

RESEARCH ARTICLE

Journal of Ecology



Negative trait-based association between abundance of nitrogen-fixing trees and long-term tropical forest biomass accumulation

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Funding information

National Science Foundation, Grant/Award Number: OIA-1632810; Montana Agricultural Experiment Station

Handling Editor: Peter Thrall

Abstract

1. Plant functional traits are thought to drive biomass production and biogeochemical cycling in tropical forests, but it remains unclear how nitrogen (N)-fixing legumes influence the functional traits of neighbouring trees and forest-wide biomass dynamics. Further, the degree to which effects of N-fixers are density-dependent and may depend on stem size and spatial scale remains largely unknown.
2. Here, we examine 30 years of stem demography data for ~20,000 trees in a lowland tropical forest in Trinidad that span a wide range of functional traits thought to drive above-ground biomass (AGB) dynamics.
3. These forests show positive but decreasing long-term net AGB accumulation resulting from constant average productivity but increasing mortality of non-fixing trees over time. We find that high abundance of N-fixing trees is associated with compositional shifts in non-fixer functional traits that confer lower competitive performance and biomass accumulation. Across tree size classes, most interactions between N-fixers and non-fixers were negative, density-dependent, and strongest at smaller spatial scales.
4. *Synthesis.* Overall, our findings suggest that local trait-based interactions between N-fixing and non-fixing trees can influence long-term carbon accumulation in tropical forests.

KEYWORDS

biomass dynamics, competition, functional traits, nitrogen fixers, size structure, spatial scale, tree demography, tropical forest

1 | INTRODUCTION

Tropical forests harbour a great diversity of tree species that vary in functional properties thought to drive biomass dynamics and biogeochemical cycling. Capturing how these traits constrain the response of the tropical forest carbon (C) cycle to disturbance and global change remains a major challenge for ecosystem models (Fisher et al., 2018; McDowell & Xu, 2017). This is especially true for the fundamental class of functional traits that affect how trees acquire growth-limiting nutrients. In many tropical forests, symbiotic

biological nitrogen (N) fixation by legumes (*Fabaceae*) is an important nutrient acquisition strategy that could potentially benefit forest-wide biomass accumulation by supplying new N for tree growth.

Recent findings question the generality of net positive effects of N-fixing legumes on tropical forest dynamics and biomass production. First, legume relative abundance (RA) and diversity vary greatly across the global tropics (Menge et al., 2019). Second, tropical legume species vary in their capacity to fix N and how they regulate N fixation in response to environmental conditions (Batterman, Wurzbarger, et al., 2013; Hedin et al., 2009; Nasto et al., 2017;

Wurzburger & Hedin, 2016). Third, N-fixing legumes can be strong competitors (e.g. for light, water, phosphorus) with neighbouring N-fixing and non-fixing trees (Nasto et al., 2017; Taylor et al., 2017). These factors likely underlie much of the wide range of reported effects of legumes on forest-wide biomass accumulation, varying from net positive (Batterman, Hedin, et al., 2013; Levy-Varon et al., 2019) to neutral (Lai et al., 2018) to net negative (Taylor et al., 2017). What is less clear is how the effects of N-fixers on forest biomass dynamics depend on the distribution of non-fixer functional traits that underpin competitive performance (e.g. height, growth rate, wood density; Kunstler et al., 2016; Rozendaal et al., 2020). Furthermore, little is known about the influence of tree size and density-dependency on such effects—information critical to realistic representation of tropical forest stand dynamics in ecosystem models (Condit et al., 2006; Fisher et al., 2018; McDowell & Xu, 2017; Purves & Pacala, 2008). Finally, several studies have documented that effects of tree diversity on forest AGB production tend to be stronger at smaller (i.e. <0.04-ha) than larger (≥1-ha) spatial grains of observation, presumably due to local niche complementarity or sampling effects (Chisholm et al., 2013; Poorter et al., 2015). Yet, it is unclear how N-fixer abundance may influence tree functional diversity itself and its consequences for biomass production, and how these interactions vary with spatial scale (but see Taylor et al., 2017).

Previous work has shown that N-fixers can influence forest AGB directly by possessing demographic advantages over their non-fixing neighbours (higher growth and survival; Menge & Chazdon, 2016) or indirectly by supplying new N to these neighbours (Brookshire et al., 2019). What remains unresolved from these studies and others is how the net effects of N-fixers on forest-wide AGB dynamics may depend on the functional attributes of the non-fixers with whom they interact. That is, while N fixation is a definable functional trait, 'non-fixer' simply denotes the absence of this ability. In reality, non-fixers comprise a diversity of species possessing a myriad of functional traits, but it remains unclear how this trait variation is influenced by the abundance of N-fixers. One possibility is that N-fixer abundance and non-fixer functional trait distributions simply associate with common resource gradients, indicating little direct effects from high N-fixer RA. Alternatively, N-fixers may filter for or against certain non-fixer traits, directly affecting forest biomass via growth and mortality.

