The Effects of Land-Cover Change and Topography on Nitrogen Cycling in a Lowland Tropical Forest of Costa Rica

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Abstract

While topographic variability and land-use change have been shown to alter nutrient availability in diverse tropical landscapes, the combined effects of these two factors on nitrogen (N) cycling are poorly understood. In all ecosystems, however, it is vital to understand controls on N availability, as N is an element that is commonly associated with nutrient limitation in ecosystems. This study investigated the effects of topographic position and land-cover type on multiple pools and fluxes associated with the N cycle in a heterogeneous wet tropical forest in southwestern Costa Rica. A suite of N cycle metrics were measured across four land-cover types (primary forest, pasture, 10-15 year successional forest, and 25-30 year successional forest), and at two topographic positions (gentle slope ~10° and steep slope ~43°). Six of seven N metrics assessed differed significantly for both topography and forest type, with the exception being $\delta^{15}N$ for topographic position and ammonium (NH_4^+) for land-cover type. Concentrations of N were higher on gentle versus steep slopes across land-cover types, and pastures exhibited the highest N concentration, with N concentrations declining through secondary forest succession. These findings suggest that differences in slope angle, likely associated with erosion rates, and anthropogenic land-cover changes that alter forest dynamics and species composition may cause spatial variation in N cycling and availability. This may have important repercussions for our understanding of nutrient availability across heterogeneous tropical landscapes that are diverse in both topography and land-cover.

Introduction

Tropical forests are notably important ecosystems due to their effects on nutrient and water cycles, high biodiversity, and role in global climate regulation (Malhi and Phillips

2004, Myers et al. 2000, Sánchez-Azofiefa et al. 2001, Cramer et al. 1999). Costa Rican

forests, alone, house over 13,403 known plant, bird, reptile, mammal and freshwater

species, with unknown species comprising an estimated 84% of total biodiversity in the

area (Myers et al. 2000, Sánchez-Azofiefa et al. 2001). The high diversity and density of

plant species (>200 species per hectare) coupled with the warm and wet climate of the

tropics cause these ecosystems to have some of the highest rates of net primary production

(i.e. the rate at which autotrophs such as plants incorporate atmospheric CO_2 into biomass)

on Earth (Cramer et al. 1999, Cain et al. 2008). The rate of CO_2 uptake from the atmosphere

that occurs in the tropics (>1000 g of carbon (C) per square meter annually) is greater than

any other biome on earth, and this, coupled with the retention of immense amounts of C in

soil and plant biomass, causes these ecosystems to be important C sinks and players in the

regulation of Earth's climate (Cramer et al. 1999).

The global benefits provided by the tropical forest biome, however, are subject to

the constraints of other nutrients within the ecosystem. Accordingly, a fundamental

question in tropical forest ecology surrounds understanding the controls on nutrient

availability and limitation. In 1941, Hans Jenny proposed an influential paradigm regarding

soil development, in which he described five state factors—climate, topography, parent

material, species composition, and time—that determine differences in soil development.

This state factor concept has subsequently been extended to broader analyses of how

ecosystems as a whole develop and function (Schelsinger and Bernhardt 2013). Time has

often been considered the most important state factor in the development of tropical soils,

as they can have some of the oldest soils on Earth (Amundson et al. 2003). As soils develop

in primary succession (i.e. succession that involves the colonization of ecosystems devoid

of life), weathering of parent material causes depletion of rock-derived elements such as phosphorus (P) (Cain et al. 2008, Davidson et al. 2004). This occurs because rock-derived elements are gradually lost through soil development due to leaching or are bound in occluded (or physically unavailable) compounds (Walker and Syers 1976, Vitousek et al. 1993). Studies in the Hawaiian islands, for instance, have shown that most rock-derived P is lost after 4 million years of soil development, and the majority of P inputs into these highly weathered soils come from dust transported through the atmosphere from Asia (Herber and Fownes 1995, Vitousek et al. 2003, Chadwick et al. 1999). In contrast to the latter pattern, studies have shown that young soils contain little or no N—a nutrient that is fixed from the atmosphere—and that concentrations of N increase through soil development (Vitousek 2004, Davidson et al. 2004). These models of soil development suggest that tropical soils should be impoverished in P and rich in N relative to other, younger ecosystems (Vitousek et al. 1993, Davidson et al. 2004, Fernandes et al. 1995).

While the above paradigm does apply to many tropical forests, it is not universally applicable due the vast heterogeneity in Jenny's five state factors found across this biome (Townsend et al. 2008). The tropics are characterized by immense variation in biotic (biological) and abiotic (physical) controlling factors, at both regional and local scales (Townsend et al. 2008, Porder et al. 2005, Vitousek and Sanford 1986). For example, the tropics contain all but one of the major soil orders and a range of soil weathering status that far exceeds those found in other ecosystems (Palm et al. 2007). Similarly, tropical forests also contain nutrient "hot" and "cold" spots in the canopy that can create islands of fertility in otherwise nutrient-poor areas (Townsend et al. 2008). This heterogeneity presents a dilemma when considering ecosystem function and trying to establish universal

rules for nutrient cycling across diverse tropical landscapes, as has been documented in many studies to date (Amundson et al. 2003, Cleveland et al. 2003, Gehring et al. 2005), including several that directly assessed nutrient limitation (Kaspari et al. 2008, Wright et al. 2011).

