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RESEARCH ARTICLE

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# Nitrogen fixer abundance has no effect on biomass recovery during tropical secondary forest succession

**Hao Ran Lai**[1,2] | **Jefferson S. Hall**[3] | **Sarah A. Batterman**[4,5] | **Benjamin L. Turner**[5] | **Michiel van Breugel**[1,2,3]

[1]Yale–NUS College, Singapore

[2]Department of Biological Sciences, National University of Singapore, Singapore

[3]ForestGEO, Smithsonian Tropical Research Institute, Panama, Republic of Panama

[4]School of Geography and Priestley International Centre for Climate, University of Leeds, Leeds, UK

[5]Smithsonian Tropical Research Institute, Panama, Republic of Panama

**Correspondence**
Michiel van Breugel
Email: mvbreugel@gmail.com

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## Abstract

1. Nitrogen-fixing trees ($N_2$ fixers) provide new nitrogen critical for rapid biomass accumulation of tropical forests during early secondary succession, but it remains unclear how the abundance of $N_2$ fixers in the forest community affects the growth of non-fixers or the primary productivity of the whole forest.
2. On the one hand, $N_2$ fixers may enhance forest productivity by providing a facilitative effect through the provision of plant-available nitrogen to non-fixing trees. On the other hand, $N_2$ fixers may suppress the growth of non-fixers by growing faster and competing more vigorously for light and other resources. A third alternative is that the growth of $N_2$ fixers themselves accumulate biomass rapidly, while having a neutral effect on non-fixers, leading to an overall increase in forest biomass.
3. We examine these alternative hypotheses using 5-year tree census data from 88 plots in 44 seasonal tropical moist secondary forests (3–32 years old) across a human-modified landscape in central Panama. We examined whether $N_2$ fixers accumulated biomass more rapidly than non-fixers, and how relative biomass of $N_2$ fixers as a functional group and as individual species influenced the growth of non-fixer and whole stand primary productivity.
4. Surprisingly, we found no evidence for either a net competitive or a facilitative effect of $N_2$ fixers as a functional group or individual species on the biomass recovery in these young forests. $N_2$ fixers did not grow faster than non-fixers. Individual mortality rates were lower among $N_2$ fixers, but biomass losses due to mortality were similar between the two groups. Overall, we found no relationship between the relative abundance of $N_2$ fixers and stand primary productivity during succession.
5. *Synthesis*. Nitrogen-fixing trees may be critical for reducing nitrogen limitation and accelerating biomass growth during tropical secondary forest succession, thereby impacting the global carbon cycle. However, our findings indicate that, in early successional seasonal tropical moist forests, nitrogen fixers provide neither a net competitive nor a facilitative effect on non-fixing trees or the whole forest stand, likely because tropical nitrogen fixers utilize facultative fixation and hence abundance poorly approximates the ecosystem function of fixation. Our results

indicate that models should not simply scale symbiotic fixation and its effects from nitrogen-fixing tree abundance.

**KEYWORDS**
biomass dynamics, carbon sequestration, competition, facilitation, nitrogen fixation, plant–plant interactions, secondary forest succession, tropical forests

## 1 | INTRODUCTION

Regrowing tropical forests on abandoned agricultural lands are potentially major carbon sinks that mitigate carbon emissions from deforestation and land degradation (Chazdon et al., 2016; Pan et al., 2011; Poorter et al., 2016; Yang, Richardson, & Jain, 2010). Yet, the net primary production and carbon sequestration of tropical forests may be constrained by nitrogen availability (LeBauer & Treseder, 2008; Wright et al., 2011; Yang et al., 2010). During early secondary forest succession, nitrogen deficiencies may be high due to the loss of biomass during deforestation and soil nutrient depletion during previous land use (Amazonas, Martinelli, Piccolo, & Rodrigues, 2011; Davidson et al., 2007; Erickson, Keller, & Davidson, 2001; Groppo et al., 2015; Powers, 2004), or the high demand for nitrogen driven by rapid rates of forest growth (Batterman, Hedin, et al., 2013; Russell & Raich, 2012).