Here, we explore how local trait-based interaction between N-fixing and non-fixing trees organizes landscape-level forest biomass dynamics. To do this, we examine stem inventory data from forests in Trinidad displaying a wide range of legume RA, high rates of individual and ecosystem-level symbiotic N fixation, and long-term net positive biomass accumulation. We take advantage of the fact that N-fixers in these forests are dominated by a single species (74% *Pentaclethra macroloba*, Table S1) that exhibits high levels of N fixation (Brookshire et al., 2019) and has been shown to be a strong resource competitor (Taylor et al., 2017). We first examine long-term trends in forest-wide biomass dynamics. Second, we use local species richness and evenness (how equal the community is numerically) as proxies for non-fixer functional diversity as well as key individual functional traits (wood density, relative growth rates, basal area, height) to analyse how the non-fixer

functional trait spectrum varies across a wide gradient of N-fixer abundance. Third, we investigate the size structure and density-dependence of N-fixer effects on net biomass accumulation in these forests. Finally, we investigate how interactions between N-fixers and non-fixers depend on the spatial scale of observation.

2 | MATERIALS AND METHODS

2.1 | Study area

We examined long-term (1983–2013) demographic data (diameter at breast height [DBH], recruitment and mortality) on 19,780 stems measured periodically (c. every 4 years) across 62 permanent sampling plots (1-ha, hereafter *plot scale*) in lowland rainforests of the Victoria-Mayaro Forest Reserve (VMFR; 540 km²; 25–80 m.a.s.l.; MAT = 27°C; MAP = ~2,000 mm) in Trinidad (Brookshire et al., 2019; Figure 1). Each plot is subdivided into 25 subplots (0.04 ha), for a total of 1,550 subplots (hereafter *neighbourhood scale*). The length of census data varied among plots from 10 to 30 years but did not vary systematically by area across the VMFR. Given natural stem mortality and recruitment, the total number of stems in our analysis varied over time; on average there were ~14,600 stems analysed across our plots in a given year. The plots contain >100 verified tree species, six of which are legumes known to fix N (Table S1).

The VMFR network of 1-ha stem inventory plots was established between 1983 and 1985 across forests that have never been logged ($n = 25$) and those potentially subject to selective cutting ($n = 37$) within a Periodic Block System (PBS) implemented in the early 1960s. In the PBS, forest blocks (~150–300 ha) are rotationally opened to selective logging (2–4 trees per ha) every ~30 years. Although the cutting and associated disturbance history of individual 1-ha plots (<3% of PBS area) are uncertain in our dataset, comparison of stem biomass and demographics revealed no long-term differences in AGB, wood density, stem growth or mortality rates between PBS and unharvested plots (Figure S1). However, plots within the PBS on average have higher total tree densities (Figure S1) and N-fixer RA compared to unharvested forests (Figure S2). We therefore account for this potential disturbance by including *Harvest* and *Plot* identity in all statistical models and normalized for stem density to isolate biomass-specific effects of N-fixers.

2.2 | Demography and biomass dynamics

We derived AGB from DBH using an allometric conversion from Colombian forests following Alvarez et al., (2012) as:

$$\ln(\text{AGB}) = 1.662 - 1.114 \ln(\text{DBH}) + 1.169 (\ln(\text{DBH}))^2 - 0.122 (\ln(\text{DBH}))^3 + 0.331 \ln(\rho), \quad (1)$$

where AGB is in kg, DBH is in cm and ρ is wood density in g/m³. Wood density was acquired from the Global Wood Density Database

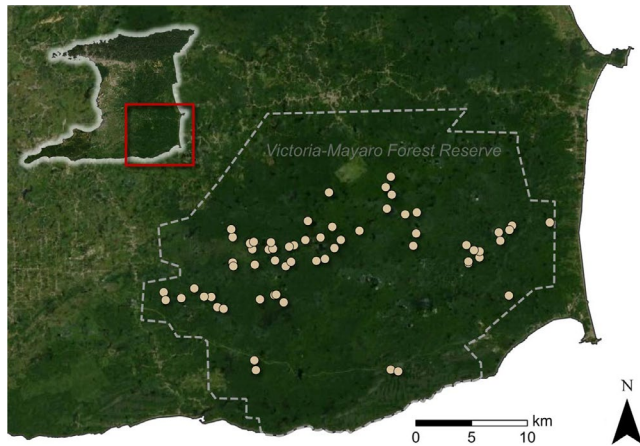


FIGURE 1 Map of the Victoria-Mayaro Forest Reserve in Southeastern Trinidad. The 62 1-ha plots are represented by white dots

(Zanne et al., 2009). We used species-specific wood density when available (76% of stems) and otherwise used the mean genus-level (22%), mean family-level (0.5%) or, in a few cases (1.5%), the South American tropical tree average wood density. The dataset was corrected for point of measurement movement between censuses, unreasonable growth, skipped censuses and measurement around basal buttressing following Brookshire et al. (2019). We accounted for the increasing probability of unmeasured recruitment and recruitment mortality with increasing census interval length by multiplying the average recruitment rate and mortality rate by the minimum-diameter biomass estimate and interval length following Brien et al. (2015). Because diameter measurements were restricted to stems >10 cm, and most AGB is comprised of stems above this diameter, we included recruits in plot-level biomass dynamics. All trees were placed into one of three different DBH size classification bins: <30, 30–60 and >60 cm. The smallest threshold of 30 cm was chosen to capture the smallest of measured stems in the VMFR, as well as a few stems that were first measured past the 20 cm DBH mark. This occurred occasionally when time-between-census intervals was long, and the tree grew >20 cm after the plot had been measured. Stems in this bin make up ~66% of all stems in the VMFR but ~18% of biomass. The upper threshold was chosen as it represents two standard deviations from the mean stem size and this bin represents ~6% of stems but ~35% of total biomass. The middle diameter bin therefore comprises ~28% of stems and almost half (~47%) of biomass.

