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CORRESPONDENCE

Can we see the nitrate from the trees? Long-term linkages between tropical forest productivity and stream nitrogen concentrations

Marcelo Ardón · Deborah A. Clark · Nicholas S. Marzolf · Alonso Ramirez · Catherine M. Pringle

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Abstract High abundance of trees capable of biological N-fixation (henceforth "N-fixers") in tropical forests has been hypothesized to drive higher stream nitrate (NO_3) concentrations compared to temperate counterparts. However, to date there have been no empirical linkages of stream NO_3 concentrations with the productivity of tropical forests. Here, we combined three unique long-term datasets from La Selva Biological Station, Costa Rica: 21 years of (1) mean annual stream NO_3-N concentrations in six stream sites within the same watershed, (2) annual growth of trees, and (3) annual leaf litterfall. We hypothesized that years of greater growth of N-fixer tree species and of greater leaf litterfall would be correlated with higher stream water NO_3-N concentrations. We also hypothesized that landscape position mediates these relationships, with growth of N-fixer trees on adjacent slopes being more strongly correlated to stream NO_3-N than the growth of such trees on upland plateau sites. We found that mean annual stream NO_3-N concentrations were consistently high (160–260 μg L^{-1}). There was substantial interannual variation in leaf litterfall (inter-year range: 5.4 to 8.1 Mg ha^{-1} $year^{-1}$), growth of N-fixers (inter-year range: 1.2 to 2.2 Mg ha^{-1} $year^{-1}$), and growth of all other tree species (inter-year range: 2.1 to 3.2 Mg ha^{-1} $year^{-1}$). To assess stream NO_3-N relationships with forest productivity, we used water conductivity to account for dilution resulting from variable discharge. We found that NO_3-N concentrations were positively related to the annual growth of the N-fixers on nearby slopes, and were negatively correlated with annual leaf litterfall. Stream NO_3-N concentrations were not related to the growth of N-fixers or other tree species in the more removed plateau areas. Using a mass balance, we estimated that symbiotic N fixation can account for 7–29% of NO_3 export. Both the growth of adjacent N-fixers and landscape-wide leaf litterfall are important drivers of

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M. Ardón (✉) · N. S. Marzolf
Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC, USA
e-mail: mlardons@ncsu.edu

D. A. Clark
Department of Biology, University of Missouri-St. Louis, St. Louis, MO, USA

N. S. Marzolf
Department of Biology, Duke University, Durham, NC, USA

A. Ramirez
Department of Applied Ecology, North Carolina State University, Raleigh, NC, USA

C. M. Pringle
Odum School of Ecology, University of Georgia, Athens, GA, USA

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the inter-annual variability of stream NO_3-N concentrations. Our results suggest that predicted changes in precipitation extremes due to climate change will alter N dynamics in tropical forests both directly, by altering discharge and export, and indirectly, by altering N-fixer tree productivity.

Introduction

Biological nitrogen fixation is an important process regulating nutrient availability for terrestrial and aquatic ecosystems (Cleveland et al. 1999). The role of biological N fixation at the watershed scale has been studied in temperate systems (Compton et al. 2003; Mineau et al. 2011; Shaftel et al. 2012), but it has not received as much attention in tropical watersheds, which have a much higher abundance of species capable of symbiotic N fixation (Menge et al. 2019). It has been hypothesized that the high abundance of N-fixer trees (species that can have symbiotic relationships with N-fixing bacteria in specialized root nodules) in many humid tropical forests drive the elevated N in soils and streams (Jenny 1950; Cleveland et al. 1999; Hedin et al. 2009). The high abundance of N-fixer trees in systems with high N in soils has been termed the N paradox (Hedin et al. 2009), given that theory would predict that trees would downregulate N fixation when N is widely available, due to the high metabolic cost of fixation to plants. N-fixation can also be carried out by free living bacteria not directly associated with plants (asymbiotic N-fixation), living in soils, leaf litter, logs, or canopy areas (Hedin et al. 2009). The biophysical controls and relative contribution of symbiotic and asymbiotic N fixation in forests has been recently identified as a frontier in N cycling research (Cleveland et al. 2022).

Symbiotic N-fixation rates change through forest succession: they are highest in young forests and decline to very low levels in old growth in tropical ecosystems (Batterman et al. 2013a; Taylor et al. 2019). Recent work has found a similar pattern in a temperate forest (Wurzburger et al. 2022), but more work is needed. In spite of the characteristically high abundance of N-fixers in old-growth tropical forests, field studies report that the N-fixers there are facultative (Menge et al. 2009) and only actively fix N after gap formation or other disturbances (Barron et al. 2011; Levy-Varon et al. 2019; McCulloch and Porder 2021). Despite the long interest in associations among tropical-forest N-fixers, N fixation, and hydrologic N losses, the lack of long-term coupled aquatic and terrestrial datasets in tropical watersheds has prevented direct examinations of their potential links and drivers.

Tropical forests in the Americas have some of the highest abundance of N-fixer trees in the world (Menge et al. 2019). The high abundance of N-fixers could lead to high N availability in soils and nearby rivers and streams through various mechanisms, which are not mutually exclusive (Brookshire et al. 2012). One mechanism is through mineralization and turnover of N-rich tissues (litter and roots) produced by N-fixers, which leads to higher N delivered to soils and nearby streams (Vitousek and Sanford 1986). N-fixers have high tissue N content regardless of whether they are actively fixing N (Hedin et al. 2009). A second mechanism posits that high N is leached from N rich tissues (roots, leaves, and stems), leading to increased soil N under the canopy of N-fixers (Osborne et al. 2017; Massmann et al. 2021). A third possibility is that species that carry out symbiotic N fixation are able to meet their growth demands without removing N from soils, therefore decreasing overall plant N uptake (Menge and Chazdon 2016). N-fixers are important facilitators of tropical-forest succession, releasing other tree species from N limitation (Menge and Hedin 2009; Batterman et al. 2013a; Taylor et al. 2019). In old-growth forests, climate and landscape position (particularly slope vs uplands) have been shown to mediate the effects of tree species on soil N content and cycling (Osborne et al. 2017), and would likely also affect hydrologic losses. The extent to which the growth and location of N-fixers explain the high hydrological N losses from tropical watersheds has not been examined.

Concentrations of N in tropical streams tend to be high compared to unpolluted temperate counterparts (Lewis et al. 1999; Gucker et al. 2016). Brookshire et al. (2012) used long-term stream NO_3 data from Maritza Biological Station in Costa Rica, and other watersheds from Costa Rica and Trinidad to show high concentrations of bioavailable (inorganic) N in tropical montane forests. Using stable isotopes and a mass-balance ecosystem model, they attributed most of the exported N to the plant-soil complex, and not from atmospheric N deposition (Brookshire et al. 2012). Working in La Selva Biological Station, Costa

Rica, Triska et al. (1993) reported that inorganic N in streams came from mineralization in the catchment. Long-term research at La Selva has concurrently examined tree dynamics and productivity (Clark et al. 1999, 2021) and stream structure and function (Pringle et al. 2016). However, terrestrial and aquatic datasets have not been directly linked. An improved understanding of how terrestrial plant productivity might affect stream N export is necessary to forecast response of tropical forests to a changing climate and alterations of the N cycle.

Here, we combined three unique long-term 21-year datasets from the same tropical forest watershed: annual growth of trees and biweekly litterfall from 10 0.5-ha plots, and mean annual stream NO_3-N concentrations from six stream sampling sites. The stream sites range from headwater streams to a fourth order river. We asked: (i) Does annual variation in tree growth (N-fixers and/or non N-fixers) drive variation in annual stream NO_3-N concentration? (ii) Does position in the landscape (slope vs flat upland plateau) influence the relationship between tree growth and stream NO_3-N concentrations? And (iii) does annual variation in leaf litterfall drive the annual changes in stream NO_3-N concentration? We hypothesized that both growth of N-fixers and leaf litterfall would be positively correlated to stream NO_3-N concentrations, as N-fixer trees produce N-rich materials (leaf litter and roots) that decompose and lead to increased N in streams. We also hypothesized that tree growth (of both non N-fixers and N-fixers) in plateau plots would not be related to stream NO_3-N concentrations.