For example, recent studies have shown that topographic variability across tropical landscapes can significantly affect nutrient cycles and availability (Fernandes et al. 1995, Cleveland et al. 2003, Porder et al. 2005, Townsend et al. 2008). Erosion on steep hillslopes can rejuvenate P supply (a rock-derived nutrient), making hillslopes P-fertile zones in otherwise P-poor landscapes—as was seen on the Hawaiian island of Kauai (Vitousek et al. 2003, Porder et al. 2005). This replenishment of P due to erosion is of great importance because P is often considered the most limiting nutrient in tropical forest systems (Walker and Syers 1976, Porder et al. 2005, Vitousek et al. 1993). However, erosion may also remove topsoil N, causing steep hillslopes to contain less N than nearby flat zones of the landscape (Amundson et al. 2003, Hilton et al. 2013, Weintraub et al. 2014). This erosion-dependent variability makes topography a factor that inherently complicates our understanding of nutrient limitation in steep landscapes, and changes in N dynamics have received far less attention to date than P.

Understanding variation in N cycling in tropical terrain with diverse topography may be further complicated by large changes in tropical land use over the last several decades. It is estimated that, as of 2005, approximately half of all land in the humid tropical forest biome had been deforested to the extent that it maintained only ~ 50% forest cover (Asner et al. 2009). In more recent decades, rates of deforestation have decreased substantially, making secondary forests an increasingly important component of the larger

tropical forest landscape (Asner et al. 2009, FAO Global Forest Resource Assessment 2005, Chazdon 2008). Forests recovering from human disturbance exhibit patterns of nutrient availability distinct from those in primary forests, which can have repercussions for ecosystem function and biodiversity recovery (Hobbs et al. 2006). For example, Davidson et al. (2004) found that successional forests growing after agricultural abandonment in the Amazon basin exhibit conservative or efficient N-cycling—similar to patterns seen in younger, N-limited temperate forest soils. To date, the interactions between forest succession and topographic variability and their effects on the N cycle have not been studied, but have the potential to be of consequence across large regions of Central America, where much of the land is a diverse mosaic of topography and land-cover.

The N cycle is an important process to understand in all ecosystems, but in the tropics has added repercussions for the global climate system. This is partly due to the relatively high concentrations of N found in tropical landscapes, as compared to more temperate latitudes, which can cause N loss pathways that create greenhouse gases (Matson and Vitousek 1990, Keller and Matson 1994). For example, some tropical forests emit large quantities of nitrous oxide (N_2O), a potent greenhouse gas with over 300 times the global warming potential per molecule then carbon dioxide (CO_2) (Matson and Vitousek 1990, Keller and Matson 1994). Furthermore, the availability of N may constrain how much C tropical forests can sequester in a changing climate (Bonan and Levis 2010, Hungate et al. 2003). These two effects on climate work in opposite ways depending on N availability: where N is found in excess, N_2O emissions are high, but where N is more limiting, it can constrain the ability of forests to store C. Thus, determining what controls N availability across the tropics is of considerable practical importance.
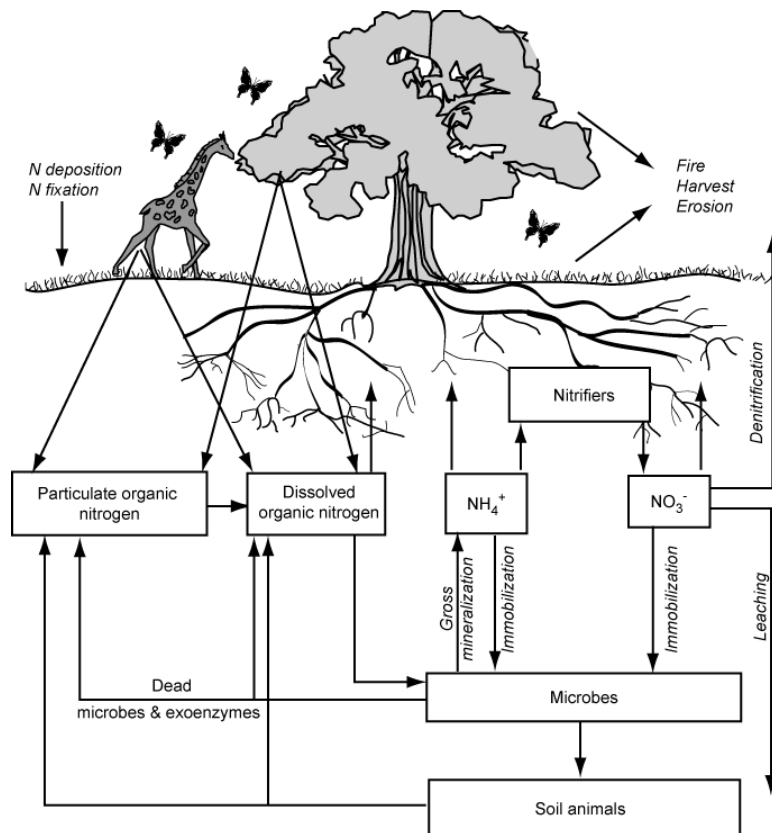
Figure 1: Conceptual model of N cycling (Schlesinger and Bernhardt 2013)

The N cycle involves complex interactions between the atmosphere and organisms living on Earth's surface (Figure 1). N enters ecosystems from the atmosphere through either N deposition or biological N fixation (Schelsinger and Bernhardt 2013). Biological fixation is an energetically expensive process, carried out by only a small proportion of organisms, and primarily occurs in situations where N is a limiting nutrient (Schelsinger and Bernhardt 2013). After N fixation, N is incorporated into organic molecules, that are eventually depolymerized (i.e. degraded into smaller pieces) and used in a series of microbial reactions, that convert organic N to forms of inorganic N, ammonium (NH_4^+) and nitrate (NO_3^-), through the processes of ammonification and nitrification, respectively (Schelsinger and Bernhardt 2013). Mineral N can then be assimilated into organic tissue, or is lost through a variety of pathways. Some of these loss pathways fractionate against

heavy N isotopes and thus leave an isotopic signature on the remaining N pool (leaching or denitrification) and others do not (volatization upon burning, agricultural harvest, and erosion) (Schelsinger and Bernhardt 2013, Robertson 1984). The different fluxes and pools of the N cycle, especially inorganic N concentrations and production rates as well as soil N isotopes, thus reflect N availability and losses in soils at various scales of time and space. The close examination of these N cycle metrics can provide insight as to the relative N-richness of a given site.