We calculated positive AGB change (hereafter *productivity*) as the difference between two census intervals divided by the length of the interval. Biomass mortality is the sum of all stems that did not reappear in a following census interval and divided by the census interval length. Finally, net AGB change was calculated as productivity minus biomass mortality. Both productivity and biomass mortality were log-transformed ($\log_e$) before statistical analyses models due to right skew. We used a mixed-effects modelling approach to account for correlation of measurements taken from the same subplot and across time. This approach allows for the conservation of the independence assumption of ordinary least squares linear regression. For

all models, we allowed the intercept to vary at random but kept the slope fixed, as this permits us to account for unmeasured variation across all groups (LME4 package in R; Bates et al., 2015; Kuznetsova et al., 2017). Biomass dynamics models at the plot and neighbourhood scales incorporated *Year* as the independent variable and *Plot* or *Plot/Subplot* nested within *Harvest* as the random effects:

$$Y = \text{Year} + (1|\text{Harvest/Plot/Subplot}), \quad (2)$$

where *Y* is the response variable (productivity, biomass mortality, or net AGB). Equation 2 calculates biomass dynamics at the neighbourhood scale, whereas the model for plot scale dynamics would lack *Subplot* as a nested effect (Equation S1). Nested random effects in mixed models allowed us to isolate the N-fixer effect size from potentially confounding effects of unmeasured site variability (e.g. edaphic characteristics) or disturbance history.

2.3 | Effects of N-fixers on functional traits

We leveraged the wide gradients of N-fixer RA at both scales to explore associations with various functional traits; namely height, BA, wood density, relative growth rate (RGR), species richness, species evenness and mortality rate. RGR was calculated as the difference between the $\log_e$ of two DBH measurements from two time intervals then divided by the interval duration. Species evenness was calculated using the Shannon evenness index. Height, BA, wood density and RGR were analysed on a per-stem basis (e.g. average stem wood density across all individual N-fixers and non-fixers within a subplot or plot) whereas richness, evenness and mortality rate were calculated at the community level (e.g. species richness per 0.04-ha or per 1-ha). Tree height data were restricted to 98 subplots ($n = 731$ trees) from one census in 1983. For direct comparison of effect size of all functional trait associations, all traits were scaled before regression (*z-score*; *scale* function in R). Similarly, because the N-fixer gradients at the plot and neighbourhood scales are different (0%–43% RA vs. 0%–97% RA), we scaled each gradient between zero (low N-fixer RA) and one (high N-fixer RA) for all trait analyses. For all analyses, we averaged traits across all years censused and used mixed-effects models with *Plot* or *Plot/Subplot* with *Harvest* as nested random effects:

$$Y = \text{RA}_{\text{Nfixer}} + (1|\text{Harvest/Plot/Subplot}), \quad (3)$$

where $\text{RA}_{\text{Nfixer}}$ is the relative abundance of N-fixers and *Y* is the functional trait of interest. Equation 3 displays the model for the neighbourhood scale, whereas at the plot scale, *Subplot* would be absent from the nested random effect (Equation S2).

2.4 | Effects of N-fixers on biomass dynamics

To examine the effects N-fixers on forest biomass dynamics, we evaluated linear slope coefficient estimates using maximum

likelihood within linear mixed-effects models. Following Equation 2, we examined how productivity, mortality and net AGB (dependent variables) varied across the gradient of N-fixer RA (independent variable) at both spatial scales. In this case, for each spatial scale and tree functional type (N-fixer vs. non-fixer) all dependent variables were scaled (z-score) before regression for direct comparison. We estimated effects of N-fixer RA on biomass dynamics by first calculating slope coefficients (i.e. effect size) without correcting for stem density. Next, to separate biomass- versus density-specific effects of N-fixers, we removed the effect of density from our results by using non-parametric LOESS regression (*loess* function in R, *span* = 1, *degree* = 2) of each biomass dynamic regressed against density (e.g. productivity ~ stem density; see Figure S4 and Supporting Information for further explanation). The residuals of the LOESS regression were then substituted in as the dependent variable and the intercept term was removed (as residuals have an intercept of 0) following Chisholm et al. (2013). This 'density-normalization' allowed us to examine whether N-fixer effects were due to local stem density or biomass-specific attributes of the N-fixers themselves. Finally, to account for effects of stem size structure, stem diameter-size bin was included in all models as an interaction with the independent variable (e.g. *N-fixer RA* × *bin*). Similar to our statistical frameworks used above, nested random effects were added to isolate the N-fixer effect size from potentially confounding effects of unmeasured site variability. Here we also account for site-wide changes across time by including *Year* as a crossed random effect:

$$Y = \text{RA}_{\text{Nfixer}} + \text{Bin} + \text{RA}_{\text{Nfixer}} : \text{Bin} + (1|\text{Harvest/Plot/Subplot}) + (1|\text{Year}). \quad (4)$$

As with the previous equations, *Subplot* is absent in the plot scale model (Equation S3). For further explanation of nested versus crossed random effects in the models, see Figure S3 and related Supporting Information.