Methods

La Selva Biological Station (10° 26′ N, 83° 59′ W), in the lowlands of Costa Rica is a 1536 ha reserve run by the Organization of Tropical Studies. The reserve is part of the last intact biological corridor on the Caribbean slope of Central America, spanning altitudes from 30 to 2900 m a.s.l. (McDade et al. 1994). La Selva receives on average 4000 mm of rainfall a year, though there is considerable year to year variation (Sanford et al. 1994). Rainfall is usually lower during January-April, though rain can still exceed 100 mm per month during those months. Annual rainfall varied from 3200 to 6550 mm per year during the study years. During the study period the mean annual temperature was 25.1 °C. Long-term meteorological data came from the Organization for Tropical Studies weather station (https://tropicalstudies.org/). Soils at the site are volcanically derived and contain higher nutrient content compared to other Neotropical forests (Powers et al. 2005; Porder et al. 2006).

La Selva's old-growth forest has more than 260 species of trees and palms (Clark et al. 1998, 1999, 2021). The distribution of these species is strongly related to the within-landscape edaphic heterogeneity (Clark et al. 1998, 1999). There are three main edaphic conditions: younger oxisol (alluvial) terrace, older oxisol plateau, and older oxisol slope (Clark et al. 2021). The younger oxisol soils have higher soil nutrients and higher leaf-litter nutrient content (Wood et al. 2006; Espeleta and Clark 2007). In this study we focused on the older oxisol soils because they dominate the old-growth landscape, comprising 68% of the landscape, while the younger oxisol (alluvial) comprise 12% (Clark et al. 1999).

Working across secondary and old growth at La Selva, Menge and Chazdon (2016) identified 37 species of potential N-fixer trees. Twenty-two of those species occur in the area used for this study (Supplementary Table S1). Across edaphic conditions, *Pentaclethra macroloba* is the dominant tree species in La Selva, accounting for 36–38% of the basal area or estimated aboveground biomass (stems ≥ 10 cm diameter) across the CARBONO plots (Clark et al. 2021). Research has shown that individual *P. macroloba* trees fix more N in early successional forest, and much less in the old growth (Taylor et al. 2019). Its seedlings and young trees fix more N when light levels are enhanced by canopy openings (Taylor and Menge 2018; McCulloch and Porder 2021). Irrespective of N-fixation rates, *P. macroloba* trees have high N in their leaves and litter (Taylor and Ostrowsky 2019; Massmann et al. 2021). The *Inga* genus contains at least 18 species that are also known to be N-fixers (Menge and Chazdon 2016; Taylor and Ostrowsky 2019); trees of this genus comprise 3–6% of the basal area across the CARBONO plots. For this study we defined the **N-fixers** of La Selva as the trees of the species: *P. macroloba*, *Balizia elegans, Stryphnodendron microstachyum, Abarema adenophors*, and nine species of the *Inga* genus, comprising 96% of N-fixer basal area of the plots used in this study (Supplementary Table 1).

La Selva is drained by two major rivers, the Sura and Salto (Pringle et al. 1993). This study focuses on the Salto watershed, which drains the old-growth section of La Selva (Fig. 1). The lower elevation portions of this watershed receive interbasin transfers of solute-rich groundwater, entering at seeps at the base of Pleistocene lava flows (Pringle and Triska 1991; Pringle et al. 1993). These regional groundwater inputs have high concentrations of solutes (PO_4^{2-}, Na^+, Cl^-, and HCO_3^-, Pringle et al. 1993), ranging from 10 to 30 times more concentrated than low solute local groundwater (Genereux et al. 2002). Similar high-solute streams are found in volcanic areas through Central and South America (Pringle et al. 1993; Ganong et al. 2015).

Measurement of stream physical and chemical parameters as part of the STREAMS project have been described in detail (Triska et al. 2006; Small et al. 2012). Monthly measurements of physical and chemical parameters started in April 1997 for 16 sites. In this study we focused on six of the sites which are all within the Salto watershed (486 ha): Salto 30, 60 and 100, which are all on the main stem of the Salto stream; Saltito 60 and 100, which are on a tributary to the main stem; and Pantano 60, which is another tributary to the Salto, which drains a swamp (number after each site name denotes relative elevation, Fig. 1). In the first week of every month, our long-term technician (who has been the same for the duration of the project) visited the sites to measure conductivity, pH, and collect and filter water samples for solute analyses. Discharge is measured monthly based on staff gage rating curves. Filtered (0.45 µm) and frozen water samples were transported to the US for chemical analyses. In this study we focus on NO_3-N concentrations which were measured using the cadmium reduction method at the UGA lab from 1997 to 2015, and at NCSU lab from 2015 to 2018 (on a AA3 Segmented Flow Analyzer, Seal Analytical, Mequon, WI). The cadmium reduction method provides results that include both NO_3 and NO_2. For the 2017–2018 samples, we also measured NO_2 using ion chromatography (Metrohm 930), but found it below detection limit. For simplicity, we report all concentrations as just NO_3-N. NH_4-N was measured using the phenate method in both labs (on a AA3 Segmented Flow Analyzer, Seal Analytical, Mequon, WI). Water samples from 2015 were analyzed in both labs, and showed high agreement between datasets (> 97% of samples were within 5% of each other). For 2017–2018 samples, we also measured dissolved organic carbon and total dissolved nitrogen (TDN)

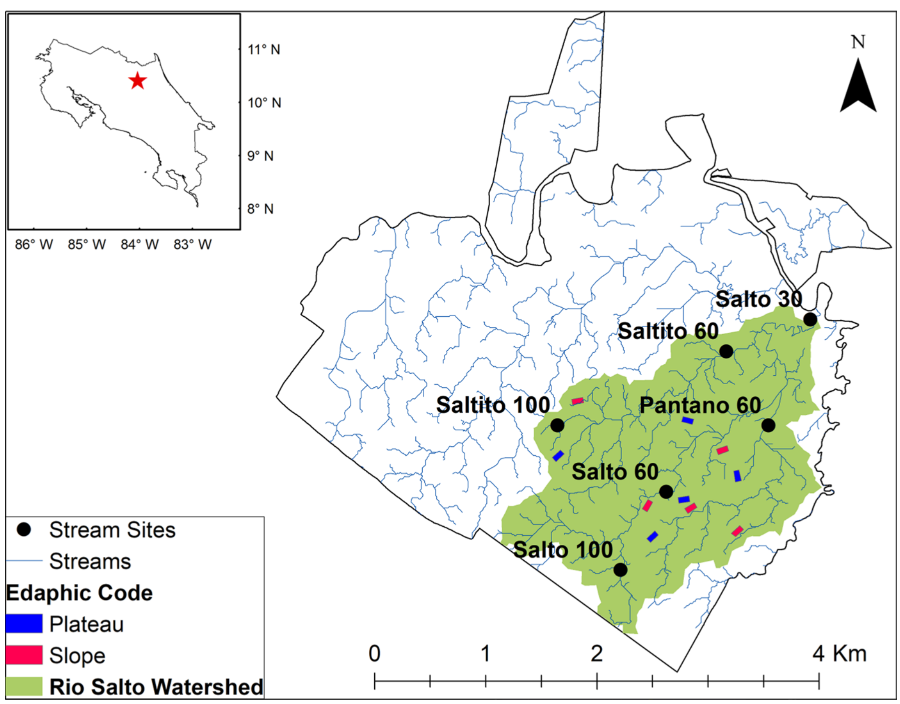


Fig. 1 Map of La Selva Biological Station showing Salto watershed (green) and six stream sites (circles) and 10 terrestrial plots (rectangles) used in this study. Insert show location of La Selva in Costa Rica

using a Teledyne TOC-TN instrument (Torch, Teledyne Tekmar, Mason OH). We calculated dissolved organic N (DON) as the difference between total dissolved N (TDN) and inorganic N (NO_3-N + NH_4-N). Due to the lack of continuous discharge data, we are unable to estimate volume weighted concentrations. As an approximation to volume weighted concentrations, we used the ratio of NO_3-N to conductivity (Likens and Bormann 1995). Conductivity in these streams decreases after rainfall events (Supplemental Fig. S1). In order to match stream datasets with the terrestrial datasets, we calculated annual means from October of year 1 through September of year 2. Long term datasets for the STREAMS project are available on the project website: http://streamslaselva.net/.