Trees capable of symbiotic dinitrogen ($N_2$) fixation ($N_2$ fixers) offer a mechanism to overcome nitrogen constraints on tropical forest carbon uptake by converting large quantities of atmospheric nitrogen into usable forms for plants. This new nitrogen would accelerate carbon accumulation during tropical forest secondary succession, enhancing the carbon sink in tropical forests and, ultimately, influencing the global carbon cycle by offsetting anthropogenic carbon emissions (Levy-Baron et al., in review).

The abundance of $N_2$ fixers varies widely across mature tropical forests (Hedin, Brookshire, Menge, & Barron, 2009; Menge, Lichstein, & Ángeles-Pérez, 2014; Sprent, 2009; ter Steege et al., 2006). Very few studies have assessed successional trends in the (relative) abundance and biomass of $N_2$ fixers in regenerating forests. Chronosequence studies in Costa Rica and Brazil suggest that the relative abundance or basal area of $N_2$ fixers increases during the first stages of succession (Gehring, Muniz, & Gomes de Souza, 2008; Gehring, Vlek, de Souza, & Denich, 2005; Menge & Chazdon, 2016; Sullivan et al., 2014), while the relative basal area of $N_2$ fixers in mature forests may be lower (Gehring et al., 2008) or higher compared with secondary forests (Menge & Chazdon, 2016; Sullivan et al., 2014). In Panama, $N_2$ fixers peak in abundance in the first few decades of succession, but remain present as a significant fraction of the community as forests mature (Batterman, Hedin, et al., 2013). Yet despite the large variation in fixer abundance and the clear importance of symbiotic $N_2$ fixation for tropical forest biomass accumulation, it remains unclear how the abundance of $N_2$-fixing trees influences the productivity of non-fixing trees and the whole forest stand.

On the one hand, one hypothesis holds that $N_2$ fixers increase forest productivity through a facilitative effect because of their ability to supply new nitrogen (Jenny, 1950). A higher abundance of $N_2$ fixers may equate to higher ecosystem levels of $N_2$ fixation, which would enhance productivity when nitrogen is limited by providing previously unavailable, newly fixed, atmospheric nitrogen to non-fixing trees in the community through the recycling of leaf litter and fine roots (Cleveland et al., 1999; Jenny, 1950; Russell & Raich, 2012; Vitousek, 1984; Vitousek et al., 2002). The observation from Panama that $N_2$-fixing trees up-regulate fixation via a carbon accumulation–nitrogen fixation feedback mechanism and supply over 50% of the nitrogen needed to support the first few decades of tropical forest biomass recovery (Batterman, Hedin, et al., 2013) suggests that fixation may provide such a facilitative effect on forest growth. If $N_2$ fixers do hold a net facilitative role, then we would expect to find a positive association between the abundance of $N_2$ fixers and the growth of other trees and the whole forest stand.

On the other hand, under nitrogen-limited conditions, access to fixed nitrogen may enhance the ability of fixers to grow and access other resources when compared to non-fixers. This may grant $N_2$ fixers a competitive advantage over non-fixers and inhibit their growth (Gehring et al., 2005), especially because competition for light is size-asymmetric (van Breugel, van Breugel, Jansen, Martínez-Ramos, & Bongers, 2012). Since non-fixers dominate tropical secondary forests in terms of biomass (Gei et al., in review), any reduction in the growth of non-fixers would subsequently reduce overall forest productivity. In support of this second hypothesis, $N_2$ fixers have been observed to have higher growth and survival rates relative to non-fixers at the early stages of secondary succession (Batterman, Hedin, et al., 2013; Menge & Chazdon, 2016) and to inhibit growth of neighbouring trees in one wet tropical site (Taylor, Chazdon, Bachelot, & Menge, 2017). If this net competition hypothesis were true, then we would expect to find a negative relationship between the abundance of $N_2$ fixers and the growth of non-fixers.