The aim of the present study was to examine how two widespread and spatially variable factors affect N availability in a tropical forest landscape: topography and land-use change. While each factor has been examined in isolation (though rarely for the effects of topography on N), we know of no other study that has integrated both factors into a single design. Building on prior work in a region of southwestern Costa Rica, this thesis used a gradient of successional to primary forest sites arrayed along a topographically complex region to do just that. This study addressed: (1) how topographic position affects N cycling in complex tropical terrain, (2) how anthropogenic land-use change and succession alter N availability in these ecosystems, and (3) what this means for the shifting paradigm of nutrient availability in tropical forests.

Methods

Study Site

This study was conducted in June of 2013 on the Osa Peninsula of southwestern Costa Rica, near the town of Los Mogos (Figure 2). This region is characterized as a wet lowland tropical forest, with heavy and seasonal rainfall (>5000 mm/yr) that peaks in the late summer and early fall (July-November), and subsides during the dry season from

January to March (Taylor et al. 2013). Approximately 75 to 40 million years ago, the Osa Peninsula was formed as a result of large seafloor volcanic events, which left some parts of land under water until more recent geologic time (Buchs et al. 2009). This sequence of events created a wide range of parent material and soil types, including highly weathered oxisols, younger ultisols, and more fertile mollisols (Buchs et al. 2009, Berrange and Thorpe 1988).
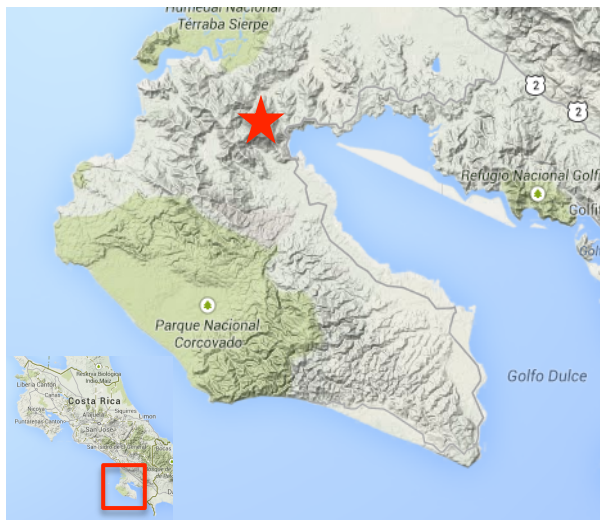

Figure 2: Map of the Osa Peninsula, with red star indicating approximate study site location

The dominant vegetation in the Los Mogos region was once lush primary tropical rainforest, but anthropogenic activities in recent decades have resulted in substantial land cover change. Today, the landscape is a mosaic of primary forest, secondary forest, and agricultural fields and pastures (Powers 2004). We conducted our study in four of these locally common land-cover types—primary forest, abandoned pasture, young (10-15 year old) successional forest, and mature (25-30 year) successional forest. The study sites were located in close proximity, with similar climates and soils developed on the same parent material. Fertilizers were not used in this region, and the pasture and successional forests examined in this study were originally cleared using slash-and-burn techniques, but were

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not burned again after the original clearing. This agricultural practice is distinct from those

commonly found in other tropical regions, such as the eastern Amazon basin, where

burning of land is repeated to prevent forest encroachment (Davidson et al. 2007,

Cleveland et al. 2003).

Field and Laboratory Methods

To study how land-cover type and landscape position affect the N cycle, two 24

meter long, contour-parallel transects were established in each forest type—one on a

gentle slope near the ridge-top and the other downslope from that transect along a steeper

slope reach (distance between transects was approximately 20 meters). The mean slope

angle for the gentle slope and the steep slope, measured at the soil surface with a hand-held

inclinometer, were 10° and 43°, respectively. Every two meters along the transect a soil

core was taken, alternating one meter above and one meter below the transect for a total of

12 samples from each transect and 24 samples from each forest type. Surface litter and

debris were removed from the sampling area and soil cores were used to extract only the

top 10 cm of soil. Samples were homogenized and kept at ambient moisture and

temperature before being treated *in situ* for extractable N, or being sent to the University of

Colorado and analyzed for a series of N cycle pools and processes.

Within one day of soil collection, soil inorganic N pools were extracted.

Approximately 8 g of fresh soil and 30 ml of 2M potassium chloride (KCl) was added to test

tubes. The tubes were then shaken vigorously for 1 minute every 30 minutes for 3 hours,

whereupon solution was left to settle for 1 hour. The solution was then filtered through 0.7

μm Whatman glass fiber filters, frozen, and transported back to the University of Colorado

at Boulder. In the laboratory, all samples were analyzed for ammonium (NH_4^+) using

colorimetric analysis on the Synergy HT microplate reader (Biotek, Highland Park, VT, USA). The samples were also colorimetrically analyzed for nitrate (NO_3^-) using an Alpkem Autoanalyzer (OI Analytical, College Station, TX, USA). Soils were oven-dried at 105°C and analyzed for soil moisture content, allowing all measurements to be provided on a dry soil weight basis.