For this study we used measurements of tree growth and leaf litterfall from a subset of the 18 plots (0.5 ha) of the CARBONO project (Clark et al. 2021). These plots were randomly located across 500 ha of old-growth forest to represent the three main edaphic conditions: younger oxisol (alluvial) terrace (6 A plots), older oxisol plateau (6 L plots), and older oxisol slope (6 P plots). Of the 18 plots, we selected 10 within the Salto river watershed (plateau plots: L2, L3, L4, L5, and L6; slope plots: P2, P3, P4, P5, and P6). The slope plots are closer to our stream sites (on average 200 m) than the plateau plots (on average 400 m away). All live woody stems ≥ 10 cm diameter were mapped and identified when the plots were installed in 1997 (Clark et al. 2021). Individual trees were measured annually for growth with a fabric diameter tape to the nearest 1 mm (rounding down). Measurements were made at a permanently marked point, usually 130 cm above the ground, or above any basal stem irregularities caused by buttresses. The same two field technicians made all the diameter measurements, with consistently high repeatability (97–100% of re-measurements were within 1 mm). Technicians measured the trees in the plots in September–November/December of each year, always in the same sequence. For more detail on the measurement protocols and quality assurance of the data see the data deposition (Clark and Clark 2021).

Each year the estimated aboveground biomass (EAB, kg) of each live tree was calculated from that year's diameter and allometric equations (Brown 1997). The annual estimated aboveground biomass increment (EABI, kg) for each individual was calculated as the difference between successive annual biomass estimates, annualized based on number of days between measurements (Clark et al. 2021). Annual estimates of plot level wood production (plot EABI), were calculated as the sum of the EABI's for all surviving individuals ≥ 10 cm diameter. We averaged the plot-level growth metrics for each edaphic condition (slope (P), plateau (L)), for two groups of trees: a) N-fixers (Supplementary Table 1); and b) all other species.

Leaf litterfall was collected at each plot biweekly from standing baskets (0.25 m^2 area, 0.8 m height) and nine ground-level "traps" (0.25 m^2 vertically projected squares demarcated on the ground) to collect leaf litterfall items > 50 cm long, which are not captured by basket traps (Villela and Proctor 1999). For this study, we used leaf litterfall from the same 10 plots as the tree growth measurements (see above). Each plot-level combined collection was sorted by category (leaves, twigs, reproductive) and oven-dried at 65° C to constant mass. The 26 bi-weekly collections each year were used to estimate annual leaf litterfall amounts by accounting for the area of collected traps and number of days between collection dates (further details in Clark 2021).

Statistical analyses

To examine relationships between annual NO_3-N concentrations and plant productivity (tree growth and leaf litterfall), we used linear models between mean annual NO_3-N concentration divided by conductivity and: (a) mean annual leaf litterfall for slope (n = 5) and plateau (n = 5) plots; (b) mean growth of N-fixers in slope (n = 5) and plateau (n = 5) plots; and (c) mean growth of non N-fixers in slope and plateau plots (n = 5 plots each). We used the average of the plots for each landscape position because we recognized that a single plot does not represent the entire watershed. In estimating annual NO_3-N concentrations, we excluded months with concentrations more than 2 standard deviations from the mean for that site (n = 2 in Salto 100, Saltito 100, and Salto 60). We tested the data and residuals of the linear models for temporal autocorrelation using the autocorrelated function and Durbin-Watson test, and didn't find any temporal autocorrelation. We used Pearson correlation coefficients to examine relationships between environmental drivers (air temperature, water temperature, discharge, and precipitation) and NO_3-N concentrations.

We used Pearson correlation coefficients to examine relationships between precipitation and leaf litterfall and growth of trees. We used one-way ANOVA followed by post-hoc Tukey to compare tree growth and leaf litterfall between edaphic conditions (slope vs plateau plots). We examined the tree growth and litterfall data for temporal autocorrelation, and also did not find any significant autocorrelation. All analyses were conducted in version 4.0.2 of the R statistical software (The R Foundation 2020).

To estimate the relative contribution of different sources of N to the watershed, we estimated input/output budgets. We estimated precipitation NO_3-N and NH_4-N inputs using the averaged concentrations for each constituent measured in rainfall collectors under the canopy (throughfall) for the period 2016–2020. We used throughfall, instead of direct precipitation because it is more representative of what reaches the streams. We multiplied the throughfall concentrations by annual precipitation, adjusted for interception using equations from (Loescher et al. 2002). We used literature values for estimates of symbiotic N-fixation (occurring within roots of N-fixers) and asymbiotic N-fixation (conducted by free-living bacteria in soils and litter) from measurements in the old-growth section at La Selva reported in Taylor et al. (2019). To estimate export from the watershed, we used average annual discharge measured at Salto 30 (bottom of the watershed) and average annual NO_3-N concentrations for that site. We recognize that using the average discharge leads to underestimation of annual export, but the goal of this exercise was to examine the potential role of N-fixers on watershed N export.

Results

Stream N dynamics

Stream NO_3-N concentrations varied temporally and spatially (Fig. 2 and Table 1). Overall mean NO_3-N concentrations were highest at Salto 100 (268 ± 11 μg L^{-1}) and decreased in downstream sites of the river that were characterized by higher discharge (Salto 30, mean $= 201 \pm 8$ μg L^{-1}, Table 1). The two sites on the Saltito had lower mean NO_3-N concentrations (~166 ± 9 μg L^{-1}, Saltito 100 and Saltito 60, Table 1). The Pantano 60 site had intermediate NO_3-N concentrations (187 ± 8 μg L^{-1}, Table 1). For 2017–18, we also measured DON and DOC concentrations (Table 1). NO_3-N was the dominant form of N in all sites (55–80% of TDN) and NH_4-N was the lowest (10–16%, Table 1). DON and NO_3-N were weakly negatively related ($r^2 = 0.20$, $p = 0.20$). DOC concentrations varied from 2.9 to 8 mg L^{-1} (Table 1). Conductivity and phosphorus (SRP) were highest in the sites that receive the interbasin groundwater inputs (Salto 30 and Saltito 60, Table 1).

There was a peak in NO_3-N concentrations in 2009–2010 in all sites (Fig. 2). The NO_3-N to conductivity ratio followed the same annual pattern as NO_3-N concentrations (Fig. 2). Note that in Salto 30 the NO_3-N to conductivity ratio is lower compared to other sites (note different scale on the second y-axis); this is due to interbasin groundwater inputs that lead to higher conductivity in this site.

Forest productivity

Leaf litterfall, the largest component of aboveground plant production in most tropical forests and at La Selva (Clark et al. 2021), was much greater than wood production in all years (Fig. 3). Mean annual leaf litterfall was similar in the slope and plateau plots (6.66 ± 0.13 Mg ha^{-1} $year^{-1}$ and 7.16 ± 0.14 Mg ha^{-1} $year^{-1}$ respectively, Fig. 3). The average annual growth of N-fixers was higher in slope than in plateau plots (1.78 ± 0.05 and 1.58 ± 0.05 Mg ha^{-1} $year^{-1}$ respectively, ANOVA and post-hoc Tukey $p = 0.01$). Growth of all other species was similar between the edaphic conditions (slope 2.60 ± 0.05 and plateau 2.75 ± 0.05 Mg ha^{-1} $year^{-1}$, ANOVA and post-hoc Tukey $p = 0.06$). Averaged across the five slope plots, annual leaf litterfall was negatively related to the annual growth of N-fixers ($r = -0.63$, $p = 0.002$) and of the non N-fixers ($r = -0.45$, $p = 0.02$). Averaged across the five plateau plots, annual leaf litterfall was negatively related with the annual growth of non N-fixers ($r = -0.55$, $p = 0.01$), and was unrelated to the annual growth of N-fixers ($r = -0.40$, $p = 0.07$). In slope plots, growth of both N-fixers and non N-fixers increased with precipitation, but declined at the highest rainfall amounts (Supplemental Fig. S2A, quadratic equation $r^2 = 0.25$, $p < 0.01$ for N-fixers, $r^2 = 0.24$, $p < 0.01$ for all other species). In plateau plots, only growth of N-fixers was related to precipitation, again increasing until

