



Biological Nitrogen Fixation Does Not Replace Nitrogen Losses After Forest Fires in the Southeastern Amazon

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ABSTRACT

Tropical forest fires have become more common due to interactions between deforestation, land clearing, and drought. Forest recovery following fires may be limited by nitrogen. Biological nitrogen fixation (BNF) is the main pathway for new nitrogen (N) to enter most ecosystems, but BNF may be constrained by other nutrients, such as molybdenum and phosphorus, which are required for the process. We studied the role of BNF 7 years into the recovery of southeastern Amazon tropical forests that were burned experimentally either annually or every 3 years between 2004 and 2010. We hypothesized that, compared with unburned primary forests, BNF in burned forests would increase due to the depletion of N in the aboveground biomass pool and that soil concentrations of molybdenum and phosphorus from ash inputs would increase, reducing their potential constraints

on BNF. Despite the fires depleting about half the aboveground N pool and rapid rates of recovery in leaf biomass and litterfall production, we found low rates of both free-living and symbiotic BNF. Higher concentrations of molybdenum and phosphorus in the mineral soils of the burned forests indicated that these elements were likely not limiting BNF. Our results suggest that despite the N demand for regrowth post-fire, substantial N stored in soils likely downregulates BNF. Overall, we found surprisingly low BNF rates ($< 1.2 \text{ kg N ha}^{-1} \text{ y}^{-1}$) in this region of the Amazon, which contrasts with the traditional paradigm that (1) tropical forests fix large quantities of N and (2) that BNF increases after forest disturbance.

Key words: symbiotic nitrogen fixation; free-living nitrogen fixation; forest fires; molybdenum (Mo); phosphorus (P); tropical forests; secondary forests.

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HIGHLIGHTS

- Fire caused large losses of nitrogen in aboveground biomass, but aboveground leaf biomass

and litterfall production rates are recovering quickly.

- Rates of free-living and symbiotic biological nitrogen fixation were very low in both unburned primary forest and burned forests 7 years post-fire.
- High soil nitrogen availability likely suppresses biological nitrogen fixation in tropical forests in the southeastern Amazon.

INTRODUCTION

Interactions between deforestation, expansion of agriculture, and droughts now increase the occurrence of fires across large areas of seasonally dry Amazon forest that historically were relatively unaffected by fires (Uhl and Kauffman 1990; Cochrane 2001). Because fires contribute a third of carbon emissions from deforestation (Aragão and others 2018), understanding controls on forest recovery post-fire is crucial as secondary forests can be large carbon sinks (Pan and others 2011). Although phosphorus (P) ultimately limits net primary productivity in most tropical forests (for example, Vitousek 1984; Hedin and others 2003), recovery and regrowth in young forests following disturbances are typically constrained by nitrogen (N) (Davidson and others 2007; Batterman and others 2013a; Wright and others 2018). During fires, N is selectively lost owing to its low temperature of volatilization in comparison with other macronutrients (Neary and others 1999) and through the various gaseous forms of N that can be produced during fires, even in low temperatures (Andreae and Merlet 2001). However, biological nitrogen fixation (BNF) may provide the new N inputs necessary to replenish N losses from fires and support recovery.

Tropical forests are thought to rebuild ecosystem N stocks relatively rapidly post-disturbance through two distinct mechanisms that contribute new N—symbiotic and free-living BNF. In the Amazon, forests have a high abundance of leguminous trees (ter Steege and others 2006) which can form root nodules that harbor N-fixing rhizobial symbionts. These plants theoretically have a competitive advantage in low N, post-fire conditions, and higher abundances of leguminous plants with associated symbiotic BNF have played an important role in secondary forest recovery (Batterman and others 2013a; Sullivan and others 2014). In seasonally dry tropical forests, leguminous plants are often even more abundant in secondary forests (Gei and others 2018). Second, the

warm and generally wet conditions of tropical forests and the relatively steady input of litter, even after fires (Rocha and others 2014), to the forest floor create the potential for high rates of heterotrophic free-living BNF associated with decomposition. In temperate forests and grasslands, BNF typically increases following fires (Hendricks and Boring 1999; Casals and others 2005; Yelenik and others 2013). However, no studies we are aware of have examined the role of BNF in forest recovery after forest fires in the tropics. In addition, few studies have directly measured BNF in the Amazon, despite its vast geographical range.

Symbiotic and free-living BNF can be constrained by P availability (Crews 1993; Crews and others 2000; Vitousek and Hobbie 2000; Batterman and others 2013b) and free-living BNF also by the availability of trace metals molybdenum (Mo) and vanadium (V) (Barron and others 2009; Wurzburger and others 2012; Darnajoux and others 2017). Phosphorus can constrain BNF either because N fixers have a higher P demand or because greater P availability can shift a system toward N limitation (Vitousek and Howarth 1991), whereas Mo and V are co-factors in the nitrogenase enzyme. The availability of Mo and P is likely low across large areas of the lowland Amazon for two reasons. First, large interior regions of the Amazon are located far from sources of ocean-derived aerosols and long-distance transport of Saharan dust that are the main new sources of P (Mahowald and others 2005), and likely Mo. Second, this region of the Amazon is situated on some of the oldest surfaces in South America (Quesada and others 2011), with highly weathered soils likely depleted in these rock-derived nutrients.

Using a large-scale fire experiment in the southeastern Amazon, 30 km north of the transition zone between forest and the Cerrado savanna, we evaluated how fire affects the availability of Mo, P, and V required for BNF, tracked changes in aboveground biomass N pools to estimate the N losses incurred by fire and the recovery of aboveground N demand post-fire, and measured free-living and symbiotic BNF during forest recovery. We hypothesized that (1) Mo and P limitation of BNF may be strong in the southeastern Amazon where soils are depleted in rock-derived nutrients and that Mo, P, and V released from aboveground biomass and the litter layer would be redeposited on the soil surface post-fire and thus more available for uptake by N-fixing bacteria; (2) fire would deplete aboveground biomass N stocks, potentially inducing temporal N limitation; and (3) both free-living and symbiotic BNF rates would be higher

several years after fires. Lastly, given that the dearth of BNF estimates from many remote regions limits our understanding of global patterns, controls, and rates of BNF, we quantified biome-specific BNF rates for this region.

METHODS

Location

The study was conducted at Fazenda Tanguro, an 800 km² working farm in eastern Mato Grosso State, Brazil. Fazenda Tanguro contains 400 km² of primary predominantly evergreen tropical forest 30 km north of the southern boundary of the Amazon forest (13° 04' S, 52° 23' W). The average annual air temperature is 27°C with less than 5°C seasonal variation. Annual rainfall is 1700 mm, with an intense dry season between May and September. Forest tree species composition represents a transition between the central Amazon forest and the more seasonal Cerrado (Ivanauskas and others 2003).

The soil is classified as a red-yellow alic dystrophic latosol (Brazilian soil classification), a relatively infertile sandy ferralsol (FAO classification), and as an oxisol (Haplustox; USDA classification). These soils located on the crystalline Brazilian Shield are among the oldest in Amazonia (Quesada and others 2011), with Tertiary and Quaternary fluvial deposits over Precambrian gneisses of the Xingu Complex (Projeto 1981).

Fire Treatments

To test tropical forest susceptibility to fire, a prescribed fire experiment was initiated at Fazenda Tanguro in 2004. Three 0.5 × 1 km (50 ha) plots were established with three treatments: (1) an unburned control, (2) a plot burned 3 years apart, or triennially, in 2004, 2007, and 2010 (Burn 3yr), and (3) a plot burned annually from 2004 to 2010 (except 2008) (Burn 1yr). Experimental fires were ignited at the end of the dry season when forests were most susceptible. Initial fires were low-intensity and slow-moving (Balch and others 2008), typical of tropical understory forest fires (Cochrane and Schulze 1999). Initial mean flame height was 31 (\pm 1) cm, with temperatures that ranged from 128°C belowground (2 cm) to 273°C at the surface to 87°C aboveground (100 cm). The fire in the Burn 3yr treatment during 2007 was more severe than the Burn 1yr treatment because of extreme dry conditions due to drought and increased fuel load from accumulated litterfall (Brando and others 2014). By 2009, the cumulative tree mortality was

63% in the Burn 3yr treatment and 50% in the Burn 1yr treatment (Brando and others 2014). Balch and others (2008) provide a further description of the site, experimental design, and fire behavior.

Symbiotic BNF

We estimated symbiotic BNF in the wet season of 2017 (between February and April), 7 years after the last fires, when we expected to find higher rates of symbiotic BNF after the immediate post-fire period when mineral N from ash would likely suppress BNF (Hobbs and Schimel 1984). In an earlier chronosequence study in Panama, Batterman and others (2013a) found the highest recruitment of putative N-fixing trees and the highest symbiotic BNF rates between 5 and 12 years after logging. Using a stratified adaptive cluster sampling approach to capture the irregular distribution of root nodules (Sullivan and others 2014), we placed four 10 × 50 m survey grids randomly within each 50 ha fire treatment plot for a total of 12 survey grids, with a minimum of 660 total cores per 50 ha treatment plot. In each survey grid, we sampled every 1 m along five 10 m transects for a total of 55 cores per plot. Each core (225 cm²) was hammered into the soil 15 cm deep. If nodules were found in a specific core, we then sampled neighboring cores until no more adjacent cores contained nodules.

Following collection of individual nodules from the survey grids, we conducted an acetylene reduction assay (ARA) (Hardy and others 1968) incubated for 1 h in the field (Sullivan and others 2014) in 125-ml gas-tight jars with lids fitted with septa (Fisher Scientific 501215190) and exposed to a 10% atmosphere of acetylene generated from calcium carbide. We took 3 sets of ethylene blanks for each batch of acetylene. We also tested for abiotic ethylene production and ethylene lost due to photodegradation during transport, but found undetectable effects.

After incubations, we collected 30 ml headspace samples and stored them in pre-evacuated 22-ml gas-tight vials (Teledyne Tekmar) fitted with thick butyl septa (Geo-Microbial Technologies, Inc.). We then dried nodules in a 65°C oven overnight to determine moisture content and dry weight. Gas samples were returned to Cornell University where we measured ethylene concentrations using a Shimadzu 8-A gas chromatograph equipped with a flame ionization detector and a Porapak-N 80/100 column at 70°C with two daily standard curves and check standards every ten samples. Ethylene blanks

from the acetylene were subtracted from sample ethylene values. For nodule samples, ethylene blanks averaged 0.1% of total ethylene production. We converted ARA rates, in units of micromoles of ethylene produced per gram dry mass of sample per hour of incubation, to BNF rates using a theoretical ratio of 3:1 (mol acetylene reduced):(mol N₂ reduced) (Hardy and others 1968).

To further examine if the putative N-fixing species formed nodules and were actively fixing N, we also conducted nodule surveys around the base of 42 putative N-fixing trees during the wet seasons of 2017 and 2018, excavating within a 2 m diameter around the base of 16 trees, and randomly sampling 12 cores (5.7 cm diameter and 10 cm deep) around 26 trees (Barron and others 2011; Wurzburger and Hedin 2016). We brought soil cores back into the laboratory to examine for nodules, which were confirmed as active using the ARA.