3 | RESULTS

3.1 | Long-term forest biomass dynamics

We document long-term increases in total AGB of $2.51 \pm 0.23 \text{ Mg ha}^{-1} \text{ year}^{-1}$ over the 30-year study period regardless of potential harvest history ($p < 0.0001$; Figure S5a). This change parallels a $0.19 \pm 0.023 \text{ m}^2 \text{ ha}^{-1} \text{ year}^{-1}$ increase in BA ($p < 0.0001$; Figure S5b), despite stem density declining by $\sim 1.5 \pm 0.26 \text{ stems ha}^{-1} \text{ year}^{-1}$ over the study period ($p < 0.0001$; Figure S5c). The sign and magnitude of plot-level changes were largely mirrored at the neighbourhood scale, with the exception of productivity which did not change at the plot scale ($p = 0.237$), but declined slightly at the neighbourhood scale ($-0.005 \pm 0.003 \text{ Mg ha}^{-1} \text{ year}^{-2}$; $p = 0.0045$; Figure 2a). In contrast, we document significant increases in biomass mortality of $0.09 \pm 0.04 \text{ Mg ha}^{-1} \text{ year}^{-2}$ at both plot and neighbourhood scales ($p < 0.0001$) scales (Figure 2b). Consequently, net AGB accumulation

declined over the 30-year period by $-0.098 \pm 0.25 \text{ Mg ha}^{-1} \text{ year}^{-2}$ (plot: $p < 0.0001$, subplot: $p < 0.0001$; Figure 2c). Across all plots, long-term total AGB was dominated by non-fixing trees (on average $229.0 \pm 103.2 \text{ Mg/ha}$ vs. $40.5 \pm 27.8 \text{ Mg/ha}$ for N-fixers) and was negatively associated with the RA of N-fixing trees, decreasing by an average of 3.2 Mg/ha at the plot scale ($p < 0.0001$) and $0.1 \text{ Mg } 0.04\text{-ha}^{-1}$ at the neighbourhood scale ($p < 0.0001$) for every 1% increase in the RA of N-fixers.

3.2 | Functional trait associations with increasing N-fixer abundance

We found support for the hypothesis that non-fixer trait distributions are associated with the RA of N-fixers and that these effects are strongest at local scales. First, we find that non-fixer species richness decreases with N-fixer RA at the neighbourhood scale ($p < 0.0001$; Figure 3c) but not the plot scale ($p = 0.446$), while non-fixer species evenness showed no relationship at either scale (plot: $p = 0.182$; subplot: $p = 0.213$; Figure 3d). Second, we find that non-fixer community-level wood density declines strongly along the N-fixer RA gradient, with decreases of 8.6% at the neighbourhood scale and 23.2% at the plot scales ($p < 0.0001$; Figure 3e). This corresponds with a 34.2% increase in stem RGR with increasing N-fixer RA at the neighbourhood scale ($p < 0.0001$; Figure 3f), although evidence was weak at the plot level ($p = 0.069$). Further, we find

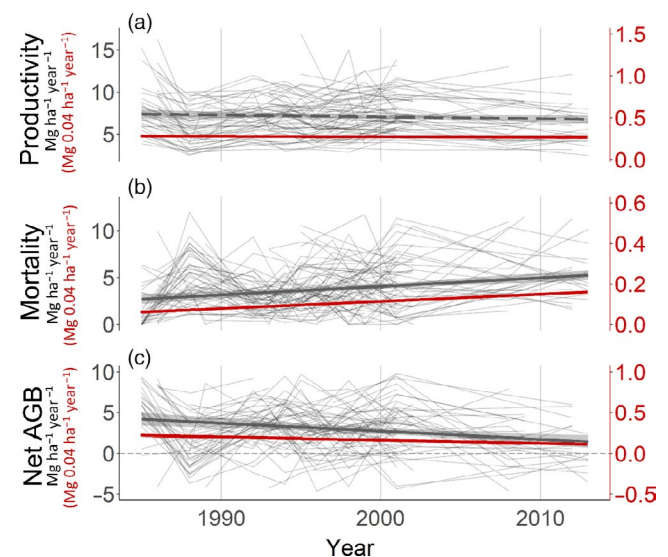


FIGURE 2 VMFR dynamics over time. Grey lines indicate dynamics at the one-hectare plot scale ($n = 62$) with red lines indicating dynamics at the 0.04-ha neighbourhood scale ($n = 1,550$). Thin background lines are individual plot changes over time. Solid lines indicate significant ($p < 0.05$) relationships as determined by linear mixed-effects models. (a) Productivity shows no significant change at the plot scale but significant decreases at the subplot scale. (b) Biomass mortality increases significantly at both scales and (c) net AGB accumulation decreases significantly at both scales

that at the neighbourhood scale, non-fixers tended to be ~2 m taller on average, (Figure S6) and that non-fixer height increased with higher N-fixer RA (plot: $p = 0.043$; subplot: $p = 0.0085$, Figure 3g).