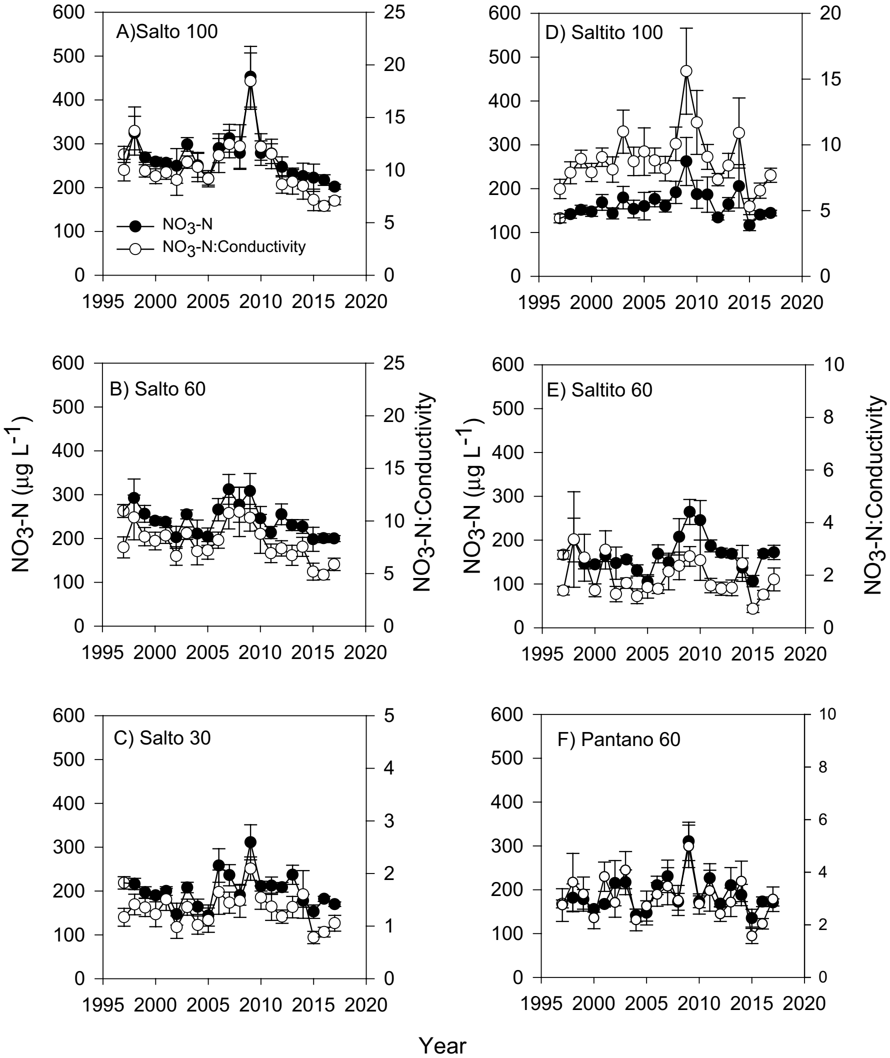


Fig. 2 Annual stream NO_3-N concentrations (filled circles) and NO_3-N: conductivity ratios (empty circles) for 6 streams sites for the period 1997–2018. Note different scales on second y-axes in **C**, **E**, and **F**

Table 1 Overall means (± standard error) of conductivity and N forms. DON and DOC means are from the year 2017, all others are means from the long-term record (1997–2018)

Site	Conductivity µS/cm	NO_3-N µg/L	NH_4-N µg/L	DON µg/L	TDN µg/L	DOC mg/L	SRP µg/L
Salto 100	27 ± 0.5	268 ± 11	36 ± 5	90 ± 17	335 ± 25	6.0 ± 1	13 ± 1
Salto 60	34 ± 1	242 ± 7	34 ± 5	98 ± 18	303 ± 20	4.2 ± 0.8	14 ± 1
Salto 30	179 ± 5	201 ± 8	51 ± 6	69 ± 12	274 ± 20	3.5 ± 0.7	77 ± 9
Saltito 100	19 ± 0.3	164 ± 6	52 ± 5	116 ± 17	319 ± 20	6.5 ± 1	7 ± 1
Saltito 60	118 ± 5	166 ± 8	33 ± 4	105 ± 18	322 ± 21	5.6 ± 0.9	49 ± 5
Pantano 60	73 ± 2	187 ± 8	57 ± 5	113 ± 20	342 ± 25	6.3 ± 1	9 ± 2

the highest rainfall amounts (Supplemental Fig. S2B, quadratic equation $r^2 = 0.31$, $p = 0.01$ for N-fixers, $r^2 = 0.11$, $p = 0.07$ for other species). Litterfall was negatively correlated to precipitation in slope plots (Supplemental Fig. S3A, $r^2 = 0.22$, $p = 0.03$), but not plateau plots (Supplemental Fig. S3B).

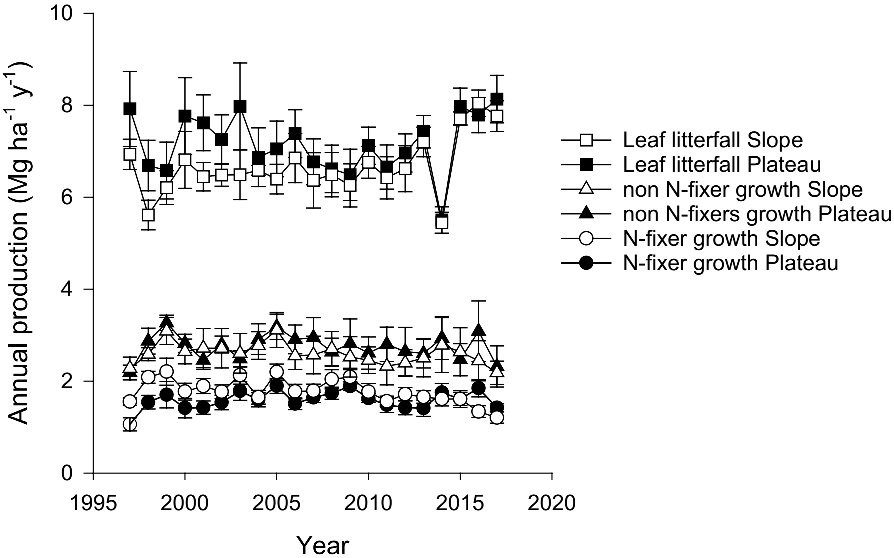


Fig. 3 Annual measurement of leaf litterfall and wood production by N-fixers and non N-fixers in slope and plateau plots from 1997 to 2018. N-fixers: Supplemental Table 1. Non N-fixers: all other species found in the plots

Stream N dynamics and meteorological conditions

There were not many environmental variables that were clearly correlated with NO_3-N and NO_3-N:conductivity ratio across the six sites (Supplementary Table 2). NO_3-N:conductivity was positively correlated with precipitation in two of the sites (Pantano 60 and Saltito 60), and with discharge in two sites (Pantano 60 and Salto 60). NO_3-N:conductivity was negatively correlated to annual mean air temperature in three of the sites (Salto 60, Saltito 60, and Pantano 60, Supplementary Table 2). NO_3-N and NO_3-N:conductivity ratio were negatively correlated to minimum air temperature in Salto 100 (Supplementary Table 2).