Free-Living BNF

We measured free-living BNF in soil and litter using the ARA method calibrated with a ¹⁵N₂ tracer (Barron and others 2009), conducted 6 and 7 years after the last fires. In the dry season of October 2016 and the wet season of March 2017, we collected 30 samples of litter by hand and 30 samples of soil (0–2 cm) using a soil corer in each treatment, for a total of 180 incubations per season. We collected samples every 50 m along three transects 250 m apart across all three plots. Approximately 15 g of litter or 25 g of fresh mineral soil were sealed in gas-tight jars described above. Litter samples were incubated overnight for 8–10 h, and soil samples were incubated for 14–18 h to accommodate lower expected rates of acetylene reduction. Litter was dried at 65°C, and soil was dried at 105°C for 2 days for dry weight and moisture measurements. We collected, stored and analyzed gas samples as described above.

Three replicates of ethylene production from litter were made per sampling day without exposure to acetylene, as litter is known to produce ethylene (Reed and others 2007). Ethylene production rates from litter averaged 17% of the total ethylene concentrations. Ethylene production from soil without exposure to acetylene was insignificant. Ethylene blanks averaged 12% of the ethylene production in litter incubations but 50% in soil incubations, due to the low rates measured.

To convert acetylene reduction rates into BNF rates, we used a site-specific ratio of 2.3:1 (mol acetylene reduced):(mol N₂ fixed) by measuring the incorporation of ¹⁵N-labeled N₂ in soil. Because

this ratio is lower than the theoretical ratio, we present free-living BNF data as a range using 2.3 as the lower and 3 as the upper bound. We used gas-tight 40-ml screw cap vials with septa (Wheaton 225310), weighed about 15 g of soil, and replaced a third of the remaining headspace with 98 atom% ¹⁵N₂ (Cambridge Isotope Laboratories). After incubating paired ARA samples with ¹⁵N₂ incubations, we terminated the ¹⁵N₂ incubations in an oven at 65°C, ground the samples, and analyzed changes in soil δ¹⁵N at the Cornell University Stable Isotope Laboratory (COIL) on a continuous flow isotope ratio mass spectrometer (Finnigan MAT Delta Plus plumbed to a Carlo Erba NC2500 elemental analyzer).

Inorganic Soil N and pH

Using the same 90 soils per season sampled for free-living BNF, we analyzed soil moisture (gravimetric moisture), pH, and 2 M KCl extractions to measure exchangeable ammonium (NH₄⁺) and nitrate (NO₃⁻) following the methods of Neill and others (1995). Immediately after initiating ARA incubations, we weighed and added 10 g of fresh soil to 50 ml of 2 mol l⁻¹ KCl for 24 h, agitating frequently, filtered samples through Whatman No. 1 filter paper and froze samples until analysis. KCl extracts were analyzed for NH₄⁺ and NO₃⁻ via colorimetric analyses at the Woods Hole Research Center, using an Astoria Pacific 303A (cartridge base) and 305D (digital detector). We measured soil pH using a 1:2 soil-to-distilled water ratio, stirred and equilibrated for 30 min with a pH meter standardized at pH 7 and 4.

Litterfall Production and Area-Based BNF Estimates

Annual litterfall production was measured to extrapolate free-living BNF rates in the litter layer from a mass basis to a per-area flux. Litterfall was estimated by collecting the production of dead organic material at least 2 cm diameter in 25 litter traps (50 × 50 cm) placed 1 m above the ground at 50-m intervals within each plot and collected every 2 weeks (Rocha and others 2014) from 2010 to 2016. We used annual litterfall production rates to scale free-living BNF rates to annual rates with a decay constant (*k* = 1.5) from Sanches and others (2008) applied to an exponential decay rate equation, $X = X_0 e^{-kt}$, where *X* = mass, *X*₀ = original annual litterfall, and *k* = decay constant, *t* is the elapsed time (years) calculating litterfall and decay for 5 years. To scale up free-living BNF rates in the soil, we assumed

BNF was occurring in the top 10 cm of soil and applied a bulk density of 1.09 g cm^{-3} from Riskin and others (2013). We calculated annual estimates assuming a 4.5 month dry season (Brando and others 2014).

To examine forest recovery indirectly through litterfall production rates, we determined litterfall N concentrations from collecting, drying, homogenizing, and grinding litter samples from 12 locations across the forest floor and analyzing for %N at COIL. We paired the site-specific litter N of 1.28% to litterfall estimates with the assumption that 68% (by weight) of litterfall was leaves (Veneklaas 1991; Rocha and others 2014; Martinelli and others 2017) and the remaining litterfall by weight was 0.215% N, using the same value as the wood N, described below.

Forest Inventory and Aboveground Wood N Demand

To estimate changes in aboveground wood N, in 2004 we tagged, mapped, measured diameter at breast height (dbh), and estimated the height of all trees at least 40 cm dbh ($n \geq 2300$ individuals across all plots) within each 50 ha treatment plot. We classified species as putative N fixers to estimate their abundances in the experimental plots (Supplementary Table 1). Trees and lianas (20–39.9 cm dbh) were sampled in belt transects ($500 \times 20 \text{ m}$) at 0, 30, 100, 250, 500, and 750 m from the forest edge ($n \geq 3400$ individuals across all plots). Nested sub-sampling ($500 \times 4 \text{ m}$) was conducted within these belt transects to measure trees and lianas (10–19.9 cm) ($n \geq 3070$ individuals across all plots). To sample 5.0–9.9 cm individuals, plots ($500 \times 2.5 \text{ m}$) were also nested within these belt transects ($n \geq 1500$ individuals across all plots).

We calculated aboveground biomass with a best-fit pantropical model (Chave and others 2014) using height, dbh measurements, and previously measured site-specific wood density (see Balch and others 2011). To estimate wood N concentrations, we grouped each of ten most dominant tree taxa (Balch and others 2008) to the nearest genus or family in a published wood N inventory (Martin and others 2014), and estimated a site-specific wood N of 0.215%, weighting the %N to dominance values of the specific tree taxa to which we applied to the summed aboveground biomass. The allometric aboveground biomass calculation (Chave and others 2014) includes an estimate of leaf biomass, which is typically less than 5% of aboveground biomass (Delitti and others 2006).

To estimate the proportion of aboveground N recovery derived from BNF, we calculated annual

aboveground N demand as the sum of the net annual change in the aboveground leaf and wood biomass pools since the last fires in 2010. Occasionally the net annual leaf and wood demands were negative due to lower LAI measurements or delayed mortality in the tree biomass pool.

Leaf Area Index (LAI) and Aboveground Leaf N Demand

We separately calculated a leaf N pool (because leaves contain disproportionately more N than wood) by using LAI, specific leaf area, and a leaf N concentration calculated from the ten most dominant species from 2004 to 2016. Seasonally, one LAI-2000 (LI-COR Instruments) was placed in an adjacent open field to measure incoming radiation with no canopy influence while below-canopy measurements were simultaneously taken (Balch and others 2008). LAI was measured at 50-m intervals within each plot ($n = 600$) four times per year.

In the wet season of 2017 (March–April), we used a slingshot to sample canopy leaves; we collected 3 branches from 3 individual trees of the 10 dominant species (Balch and others 2008) in each plot, for a total of 90 individual trees. We weighed the leaves after drying for 2 days at 65°C and analyzed the surface area of the leaves using a LI-COR LI-3100C Area Meter to calculate a specific leaf area. Dried leaf samples were ground and measured for bulk carbon (C), N, and $\delta^{15}\text{N}$ on a Delta Plus, ThermoQuest-Finnigan analyzer at Centro de Energia Nuclear na Agricultura (CENA) at the University of São Paulo, Piracicaba. We calculated the mass of N (kg ha^{-1}) by combining LAI ($\text{m}^2 \text{ m}^{-2}$) with specific leaf area (g m^{-2}) and the %N of the leaves, again weighting the specific leaf area and %N to dominance values of the specific tree taxa at our site.

Soil Analysis

In January 2016, 6 years after the last fires, we collected 12 soil cores to depths of 0–2 cm and 2–10 cm in each treatment plot. We ground the soil in an alumina ceramic ring and puck shatterbox. Samples were oven-dried at 105°C , ashed at 550°C for 4 h, and digested in concentrated nitric acid (HNO_3) using the 3052 EPA microwave-assisted acid digestion of siliceous and organically based matrices. We centrifuged, filtered, and diluted digests prior to Mo and V analysis on a Finnigan Element 2 inductively coupled plasma mass spectrometer (ICP-MS) and P analysis on a Spectroblue

inductively coupled plasma optical emission spectroscopy (ICP-OES). We digested a certified natural sample (SDO-1, a Devonian Ohio shale from the USGS), run in parallel with the samples. The remaining subsamples were used to analyze bulk C, N, and $\delta^{15}\text{N}$ at COIL. Based on analyses of duplicate samples and standards, we estimate that the precision on the ICP-MS analysis was approximately 5% relative standard deviation. Although the concentrated nitric acid digestion is not a total digestion, we used this method to most consistently compare concentrations of Mo and P at our site to Mo and P concentrations measured in other BNF studies (Barron and others 2009; Wurzburger and others 2012; Jean and others 2013).

Statistical Analyses

We compared soil $\delta^{15}\text{N}$, Mo, and V using a two-way ANOVA with soil depth and treatment as factors, and foliar C, N, and $\delta^{15}\text{N}$ between burn treatments using a one-way ANOVA. We analyzed soil C, N, C/N, and P, and foliar C/N and specific leaf area (g m^{-2}) by ln-transforming the data and also using a two-way ANOVA for soil, and a one-way ANOVA for foliage. Although soil pH was not transformed, NH_4^+ and NO_3^- were analyzed by ln-transforming (+0.1) the data and using a two-way ANOVA with season and treatment as factors, including their interaction. Post-hoc analysis was conducted with a Tukey test. Free-living BNF in soil and litter violated assumptions of parametric tests, so we used the Kruskal–Wallis test separately for each season with treatment as the predictor variable. Post-hoc analysis was conducted with the Dunn's test. We tested the relationship between BNF and moisture using a Theil–Sen estimator. We inspected residuals visually by examining QQ plots to check for normal distribution and to investigate heteroscedasticity. Statistical analyses were performed using R software v.3.0.3 (R Development Core Team).

RESULTS

N Fixer Abundance and Symbiotic BNF

Putative N fixer abundance increased after fires tenfold, but associated symbiotic BNF did not increase to those levels. In 2016, basal area of putative N fixers was 0.76% in the Control. In contrast, the basal area of putative N fixers increased to 9.8% and 9.0% in the Burn 3yr and Burn 1yr treatments, respectively.

