al. 1982). However, as our study site is underlain by greywackes, it is reasonable to suspect that chemical dissolution rates may be on the high end, hence our 50% physical-to-chemical denudation estimate. Total soil erosion N losses were then added to litter and DON fluxes (mean from the three slope positions) to project total overland N losses in the watershed incision zone. On the input side, modeled atmospheric N deposition rates of 400 mg N m$^{-2}$ yr$^{-1}$ (Dentener et al. 2006) were added to the slope-wide mean estimate for total BNF.

While there are many limitations and caveats to this simple extrapolation exercise, N inputs and outputs estimated in this way are comparable, with a slight tendency toward net N loss (Figure 5.5). Further, subsurface leaching and gaseous N losses are missing from our

![Figure 5.5 Balance between total N inputs and total erosive losses estimated for the watershed incision zone. BNF = biological N fixation, DON = dissolved organic N.](image-url)
estimates – if these pathways are included, losses in the incision zone would almost certainly be greater than inputs. The fact that N inputs and erosive losses are of similar magnitude and lean toward N depletion in eroding zones, both on annual timescales as well as in the longer-term, makes a compelling case for the importance of geomorphic and hydrologic controls on the N cycle in our study region. If losses continue to outpace inputs, steep reaches could ultimately face strongly N-limited conditions. Ecological theory suggests biological N\textsubscript{2} fixation should be up-regulated under these circumstances, which may explain higher leaf litter BNF rates on the upper slope. And yet, we did not observe up-regulation of free-living inputs on the steep slope, which could be due to limitation by some other resource (Reed et al. 2011). While up-regulation of N fixation is one possibility, the other is that geomorphically-driven nutrient variation could act as filter (Tiessen et al. 1994, Condit et al. 2013), selecting for species with lower N requirements. This could perpetuate low-N conditions through plant-soil feedbacks. The question of how biota respond to geologically-mediated variations in nutrient availability is an intriguing one (Higgins et al. 2011, Hahm et al. 2014) and deserves further study.

Variations in the total amounts as well as relative dominance of runoff, slopewash, and litterwash nitrogen loss conformed to our expectations in some ways but not others. That overland transport from the ridge was a small fraction of the slopes (Figure 5.2) was predictable, given the flatness of this geomorphic zone. Here, we expected surface erosion processes to be transport-limited (Stallard 1985, Anderson et al. 2002), thus is was not surprising that dissolved N in runoff dominated surficial N losses (Figure 5.3) and that total overland N losses were small, equaling only a small fraction of BNF inputs (Figure 5.4). On the steep slope, overland N losses were of greater consequence, large enough to help maintain conservative N cycling. This general
finding was as predicted, but the mechanism was unforeseen. Slopewash soil N loss equaled ~
20% of wet-season BNF inputs – not enough alone to substantially impact N accumulation and
constrain the N cycle. Yet, notable amounts of N were associated with the transport of surface
litter, and when combined with soil and dissolved losses, on a per-area basis they roughly
equaled BNF inputs (Figure 5.4). Dynamics at the mid-slope positions also diverged somewhat
from expectations. While N losses were generally intermediate to the flat ridge and steep lower
slope, slopewash losses on the shoulder were greater than anticipated based solely on the local
slope angle (Figure 5.3), while the upper slope lost a lower fraction of BNF inputs (Figure 5.4)
than expected. These findings can all be linked to varied leaf litter dynamics along the hillslope.