Stream N dynamics and forest productivity

In all six sites, the NO_3-N to conductivity ratio increased in years when N-fixer trees exhibited higher growth (Fig. 4, Table 2). This relationship explained 20 to 46% of the variation at these sites (Fig. 4, Table 2), with the most variation explained in an intermediate-size stream (Salto 60). In Salto 30 the trend was similar, with increasing NO_3-N:conductivity in years with higher N-fixer growth, although only 20% of the variation was explained by N-fixer growth (Table 2). In contrast, there was no significant relation between the NO_3-N:conductivity and the growth of either the non N-fixers in the slope plots (Supplemental Fig. S4) or of the N-fixers in the (more removed) plateau (L) plots (except for Saltito 100, Supplemental Fig. S5). Growth of just *P. macroloba* explained 21 to 42% of the variation in the NO_3-N:conductivity ratio at four out of the six sites (Supplemental Fig. S6). At all six stream sites, the NO_3-N:conductivity ratio was negatively related with annual leaf litterfall in both slope and plateau plots (Fig. 5, Table 2). The relationships explained 25 to 43% of the inter-annual variation (Fig. 5, Table 2), with a higher percentage of the variation explained in intermediate size streams (Salto 60 and Saltito 60).

Partial mass balance budget

NO_3-N rainfall (throughfall) inputs were the main source of nitrogen to this watershed, ranging from 5.0 to 10.0 kg ha^{-1} year^{-1} (Fig. 6). NH_4-N rainfall (throughfall) inputs were smaller, ranging from 0.14 to 0.3 kg ha^{-1} year^{-1} (Fig. 6). Watershed export varied between 4.0 and 17 kg NO_3-N ha^{-1} year^{-1} (Fig. 6). Asymbiotic N fixation reported in Taylor et al. (2019) was 2.82 kg ha^{-1} year^{-1} and symbiotic N fixation was reported to range from 0.56 (geometric mean) to 2.33 (arithmetic mean) kg ha^{-1} year^{-1}. Based on these values, we estimate that symbiotic N-fixation could contribute on average 7 to 29% (range 4–57%) of the inter-annual watershed NO_3-N

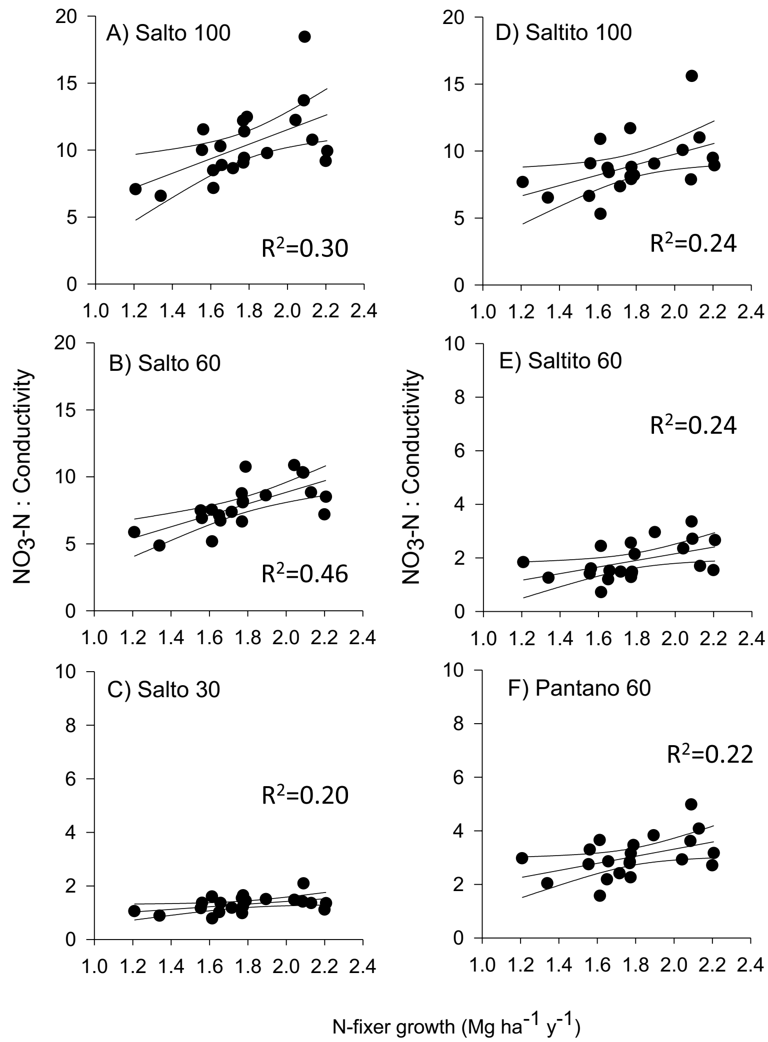


Fig. 4 Relationships (regression and 95% confidence intervals) between annual stream NO_3-N: conductivity ratios and annual growth of N fixers in slope plots during the period 1997–2018. R^2 are from simple linear regression, other model parameters are reported in Table 2. Note different scales on y-axes in **C**, **E**, and **F**

export depending on which estimate of symbiotic N-fixation was used (Fig. 6B).

Discussion

Our study is the first to link interannual variation in growth of N-fixer trees with NO_3-N concentrations in streams draining an old growth tropical forest. Previous results in tropical, temperate, and boreal areas have used percent basal cover and stream NO_3-N concentrations (Table 3), exploring spatial patterns, but not temporal patterns as we do here. Results over the two-decade study show that years with high growth of N-fixers are also years with higher stream NO_3-N concentrations, after accounting for dilution (Fig. 4). The same pattern was not evident for growth of non N-fixer trees (Fig. S4), or growth of N-fixer trees in the more removed upland plateau sites (Fig. S5). Our findings strongly suggest that the growth of a subset of the tree species (13 species), a critical functional group (N-fixers, i.e. Supplementary Table 1), are important drivers of stream N concentrations in this species-rich tropical forest (> 260 tree species). Our results agree with the long-standing hypothesis that N-fixers drive the high bioavailable concentrations of N in tropical forest streams (Jenny 1950; Vitousek and Sanford 1986; Hedin et al. 2009). However, our hypothesis regarding the positive relationship between leaf litterfall and stream NO_3-N was not supported, suggesting that decomposition of N rich litter might not be as important as previously thought.

Table 2 Model outputs of general linear regressions of NO_3-N: Conductivity ratio vs growth of trees (N fixers and non-N fixers in slope and plateau plots) and leaf litterfall

Site	Independent variable/location	Parameter estimate	AIC	R^2	RSE	p value
Salto 100	**N fixers/slope**	**5.42**	**97.54**	**0.30**	**2.25**	**0.008**
	Non N-fixers/slope	− 0.11	105.32	0.01	2.70	0.96
	N fixers/plateau	3.08	104.14	0.05	2.63	0.30
	Non N-fixers/plateau	1.05	105.06	0.01	2.69	0.63
	Litterfall/slope	**− 2.22**	**98.34**	**0.28**	**2.29**	**0.01**
	Litterfall/plateau	**− 1.74**	**100.96**	**0.18**	**2.44**	**0.04**
Salto 60	**N-fixers/slope**	**4.28**	**73.61**	**0.46**	**1.27**	**0.0006**
	Non N-fixers/slope	1.31	86.08	0.03	1.71	0.43
	N-fixers/plateau	1.52	86.07	0.03	1.72	0.44
	Non N-fixers plateau	0.62	86.55	0.01	1.73	0.66
	Litterfall/slope	**− 1.77**	**74.68**	**0.43**	**1.305**	**0.001**
	Litterfall/plateau	**− 1.16**	**82.04**	**0.20**	**1.55**	**0.04**
Salto 30	**N-fixers/slope**	**0.49**	**8.87**	**0.20**	**0.27**	**0.04**
	Non N-fixers/slope	0.01	13.65	0.001	0.30	0.95
	N-fixers/plateau	0.30	12.72	0.04	0.30	0.36
	Non N-fixers/plateau	0.09	13.48	0.008	0.31	0.69
	Litterfall/slope	**− 0.26**	**5.32**	**0.32**	**0.25**	**0.006**
	Litterfall/plateau	**− 0.24**	**6.82**	**0.27**	**0.25**	**0.01**
Saltito 100	**N-fixers/slope**	**3.90**	**91.36**	**0.24**	**1.94**	**0.02**
	Non N-fixers/slope	1.50	96.50	0.02	2.19	0.48
	N-fixers/plateau	**5.53**	**91.56**	**0.26**	**1.91**	**0.01**
	Non N-fixers/plateau	1.17	96.57	0.02	2.19	0.51
	Litterfall/slope	**− 1.71**	**91.06**	**0.25**	**1.92**	**0.02**
	Litterfall/plateau	**− 1.63**	**91.18**	**0.24**	**1.93**	**0.02**
Saltito 60	**N-fixers/slope**	**1.23**	**43.03**	**0.24**	**0.61**	**0.02**
	Non N-fixers/slope	0.49	48.16	0.02	0.69	0.47
	N-fixers/plateau	0.65	47.97	0.03	0.69	0.40
	Non N-fixers/plateau	0.31	48.41	0.01	0.69	0.58
	Litterfall/slope	**− 0.68**	**37.98**	**0.40**	**0.54**	**0.002**
	Litterfall/plateau	**− 0.52**	**42.69**	**0.25**	**0.6**	**0.02**
Pantano 60	**N-fixers/slope**	**1.32**	**48.08**	**0.22**	**0.69**	**0.03**
	Non N-fixers/slope	0.05	53.27	0.002	0.78	0.94
	N-fixers/plateau	0.94	51.95	0.01	0.76	0.28
	Non N-fixers/plateau	0.03	53.27	0.0003	0.78	0.93
	Litterfall/slope	**− 0.72**	**43.9**	**0.36**	**0.63**	**0.004**
	Litterfall/plateau	− 0.45	49.81	0.15	0.72	0.08