In 2017, symbiotic BNF was low or nonexistent across the experimental area (Figure 1). We found no presence of symbiotic BNF in the Control and

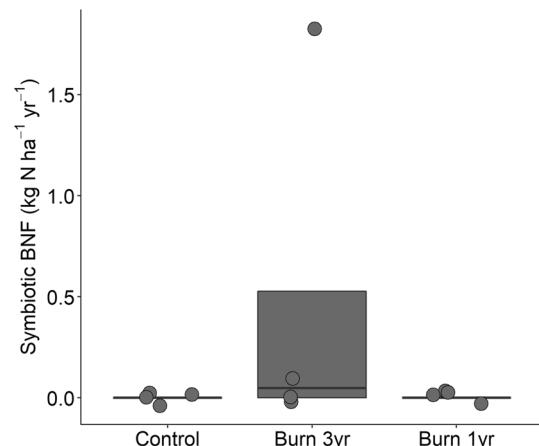


Figure 1. Annual average symbiotic nitrogen fixation rates ($\text{kg N ha}^{-1} \text{yr}^{-1}$) across the unburned (Control), triennially burned (Burn 3yr), and annually burned (Burn 1yr) forest treatments in the southeastern Amazon, measured 7 years after the last fire; $n = 4$ survey plots per treatment, black line indicates median and gray shading indicates interquartile range

Burn 1yr treatment, and an average of $0.48 \text{ kg N ha}^{-1} \text{yr}^{-1}$ in the Burn 3yr treatment (Table 1). This annual rate was calculated based on two of four survey grids within the Burn 3yr plot that contained root nodules. Using metrics defined by Winbourne and others (2018b), the proportion of cores within the four survey grids in the Burn 3yr treatment that contained nodules, p , was 0.0732, whereas the variation among nodule weights, v , was 0.731. In one survey grid, we found $0.031 \text{ g nodule m}^{-2}$ and an average ARA rate of $7.61 \mu\text{mol ethylene g}^{-1} \text{h}^{-1}$, and in the second, we found $0.097 \text{ g nodule m}^{-2}$ and an average ARA rate of $46.0 \mu\text{mol ethylene g}^{-1} \text{h}^{-1}$. To confirm the low rates of symbiotic BNF, we also used a tree-based sampling approach which revealed that only two of the 42 sampled N-fixing trees, both *Inga thibaudiana*, harbored nodules.

Free-Living BNF

Overall, BNF rates in the litter and soil were higher across the wet season, and higher in the Control relative to the Burn 3yr and Burn 1yr treatments (Figure 2). Free-living BNF rates in the litter layer, on a mass basis, were 5 times higher in the Control than in the Burn 3yr treatment ($p = 0.05$) and 13 times higher in the Control than in the Burn 1yr treatment ($p = 0.00016$) (Table 1). Wet season free-living BNF rates in mineral soil in the Control averaged 50% higher than rates found in the Burn 3yr and the Burn 1yr treatments (Table 1). However, this difference was only marginally higher in

Table 1. Mean Free-Living Nitrogen Fixation Measured Through Nitrogenase Activity (Acetylene Reduction Activity, ARA) in Litter and Soil on a Mass Basis During the Dry and Wet Season, and Mean Nitrogen Fixation Fluxes on an Area Basis

	Litter ARA rate (nmol C ₂ H ₄ h ⁻¹ g ⁻¹)		Soil ARA rate (nmol C ₂ H ₄ h ⁻¹ g ⁻¹)		Annual free-living BNF (kg N ha ⁻¹ y ⁻¹) ¹		Annual symbiotic BNF (kg N ha ⁻¹ y ⁻¹) ¹		Annual total BNF (kg N ha ⁻¹ y ⁻¹)
	Dry	Wet	Dry	Wet	Annual free-living BNF (kg N ha ⁻¹ y ⁻¹) ¹	Annual symbiotic BNF (kg N ha ⁻¹ y ⁻¹) ¹	Annual free-living BNF (kg N ha ⁻¹ y ⁻¹) ¹	Annual symbiotic BNF (kg N ha ⁻¹ y ⁻¹) ¹	
Control	0.062 (0.045) ^a	0.288 (0.210) ^a	0.0014 (0.0009) ^a	0.00425 (0.0005) ^a	0.27–0.33	0.27–0.33	0	0	0.27–0.33
Burn 3yr	0.014 (0.059) ^a	0.064 (0.022) ^b	0.000019 (0.00002) ^a	0.00528 (0.001) ^a	0.16–0.21	0.48 (0.36–0.96) ²	0.52–1.21	0	0.52–1.21
Burn 1yr	0.038 (0.015) ^a	0.022 (0.010) ^c	0.00065 (0.0002) ^a	0.00358 (0.0006) ^a	0.12–0.16	0	0.12–0.16	0	0.12–0.16

¹The range is derived from the site specific (2.3:1) and theoretical ratio (3:1) (mol acetylene reduced):(mol N₂ fixed).
²From Winbourne and others (2018b) error estimates for symbiotic N fixation are: $p = 0.0732$, and $v = 0.731$. p is the proportion of soil cores containing nodules, and v is the standard deviation of nodule biomass divided by the nodule biomass mean found in cores with nodules. Range is estimated from p , v , and the minimum sample size ($n = 220$). Data are means ± 1 standard error; $n = 30$. Letters indicate significant differences ($p < 0.05$).

the Burn 1yr treatment ($p = 0.052$), and there were no significant differences between the Control and the Burn 3yr treatment ($p = 0.29$). Dry season free-living BNF rates in mineral soils in the Control treatment averaged 7 times higher than the Burn 3yr treatment and 2 times higher than the Burn 1yr treatment, but high variation precluded detection of significant differences ($p = 0.12$ for Burn 3yr $p = 0.086$ for Burn 1yr) (Table 1).

When we grouped the treatments together by season, free-living BNF rates were significantly higher in the wet season compared to the dry season in the litter layer ($p < 0.0001$) and mineral soil ($p < 0.0001$). Rate and moisture were significantly correlated in the mineral soil ($p < 0.0001$) and in the litter layer ($p < 0.0001$).

Free-living BNF rates in both litter and soils were highly variable across sampling locations and between seasons, with a small number of samples that represented disproportionately high rates. In the wet season litter layer, 1% of the measurements accounted for 57% of the free-living BNF in litter across all treatments, and 4% accounted for 68%. In the dry season litter layer, 6% of the measurements accounted for 71% of the BNF. For mineral soils in the dry season, 2% of the measurements accounted for 60% of the BNF, but this effect was less pronounced in the wet season, where 3% of the measurements accounted for 16% of the BNF (Figure 2).

Scaled to an annual per-area flux, all free-living BNF rates were no more than 0.33 kg ha⁻¹ y⁻¹ (Table 1).

Inorganic Soil N and pH

Surface soil nitrate and ammonium concentrations were higher in the Control than the burned treatments in both the wet and dry seasons, whereas soil pH was higher in the burned treatments (Figure 3). In the dry season, ammonium concentrations in the Burn 3yr and Burn 1yr treatments were both significantly lower than the Control ($p = 0.0061$ for Burn 3yr, $p = 0.0004$, for Burn 1yr), with concentrations about 2 times higher in the Control than the burned treatments (Figure 3, Supplementary Table 4). In the wet season, ammonium concentrations were significantly lower in the Burn 1yr treatment than in the Control ($p = 0.0008$), with concentrations about 1.5 times lower, whereas the Burn 3yr and Control did not differ ($p = 0.18$). Wet and dry season concentrations of ammonium were significantly different within all treatments ($p < 0.0001$ for Control,

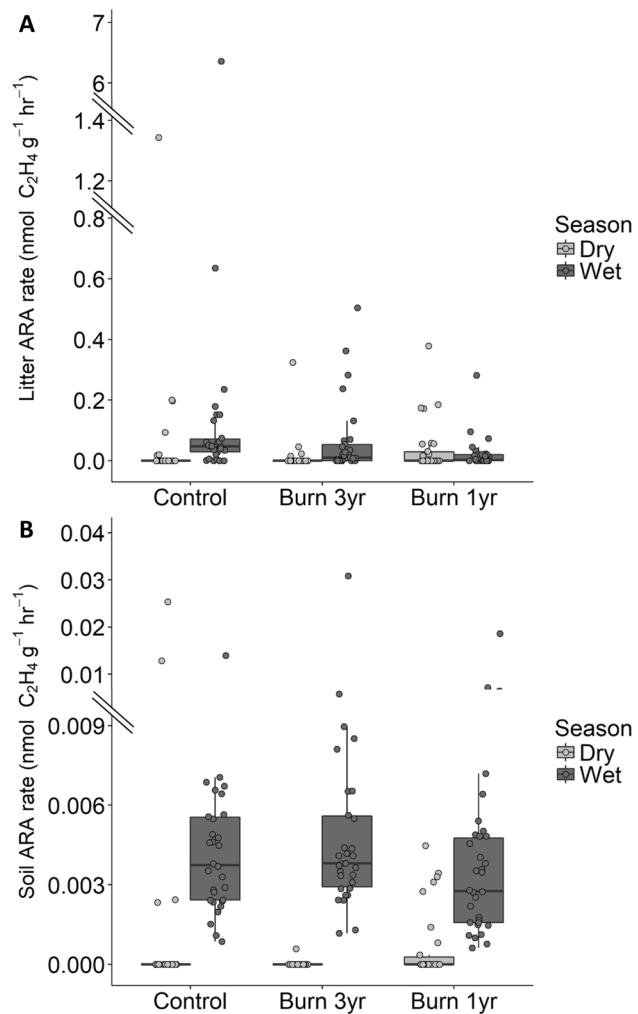


Figure 2. Free-living nitrogen fixation measured through nitrogenase activity (acetylene reduction activity, ARA) in the litter layer (**A**) and mineral soil (**B**) across the unburned (Control), triennially burned (Burn 3yr), and annually burned (Burn 1yr) forest treatments in the southeastern Amazon measured 6 and 7 years after the last fires; $n = 30$ per treatment, black line indicates median and gray shading indicates interquartile range.

$p = 0.0002$ for Burn 3yr, $p < 0.0001$ for Burn 1yr (Supplementary Table 4).

Consistent with ammonium, nitrate concentrations in both the wet and dry season were significantly lower in the Burn 3yr and Burn 1yr treatments relative to the Control ($p = 0.0002$, $p = 0.0168$ in the wet season, respectively, and $p < 0.0001$, $p < 0.0001$ in the dry season, respectively), with concentrations over 2 times higher in the Control than the burned treatments (Figure 3). Dry season concentrations of ammonium and nitrate were higher across all treatments relative to the wet season ($p < 0.0001$ for all treatments). Ammonium dominated over nitrate across the wet and dry seasons in all treatments (Figure 3; Supplementary Table 4).