Fresh soils transported to the University of Colorado were also analyzed for their nitrification potential, or the rate at which NO_3^- is produced when the nitrification substrate (NH_4^+) is non-limiting (Schelsinger and Bernhardt 2013). In the laboratory, approximately 10 g of each soil sample was added to an Erlenmeyer flask with 100 ml of 0.5 mM NH_4^+ buffer solution (Stark and Firestone 1996). Each sample was swirled to mix and then 15 ml was immediately removed, filtered, and frozen to be later analyzed colorimetrically for NO_3^-, as described above. The remaining mixture of soil and buffer was capped with a porous plug that allowed oxygen (O_2) diffusion and incubated in the dark on an orbital shaker at 150 rmp for 24 hours. At the end of the incubation period, solutions were filtered and frozen for subsequent colorimetric NO_3^- analysis. The nitrification potential rate was calculated as the difference between the final and initial NO_3^- concentration divided by the soil weight and incubation time.

In the laboratory, dry soil subsamples were ground to a fine powder, packed into aluminum capsules, and shipped to the Center for Stable Isotope Biogeochemistry at the University of California, Berkeley for %C, %N, and $\delta^{15}N$ analysis. The natural abundance of soil $\delta^{15}N$ has been widely used as an integrated metric of environmental N cycling because $\delta^{15}N$ values are sensitive to different pathways of N loss and the rates at which these losses occur (Amundson et al. 2003, Hilton et al. 2013). This makes $\delta^{15}N$ a very useful

measurement to understand how topography and land-use affect the N cycle in this system, as varying quantities of $\delta^{15}N$ imply different pathways of N loss and availability over long timescales (Amundson et al. 2003, Hilton et al. 2013, Weintraub et al. 2014). The natural abundance of soil $\delta^{15}N$ ($^0/_{00}$) is equal to $[(^{15}N/^{14}N_{sample})/(^{15}N/^{14}N_{standard})]-1 \times 1000$, where the standard is atmospheric N_2.

Statistical Analyses

In order to determine whether N cycle pools and process rates were affected by topography or land-cover type, two-way ANOVAs were used to analyze the data in the R statistical analysis program (R Core Development Team, 2011). Linear models were constructed with topography and forest type as the possible predictor variables. Initially, all models were tested with an interaction term between the two fixed factors. The significance of interaction coefficients was then examined, and if there was no significance the model was simplified, meaning the interaction term was dropped. This data showed no significance for interaction coefficients so all of the models were simplified. Tukey post-hoc tests were then used to examine pairwise significant differences between land-cover types. For example, the final code for nitrification potential was as follows:

```
Model 1<-lm (Npot~position*age)
      aov (Model 1)
      summary (Model 1)
Model 2<-aov (Npot~position+age)
      aov (Model 2)
      summary (Model 2)
      TukeyHSD (Model 2)
```

Results

We observed a number of significant effects of both topography and land-cover type on aspects of the nitrogen (N) cycle in the study sites. Table 1 shows the mean and

standard deviation of each transect for the different biogeochemical parameters measured, including both landscape position (gentle vs. steep slope) and land-cover type. Results of the linear models indicate that six of the seven parameters differed significantly for both topography and forest type, with the exception being $\delta^{15}N$ for topographic position and NH_4^+ for forest type. Soil moisture for the four different sites ranged from 35.44% to 46.19% moisture, with no significant differences between the different sites.

	Primary Forest		Pasture		10-15 Year Successional		25-30 Year Successional	
	GS	SS	GS	SS	GS	SS	GS	SS
NH_4^+ (µg N/g Soil)	4.80 (0.360)	4.84 (1.07)	5.35 (0.900)	4.82 (0.863)	5.40 (0.976)	4.43 (1.12)	5.53 (1.38)	4.41 (0.289)
N. Potential (µg N/g Soil/day)	10.24 (8.05)	2.89 (3.12)	15.75 (12.87)	9.09 (8.02)	8.83 (4.69)	7.35 (3.00)	2.65 (1.85)	1.37 (0.866)
NO_3^- (µg N/g Soil)	1.05 (0.732)	0.229 (0.358)	2.06 (2.30)	0.939 (0.979)	0.319 (0.330)	0.451 (0.329)	0.0765 (0.147)	0.0217 (0.0549)
$\delta^{15}N$ ($^0/_{00}$)	3.72 (0.711)	3.68 (1.35)	4.84 (0.259)	4.90 (0.459)	4.68 (0.244)	4.79 (0.594)	2.67 (0.808)	3.27 (1.04)
Soil C (%)	4.75 (1.35)	2.84 (1.34)	4.99 (1.17)	4.35 (1.52)	5.01 (0.787)	3.33 (0.750)	6.62 (1.94)	4.42 (1.72)
Soil N (%)	0.415 (0.103)	0.246 (0.0963)	0.506 (0.114)	0.418 (0.120)	0.406 (0.0533)	0.313 (0.0528)	0.502 (0.105)	0.344 (0.0970)
C:N	11.38 (0.753)	11.18 (1.83)	9.81 (0.341)	10.25 (0.946)	12.35 (1.72)	10.58 (0.775)	12.95 (1.37)	12.61 (1.72)