Forest floor fine litter emerged as an important player in multiple aspects of the overland
N story, a finding that supports and expands upon the myriad roles of leaf litter in tropical forest
nutrient supply and biogeochemical dynamics detailed in previous studies (Cuevas and Medina
slope, low forest floor litter densities (Figure A2) contributed to high runoff and slopewash rates,
even though the local slope angle was fairly gentle. The capacity of surface litter to buffer the
soil from rainsplash and promote infiltration is well known (Renard et al. 1997) – though we are
not certain why the shoulder slope had lower litter densities, this almost certainly had a
meaningful impact on slopewash erosion rates, similar to studies reviewed in Sayer (2006). On
the mid-slope, moderate rates of overland N loss were dwarfed by very high rates of leaf litter
BNF, higher than any other microsite measured along the hillslope (Table 5.2). Again, while we
are not certain why litter here was such an N2-fixation hotspot (as the C:N ratio was not higher
than other landscape positions, data not shown), it had meaningful consequences for the
relationship between biological N inputs and overland N outputs during the study period. On the
lower slope, leaf litter served as the main source of N transported overland. As leaf litter clearly serves many important functions in tropical forests (Cuevas and Medina 1988, Sayer 2006) and plays diverse roles in tropical N cycling in dissected terrain, environmental and land-use changes that affect litterfall quantities and temporal patterns could have notable affects on tropical nutrient cycling and surface erosion dynamics. This could be especially true if such changes coincide with altered hydrologic regimes. On a natural history note, we collected many seeds in the erosion troughs, and wonder about the role of “seedwash” as a dispersal mechanism for trees growing in hilly catchments.

While surface transport matters for steep slope N cycling, slopewash is still only a small fraction of total hillslope erosion. This is likely linked to the low percentage of rainfall that becomes surface runoff – in our study, this value was ≤ 1%, similar to results from Puerto Rico in Larsen et al. (1999). This suggests that even though rainfall is highly erosive (Vahrson 1990), if soils have rapid infiltration capacity then surface flowpaths activate infrequently. We calculated very small contributing areas for the unbounded erosion collectors (mean = 8 m²), and contributing areas did not increase with distance from the divide. These findings imply that the hillslope itself is a complex drainage network, with many “micro-catchments” isolated from each other by local divides and areas of re-infiltration. Evidence for diverse links between organisms and hillslope processes in Earth’s critical zone are increasing (Amundson et al. 2007), and it is quite possible that organisms help shape surface microtopography and erosion patterns. For example, treefalls have been shown to create a “pit-and-mound” morphology, which enables mid-slope sediment trapping and promotes a less coherent drainage network (Embleton-Hamann 2004, Hancock et al. 2012). Tree buttresses parallel to slope contours may also trap slopewash and promote infiltration of surface runoff (Herwitz 1988). The hillslope micro-catchments
created by dynamics such as these likely become connected only during the largest rain events when otherwise isolated zones become hydrologically linked (i.e. the “fill and spill” model, McDonnell et al. 2013). Hillslope micro-catchments created by organisms may thus act to reduce surface erosion susceptibility on steep hillslopes, offsetting some overland nutrient loss and promoting nutrient retention. As Larsen et al (1999) similarly surveyed very small areas (mean = 15 m²) for unbounded erosion troughs in Puerto Rico, it is probable that these patterns apply to steep, wet, high-biomass forests beyond the one examined in this study.

The results presented here suggest soil and organic matter erosion can be an important pathway for N loss in steep tropical terrain. Erosive N outputs in steep regions of the landscape are on par with N inputs, suggesting a role for these processes in conservative N cycling. While geomorphologic and hydrologic processes promote significant N loss, feedbacks between organisms and surface micro-topography serve to counteract some of this dynamic. This study supports a growing body of work linking geomorphology to the ecosystem ecology of forests – an exciting forefront of research, especially in the less-studied wet tropics.
CHAPTER 6: CONCLUSIONS

In this thesis, I have explored how changes in organic matter inputs, tree species and topography can affect tropical forest nutrient cycling. Together, my work underscores the biogeochemical complexity of tropical soil nutrient dynamics and their susceptibility to forest and environmental change. Specifically, my findings suggest that the commonly-held assumption that tropical forests are rich in N and cycle this element in excess should be approached with caution. Variations in the quantity of leaf litter, the types of tree species, and the shape (and erosional susceptibility) of the landscape can all affect relative N richness. In the context of previous findings (Cleveland and Townsend 2006, Kaspari et al. 2008, Wright et al. 2011, Alvarez-Clare et al. 2013), my work supports the idea that N limitation of growth or other ecosystem processes may be more widespread in lowland tropical forest than previously thought.