The fires raised mineral soil pH in both burned treatments relative to the Control in both the dry and wet seasons (Figure 3). In the dry season, soil pH of the Control ($\text{pH} = 3.96$) was significantly lower than in the Burn 3yr ($\text{pH} = 4.49$, $p < 0.0001$) and Burn 1yr treatments ($\text{pH} = 4.38$, $p < 0.0001$) (Figure 3). In the wet season, soil pH of the Control ($\text{pH} = 4.12$) was also significantly lower than the Burn 3yr ($\text{pH} = 4.53$, $p < 0.0001$) and Burn 1yr ($\text{pH} = 4.43$, $p < 0.0001$) treatments (Figure 3). Within each treatment, soil pH was generally higher during the wet season, but only significantly different in the Control ($p = 0.023$ for Control, $p = 0.52$ for Burn 3yr, $p = 0.39$ for Burn 1yr) (Supplementary Table 4).

Aboveground N Losses and Demand

The fires depleted aboveground N stocks through immediate and delayed mortality, leaving 49% of total aboveground N in the Burn 3yr treatment, and 57% of total aboveground N in the Burn 1yr treatment relative to 2004 (Supplementary Figure 1). Leaf N biomass in the Burn 3yr and Burn 1yr treatments recovered from 30 and 34% of the Control in 2013 to 53 and 48% of the Control in 2016 (Supplementary Figure 1), averaging 5 kg N $\text{ha}^{-1} \text{y}^{-1}$ from 2011 to 2016 in both treatments. Aboveground N demand was estimated by calculating the annual net changes in wood and leaf N pools. Although the regression line for the Control treatment had a slope of 0, the net changes in the Burn 3yr and Burn 1yr treatments had a positive slope of 7.9 and 3.4 for the Burn 3yr and Burn 1yr treatments, respectively, despite the annual variability (Figure 4).

Litterfall N production rates were at their lowest in 2011 at 35 and 53 kg N $\text{ha}^{-1} \text{y}^{-1}$ in the Burn 3yr

and Burn 1yr treatments, but increased to 135 and 113 kg N $\text{ha}^{-1} \text{y}^{-1}$ in the Burn 3yr and Burn 1yr treatments by 2015, respectively, or 89% and 84% of the Control (Figure 4).

Foliar N and $\delta^{15}\text{N}$

The fire treatments did not affect foliar %N or the $\delta^{15}\text{N}$ in the burned treatments relative to the Control. The %N in leaves was similar in the Control, Burn 3yr, and Burn 1yr treatments ($p = 0.11$), and foliar $\delta^{15}\text{N}$ did not differ across treatments ($p = 0.70$) (Table 2; Supplementary Table 3). The C/N ratios were marginally lower in the Burn 1yr treatment compared to the Control ($p = 0.08$), but no differences were observed between the Control and the Burn 3yr treatment ($p = 0.73$).

Soil Nutrients and Characteristics

In the burned treatments, Mo and P generally increased by 50% in the top mineral soil (0–2 cm),

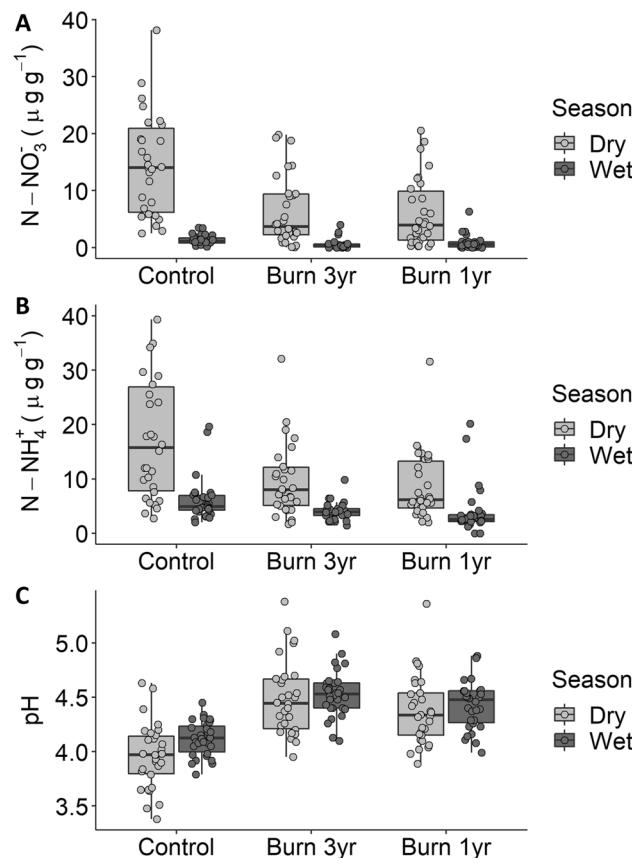


Figure 3. Nitrate (NO_3^-) (A), Ammonium (NH_4^+) (B), and pH (C) of mineral soils (0–2 cm) across the wet and dry seasons and the unburned (Control), triennially burned (Burn 3yr), and annually burned (Burn 1yr) forest treatments in the southeastern Amazon; $n = 30$ per treatment, black line indicates median and gray shading indicates interquartile range.

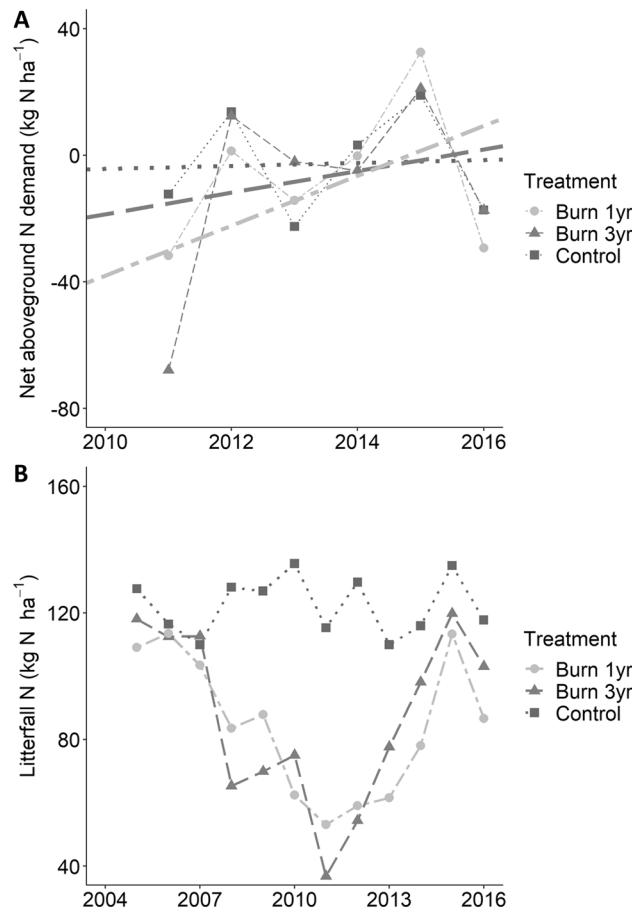


Figure 4. Annual aboveground N demand from net changes in the wood and leaf N pools across the unburned (Control), triennially burned (Burn 3yr), and annually burned (Burn 1yr) forest treatments in the southeastern Amazon, with the last fires in 2010 (A) and annual litter N fluxes from litter production (kg N ha⁻¹ y⁻¹). The thicker lines, representing the regression of the net changes for the unburned (Control; dotted), triennially burned (Burn 3yr; long dash), and annually burned (Burn 1yr; two dash), are calculated from 2010 to 2016.

whereas V decreased by a third. The surface mineral soil Mo concentration was significantly higher in the Burn 3yr treatment compared to the Control ($p = 0.0027$) but not in the Burn 1yr treatment ($p = 0.33$). The soil P concentration was significantly higher in the Burn 1yr treatment ($p = 0.034$) but not the Burn 3yr treatment ($p = 0.71$). The soil V concentrations were significantly lower in the Burn 3yr ($p = 0.0015$) and Burn 1yr treatments ($p = 0.0012$) relative to the Control. The soil P concentrations were higher in the surface soils (0–2 cm) compared to the deeper soils (2–10 cm) in both the Burn 3yr ($p = 0.002$) and Burn 1yr treatments ($p = 0.0001$), but not in the Control ($p = 0.12$). The same pattern was found for Mo, where surface (0–2 cm) Mo concentrations were higher than the deeper soils (2–10 cm) for both the Burn 3yr ($p = 0.028$) and Burn 1yr treatments

($p = 0.0004$) but not in the Control ($p = 0.10$) (Figure 5, Supplementary Table 3).

The fire treatments did not affect mineral soil %C or %N. There were no differences in the mineral soils between the Control and the Burn 3yr and Burn 1yr treatments in %C ($p = 0.99$, $p = 0.89$, respectively), %N ($p = 0.054$, $p = 0.79$, respectively), or soil $\delta^{15}\text{N}$ ($p = 0.98$, $p = 0.48$, respectively) (Table 2). However, the C/N ratio was significantly higher in the Burn 1yr treatment ($p = 0.0024$) compared to the Control (Table 2).

DISCUSSION

Low Rates of Biological Nitrogen Fixation Post-fire

Contrary to our hypothesis that both symbiotic and free-living BNF would increase post-fire, we found no evidence of increased BNF in the burned forests.

Table 2. Soil and Foliar Nitrogen and Carbon Across the Experimental Burn Treatments

Treatment	Soil Depth (cm)	Foliation					
		%N ¹	δ ¹⁵ N	%C ¹	C/N ¹	δ ¹⁵ N	%C
Control	0–2	0.28 (0.03) ^{a,†}	4.19 (0.20) ^{a,†}	4.77 (0.65) ^{a,†}	16.63 (0.53) ^{b,†}	2.11 (0.05) ^a	3.16 (0.16) ^a
	2–10	0.17 (0.01) ^{A,‡}	5.30 (0.19) ^{A,‡}	2.63 (0.22) ^{A,‡}	15.28 (0.20) ^{A,‡}	2.07 (0.07) ^a	3.33 (0.26) ^a
Burn 3yr	0–2	0.27 (0.04) ^{a,†}	3.88 (0.19) ^{a,†}	4.75 (0.84) ^{a,†}	17.04 (0.66) ^{b,†}	2.07 (0.07) ^a	50.19 (0.47) ^a
	2–10	0.14 (0.02) ^{AB,‡}	5.34 (0.26) ^{A,‡}	2.12 (0.28) ^{AB,‡}	15.17 (0.34) ^{A,‡}	1.94 (0.06) ^a	24.96 (0.79) ^{ab}
Burn 1yr	0–2	0.24 (0.02) ^{a,†}	4.15 (0.11) ^{a,†}	4.55 (0.43) ^{a,†}	19.01 (0.59) ^{a,†}	3.07 (0.23) ^a	50.02 (0.53) ^a
	2–10	0.11 (0.01) ^{B,‡}	5.73 (0.17) ^{A,‡}	1.76 (0.15) ^{B,‡}	15.52 (0.31) ^{A,‡}	26.26 (0.72) ^a	126.3 (5.0) ^{ab}

¹Indicates if group was ln-transformed prior to statistical tests. Data are means \pm 1 standard error; $n = 12$ for soils and $n = 30$ for foliage. Lower-case letters indicate significant groupings in mineral soils (0–2 cm) and foliage; capital letters indicate significant groups in deeper soils (2–10 cm), and daggers (†,‡) indicate if there are differences between soil depths between each group ($p < 0.05$).