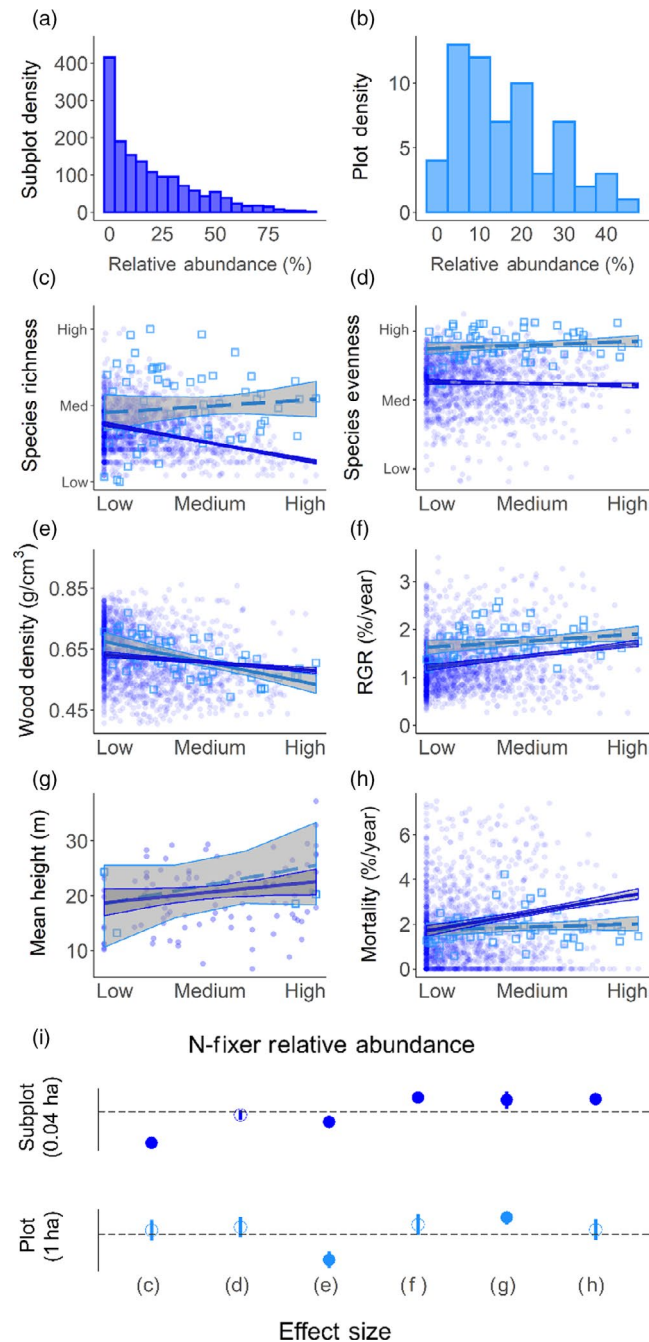


FIGURE 3 Non-fixer traits across a gradient of N-fixers at the neighbourhood (dark blue, 0.04-ha) and plot (light blue, 1-ha) scale. (a) Distribution of N-fixers at the neighbourhood scale range from zero to 80% of basal area whereas (b) distribution of N-fixers at the plot scale range from zero to 45% of basal area. Because N-fixers span different ranges at the different scales, N-fixer gradients were scaled between 0 (low) and 1 (high) for comparison. (c) N-fixer effects on relativized species richness (d) and species evenness. (e) N-fixer influence on woody density, (f) RGR, (g) average canopy height, and (h) percent mortality. For direct comparison, (i) we scaled each variable and compared slope coefficients and the neighbourhood and plot scale

Finally, we document a 65% increase in non-fixer mortality at the neighbourhood scale across the gradient of N-fixer RA ($p < 0.0001$, Figure 3h), but no relationship at the plot scale ($p = 0.39$). Thus, at smaller spatial grains, we find strong evidence that increasing N-fixer RA is associated with lower wood density, faster growing, taller and higher-mortality non-fixers.

We next examined how increasing N-fixer abundance influenced their own functional trait distributions (Figure 4). Most significant associations were evident only at the neighbourhood scale. First, we find that N-fixer species richness increases with increasing N-fixer RA ($p < 0.0001$, Figure 4b), although the effect size was small. Second, we find that N-fixer stem mortality increases