Each of the experiments presented in this thesis used a common approach, wherein all environmental variables were held constant except the one of core interest. This technique, widespread in ecosystem ecology (Vitousek 2004, Chapin et al. 2011), is useful because it facilitates mechanistic examination of how single variables affect nutrient cycling processes. Yet in reality, the factors that control ecosystem structure and function often change in concert over space and time, and this is a great challenge for ecosystem ecologists in this era of global change. New and creative ways to probe the effects of multiple, interacting controlling factors on biogeochemical processes are needed in order to predict global change consequences across large and variable portions of the tropical forest biome. This includes the use of powerful new remote sensing tools (Asner et al. 2010, Baccini et al. 2012) as well as networks of large-plot, globally distributed monitoring sites (ter Steege et al. 2006, Chave et al. 2008). Such tools are already being used to explore forest community and carbon dynamics; there is much potential for
biogeochemists to engage with these efforts, in order to connect trends in carbon cycling to those of nutrient availability and limitation in diverse tropical forests.
REFERENCES


Larsen, M. C., Z. Liu, and X. Zou. 2012. Effects of Earthworms on slopewash, surface runoff, and fine-litter transport on a humid tropical forested hillslope in eastern Puerto Rico. Pages


### APPENDIX

*Table A1, Chapter 2* Mean values with one standard error in parentheses of soil enzyme activities expressed per gram soil (a), per gram soil carbon (b), and per milligram microbial biomass carbon (c) in control and treatment plots (1× = control, 0× = litter removal, 2× = double litter, -50% = fifty percent throughfall reduction). Below are ANOVA results (F, P) from linear mixed effects models examining treatment effects on soil enzyme activities. Date effects are not shown. Letters indicate significant differences between treatments.

#### a) nmol g dry soil⁻¹ h⁻¹

<table>
<thead>
<tr>
<th></th>
<th>aP</th>
<th>NAG</th>
<th>LAP</th>
<th>BG</th>
<th>BX</th>
<th>CBH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1×</td>
<td>652&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16</td>
<td>143&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(37)</td>
<td>(7)</td>
<td>(2)</td>
<td>(14)</td>
<td>(4)</td>
<td>(4)</td>
</tr>
<tr>
<td>0×</td>
<td>578&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13</td>
<td>76&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(6)</td>
<td>(4)</td>
<td>(2)</td>
<td>(6)</td>
<td>(3)</td>
<td>(1)</td>
</tr>
<tr>
<td>2×</td>
<td>875&lt;sup&gt;c&lt;/sup&gt;</td>
<td>153&lt;sup&gt;c&lt;/sup&gt;</td>
<td>25</td>
<td>201&lt;sup&gt;c&lt;/sup&gt;</td>
<td>63&lt;sup&gt;b&lt;/sup&gt;</td>
<td>58&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(61)</td>
<td>(12)</td>
<td>(5)</td>
<td>(14)</td>
<td>(6)</td>
<td>(6)</td>
</tr>
<tr>
<td>-50%</td>
<td>686&lt;sup&gt;b&lt;/sup&gt;</td>
<td>76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17</td>
<td>133&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(23)</td>
<td>(9)</td>
<td>(3)</td>
<td>(11)</td>
<td>(3)</td>
<td>(4)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1×</td>
<td>6.53</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>0×</td>
<td>19.93</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2×</td>
<td>2.45</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>-50%</td>
<td>23.59</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>5.69</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