Recovery of the leaf biomass pools and litterfall production rates were rapid, with a net increase in aboveground N demand for leaf production of about 5 kg N ha⁻¹ y⁻¹ in the burned forests (Figure 6, Supplementary Figure 1). However, BNF inputs were less than 1.2 kg N ha⁻¹ y⁻¹. Although the symbiotic BNF rates in the triennially burned forest were higher than in the unburned forest, which had no detectable symbiotic BNF, the rates were still very low (mean, 0.48 kg N ha⁻¹ y⁻¹). Tree species capable of supporting symbiotic BNF increased tenfold in the burned plots relative to unburned forest, but the trees supported very little symbiotic BNF. Surprisingly, free-living BNF rates were generally higher in the unburned forest, likely because the litter and soil stay moist for a longer time, compared to the burned forests which dry out as a result of lower canopy coverage and more exposure to direct sunlight.

Overall, free-living and symbiotic BNF rates were markedly low compared to other tropical forests. BNF rates (0.16–1.2 kg N ha⁻¹ y⁻¹ across all treatments) were at least fivefold lower than average estimates for tropical forests as a whole (5.7 kg N ha⁻¹ y⁻¹) (Sullivan and others 2014). The levels of symbiotic BNF were substantially lower than other previous estimates in secondary and mature wet and dry tropical forests (Sylvester-Bradley and others 1980; Batterman and others 2013a; Gei 2014; Winbourne and others 2018a; Brookshire and others 2019; Taylor and others 2019). Free-living BNF in the mineral soil and litter layer (≤ 0.33 kg N ha⁻¹ y⁻¹) were also substantially lower than reported values from other studies (Jordan and others 1983; Russell and Vitousek 1997; Ley and D'Antonio 1998; Reed and others 2007).

We consider four possible explanations for the markedly low BNF rates, even in the secondary burned forests, compared to other tropical forests. First, the low symbiotic BNF rates could in part be explained by the specific N-fixing species at our site. Recent work has demonstrated that taxonomic identity, rather than soil properties, is the most important predictor in determining BNF rates across a landscape (Wurzburger and Hedin 2016). Although the putative N fixer abundance increased tenfold in the burned forests compared to the unburned forest, the specific species at our site may not be these previously identified 'superfixers,' species that will consistently fix high rates of symbiotic BNF (Wurzburger and Hedin 2016). Some of the previously identified superfixers include *Pentaclethra macroloba*, which dominate in forests of Trinidad and Costa Rica (Brookshire and

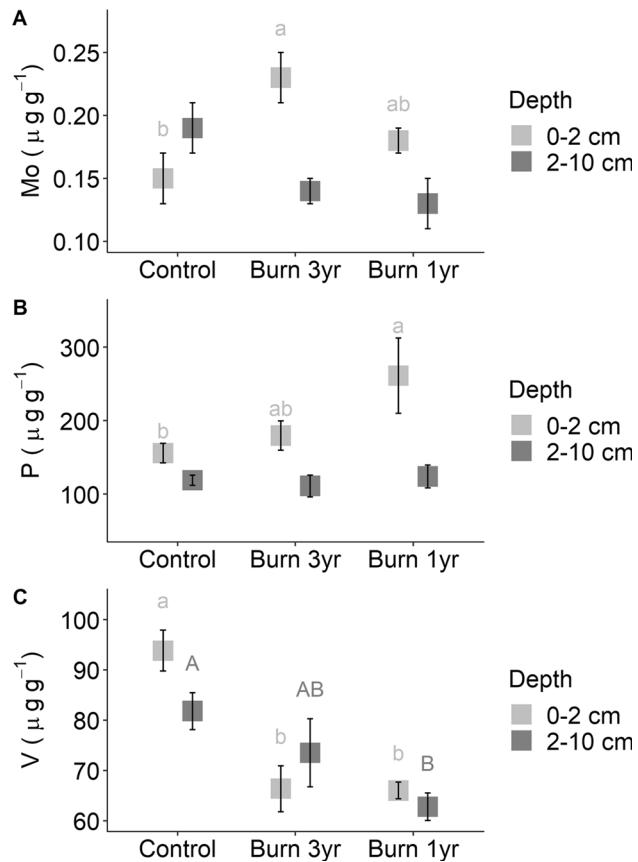


Figure 5. Total molybdenum (Mo) (**A**), phosphorus (P) (**B**), and vanadium (V) (**C**) soil concentrations ($\mu\text{g g}^{-1}$ dry weight soil) across the unburned (Control), triennially burned (Burn 3yr), and annually burned (Burn 1yr) forest treatments in the southeastern Amazon; data are means \pm 1 standard error; $n = 12$. Lower-case letters indicate significant groupings in mineral soils (0–2 cm), capital letters indicate significant groupings in deeper soils (2–10 cm), and no letters indicate no significant differences ($p < 0.05$).

others 2019; Taylor and others 2019); *Tachigali versicolor*, a monocarpic species; and certain species within the *Inga* genus (Wurzburger and Hedin 2016). However, perhaps the species and not the genera determines an individual's superfixer status. Although species in the *Tachigali* and *Inga* genera were at our site, they were not the same species as previously identified superfixers. These differences in part, could explain the low symbiotic BNF rates measured.

Second, the low symbiotic BNF rates across the treatments could also be explained by suppression by high levels of soil N at our site, because many species in the tropics are facultative N fixers, decreasing symbiotic BNF when N is available (Barron and others 2011; Menge and others 2014). Substantial deep soil inorganic N pools have been found at our site, up to 228 kg N- $\text{NO}_3^- + \text{NH}_4^+$ ha^{-1} in the top 4 m of the soil profile (Jankowski and others 2018). This deep soil N could potentially be available to deep-rooted trees, which

are common across Amazonia (Nepstad and others 1994), particularly in the southern and eastern regions where evergreen forests require deep soil water during the dry season. Because BNF is energetically expensive, the availability of this high N pool could lead to low rates of symbiotic BNF. Large inorganic N pools have also been found in the central and southern Amazon (Schroth and others 1999; Feldpausch and others 2010), making these forests across the Amazon relatively unique compared to other tropical forests where BNF has been quantified. The surface inorganic N concentrations in the unburned forest were also relatively high at our site in the southeastern Amazon, averaging a total of 37 μg and 7.5 μg $\text{NO}_3^- + \text{NH}_4^+$ g^{-1} (dry and wet seasons, respectively; or 4.1 kg $\text{NO}_3^- + \text{NH}_4^+$ ha^{-1}), which could also potentially explain the low free-living BNF rates.

Other N cycle indicators, such as foliar %N, foliar $\delta^{15}\text{N}$, and static soil N pools also indicate that N is relatively abundant in these forest soils. The higher

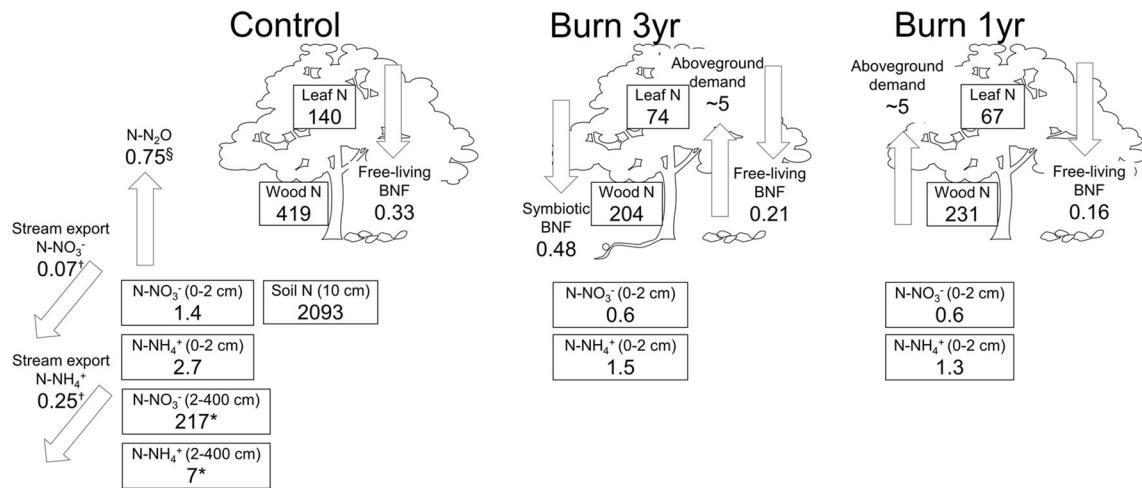


Figure 6. Diagram of the pools of N (outlined in black boxes) in 2016, 6 years after the last fires, and fluxes (gray arrows) in 2016 and 2017, 6 and 7 years after the last fires across the unburned (Control), triennially burned (Burn 3yr), and annually burned (Burn 1yr) forest treatments in the southeastern Amazon. Components include leaf, wood, nitrate (N-NO₃⁻), and ammonium (N-NH₄⁺). Fluxes include inputs through free-living (upper estimate) and symbiotic BNF, and aboveground N demand from leaf production. On the left, soil stocks, inputs, and outputs measured at Tangaro are also presented for the unburned forest. All values are presented as kg N ha⁻¹ for pools and kg N ha⁻¹ y⁻¹ for fluxes. See discussion on estimated dinitrogen (N₂), nitric oxide (NO), and dissolved organic N (DON) fluxes, and ammonia (NH₃) deposition. \$O'Connell (2015), †Riskin and others (2017), *Jankowski and others (2018).

foliar %N and $\delta^{15}\text{N}$ across all treatments, ranging from 1.9–2.1%N, and 2.1–3.2‰ (Table 2), more closely resembled those of mature tropical forests (1.9%N, and 3.7‰, respectively; Martinelli and others 1999) rather than those of younger forests (1.4%N, and – 0.5‰, respectively; Davidson and others 2007). Although a static soil pool of N cannot indicate availability (Vitousek and Sanford 1986), at a site most similar to ours in the central Amazon, Davidson and others (2007) concluded that the top 10 cm of mineral soil contains enough organic-N stocks (≥ 1000 kg N ha⁻¹) to supply the regrowing forest even if only a small fraction is gradually mineralized to a bioavailable form. Although Winbourne and others (2018a) found relatively high rates of BNF in the Atlantic Forest of Brazil, they made the same conclusion, estimating that the top 10 cm of mineral soil contained approximately 1900 kg N ha⁻¹ to support a majority of the forest recovery. In these southeastern Amazon forests, soils contained approximately 2100 kg N ha⁻¹ in the top 10 cm, further supporting the conclusion that large stocks of soil N could support regrowing biomass after fire.