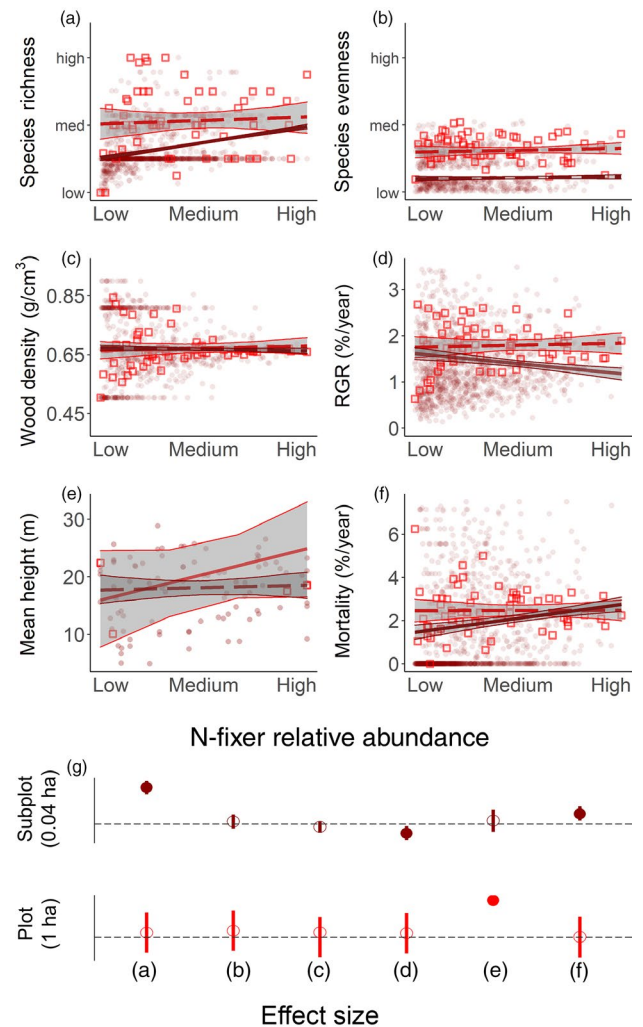


FIGURE 4 N-fixer traits across a gradient of N-fixers at the neighbourhood (dark red) and plot (light red) scale. Solid lines indicate significant ($p < 0.05$) relationships. Abundance is mapped from low to high by scaling the relative abundance of N-fixers at the plot (range: 0%–43%) and subplot (0%–97%) scale. (a) N-fixer effects on relativized species richness (b) and species evenness. (c) N-fixer influence on woody density, (d) RGR, (e) average canopy height, and (f) percent mortality. For direct comparison, (g) we scaled each variable and compared slope coefficients at the neighbourhood and plot scale

($p = 0.0045$, Figure 4d) while RGR declines with increasing N-fixer RA ($p = 0.011$, Figure 4f), suggesting higher intraspecific competition with increasing N-fixer abundance. Finally, N-fixer mean height increased slightly with increasing RA at the plot scale ($p = 0.0143$, Figure 4e).

3.3 | Effects of N-fixers on forest biomass accumulation

Our analysis revealed strong associations between N-fixer RA and AGB dynamics, but that density-dependent effects prevail (i.e. the strongest effects depend on N-fixer stem density rather than N-fixer biomass) across tree sizes and spatial scales. Although the effect of increasing N-fixer stem density on N-fixer and non-fixer biomass is expected numerically, the existence of density-normalized effects indicates biomass-specific interactions. Further, we find that all biomass-specific effects are evident only at neighbourhood scales (Figure 5a,c). We show that increasing N-fixer RA is significantly negatively associated with non-fixer biomass (decreased productivity and increased mortality; Figure 5a) and positively associated with N-fixer biomass (increased productivity and decreased mortality; Figure 5c), with the strongest effects on the largest stems. However, for the largest N-fixers (>60 cm), there was never more than one per subplot, thus the density-normalized versus unnormalized results were the same. Overall, we find positive density-normalized

effects of N-fixers on N-fixer biomass and negative effects on non-fixer biomass.

4 | DISCUSSION

Our results complement the growing body of research on the functional roles of N-fixing legumes in tropical forests. By leveraging long-term stem demographic data distributed across a wide range of N-fixer stem density in a common landscape, our study uniquely offers an examination of how trait-based trade-offs among non-fixing neighbours affect long-term biomass dynamics and the scale at which these effects emerge.

Forests of the VMFR show positive but decreasing net AGB accumulation over the last 30 years, consistent with findings across Amazonia (Brienen et al., 2015). We show that this results from constant average productivity but increasing mortality of non-fixers over time. While the ultimate drivers of this decline remain uncertain for our forests, our findings suggest that local interactions between N-fixing and non-fixing trees may influence this trend. Despite evidence that symbiotic N fixation can facilitate tree growth in tropical forests (Batterman, Hedin, et al., 2013; Levy-Varon et al., 2019), we find that high RA of N-fixing trees across our 62 plots is associated with compositional shifts in non-fixer functional traits that confer lower competitive performance and biomass accumulation. Across tree size classes, most interactions