Table 1: Soil C and N values at the 8 different transect locations; GS=gentle slope ($\sim10^0$) and SS=steep slope ($\sim43^0$), values are means with standard deviation in parenthesis.

	p-value		F-value	
	Forest Type	Topography	Forest Type	Topography
NH_4^+ (µg N/g Soil)	0.74970	0.00034	0.405	13.864
N. Potential (µg N/g Soil/day)	<0.0001	0.00152	13.38	10.69
NO_3^- (µg N/g Soil)	<0.0001	0.00305	19.809	9.273
$\delta^{15}N$ ($^0/_{00}$)	<0.0001	0.33	30.659	0.958
Soil C (%)	0.000412	<0.0001	6.652	33.849
Soil N (%)	<0.0001	<0.0001	9.033	41.517
C:N	<0.0001	0.0452	21.267	4.124

Table 2: Results of ANOVA for 7 different biogeochemical parameters; degrees of freedom for forest type=3 and for topography =2.

While NH_4^+ concentrations showed no significant difference between land-cover types, there was a statistically significant difference between extractable NH_4^+ levels in transects at different topographic positions (P<0.001, F-value=13.864;Table 2 and Figure

3A). Apart from the primary forest, where pools did not vary by position, gentle slopes had an average of 0.87 μg/g soil more NH_4^+-N than the steep slopes. Conversely, nitrification potential varied greatly with both land-cover type (P<0.001, F-value=13.38; Table 2 and Figure 3B) and topography (P<0.01, F-value=10.69; Table 2 and Figure 3B). There were statistically significant differences in the nitrification potential between the primary forest and the pasture (P=0.004), the 25-30 year successional forest and the pasture (P<0.001), and the 25-30 year successional forest and the 10-15 year successional forest (P<0.001). The lowest values for nitrification potential were found in the 25-30 year successional forest and the steep slope of the primary forest, while the pasture, 10-15 year successional forest, and primary forest gentle slope tended to have high nitrification potential. Within three out of four land-cover types, the gentle slopes had consistently higher nitrification potentials than values found on the steep slopes, a pattern consistent across almost all N parameters measured.
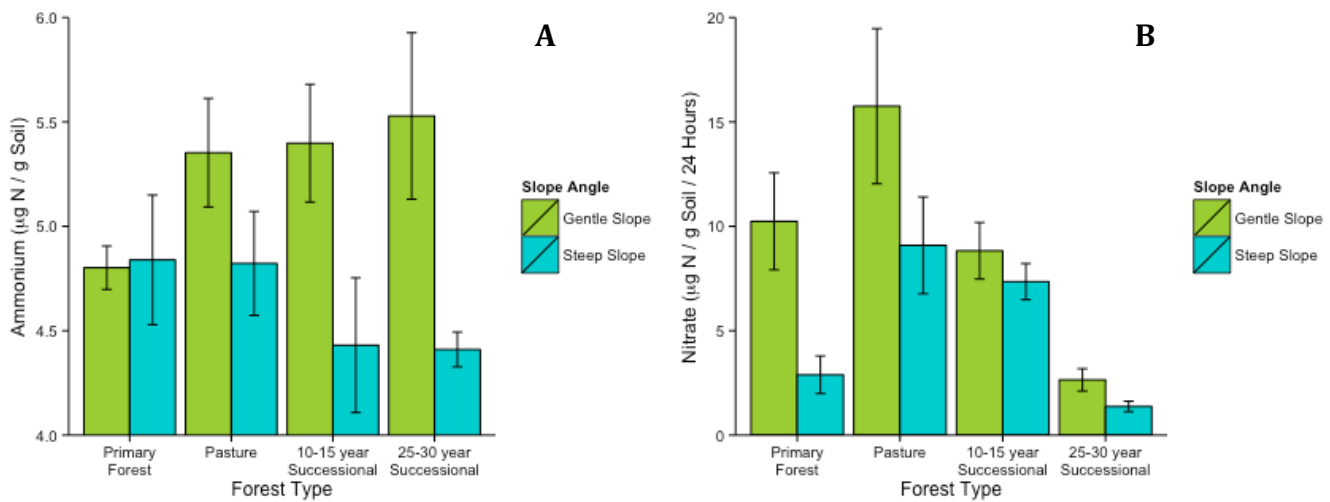

Figure 3(A) and Figure 3(B): A—mean soil NH_4^+ (μg NH_4^+/g Soil) for each transect in the Los Mogos region of Costa Rica, with error bars depicting ± 1 standard error of mean. B—mean soil nitrification potential (μg NO_3^- / g Soil*day) for each transect in the Los Mogos region of Costa Rica, with error bars depicting ± 1 standard error of mean.