Third, the low BNF rates that we measured in the burned forests relative to previously studied, clear-cut tropical secondary forests could be attributed to the observation that less aboveground N was lost in comparison to clear-cut forests. Although the fires led to a loss of approximately half of the original

aboveground N, likely lowering the N/P stoichiometry in this forest, perhaps the N losses still did not induce N limitation or increase light availability through the canopy that would typically facilitate higher BNF rates (Batterman and others 2013a; Taylor and Menge 2018). The fires led to a loss of 51% of total aboveground N in the triennially burned forest, and 43% of total aboveground N in the annually burned forest relative to the unburned forest. The triennially burned forest lost more N than the annually burned forest due to increased fuel loads that accumulated during the non-burned years (Brando and others 2014), which could potentially explain the detection of symbiotic BNF in the triennially burned forest (mean, 0.48 kg N ha⁻¹ y⁻¹) compared to the annually burned forest (0 kg N ha⁻¹ y⁻¹). Alternatively, the annually burned forest also had more grass invasion (Silvério and others 2013) which reduced tree coverage and could also explain the lower symbiotic BNF rate. Despite the large N losses post-fire, our results were consistent with another study conducted in warm temperate longleaf pine savannas where BNF also did not increase to replace the N losses from fires, also likely due to high N availability (Tierney and others 2019).

Our final consideration for why BNF rates were low at our site in the southeastern Amazon is that patterns of BNF can be difficult to constrain due to hot spots, defined as disproportionately high rates

relative to the surrounding area (McClain and others 2003) in space and time. The hot spots of free-living BNF in the soil and litter across our experiment may affect how we scaled up estimates and could account for a missing source of N inputs. And although most BNF occurs on the forest floor, there may have been free-living BNF in the canopy (Reed and others 2008), and BNF associated with ants (Pinto-Tomás and others 2009) and termites (Yamada and others 2006), bryophytes, lichens, and decaying wood (Matzek and Vitousek 2003) unaccounted for. Because our study only measured BNF at the 6- and 7-year mark post-fire, we do not have a complete temporal picture of BNF across time during forest recovery. Our use of a theoretical ratio of (mol acetylene reduced):(mol N₂ reduced) to scale up our symbiotic BNF rates to annual fluxes may have also increased uncertainty in the final flux estimates (Anderson and others 2004). Lastly, because symbiotic BNF is notoriously difficult to scale up, our survey may not have sampled enough cores to accurately represent the forest (Winbourne and others 2018b). Future work is needed in the field to constrain error estimates and improve scaling accuracy.

Increases of Soil Molybdenum, Phosphorus, and pH, and Decreases in Vanadium Post-fire

Molybdenum and P concentrations at our site in the southeastern Amazon were relatively low, which makes the lack of response in BNF to increased Mo and P post-fire more surprising. Due to nonexistent parent material weathering and low atmospheric inputs to these southeastern Amazon forests, average Mo and P concentrations were low in the unburned mineral soils, averaging 0.145 and 157 µg g⁻¹, respectively, and substantially depleted with respect to their average crustal abundance worldwide (1–2 and 700 µg g⁻¹, respectively; Taylor and McLennan 1995). The scarcity of tropical soil Mo values generally makes it difficult to assess these concentrations in a broader context, but we note that the mineral soil Mo concentrations at this site are lower than concentrations found at other tropical sites (for example, Reed and others 2013; Winbourne and others 2017) and that total P concentrations in these soils were also low compared to other tropical sites (for example, Wurzburger and others 2012; Sullivan and others 2014). Even at higher soil Mo and P concentrations, Mo and P limitation have been demonstrated for free-living BNF (Wurzburger and others 2012; Reed and others 2013; Winbourne and others 2017).

Despite the generally low concentrations of both rock-derived nutrients compared to other tropical forests, Mo and P limitations are likely not the main reasons for low BNF rates at our site in the southeastern Amazon. Because the fires increased average concentrations of Mo and P in the mineral soils to 0.23 and 0.18 µg Mo g⁻¹ and 178 and 261 µg P g⁻¹ in the triennially burned and annually burned forests, if BNF was limited by Mo and/or P, the increased concentrations of soil Mo and P should have facilitated higher free-living BNF rates. However, because free-living BNF did not increase post-fire, we suspect that Mo and P are not the major contributors to the low BNF rates measured.

Furthermore, we also hypothesized that the increased soil pH in the burned plots also could theoretically reduce pH effects on Mo and P availability, making them more available for microbial activity. The forest soils in the southeastern Amazon are highly acidic at pH 4, which affects Mo and P formation of chemical complexes and sorption to soils (Haynes 1982; Reddy and Gloss 1993). We found that burning did increase pH, as ash is deposited on the soil surface and more cations are released, which raises the base saturation and thus the soil pH (Kauffman and others 1992; Nardoto and Bustamante 2003). Regardless, despite the increase in soil pH, as well as the increase in Mo and P concentrations, we still did not see an increase in free-living BNF 7 years following the last fires.

Biological Nitrogen Fixation and Nitrogen Cycling in the Southeastern Amazon

In the tropics, BNF rates are thought to be high in part to combat high hydrologic and gaseous N losses (Hedin and others 2009). But compared to other tropical forests, measurements at our site in the southeastern Amazon indicate that hydrologic and gaseous N losses are generally very low. Previous work at this site found low N losses through leaching and to the atmosphere as nitrous oxide (N₂O). Riskin and others (2017) estimated N leaching losses from mature forest sites to average $0.07 \pm 0.02 \text{ kg NO}_3^- \text{-N ha}^{-1} \text{ y}^{-1}$ and $0.25 \pm 0.10 \text{ kg NH}_4^+ \text{-N ha}^{-1} \text{ y}^{-1}$, in contrast to higher hydrologic losses of N found at other tropical sites: for example, losses of nitrate totaling 4–6 kg NO₃⁻-N ha⁻¹ y⁻¹ in Costa Rica (Newbold and others 1995). At our site, gaseous N losses were estimated to be 0.75 kg N₂O-N ha⁻¹ y⁻¹ (O'Connell 2015), and dinitrogen emissions are likely low because the soils are well-drained and because of

the long dry season (Figueira and others 2016). The gaseous losses of N at our site are much lower than the estimates of gaseous losses ranging from 2 to 9 kg N ha⁻¹ y⁻¹ across the tropics (Houlton and others 2006). Nitric oxide (NO) emissions may be a more important loss at our site, since NO emissions were found to exceed N₂O emissions in the nearby Cerrado region on similar soils with a slightly drier climate (Varella and others 2004; Figueira and others 2016). Dissolved organic N (DON) can account for the majority of nitrogen losses from some tropical forests (Perakis and Hedin 2002), but while DON has not yet been measured, at a similar site in central Rondônia, Brazil, DON, made up 54–65% of total N exports in forest streams (Neill and others 2001). In addition, N deposition is likely low in this region, about < 1 kg N ha⁻¹ y⁻¹ according to estimates found by Germer and others (2009). In summary, the relatively low losses of N from this region indicate that aside from disturbance, relatively little excess N from BNF is required. We conclude that this region of the southeastern Amazon is characterized by a relatively 'closed' N cycle with low inputs from BNF and low hydrologic and gaseous losses (Figure 6).

Recent studies that have noted the heterogeneity of tropical forests (Townsend and others 2008) where some forests do not follow the conventional N-rich paradigm of tropical N biogeochemistry (Soper and others 2017). Within the Amazon, our results were consistent with the conclusions of Davidson and others (2007) who did not find evidence of increased BNF during forest recovery, and the conclusion of Nardoto and others (2014) that most *Fabaceae* species that are capable of nodulating do not fix N in Amazonia. Nardoto and others (2014) suggest that soil N stocks may have built up over the millennia, so while inputs and outputs are currently low, the mass of N stored in the soils remains high. Nardoto and others (2014) further suggest that *Fabaceae* proliferated in South America 50 Mya, and likely historically fixed N which led to an accrual of N stocks, which could suppress BNF in mature forests, since most legumes are facultative fixers (Barron and others 2011; Menge and others 2014). The highly weathered, deep oxisols at our site (Jankowski and others 2018) and also found in the central and southern Amazon (Schroth and others 1999; Feldpausch and others 2010), and the high concentrations of deep soil nitrate suggest that the soils have developed anion exchange capacity, allowing storage of large quantities of nitrate. In contrast to less weathered tropical soils where nitrate is found to leach into stream at higher quantities, the low streamwater concentrations of

nitrate at our site also suggest that differences due to geologic history could allow large N stocks to accrue. Perhaps the geologic history in this region and across the lower Amazon as a whole can reconcile why, for example, Panama, which has a relatively similar soil N percentage (Batterman and others 2013a), and Costa Rica and Trinidad, with high surface soil nitrate and ammonium concentrations (Brookshire and others 2019; Taylor and others 2019), are still characterized by high BNF rates.

The aboveground N recovery and relatively abundant soil inorganic N we observed highlight the potential resilience and lack of severe nutrient limitation following fire disturbance in some tropical forests. The relatively rapid recovery of nutrient cycling is consistent with findings by a recent meta-analysis looking at patterns of biogeochemical recuperation (Sullivan and others 2019). However, this study raises new questions: are low BNF rates more common in some tropical forests than previously thought? What drives some systems to exhibit a more closed N cycle, while others exhibit leakier cycling? What controls patterns of BNF that can reconcile the variation in rates measured across the tropics? Further studies will shed light on patterns of BNF and the possible role of geologic history as suggested by Nardoto and others (2014) in explaining broader biogeochemical processes across ecosystems.

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REFERENCES

Anderson MD, Ruess RW, Uliassi DD, Mitchell JS. 2004. Estimating N₂ fixation in two species of *Alnus* in interior Alaska using acetylene reduction and ¹⁵N₂ uptake. *Ecoscience* 11:102–12.

Andreae MO, Merlet P. 2001. Emission of trace gases and aerosols from biomass burning. *Glob Biogeochem Cycles* 15:955–66.

Aragão LEOC, Anderson LO, Fonseca MG, Rosan TM, Vedovato LB, Wagner FH, Silva CVJ, Silva Junior CHL, Arai E, Aguiar AP, Barlow J, Berenguer E, Deeter MN, Domingues LG, Gatti L, Gloor M, Malhi Y, Marengo JA, Miller JB, Phillips OL, Saatchi S. 2018. 21st century drought-related fires counteract the decline of Amazon deforestation carbon emissions. *Nat Commun* 9:1–12.

Balch JK, Nepstad DC, Brando PM, Curran LM, Portela O, de Carvalho O, Lefebvre P. 2008. Negative fire feedback in a transitional forest of southeastern Amazonia. *Glob Change Biol* 14:2276–87.

Balch JK, Nepstad DC, Curran LM, Brando PM, Portela O, Guilherme P, Reuning-Scherer JD, de Carvalho Jr O. 2011. Size, species, and fire behavior predict tree and liana mortality from experimental burns in the Brazilian Amazon. *For Ecol Manag* 261:68–77.

Barron AR, Purves DW, Hedin LO. 2011. Facultative nitrogen fixation by canopy legumes in a lowland tropical forest. *Oecologia* 165:511–20.