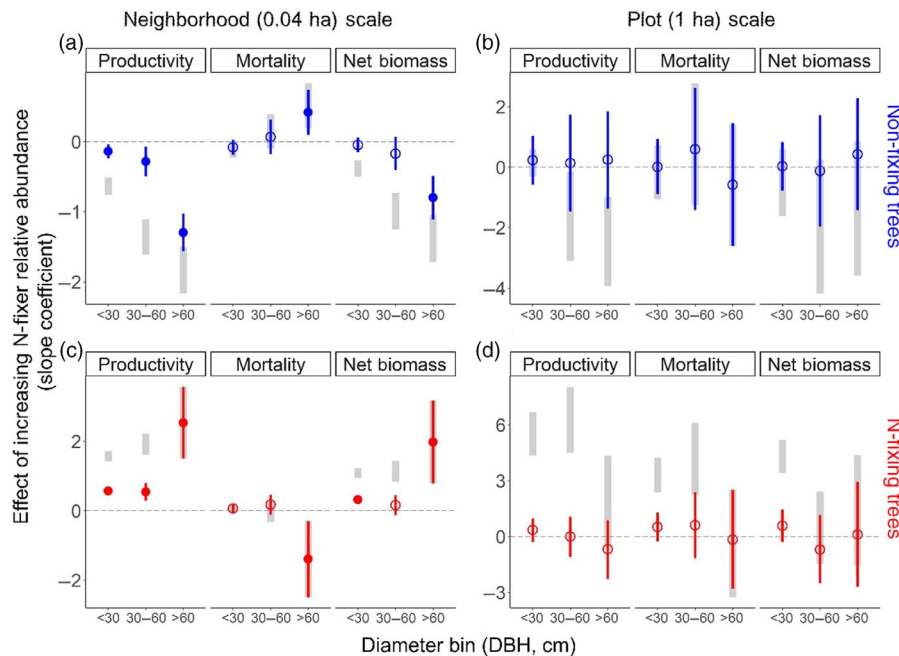


FIGURE 5 Effect of N-fixers on forest biomass dynamics at the neighbourhood and plot scales across three diameter class sizes. The effect size is the slope coefficient from a mixed-effects model where N-fixer relative basal area is the independent variable and the normalized biomass dynamic is the dependent variable which can be negative, positive or neutral. Grey bars indicate the analysis run without correcting for N-fixer stem density, whereas the coloured circles and accompanying line (95% confidence interval) have been adjusted for density effects. Circles below the zero line are negatively associated with N-fixer abundance and circles above the line display a positive association. Open circles on the line indicate no significant relationship. (a) N-fixers effects on the non-fixers at the neighbourhood scale and (b) plot scale. (c) N-fixer effects on N-fixers at the neighbourhood scale and (d) plot scale

between N-fixers and non-fixers were negative, density-dependent and strongest at smaller spatial scales. At both spatial scales, higher densities of N-fixers tended to promote their own biomass production at the expense of non-fixers, suggesting that competitive effects may outweigh the benefits of high N fixation on net biomass accumulation.

We identify that shifts in two 'fast-slow' demographic trade-offs underpin tree dynamics as N-fixer abundance increases—growth versus survival and construction cost versus longevity (Reich, 2014). Across our plots, species richness of non-fixers declines with increasing N-fixer RA. Although this pattern is expected numerically, it also implies lower non-fixer functional diversity. We show that this is associated with a directional shift toward 'fast' species that have lower wood density (i.e. lower construction cost), grow faster and turnover more rapidly (i.e. lower longevity/survival). Although these traits suggest higher productivity, they also imply a lower tolerance of competition and low competitive effect on neighbours (Kunstler et al., 2016; Reich, 2014; Rozendaal et al., 2020; Rüger et al., 2020; Wright et al., 2010). Notably, while non-fixing trees are on average ~2 m taller than N-fixers across our plots, we also find that their mean height increases with increasing N-fixer RA. We hypothesize that this reflects trait coordination across the growth-survival trade-off with increasing N-fixer competition, i.e. fast growing non-fixer species attain canopy dominance in competition for light but do not persist. That non-fixer species evenness does not change with increasing N-fixer RA indicates that these patterns are not driven by a consistent dominance by a handful of species, but rather a coherent shift in functional trait composition (towards faster growth and lower survival). In addition to competitive effects on non-fixers, the fact that N-fixer productivity and survival decline with increasing N-fixer RA at the neighbourhood scale also supports the existence of strong 'intra-specific' competition between N-fixing trees.

Our results suggest that N-fixer effects on the trait space of neighbouring non-fixers depend strongly on spatial scale. Many effects of N-fixer RA on non-fixer AGB dynamics at the neighbourhood scale were due to increases in N-fixer biomass (as measured by BA) rather than N-fixer stem densities, while effects at the plot scale were entirely density-dependent. These findings support results from biodiversity-function studies showing that species richness generally has the strongest effects on biomass production at smaller spatial grains (Chisholm et al., 2013; Poorter et al., 2015). This is expected due to stronger complementarity and sampling effects at local scales and pattern dampening at larger aggregate scales (Chisholm et al., 2013). In our case, however, rather than examining productivity as a function of species richness or diversity, we instead examined it across a gradient of increasing dominance of one functional group—N-fixers. We justify this functional grouping by the fact that the N-fixer community is relatively species poor and is dominated by *Pentaclethra*. This species exhibits high individual-level N fixation (Brookshire et al., 2019; Taylor et al., 2019), potentially one mechanism by which the species could effectively compete with neighbouring non-fixers if their growth is limited by N availability. We have

previously documented relatively high plot-level N fixation in these forests at levels *sufficient* to supply much of net AGB production, but it remains unclear if these N inputs are *necessary* to maintain long-term positive net AGB accumulation (Brookshire et al., 2019). Our results here suggest that documented negative effects of competition may outweigh facilitative effects of N supply. It is also possible that N-fixers place high demands on resources such as water, phosphorus and light (Taylor et al., 2017) and thereby inhibit growth of neighbouring non-fixers, a pattern consistent with higher density-normalized N-fixer biomass production with higher N-fixer RA at the neighbourhood scale.