Soil NO_3^- concentrations varied significantly between topographic positions (P<0.01, Table 2 and Figure 4A) and land-cover types (P<0.001; Table 2 and Figure 4A). Most land-cover types had unique nitrate values—there were statistically significant differences between the pasture and 25-30 year successional forest (P<0.0001), the primary forest and the 25-30 year successional forest (P=0.0002), the 10-15 year successional forest and the 25-30 year successional forest (P=0.001), the primary forest and the pasture (P=0.006), and the 10-15 year successional forest and the pasture (P=0.001). In fact, the 10-15 year successional forest and the primary forest were the only two groups for which mean nitrate concentrations did not differ significantly. The pasture had the highest amount of NO_3^-, with the 25-30 year successional forest having the lowest values. Similar to other N metrics, for three of the four land-use types the gentle slope transects had higher nitrate concentrations than the steep slopes.
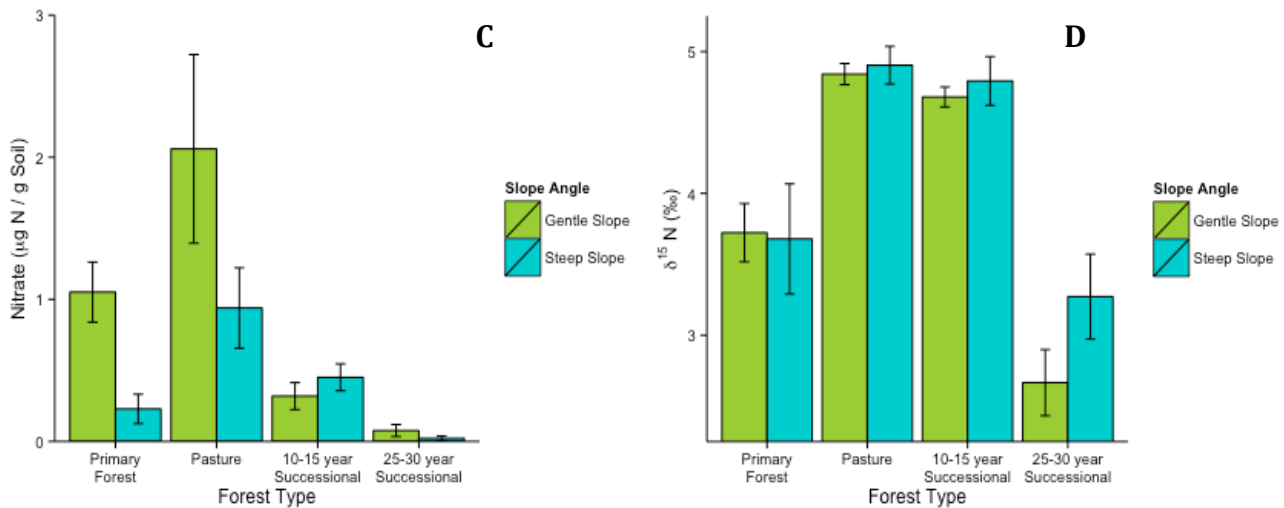

Figure 4(A) and Figure 4(B): C—mean soil NO_3^- ($\mu g\ NO_3^-$ / g Soil) for each transect in the Los Mogos region of Costa Rica, with error bars depicting ± 1 standard error of mean. D—mean soil $\delta^{15}N$ ($^0/_{00}$) for each transect in the Los Mogos region of Costa Rica, with error bars depicting ± 1 standard error of

Surprisingly, $\delta^{15}N$ showed no statistically significant difference between topographic positions (P>0.05, Table 2 and Figure 4B); however, there was a statistically

significant difference between various land-cover types (P<0.001, Table 2 and Figure 4B). The highest values of $\delta^{15}N$ were found in the pasture and the 10-15 year successional forest, and the lowest values were fond in the 25-30 year successional forest. There were statistically significant differences between the pasture and the 25-30 year successional forest (P<0.0001), the primary forest and the 25-30 year successional forest (P=0.006), the 10-15 year successional forest and the 25-30 year successional forest (P<0.0001), the primary forest and the pasture (P<0.0001), and the 10-15 year successional forest and the primary forest (P=0.0001)—only in the 10-15 year successional forest and the pasture are the $\delta^{15}N$ values statistically similar.

Discussion

Topography and N cycling

Many influential studies of tropical forest nutrient limitation have been restricted to the analysis of geomorphically stable and minimally eroded surfaces—thus establishing a paradigm of soil nutrient availability that may not always apply to more dissected landscapes (Walker and Syers 1976, Vitousek et al. 2003, Weintraub et al. 2014, Porder et al. 2005). The results presented here suggest that nutrient cycling dynamics and patterns of relative nitrogen (N) limitation may vary at regional and landscape scales, where topographic variation can alter N availability. The heterogeneous topography of the Osa Peninsula of Costa Rica exhibits substantial variation in N availability across small distances, with higher N concentrations found on the ridge-tops compared to steep hillslopes.

While this pattern could be the result of various factors, the most plausible one surrounds the high rates of topsoil and organic matter erosion on steeper hillslopes, which

in turn prevent the chronic accumulation of N over long timescales. While the influence of erosion on nutrient availability has been known for some time for rock-derived elements like P and potentially calcium (Ca) (e.g. Porder et al. 2005, Vitousek et al. 2003, Bern et al. 2005), the effects on N are not widely known. Work that motivated this thesis was the first to show potential links between erosion and the N cycle in the lowland tropics (Weintraub et al. 2014), and other evidence only reported from California (Amundson et al. 2003) and montane tropical forests of Taiwan (Hilton et al. 2013).