Barron AR, Wurzburger N, Bellenger JP, Wright SJ, Kraepiel AML, Hedin LO. 2009. Molybdenum limitation of asymbiotic nitrogen fixation in tropical forest soils. *Nat Geosci* 2:42–5.

Batterman SA, Hedin LO, Van Breugel M, Ransijn J, Craven DJ, Hall JS. 2013a. Key role of symbiotic dinitrogen fixation in tropical forest secondary succession. *Nature* 502:224–7.

Batterman SA, Wurzburger N, Hedin LO. 2013b. Nitrogen and phosphorus interact to control tropical symbiotic N₂ fixation: a test in *Inga punctata*. *J Ecol* 101:1400–8.

Brando PM, Balch JK, Nepstad DC, Morton DC, Putz FE, Coe MT, Silvério D, Macedo MN, Davidson EA, Nóbrega CC, Alencar A, Soares-Filho BS. 2014. Abrupt increases in Amazonian tree mortality due to drought–fire interactions. *Proc Natl Acad Sci USA* 111:6347–52.

Brookshire ENJ, Wurzburger N, Currey B, Menge DNL, Oatham MP, Roberts C. 2019. Symbiotic N fixation is sufficient to support net aboveground biomass accumulation in a humid tropical forest. *Sci Rep* 9:1–10.

Casals P, Romanya J, Vallejo VR. 2005. Short-term nitrogen fixation by legume seedlings and resprouts after fire in Mediterranean old-fields. *Biogeochemistry* 76:477–501.

Chave J, Réjou-Méchain M, Búrquez A, Chidumayo E, Colgan MS, Delitti WBC, Duque A, Eid T, Fearnside PM, Goodman RC, Henry M, Martínez-Yrízar A, Mugasha WA, Muller-Landau HC, Mencuccini M, Nelson BW, Ngomanda A, Nogueira EM, Ortiz-Malavassi E, Pélassier R, Ploton P, Ryan CM, Saldaña-Jaén JG, Vieilledent G. 2014. Improved allometric models to estimate the aboveground biomass of tropical trees. *Glob Change Biol* 20:3177–90.

Cochrane MA. 2001. Synergistic interactions between habitat fragmentation and fire in evergreen tropical forests. *Conserv Biol* 15:1515–21.

Cochrane MA, Schulze MD. 1999. Fire as a recurrent event in tropical forests of the Eastern Amazon: effects on forest structure, biomass, and species composition. *Biotropica* 31:2–16.

Crews TE. 1993. Phosphorus regulation of nitrogen fixation in a traditional Mexican agroecosystem. *Biogeochemistry* 21:141–66.

Crews TE, Farrington H, Vitousek PM. 2000. Changes in asymbiotic, heterotrophic nitrogen fixation on leaf litter of *Metrosideros polymorpha* with long-term ecosystem development in Hawaii. *Ecosystems* 3:386–95.

Darnajoux R, Zhang X, McRose DL, Miadlikowska J, Lutzoni F, Kraepiel AML, Bellenger JP. 2017. Biological nitrogen fixation by alternative nitrogenases in boreal cyanolichens: importance of molybdenum availability and implications for current biological nitrogen fixation estimates. *New Phytol* 213:680–9.

Davidson EA, de Carvalho CJR, Figueira AM, Ishida FY, Ometto JPHB, Nardoto GB, Sába RT, Hayashi SN, Leal EC, Vieira ICG, Martinelli LA. 2007. Recuperation of nitrogen cycling in Amazonian forests following agricultural abandonment. *Nature* 447:995–8.

Delitti WBC, Meguro M, Pausas JG. 2006. Biomass and mineral mass estimates in a ‘cerrado’ ecosystem. *Braz J Bot* 29:531–40.

Feldpausch TR, Couto EG, Rodrigues LC, Pauleto D, Johnson MS, Fahey TJ, Lehmann J, Riha SJ. 2010. Nitrogen aboveground turnover and soil stocks to 8 m depth in primary and selectively logged forest in southern Amazonia. *Glob Change Biol* 16:1793–805.

Figueira AMS, Davidson EA, Nagy RC, Riskin SH, Martinelli LA. 2016. Isotopically constrained soil carbon and nitrogen budgets in a soybean field chronosequence in the Brazilian Amazon region. *J Geophys Res Biogeosci* 121:2520–9.

Gei M, Rozendaal DMA, Poorter L, Bongers F, Sprent JI, Garner MD, Aide TM, Andrade JL, Balvanera P, Becknell JM, Brancalion PHS, Cabral GAL, César RG, Chazdon RL, Cole RJ, Colletta GD, De Jong B, Denslow JS, Dent DH, Dewalt SJ, Dupuy JM, Durán SM, Do Espírito Santo MM, Fernandes GW, Nunes YRF, Finegan B, Moser VG, Hall JS, Hernández-Steфанoni JL, Junqueira AB, Kennard D, Lebrija-Trejos E, Letcher SG, Lohbeck M, Marín-Spiotta E, Martínez-Ramos M, Meave JA, Menge DNL, Mora F, Muñoz R, Muscarella R, Ochoa-Gaona S, Orihuela-Belmonte E, Ostertag R, Peña-Claros M, Pérez-García EA, Piotto D, Reich PB, Reyes-García C, Rodríguez-Velázquez J, Romero-Pérez IE, Sanaphre-Villanueva L, Sanchez-Azofeifa A, Schwartz NB, De Almeida AS, Almeida-Cortez JS, Silver W, De Souza Moreno V, Sullivan BW, Swenson NG, Uriarte M, Van Breugel M, Van Der Wal H, Veloso MDDM, Vester HFM, Vieira ICG, Zimmerman JK, Powers JS. 2018. Legume abundance along successional and rainfall gradients in Neotropical forests. *Nat Ecol Evol* 2:1104–11.

Gei MG. 2014. Biological nitrogen fixation in tropical dry forests of Costa Rica: patterns and controls. University of Minnesota, PhD dissertation.

Germer S, Neill C, Vetter T, Chaves J, Krusche AV, Elsenbeer H. 2009. Implications of long-term land-use change for the hydrology and solute budgets of small catchments in Amazonia. *J Hydrol* 364:349–63.

Hardy RWF, Holsten RD, Jackson EK, Burns RC. 1968. The acetylene–ethylene assay for N_2 fixation: laboratory and field evaluation. *Plant Physiol* 43:1185–207.

Haynes RJ. 1982. Effects of liming on phosphate availability in acid soils—a critical review. *Plant Soil* 68:289–308.

Hedin LO, Brookshire ENJ, Menge DNL, Barron AR. 2009. The nitrogen paradox in tropical forest ecosystems. *Annu Rev Ecol Evol Syst* 40:613–35.

Hedin LO, Vitousek PM, Matson PA. 2003. Nutrient losses over four million years of tropical forest development. *Ecology* 84:2231–55.

Hendricks JJ, Boring LR. 1999. N_2 -fixation by native herbaceous legumes in burned pine ecosystems of the southeastern United States. *For Ecol Manag* 113:167–77.

Hobbs NT, Schimel DSS. 1984. Fire effects on nitrogen mineralization and fixation in mountain shrub and grassland communities. *J Rangel Manag* 37:402–5.

Houlton BZ, Sigman DM, Hedin LO. 2006. Isotopic evidence for large gaseous nitrogen losses from tropical rainforests. *Proc Natl Acad Sci USA* 103:8745–50.

Ivanauskas NM, Monteiro R, Rodrigues RR. 2003. Alterations following a fire in a forest community of Alto Rio Xingu. *For Ecol Manag* 184:239–50.

Jankowski KJ, Neill C, Davidson EA, Macedo MN, Costa C, Galford GL, Maracahipes Santos L, Lefebvre P, Nunes D, Cerri CEP, McHorney R, O'Connell C, Coe MT. 2018. Deep soils modify environmental consequences of increased nitrogen fertilizer use in intensifying Amazon agriculture. *Sci Rep* 8:1–11.

Jean ME, Phalyvong K, Forest-Drolet J, Bellenger JP. 2013. Molybdenum and phosphorus limitation of asymbiotic nitrogen fixation in forests of Eastern Canada: influence of vegetative cover and seasonal variability. *Soil Biol Biochem* 67:140–6.

Jordan C, Caskey W, Escalante G, Herrera R, Montagnini F, Todd R, Uhl C. 1983. Nitrogen dynamics during conversion of primary Amazonian rain forest to slash and burn agriculture. *Oikos* 40:131–9.

Kauffman JB, Till KM, Shea RW. 1992. Biogeochemistry of deforestation and biomass burning. In: O'Brien D, Ed. *The science of global change*. Washington: ACS Symposium Series. p 428–56.

Ley RE, D'Antonio CM. 1998. Exotic grass invasion alters potential rates of N fixation in Hawaiian woodlands. *Oecologia* 113:179–87.

Mahowald NM, Artaxo P, Baker AR, Jickells TD, Okin GS, Randerson JT, Townsend AR. 2005. Impacts of biomass burning emissions and land use change on Amazonian atmospheric phosphorus cycling and deposition. *Glob Biogeochem Cycles* 19:1–15.

Martin AR, Erickson DL, Kress WJ, Thomas SC. 2014. Wood nitrogen concentrations in tropical trees: phylogenetic patterns and ecological correlates. *New Phytol* 204:484–95.

Martinelli LA, Lins SRM, dos Santos-Silva JC. 2017. Fine litter-fall in the Brazilian Atlantic Forest. *Biotropica* 49:443–51.

Martinelli LA, Piccolo MC, Townsend AR, Vitousek PM, Cuevas E, McDowell W, Robertson GP, Santos OC, Treseder K. 1999. Nitrogen stable isotopic composition of leaves and soil: tropical versus temperate forests. *Biogeochemistry* 46:45–65.

Matzek V, Vitousek P. 2003. Nitrogen fixation in bryophytes, lichens, and decaying wood along a soil-age gradient in Hawaiian montane rain forest. *Biotropica* 35:12–19.

McClain ME, Boyer EW, Dent CL, Gergel SE, Grimm NB, Groffman PM, Hart SC, Harvey JW, Johnston CA, Mayorga E, McDowell WH, Pinay G. 2003. Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems. *Ecosystems* 6:301–12.

Menge DNL, Lichstein JW, Ángeles-Pérez G. 2014. Nitrogen fixation strategies can explain the latitudinal shift in nitrogen-fixing tree abundance. *Ecology* 95:2236–45.

Nardoto GB, Bustamante MMDC. 2003. Effects of fire on soil nitrogen dynamics and microbial biomass in savannas of Central Brazil. *Pesqui Agropecu Bras* 38:955–62.

Nardoto GB, Quesada CA, Patiño S, Saiz G, Baker TR, Schwarz M, Schrodert F, Feldpausch TR, Domingues TF, Marimon BS, Marimon Junior B-H, Vieira ICG, Silveira M, Bird MI, Phillips OL, Lloyd J, Martinelli LA. 2014. Basin-wide variations in Amazon forest nitrogen-cycling characteristics as inferred from plant and soil ^{15}N : ^{14}N measurements. *Plant Ecol Divers* 7:173–87.