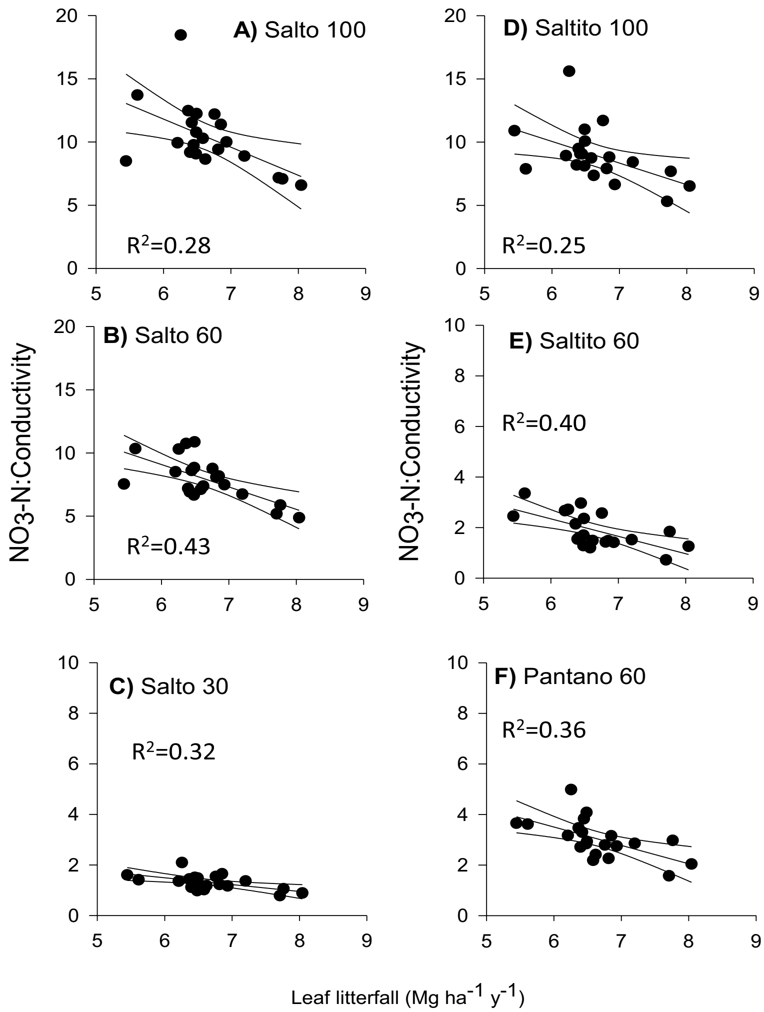
AIC Akaike's information criteria, *RSE* residual standard error

Overall, our results indicate that productivity, species identity, and location are important determinants of overall N export in this diverse wet, tropical forest.

Stream N dynamics

Stream NO_3 concentrations were similar in streams that receive interbasin groundwater transfers and streams that do not (Table 1). Previous studies have documented that N is not limiting for algae (Pringle et al. 1986) or microbes (Stallcup et al. 2006) in La Selva streams. Of all the solutes that we have studied in these streams (P, pH, conductivity, SO_4), NO_3 is the least affected by the interbasin groundwater transfers (Table 1). This makes for interesting examinations of the relative importance of in-stream vs

Fig. 5 Relationships (regression and 95% confidence intervals) between annual stream NO_3-N: conductivity ratios and annual leaf litterfall in slope plots in the period 1997–2018. R^2 are from simple linear regression, other model parameters are reported in Table 2. Note different scales on y-axes in **C**, **E**, and **F**



terrestrial processes in driving stream N concentrations. If N fixation in aquatic ecosystems is P limited, as has been shown in terrestrial systems (Batterman et al. 2013b), we would expect higher N concentrations in areas receiving the interbasin inputs (Salto 30), but we did not observe that. This suggests that processes occurring outside the stream channel, such as N fixation by symbiotic and asymbiotic processes are strong enough to mask changes due to in-stream processes.

Stream NO_3-N concentrations peaked in 2009–2010 in all sites, and at this point we are unable to explain that peak. Those years were not drier or wetter than other years. Those years did have high N-fixer growth (highest points in Fig. 4A and D) and low leaf litterfall production (highest points Fig. 5A and D), supporting our overall mechanisms. However, there are likely instream processes, such as nitrification, and watershed processes (N deposition) that we have not accounted for. Annual NO_3-N concentrations were not related to our estimated throughfall deposition (Supplemental Fig. S7), though we lack direct measurements of throughfall concentrations for the entire record. Our previous research in two smaller watersheds at La Selva found a positive relationship between stream NO_3-N concentrations and discharge, using two years of continuous discharge and weekly water samples (Ganong et al. 2015). Our longer-term record using monthly measurements did not show the same patterns, with only two sites showing a positive relationship between NO_3-N to conductivity ratio and discharge (Supplementary Table 2). Previous studies have shown differences in concentration versus discharge relationships in higher frequency vs