The higher amount of erosion on steep hillslopes constantly rejuvenates the soil and 'resets the clock' for soil development (Porder et al. 2005, Amundson et al. 2003, Vitousek et al. 2003, Weintraub et al. 2014). As noted above, several studies have demonstrated the effects of erosion on phosphorus (P) availability in diverse tropical landscapes, where concentrations of this nutrient on steeper hillslopes approach those found on younger and more fertile soils (Porder et al. 2005, Vitousek et al. 2003, Silver et al. 1994, Crews et al. 1995). In the present study, however, we focused on N, which is often assumed to cycle in relative excess in lowland tropical forests (i.e. N availability is relatively high, causing N cycling to be less efficient) (Vitousek et al. 2003, Walker and Syers 1976, Porder et al. 2005). However, the results of our study suggest that topography can also have a strong impact on the N cycle. However, because N is fixed from the atmosphere and accumulates in the soil over time, the pattern for N is the opposite of that for P. Rather than rejuvenating nutrient availability, as seen for P and other rock-derived nutrients, erosion removes accumulated soil N as well as N-rich leaf litter, making N more likely to be limiting on steep hillslopes. This pattern is consistently present in all but one of the N cycle pools and fluxes measured in our project, and is also seen in the findings of a study done in the montane

tropics (Hilton et al. 2013) and in a primary forest near the study sites examined in this thesis (Weintraub et al. 2014). For the latter study, relatively N-poor conditions were documented on steep hillslopes in contrast to comparatively N-rich conditions on ridge-tops with low slope angles.

The only N cycle parameter where the patterns documented here were not consistent with previous research was $\delta^{15}N$. Higher levels of $\delta^{15}N$ were expected on the lower slope angles because of the hypothesized N-enriched state and because topographic positions subject to water collection and anaerobic conditions, such as those found on low-angle ridge-tops, may also exhibit higher levels of denitrification and subsequent residual accumulation of ^{15}N (Amundson et al. 2003, Hilton et al. 2013, Weintraub et al. 2014). In contrast to this pattern, higher rates of erosion commonly found on steep hillslopes cause non-fractionating losses of N (Hilton et al. 2013, Weintraub et al. 2014). Because erosion limits N accumulation, it is thought to prevent enrichment of $\delta^{15}N$ values by fractionating pathways that require anoxic conditions and high N availability (Amundson et al. 2003, Hilton et al. 2013, Weintraub et al. 2014).

However, the sites studied in this thesis did not follow this expected pattern, as $\delta^{15}N$ did not very with slope angle. The most probable explanation may lie in the overall geomorphic differences between the study sites examined in the present study and those which led to the above citations, including the Weintraub et al. study on the southern Osa Peninsula (2014). There, younger overall landscapes exhibit a clear patter of geomorphic disequilibrium, in which steep slopes result from the gradual upcutting of stream courses into a remnant landscape on which substantial wide, flat ridges still remain (Weintraub et al. 2014, Buchs et al. 2009). In the Los Mogos region, the underlying substrate age is much

older, and the resultant landscape consists of very few broad flat areas along the high elevation portions of the region (Buchs et al. 2009, Berrange and Thorpe 1988). Instead, nearly everything exists on some degree of slope, as evidenced by the fact that even our "gentle" slope sites were considerably steeper than those used in the Weintraub et al. (2014) topographic transects. As such, the erosive losses are likely more similar across our sampling sites, perhaps leading to fewer geomorphically-induced differences in N-cycle metrics that are only altered over long timescales, such as $\delta^{15}N$.

Land-Cover and N cycling

The spatial heterogeneity in N cycling created by topographically dissected tropical landscapes is further complicated by land-use change that alters forest composition, structure, and dynamics. Our data concur with recent studies, which found that secondary forests created by human disturbances have distinct patterns of nutrient availability and limitation from the mature primary forests of which they were derived (Fernandes et al. 1995, Reiners et al. 1994, Davidson et al. 2004, Sullivan et al. 2014, Gehring et al. 2005). Though, studies in the Amazon basin revealed different patterns than our own, possibly due to differences in soil composition and agricultural practices found in that region (Davidson et al. 2004, Gehring et al. 2005). Our results suggest that pasture soils on the Osa Peninsula tended to have higher mineral N contents than primary forest soils, and that succession to mature land-cover types, such as old successional forests, tends to reduce N availability. We believe that this pattern is a direct consequence of land-use change, and the related variations in tree species composition and growth rates, soil microbial communities and rates of biological N fixation in pastures and secondary forests.

In a study done in Costa Rica, Reiners et al. (1994) found that species richness and biomass increased with pasture abandonment, due to the conversion from herb-dominated pasture to tree-dominated secondary forest. Because pastures have significantly fewer and smaller taxa than secondary forests (Reiners et al. 1994, Fernandes et al. 1995), inorganic N accumulates in pasture soils as the plant communities growing there have a lower N uptake capacity than more mature forests (Russell and Raich 2012). Our findings support this notion that higher concentrations of inorganic N in pasture and young successional forest soils are due to differences in plant communities and their ability to take up N. Our results imply that after removal of large amounts of plant biomass during deforestation, a large amount of N accumulated in soils. Yet, the increase in species diversity and high rates of tree growth following agricultural abandonment in secondary forests reduces N availability in these ecosystems, a pattern consistent with several studies to date, including ours (Fernandes et al. 1995, Reiners et al. 1994, Davidson et al. 2004, Sullivan et al. 2014, Gehring et al. 2005). The decrease in N availability with secondary succession (Davidson et al. 2004) likely results from fast-growing plants assimilating large amounts of N from soils.

The role of N_2-fixation in alleviating N constraints in successional forests is an interesting question. Davidson et al. (2004) found that dominant species in regenerating Amazonian forests were not N fixers and that soil properties—including P limitation and N abundance—prevented N fixers from having a significant advantage, as biological N fixation is an energetically expensive process. However, in a study performed on the Osa Peninsula by Sullivan et al. (2014), secondary forests did have higher total biological N_2-fixation inputs than primary forests, and rates of fixation increased through secondary succession. The low $\delta^{15}N$ values measured in our 25-30 year successional forest site as

compared to the less mature land-cover types is consistent with this finding, as the decline in $\delta^{15}N$ enrichment in the old successional forest probably resulted from more N being fixed from the atmosphere (which is isotopically light).