Neary DG, Klopatek CC, DeBano LF, Ffolliott PF. 1999. Fire effects on belowground sustainability: a review and synthesis. *For Ecol Manag* 122:51–71.

Neill C, Deegan LA, Thomas SM, Cerri CC. 2001. Deforestation for pasture alters nitrogen and phosphorus in small Amazonian streams. *Ecol Appl* 11:1817–28.

Neill C, Piccolo MC, Steudler PA, Melillo JM, Feigl BJ, Cerri CC. 1995. Nitrogen dynamics in soils of forests and active pastures in the western Brazilian Amazon Basin. *Soil Biol Biochem* 27:1167–75.

Nepstad DC, de Carvalho CR, Davidson EA, Jipp PH, Lefebvre PA, Negreiros GH, da Silva ED, Stone TA, Trumbore SE, Vieira S. 1994. The role of deep roots in the hydrological and carbon cycles of Amazonian forests and pastures. *Nature* 372:666–9.

Newbold JD, Sweeney BW, Jackson JK, Kaplan LA. 1995. Concentrations and export of solutes from six mountain streams in northwestern Costa Rica. *J N Am Benthol Soc* 14:21–37.

O'Connell CS. 2015. Ecological tradeoffs to an agricultural Amazonia: investigating the effects of increased agricultural production on Amazonia's contribution to global climate and nitrogen. University of Minnesota, PhD dissertation.

Pan Y, Birdsey RA, Fang J, Houghton R, Kauppi PE, Kurz WA, Phillips OL, Shvidenko A, Lewis SL, Canadell JG, Ciais P, Jackson RB, Pacala SW, McGuire AD, Piao S, Rautiainen A, Sitch S, Hayes D. 2011. A large and persistent carbon sink in the world's forests. *Science* 333:988–93.

Perakis SS, Hedin LO. 2002. Nitrogen loss from unpolluted South American forests mainly via dissolved organic compounds. *Nature* 415:416–19.

Pinto-Tomás AA, Anderson MA, Suen G, Stevenson DM, Chu FST, Cleland WW, Weimer PJ, Currie CR. 2009. Symbiotic nitrogen fixation in the fungus gardens of leaf-cutter ants. *Science* 326:1120–3.

Projeto R. 1981. Folha SD.22 Goiás. In: *Geologia, geomorfologia, pedologia, vegetação, uso potencial da terra*. Rio de

Janeiro: Ministério das Minas e Energia, Departamento Nacional de Mineral, Fundação.

Quesada CA, Lloyd J, Anderson LO, Fyllas NM, Schwarz M, Czimczik CI. 2011. Soils of Amazonia with particular reference to the RAINFOR sites. *Biogeosciences* 8:1415–40.

Reddy KJ, Gloss SP. 1993. Geochemical speciation as related to the mobility of F, Mo and Se in soil leachates. *Appl Geochem* 8:159–63.

Reed SC, Cleveland CC, Townsend AR. 2007. Controls over leaf litter and soil nitrogen fixation in two lowland tropical rain forests. *Biotropica* 39:585–92.

Reed SC, Cleveland CC, Townsend AR. 2008. Tree species control rates of free-living nitrogen fixation in a tropical rain forest. *Ecology* 89:2924–34.

Reed SC, Cleveland CC, Townsend AR. 2013. Relationships among phosphorus, molybdenum and free-living nitrogen fixation in tropical rain forests: results from observational and experimental analyses. *Biogeochemistry* 114:135–47.

Riskin SH, Neill C, Jankowski K, Krusche AV, McHorney R, Elsenbeer H, Macedo MN, Nunes D, Porder S. 2017. Solute and sediment export from Amazon forest and soybean headwater streams. *Ecol Appl* 27:193–207.

Riskin SH, Porder S, Neill C, Silva Figueira AM, Tubbesing C, Mahowald N. 2013. The fate of phosphorus fertilizer in Amazon soya bean fields. *Philos Trans R Soc B Biol Sci* 368:1–10.

Rocha W, Metcalfe DB, Doughty CE, Brando P, Silvério D, Halladay K, Nepstad DC, Balch JK, Malhi Y. 2014. Ecosystem productivity and carbon cycling in intact and annually burnt forest at the dry southern limit of the Amazon rainforest (Mato Grosso, Brazil). *Plant Ecol Divers* 7:25–40.

Russell AE, Vitousek PM. 1997. Decomposition and potential nitrogen fixation in *Dicranopteris linearis* litter on Mauna Loa, Hawai'i. *J Trop Ecol* 13:579–94.

Sanches L, Valentini CMA, Pinto Júnior OB, Nogueira JS, Vourlitis GL, Biudes MS, da Silva CJ, Bambi P, Lobo FA. 2008. Seasonal and interannual litter dynamics of a tropical semideciduous forest of the southern Amazon Basin, Brazil. *J Geophys Res Biogeosci* 113:1–9.

Schroth G, da Silva LF, Seixas R, Geraldes W, Macêdo JLV, Zech W. 1999. Subsoil accumulation of mineral nitrogen under polyculture and monoculture plantations, fallow and primary forest in a ferrallitic Amazonian upland soil. *Agric Ecosyst Environ* 75:109–20.

Silvério DV, Brando PM, Balch JK, Putz FE, Nepstad DC, Oliveira-Santos C, Bustamante MC. 2013. Testing the Amazon savannization hypothesis: fire effects on invasion of a neotropical forest by native cerrado and exotic pasture grasses. *Philos Trans R Soc B Biol Sci* 368:1–8.

Soper FM, Taylor PG, Wieder WR, Weintraub SR, Cleveland CC, Porder S, Townsend AR. 2017. Modest gaseous nitrogen losses point to conservative nitrogen cycling in a lowland tropical forest watershed. *Ecosystems* 21:901–12.

ter Steege H, Pitman NCA, Phillips OL, Chave J, Sabatier D, Duque A, Molino JF, Prévost MF, Spichiger R, Castellanos H, Von Hildebrand P, Vásquez R. 2006. Continental-scale patterns of canopy tree composition and function across Amazonia. *Nature* 443:444–7.

Sullivan BW, Nifong RL, Nasto MK, Alvarez-Clare S, Dencker C, Soper FM, Shoemaker KT, Ishida FY, Zaragoza-Castells J, Davidson EA, Cleveland CC. 2019. Biogeochemical recuperation of lowland tropical forest during succession. *Ecology* 100:1–14.

Sullivan BW, Smith WK, Townsend AR, Nasto MK, Reed SC, Chazdon RL, Cleveland CC. 2014. Spatially robust estimates of biological nitrogen (N) fixation imply substantial human alteration of the tropical N cycle. *Proc Natl Acad Sci USA* 111:8101–6.

Sylvester-Bradley R, de Oliveira LA, de Podestá Filho JA, St. John TV. 1980. Nodulation of legumes, nitrogenase activity of roots and occurrence of nitrogen-fixing *Azospirillum* spp. in representative soils of central Amazonia. *Agro-Ecosystems* 6:249–66.

Taylor BN, Chazdon RL, Menge DNL. 2019. Successional dynamics of nitrogen fixation and forest growth in regenerating Costa Rican rainforests. *Ecology* 100:1–13.

Taylor BN, Menge DNL. 2018. Light regulates tropical symbiotic nitrogen fixation more strongly than soil nitrogen. *Nat Plants* 4:655–61.

Taylor SR, McLennan SM. 1995. The geochemical evolution of the continental crust. *Rev Geophys* 33:241.

Tierney JA, Hedin LO, Wurzburger N. 2019. Nitrogen fixation does not balance fire-induced nitrogen losses in longleaf pine savannas. *Ecology* 100:1–15.

Townsend AR, Asner GP, Cleveland CC. 2008. The biogeochemical heterogeneity of tropical forests. *Trends Ecol Evol* 23:424–31.

Uhl C, Kauffman JB. 1990. Deforestation, fire susceptibility, and potential tree responses to fire in the eastern Amazon. *Ecology* 71:437–49.

Varella RF, Bustamante MMC, Pinto AS, Kisselle KW, Santos RV, Burke RA, Zepp RG, Viana LT. 2004. Soil fluxes of CO₂, CO, NO, and N₂O from and old pasture and from native savanna in Brazil. *Ecol Appl* 14:S221–31.

Veneklaas EJ. 1991. Litterfall and nutrient fluxes in two montane tropical rain forests. *J Trop Ecol* 7:319–36.

Vitousek P, Hobbie S. 2000. Heterotrophic nitrogen fixation in decomposing litter: patterns and regulation. *Ecology* 81:2366–76.

Vitousek PM. 1984. Litterfall, nutrient cycling, and nutrient limitation in tropical forests. *Ecology* 65:285–98.

Vitousek PM, Howarth RW. 1991. Nitrogen limitation on land and in the sea: how can it occur? *Biogeochemistry* 13:87–115.

Vitousek PM, Sanford RL. 1986. Nutrient cycling in moist tropical forest. *Annu Rev Ecol Syst* 17:137–67.

Winbourne JB, Brewer SW, Houlton BZ. 2017. Iron controls over di-nitrogen fixation in karst tropical forest. *Ecology* 98:773–81.

Winbourne JB, Feng A, Reynolds L, Piotto D, Hastings MG, Porder S. 2018a. Nitrogen cycling during secondary succession in Atlantic Forest of Bahia, Brazil. *Sci Rep* 8:1–9.

Winbourne JB, Harrison MT, Sullivan BW, Alvarez-Clare S, Lins SR, Martinelli L, Nasto M, Piotto D, Rolim S, Wong M, Porder S. 2018b. A new framework for evaluating estimates of symbiotic nitrogen fixation in forests. *Am Nat* 192:618–29.

Wright SJ, Turner BL, Yavitt JB, Harms KE, Kaspari M, Tanner EVJ, Bujan J, Griffin EA, Mayor JR, Pasquini SC, Sheldrake M, Garcia MN. 2018. Plant responses to fertilization experiments in lowland, species-rich, tropical forests. *Ecology* 99:1129–38.

Wurzburger N, Bellenger JP, Kraepiel AML, Hedin LO. 2012. Molybdenum and phosphorus interact to constrain asymbiotic nitrogen fixation in tropical forests. *PLoS ONE* 7:1–7.

Wurzburger N, Hedin LO. 2016. Taxonomic identity determines N₂ fixation by canopy trees across lowland tropical forests. *Ecol Lett* 19:62–70.

Yamada A, Inoue T, Wiwatwitaya D, Ohkuma M, Kudo T, Sugimoto A. 2006. Nitrogen fixation by termites in tropical forests, Thailand. *Ecosystems* 9:75–83.

Yelenik S, Perakis S, Hibbs D. 2013. Regional constraints to biological nitrogen fixation in post-fire forest communities. *Ecology* 94:739–50.