Negative effects of increasing N-fixer abundance on non-fixer productivity, survival and net AGB accumulation were strongest at the neighbourhood scale and most were most pronounced for the largest stems. This pattern contrasts with the finding that trait-based interactions are most intense at the sapling stage (Wright et al., 2010). While we lack data for stems <10 cm necessary to explore such interactions, we attribute this difference to the fact that non-fixers are on average larger and more productive than N-fixers (Brookshire et al., 2019), and dominate total forest AGB. As such, forest biomass declines with increasing N-fixer RA, especially for the largest and most productive non-fixing stems. This leads to lower marginal AGB gains by non-fixers, despite neighbourhood-level increases in non-fixer RGR with increasing N-fixer abundance.

Our work is most directly comparable to recent work in Costa Rican forests (e.g. Menge & Chazdon, 2016; Taylor et al., 2017, 2019). These forests display a wide range of N-fixer RA and the N-fixer community is also dominated by *Pentaclethra*, although in contrast to Trinidad, *Pentaclethra* in Costa Rica is a canopy-dominant species. This key difference may partially explain why N-fixing trees in Costa Rican forests display strong demographic advantages (higher growth and survival) over neighbouring non-fixing trees. However, with increased crowding of N-fixers, these advantages were offset by strong competitive effects on their neighbours, resulting in a net negative effect on forest-wide AGB accumulation (Taylor et al., 2017). Our work in Trinidadian forests largely supports net negative effects of N-fixer crowding but uniquely provides an alternative hypothesis that shifts in non-fixer functional traits may underlie diminished net forest AGB accumulation with increasing N-fixer RA.

It is possible that some spatial associations of N-fixer and non-fixer demographics and biomass dynamics partly track unknown environmental gradients (e.g. disturbance history and soil nutrient availability). In particular, several of our documented responses of non-fixing trees to increasing N-fixer RA (e.g. lower wood density, faster growth) could be attributed to direct effects of cutting (e.g. increased light availability) rather than N-fixers themselves. Indeed, such trait-based shifts and their competitive consequences are common during forest succession (Kunstler et al., 2016). In addition, given high rates of N fixation across the VMFR (Brookshire et al., 2019) and observed higher N-fixer RA in plots exposed to selective harvest, it is also possible that enhanced N supply itself (or another nutrient such as phosphorus)

could promote the shift toward faster growth and lower survival among neighbouring trees, consistent with predictions of nutrient supply on plant trait distributions (Reich, 2014).

While we cannot entirely exclude these possibilities, we find no evidence that non-fixer functional responses differ systematically between harvested and unharvested areas. All tests of N-fixer RA on functional traits and biomass dynamics remained strong after accounting for *Harvest*, *Plot* and *Subplot* as random effects in statistical models. Further, although N-fixer effects on soil fertility could potentially explain some of our results, our previous work in the VMFR showed that soil N availability did not vary with N fixation (Brookshire et al., 2019) and recent findings from *Pentaclethra*-dominated Costa Rican forests indicate that N fixation actually decreases with increasing N-fixer RA (Taylor et al., 2019). Finally, that the strongest effects of N-fixers occurred at small spatial scales suggests that net negative effects of N-fixer RA on non-fixer biomass dynamics emerge from local competitive interactions regardless of disturbance history.

Our evidence for coherent spatial shifts in tree functional traits in response to N-fixer abundance suggests that local trait-based interactions between N-fixing and non-fixing trees can influence long-term C accumulation in tropical forests. As the sign and strength of tree interactions and their effects on biomass dynamics depended strongly on spatial scale, stem size structure and stem density, our results have important implications for how tropical N-fixers are represented in the next generation of trait-based dynamic vegetation models.

ACKNOWLEDGEMENTS

We would like to thank Carlton Roberts and the Trinidadian Forestry Division for sharing decades of forest inventory data. We thank two anonymous reviewers for comments that improved this manuscript. We also thank Hannah Duff, Justin Gay, Sasha Loewen and Shealyn Malone for useful comments on early drafts of the manuscript. Funding to E.N.J.B. came from the Montana Agricultural Experiment Station and the US National Science Foundation award OIA-1632810.

AUTHORS' CONTRIBUTIONS

B.C. and E.N.J.B. designed the study; M.P.O. acquired and provided the data; B.C. analysed the data; B.C. and E.N.J.B. led the writing with critical contributions from M.P.O.

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/1365-2745.13524>.

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository <https://doi.org/10.5061/dryad.jsxksn07j> (Currey et al., 2020).

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How to cite this article: Currey B, Oatham MP, Brookshire ENJ.

Negative trait-based association between abundance of nitrogen-fixing trees and long-term tropical forest biomass accumulation. *J Ecol*. 2021;109:966–974. <https://doi.org/10.1111/1365-2745.13524>