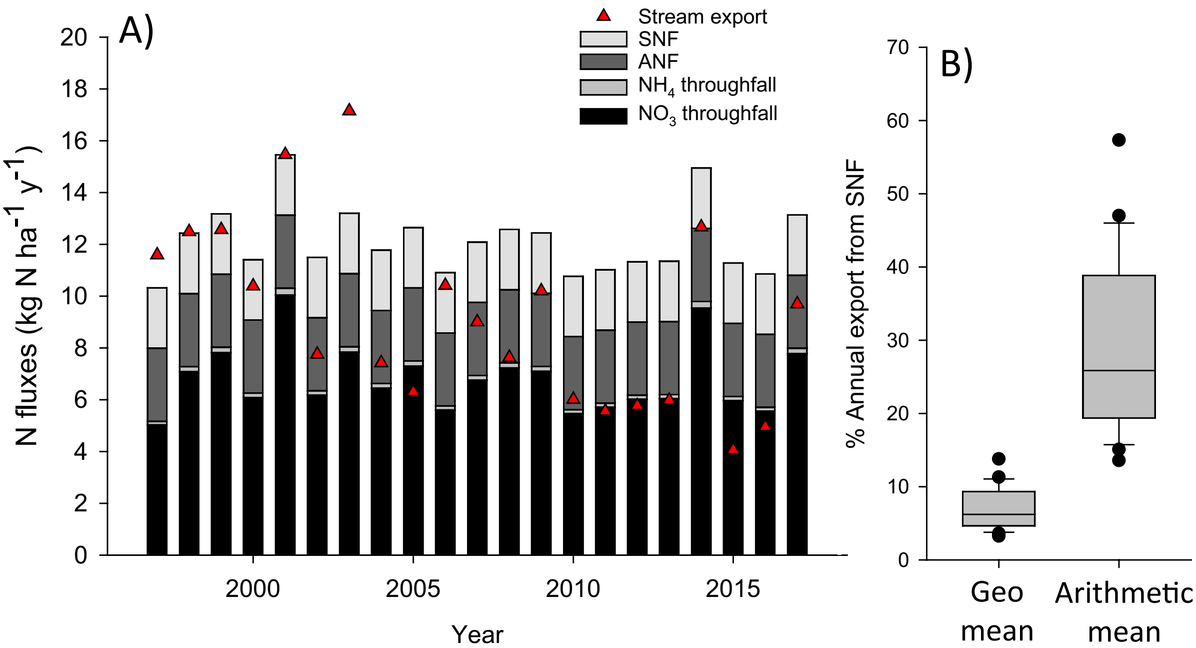


Fig. 6 Annual inputs (bars) and export (red triangles) from the Salto watershed for the period 1997–2018 (**A**). SNF = symbiotic N fixation, ANF = asymbiotic N fixation. Percent of NO_3-N export that could be attributed to symbiotic N fixation (SNF) using low (geometric mean) and high (arithmetic mean) estimates of N fixation (**B**) in old growth forest in La Selva reported in Taylor et al. (2019)

Table 3 Summary of studies that report basal area of N-fixers and nearby stream NO_3 concentrations in tropical, temperate, and boreal biomes

Tree species	Biome	N fixers % basal area	NO_3-N (µg/L)	Tree productivity	Location	Citation
Multiple	Tropical	38%	100–500	Yes	La Selva, Costa Rica	1
Multiple	Tropical	10–20%	200–300	No	Guanacaste, Costa Rica	2, 3
Red alder (Alnus rubra)	Temperate	6–52%	74–2043	No	Salmon River Basin, Oregon	4
Red alder (Alnus rubra)	Temperate	69–76%	186–2200	No	Willamette River Basin, Oregon	5
Alder (Alnus spp)	Boreal	0–27%	1–1605	No	Kenai Peninsula, Alaska	6
Alder (Alnus spp)	Boreal	0–28%	3–1560	No	Kenai Peninsula, Alaska	7

Citations: (1) this paper; (2) Brookshire et al. (2012); (3) Gei et al. (2018); (4) Compton et al. (2003), (5) Compton et al. (2020), (6) Hiatt et al. (2017), (7) Shaftel et al. (2012)

lower frequency datasets (Fazekas et al. 2020). Previous work in La Selva suggested that most of the NO_3 in stream was from nitrification and mineralization (Triska et al. 1993; Duff et al. 1996). Our results agree with these previous studies, and advance our understanding by illustrating how productivity of N-fixers can also drive NO_3-N concentrations.

Forest productivity

Growth of all trees in slope plots, and N-fixers in plateau plots, increased with increasing precipitation up to a point, and then declined (Supplemental Fig. S2). In contrast, leaf litterfall was negatively correlated to precipitation in slope plots (Supplemental Fig. S3). This might seem to contradict results from Clark et al. (2021), which did not find relationships between precipitation and growth of all trees or leaf litterfall. However, since they were working with all species combined, and with the complete network of 18 plots, and we are using a subset of their plots (10 plots) we do not think our results contradict their findings. Future studies should examine if N-fixers across all edaphic conditions at La Selva are more vulnerable to changes in precipitation, as suggested by our analyses.

Long-term research at La Selva suggest that while total rainfall has not changed, the number of extreme rainfall events (>62 mm of rainfall in one day) are becoming more frequent (Salcido et al. 2020). This increase in precipitation could lead to more favorable tree growth conditions, but if precipitation is too high, growth of N-fixers goes down (Supplemental Fig. S2). Increase in extreme events could also lead to more N export. Climate change has also increased night time temperature, which has been linked to lower growth of all tree species (Clark et al. 2021), suggesting that complex interactions between changes in precipitation and temperature will have effects on plant productivity and N concentrations in streams that are difficult to forecast.

Forest productivity and stream N dynamics

The mechanisms driving the observed patterns between N-fixer growth and stream NO_3-N concentrations are not yet clear. Few studies have directly examined links between N fixation rates and growth of mature N fixers. Working in the same area of our study, but focusing mostly on secondary forests (Taylor et al. 2019), found no relationship between tree growth and measurements of symbiotic N fixation. However, this was done in one year across four secondary forests and one old growth forest. In a greenhouse experiment, Taylor and Menge (2021) found that higher rates of N fixation led to higher growth of *Pentaclethra* seedlings. It remains to be seen if future multi-year studies in old growth forests, which is what we studied here, find relationships between inter-annual tree growth and rates of symbiotic N fixation, and how that N might make it into nearby streams (Fig. 7). N fixation at the landscape scale is very challenging to estimate, requiring thousands of measurements, which is rarely done (Winbourne et al. 2018).

Production and decomposition of fine root biomass is another mechanism that could explain the patterns reported here. Increased production and decomposition of fine root biomass could lead to higher soil N (Osborne et al. 2017), and eventually more leaching to nearby streams. Previous research at La Selva has shown that higher soil N content leads to higher fine root biomass (Espeleta and Clark 2007). If N-fixer trees produce more fine root biomass in years in with higher stem growth, when the fine roots die and decompose there would be more NO_3 in soils that could leach into the streams. However, allocation of growth among different tissue compartments (stem, leaves, roots) in both N-fixers and non N-fixer tropical tree species is still not well understood (Taylor and Menge 2021). Future research should examine

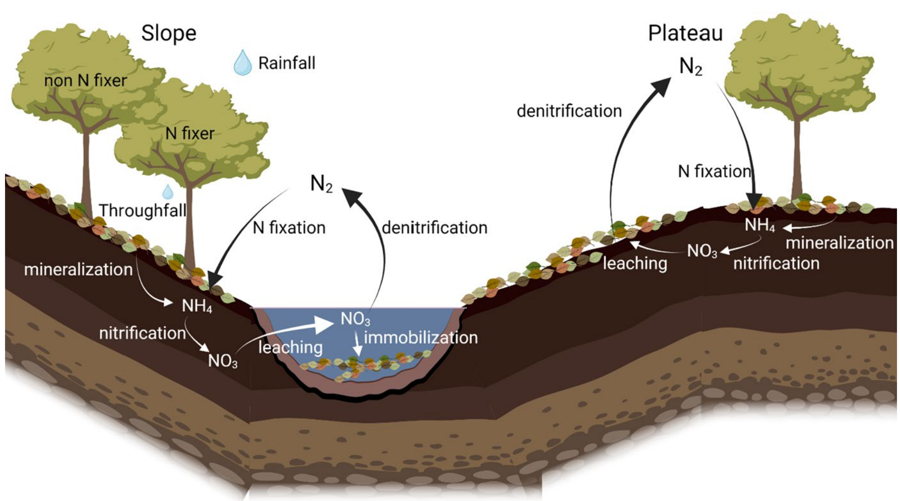


Fig. 7 Conceptual figure of landscape moderation of the effects of N-fixers and non N-fixers tree growth on stream NO_3 concentrations. Growth of N-fixers in nearby slope plots is related to stream NO_3 concentrations, suggesting strong linkages between terrestrial productivity and stream N. Growth of N-fixers in farther removed plateau areas is not related to stream NO_3 concentrations, suggesting more localized N recycling

if turnover of fine root biomass, and N content of fine roots might be related to N concentrations in the streams.

Leaching of N from N-rich tissues could also be a mechanism driving the patterns observed here. *Pentaclethra macroloba* has high tissue N; the average N of the canopy foliage increases with greater local abundance of this species (Wood et al. 2006; Massmann et al. 2021). A study using 16 year old plantations in La Selva, found that soils underneath planted *Pentaclethra macroloba* had the highest rates of soil N losses (110 kg ha^{-1} $year^{-1}$, average across 4 species was 49 ± 83 kg Ha^{-1} $year^{-1}$, Russell and Raich 2012). These losses include hydrologic losses and denitrification, which we do not account for in our budget estimates. The reported total N losses from this young plantation are much higher than hydrologic losses we report (Fig. 6) and the soil leaching losses reported from other sites in the La Selva old-growth forest (9 kg ha^{-1} $year^{-1}$, Schwendenmann and Veldkamp 2005). Old-growth forests usually cycle nutrients more closely than young forests (Hedin et al. 2003). At La Selva, we would expect that years with higher rainfall lead to both higher growth of N-fixers and higher leaching of N into soils and streams.