#### b) nmol g soil C⁻¹ h⁻¹

<table>
<thead>
<tr>
<th></th>
<th>aP</th>
<th>NAG</th>
<th>LAP</th>
<th>BG</th>
<th>BX</th>
<th>CBH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1×</td>
<td>12,091&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1,391&lt;sup&gt;a&lt;/sup&gt;</td>
<td>325</td>
<td>2,599&lt;sup&gt;a&lt;/sup&gt;</td>
<td>825</td>
<td>592&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(638)</td>
<td>(88)</td>
<td>(40)</td>
<td>(298)</td>
<td>(50)</td>
<td>(67)</td>
</tr>
<tr>
<td>0×</td>
<td>15,212&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1,274&lt;sup&gt;a&lt;/sup&gt;</td>
<td>314</td>
<td>2,045&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1017</td>
<td>372&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(873)</td>
<td>(96)</td>
<td>(33)</td>
<td>(199)</td>
<td>(71)</td>
<td>(36)</td>
</tr>
<tr>
<td>2×</td>
<td>12,091&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2,091&lt;sup&gt;b&lt;/sup&gt;</td>
<td>334</td>
<td>2,774&lt;sup&gt;a&lt;/sup&gt;</td>
<td>874</td>
<td>784&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(607)</td>
<td>(106)</td>
<td>(46)</td>
<td>(177)</td>
<td>(53)</td>
<td>(63)</td>
</tr>
<tr>
<td>-50%</td>
<td>13,258&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1,380&lt;sup&gt;a&lt;/sup&gt;</td>
<td>306</td>
<td>2,519&lt;sup&gt;a&lt;/sup&gt;</td>
<td>845</td>
<td>594&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(689)</td>
<td>(122)</td>
<td>(48)</td>
<td>(224)</td>
<td>(35)</td>
<td>(62)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1×</td>
<td>4.18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>0×</td>
<td>10.76</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2×</td>
<td>0.20</td>
<td>0.892</td>
</tr>
<tr>
<td>-50%</td>
<td>3.08</td>
<td>0.040</td>
</tr>
<tr>
<td></td>
<td>1.47</td>
<td>0.240</td>
</tr>
<tr>
<td></td>
<td>9.40</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Table A2, Chapter 4 Mean values with standard errors in parentheses of eight soil N cycle metrics at the three landscape positions. Values are means ± SE, and letters indicate significant differences between positions at α = 0.05.

<table>
<thead>
<tr>
<th></th>
<th>aP</th>
<th>NAG</th>
<th>LAP</th>
<th>BG</th>
<th>BX</th>
<th>CBH</th>
</tr>
</thead>
<tbody>
<tr>
<td>$1 \times$</td>
<td>1.252&lt;sup&gt;a&lt;/sup&gt;</td>
<td>148&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31</td>
<td>281&lt;sup&gt;a&lt;/sup&gt;</td>
<td>86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>63&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(72)</td>
<td>(13)</td>
<td>(4)</td>
<td>(39)</td>
<td>(7)</td>
<td>(8)</td>
</tr>
<tr>
<td>$0 \times$</td>
<td>1.654&lt;sup&gt;b&lt;/sup&gt;</td>
<td>142&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34</td>
<td>224&lt;sup&gt;a&lt;/sup&gt;</td>
<td>109&lt;sup&gt;b&lt;/sup&gt;</td>
<td>42&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(73)</td>
<td>(6)</td>
<td>(5)</td>
<td>(19)</td>
<td>(8)</td>
<td>(4)</td>
</tr>
<tr>
<td>$2 \times$</td>
<td>1.461&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>261&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40</td>
<td>334&lt;sup&gt;b&lt;/sup&gt;</td>
<td>104&lt;sup&gt;b&lt;/sup&gt;</td>
<td>95&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(90)</td>
<td>(15)</td>
<td>(8)</td>
<td>(25)</td>
<td>(8)</td>
<td>(8)</td>
</tr>
<tr>
<td>-50%</td>
<td>1.351&lt;sup&gt;a&lt;/sup&gt;</td>
<td>136&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29</td>
<td>257&lt;sup&gt;a&lt;/sup&gt;</td>
<td>84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>61&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(39)</td>
<td>(10)</td>
<td>(6)</td>
<td>(23)</td>
<td>(3)</td>
<td>(8)</td>
</tr>
</tbody>
</table>

| F        | 5.30 | 18.54 | 0.75 | 4.96 | 4.59 | 10.23 |
| P        | 0.004 | <0.001 | 0.530 | 0.006 | 0.008 | <0.001 |

Notes: Nit = nitrification, min = mineralization. Numerator degrees of freedom equals two for all statistical tests. Denominator degrees of freedom equals 18 for all tests except net nitrification, net N mineralization, and net nitrification: net N mineralization, which have 16 denominator degrees of freedom.
Figure A1, Chapter 5 Erosion trough water volumes for each collection period, with the date of roof additions indicated by the dashed line.
Figure A2, Chapter 5 Standing forest floor fine litter mass (n = 5) along the study hillslope during the 2012 wet season. Mixed model analysis indicates positions differed significantly ($F_{3,211} = 13.01, P < 0.001$) and also varied with time ($F_{12,211} = 12.02, P < 0.001$). Lines are standard errors of the mean.