Gehring et al. (2005) found a similar pattern in a Central Amazonian rainforest, where biological N fixation rates were high during secondary succession and low in primary forests, suggesting sufficiently high N-availability in the primary forest to make N_2-fixation unfavorable. This conclusion is further supported by evidence that shows a shift in legume abundance through succession (Sullivan et al. 2014). Because N_2-fixation by legume-rhizobia symbiosis is believed to be the main pathway for secondary forests to recuperate losses in soil N, the increase in legume abundance implies that N is limiting in old successional forests, and N_2-fixing plants are advantageous during forest succession (Sullivan et al. 2014, Batterman et al. 2013, Gehring et al. 2005).

The observed patterns of altered N availability with land-cover type could also be linked to differences in microbial communities. For instance, Cleveland et al. (2003) found that conversion of forest to pasture can lead to dramatic decreases in the overall size of the microbial community, as deforestation causes significant changes in the soil environment such as soil carbon sources, nutrient availability, pH, and bulk density. This change may contribute to elevated inorganic N concentrations in pasture soils. Further, differences in soil microbe community composition may also be relevant, as N cycling involves complex interactions between microbes, including nitrification and denitrification (Schelsinger and Bernhardt 2013). Differences in microbial communities following land-use change could, thus, affect the N parameters tied to these microbially-mediated processes, particularly concentrations of NH_4^+ and NO_3^-, nitrification potential, and $\delta^{15}N$ (Cleveland et al. 2003).

Greater Consequences and Future Study

Our results add to a growing body of work suggesting that not all tropical forests are as rich in N relative to other ecosystems as dominant paradigms have suggested (Townsend et al. 2008). This has important implications for tropical forest restoration, as nutrient limitation to plant growth and ecosystem function could involve not only P limitation, as has traditionally been assumed, but also relative N limitation on both steep hillslopes and in successional forests. Recent restoration projects targeting severely degraded landscapes have begun to focus on the success of a "nucleation model" of forest restoration, which involves planting tree seedlings in islands, rather than uniformly over a landscape (Cole et al. 2010, Holl et al. 2010, Zahawi et al. 2013). When considering forest restoration practices, it is important to develop strategies that are both economically and ecologically viable, and the nucleation model has shown to have both advantages and disadvantages in several studies (Cole et al. 2010, Holl et al. 2010, Zahawi et al. 2013). For instance, a recent study identified a cheap and relatively simple nucleation strategy to support seedling survival, but there were two issues—damage to seedlings in early development and lower seedling growth rates, thought to be a result of less N_2-fixing species grown in the islands than plantation-style restoration plots (Holl et al. 2010).

Several studies have shown that planting fast-growing biological N_2-fixing tree species enhances the growth of other species in forest restoration projects of Costa Rica (Holl et al. 2010, Carpenter et al. 2004, Siddique et al. 2008, Nicholas and Carpenter 2006). Similarly, increased legume abundance and N_2-fixiation rates of naturally aggrading forests in Costa Rica suggest that in late successional forests in the tropics and particularly on

steep hillslopes, N can be a limiting nutrient and biological N fixation can be facultatively regulated, or species composition can change to meet N demand (Sullivan et al. 2014, Barron et al. 2011, Gehring et al. 2005, Menge and Hedin 2009). Again, this suggests that N limitation plays an important role in plant growth during secondary succession, and must be considered. In the future, it would be beneficial to study how different restoration strategies—including what seedlings are planted, in which pattern, and in what steepness of terrain—affect nutrient cycles and promote the most growth in restored areas. It is important to understand not only the mechanisms responsible for inhibiting growth during restoration projects, but also the diverse ecological consequences and affects on nutrient cycling of different management techniques across these diverse landscapes. Therefore, I suggest that N constraints should be explicitly considered in nucleation project planning, and addressed through the promotion of N_2-fixing trees in islands.

Conclusion

The early paradigm of soil development and nutrient availability in lowland tropical forests characterized their soils as being nitrogen (N) rich and phosphorus (P) poor (Walker and Syers 1976, Vitousek 2004, Fernandes et al. 2005, Vitousek et al. 1993, Davidson et al. 2004). The results of this thesis agree with others that suggest this paradigm is not universal across tropical landscapes, as they are incredibly heterogeneous in all of Jenny's five state-factors, including climate, topography, parent material, species composition, and time (Jenny 1941, Townsend et al. 2008). The aim of the present study was to examine how topographic variability and land-use change, two widespread and spatially variable factors on the Osa Peninsula of Costa Rica, affect N availability in a lowland tropical forest landscape. Our results suggest that steep hillslopes exhibit lower

availabilities of N, which is likely linked to higher rates of erosion in these areas and removal of N-rich soil and organic matter.

In terms of the effects of land-use change on the N cycle, our data suggest that abandoned pastures had higher availabilities of soil N than primary forests, and that N concentrations declined through forest succession. This is most likely due to a combination of land clearing creating an initial pulse of N, for which there is much less demand than in primary forests, followed by shifts in species composition and N demand over time, as well as by substantial differences in biological N fixation rates through forest succession (Sullivan et al. 2014, Barron et al. 2011, Cleveland et al. 2003, Gehring et al. 2005, Menge and Hedin 2009). These results have important implications on our understanding of nutrient cycling and availability in tropical forest landscapes, and could prove useful when considering and evaluating methods of restoration in degraded tropical rainforest ecosystems that are limited by N availability (Holl et al. 2010).

Acknowledgements

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