Our findings illustrate the importance of topography (and/or proximity to stream) in mediating the effect of N-fixers on N cycling. The growth of N-fixers in the (more removed) high plateau areas (L plots) was not correlated with stream NO_3-N (Supplemental Fig. S5), suggesting that landscape position is important for the effect of N-fixer growth on stream NO_3-N concentrations (Fig. 7). The plots located on slopes are closer to the streams, and are thus more likely to drain into stream surface waters, and by proximity, the growth of N-fixers in slope plots would be more closely linked to stream NO_3-N. The slopes at La Selva also experience higher rates of erosion than plateau areas (Porder et al. 2006). Our results suggest that N might be cycled and lost to nearby rivers and streams from slope plots, while it is recycled in place in plateau sites (Fig. 7), which is similar to what was observed by Osborne et al. (2017, 2020) in the Osa Peninsula of Costa Rica. Research in the Osa peninsula of Costa Rica has also shown the importance of topography and climate in driving (soil) N-availability (Osborne et al. 2017). N-rich canopies produced islands of higher soil N fertility (Osborne et al. 2017, 2020). While long-term research at La Selva has shown that landscape position is an important determinant of tree growth (Clark et al. 2019, 2021), our results are the first to show that this is relevant for stream NO_3-N concentration. More direct examinations of tree growth and soil N in different areas of the landscape (slope vs upland plateau), and nearby stream NO_3-N concentrations could help elucidate aquatic-terrestrial linkages in the N cycle across tropical watersheds.

The negative relationship we found between average annual stream NO_3 concentrations and annual leaf litterfall (Fig. 5) was contrary to what we expected. However, a similar pattern was reported in Puerto Rican streams, where NO_3-N concentrations were negatively related to leaf litterfall after accounting for dilution (McDowell and Asbury 1994). This pattern has also been reported in temperate streams, where NO_3-N concentrations are lowest during the peak litterfall period in autumn (Lutz et al. 2011). The negative relationship between leaf litterfall and the stream NO_3-N concentrations does not contradict the mechanism of decomposition of N-rich litter leading to higher N concentrations in streams. Given that the leaf litterfall was not separated by species in this study, we are unable to differentiate litterfall of N fixing versus non-N fixing species.

We hypothesize that microbial immobilization of NO_3-N during the decomposition of leaf litter in the stream channel is driving the negative correlations between annual leaf litterfall and annual stream water NO_3-N (Fig. 5). We have documented increases in leaf-litter N during decomposition in streams (Ardón et al. 2006; Stallcup et al. 2006; Ardón and Pringle 2008), which is evidence that NO_3 is immobilized as heterotrophic microbes breakdown litter. Experimental additions of leaf litter, or of labile carbon could be used to examine if this mechanism can decrease stream water N concentrations (Bernhardt and Likens 2002). If N in streams is immobilized during the decomposition process, it could later be exported as particulate organic N (PON). While we did not measure PON, it has been shown to be substantial in a small watershed in southwest Costa Rica (Taylor et al. 2015).

Another potential mechanism explaining lower NO_3-N in years with higher leaf litterfall could be increased denitrification in riparian soils and stream channels. Greater carbon availability from decomposing leaf litter could fuel denitrification rates

(Mulholland et al. 2008). Denitrification rates in La Selva streams were reported to be lower compared to measurements from temperate streams, ranging between 1.6 to 24 mg N m^{-2} day^{-1} (Duff et al. 1996; Small et al. 2013). Rates were not related to stream water P and were only weakly related to sediment organic matter (Small et al. 2013). Measurements of denitrification and mineralization across the Pantano river suggested that it was a net source of NO_3-N to downstream (Duff et al. 1996). An improved understanding of denitrification rates, both in the stream and in nearby riparian areas is necessary to gain a more complete picture of the N cycle at the watershed scale.

Partial mass balance budget

Our partial mass balance calculations suggest that symbiotic N fixation by N-fixer trees could account for, on average, 7 to 29% of the stream NO_3-N export from this watershed. Our estimated N export (4–17 kg ha^{-1} $year^{-1}$, Fig. 6) is high compared to other sites (0.05–1.4 kg ha^{-1} $year^{-1}$, Gucker et al. 2016), but is within the range of previous results from smaller watersheds in La Selva (ranges 3.9–26.4 kg ha^{-1} $year^{-1}$, Ganong et al. 2015). Our estimates of precipitation N inputs (5–10 kg ha^{-1} $year^{-1}$) also agree with previous measurements from La Selva (4.4–8.8 kg ha^{-1} $year^{-1}$, Eklund et al. 1997) and measurements from the Osa Peninsula in Costa Rica (8 kg ha^{-1} $year^{-1}$, Taylor et al. 2015). Whether the percentage of export explained by symbiotic N fixation is high or low depends on the perspective. On the one hand, the contribution of symbiotic N fixation to overall N fluxes could be considered low (explaining around 30% of the total export) illustrating that rainfall inputs, asymbiotic N fixation, and mineralization of annual litterfall (which we did not account for in our budget calculations) are all important components of this watershed's N cycle. On the other hand, the 7 to 29% proportion could be viewed as relatively high, because N-fixer trees in old-growth tropical forests are considered to only actively fix N after canopy gap openings or other disturbances (Barron et al. 2011; McCulloch and Porder 2021), and their N-fixation rates are much lower than in secondary forests (Batterman et al. 2013a; Taylor et al. 2019). Most of the literature suggests that the role of N-fixer trees is important in early successional forests, and almost negligible in tropical old-growth forests (Batterman et al. 2013a; Levy-Varon et al. 2019; Taylor et al. 2019). Our results suggest that they can still be contributing up to a fourth of the NO_3-N being exported from the La Selva old growth (Fig. 6). Research in Alaska and Oregon has illustrated that N-fixation by alder can increase NO_3-N concentrations across watersheds (Compton et al. 2003, 2020; Shaftel et al. 2012; Lin et al. 2019; Table 3). Our study presents a first approximation of how much NO_3-N export could be coming from N-fixers in a tropical watershed, and clearly suggests that we need a better understanding of annual tree growth, litterfall production, and rates of N-fixation across different edaphic conditions and over multiple years.

Conclusion

Our study demonstrates linkages between the inter-annual variation in N-fixer tree growth and in stream NO_3 concentrations in an old-growth tropical rainforest. While our findings do not prove that N-fixers drive N hydrologic losses at the watershed scale, they point to potential mechanisms. Our results illustrate that the annual growth of a few N-fixer tree species seems to be an important contributor to annual stream N concentrations, which is surprising given the high diversity of trees in this site. Better documentation of N processes in terrestrial and aquatic systems will help in understanding the response of tropical forests to anthropogenic climate change and alterations of the N cycle.

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Data Availability All data used in this study are publicly available: STREAMS data: Pringle, C.M. 2021. STREAMS Project: Emergent landscape patterns in stream ecosystem processes resulting from groundwater/surface water interactions ver 537849. Environmental Data Initiative. https://doi.org/10.6073/pasta/6cb25ffd722e79690090e387efd2cfe7. CARBONO data: Clark, D. A. 2021. Biweekly fine litterfall in the 18 CARBONO Project plots, La Selva Biological Station, October 1997-October 2018. Dryad. https://doi.org/10.5061/dryad.dfn2z351r. Clark, D. A., and D. B. Clark. 2021. Two decades of annual landscape-scale tree growth and dynamics in old-growth tropical rainforest in the CARBONO Project, La Selva Biological Station, 1997–2018. Dryad. https://doi.org/10.5061/dryad.51c59zw8n

Declarations

Competing Interests No competing interests.

Ethical approval Not applicable.

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