In reality, the effect of fixer abundance will likely integrate both facilitative and competitive effects. The net effect on non-fixer and whole forest growth will depend on the degree to which the benefit of the extra soil-available nitrogen is offset by competition of $N_2$ fixers for other limiting resources (Gehring et al., 2005). In other words, the net effect of $N_2$ fixers on non-fixer and forest growth depends on the relative strength of the competitive effects and facilitative effects of $N_2$ fixers. This can lead to various combinations of patterns that contrast and link growth of $N_2$ fixers and non-fixers: (1) If facilitation outweighs competition, we expect $N_2$ fixers to grow faster than non-fixers *and* a positive association between $N_2$ fixer relative abundance and growth of non-fixers and the whole stand. (2) If the effects of facilitation and competition are comparable, we expect no association between the relative abundance of $N_2$ fixers and non-fixer growth. At the same time, the "extra" growth of $N_2$ fixers themselves would lead to a positive net effect of the relative abundance of $N_2$ fixers on whole stand growth. (3) Finally, if competition outweighs facilitation, the net effect on whole stand growth depends on the balance between the "extra" growth of $N_2$ fixers, and their net-negative effect on non-fixer growth.

This balance between facilitative and competitive effects of $N_2$ fixers has been observed in agroforestry systems. $N_2$ fixers are often included in agroforestry systems under the assumption that $N_2$ fixers will supply the non-fixing focal crops with additional nitrogen. Most of these systems require the pruning and mulching of $N_2$ fixer leaves and branches to make the fixed nitrogen available to the focal crops (i.e. facilitative effect; Forrester, Bauhus, Cowie, & Vanclay, 2006; Young, 1997). However, such pruning also serves to reduce competition for light and soil resources (i.e. reducing the competitive effect; Beer, Muschler, Kass, & Somarriba, 1998; Nichols, Rosemeyer, Carpenter, & Kettler, 2001; Russo, 2005). Without such control measures, competition by $N_2$-fixing trees for common resources could be stronger than their facilitative effects of supplying newly fixed nitrogen (Boyden, Binkley, & Senock, 2005; Schroth, Lehman, Rodrigues, Barros, & Macedo, 2001). In natural systems, the net outcome of both facilitative and competitive effects remains unclear.

Here, we examine the hypotheses that $N_2$ fixers facilitate stand-level biomass accumulation and/or limit forest growth through competition using 5-year annual census data of 88 plots in 44 young secondary forest sites established across a tropical seasonal moist human-modified landscape in the Agua Salud Project, Panama. First, we evaluated whether the growth and mortality of $N_2$ fixers differed from other trees in order to identify whether $N_2$ fixers possessed any competitive advantage over non-fixers. Second, we examine whether the relative net above-ground biomass accumulation of other trees or the whole stand was associated with the relative abundance of $N_2$ fixers. In our analyses, we assess the effects of individual $N_2$ fixer species as well as the $N_2$ fixer functional group, since it is becoming increasingly clear that $N_2$-fixing species may differ in their fixation function (Batterman, Hedin, et al., 2013; Wurzburger & Hedin, 2016).

## 2 | MATERIALS AND METHODS

### 2.1 | Study area

The study was part of the Agua Salud Project's Secondary Forest Dynamics Network, situated in the central part of the Panama Canal Watershed, adjacent to Soberania National Park (9°13′N, 79°47′W). The area receives 2,700 mm of annual rainfall with a dry season from mid-December to early May (Ogden, Crouch, Stallard, & Hall, 2013). Soils in the study area include Oxisols (Inceptic Hapludox) and Inceptisols (Oxic and Typic Dystrudepts) and are typical of soils developed on basalt in the region (B. Turner, I. Baillie & J.S. Hall, unpubl. data). They are strongly weathered, infertile, and well-drained, with little variation in topsoil texture (silty clays to clays) and soil nutrient concentrations (Appendix S1). Soils, topography and hydrology in the study area are further described in a range of papers (Hassler, Zimmermann, van Breugel, Hall, & Elsenbeer, 2011; Neumann-Cosel, Zimmermann, Hall, van Breugel, & Elsenbeer, 2011; Ogden et al., 2013; Zimmermann et al., 2013). Land use is a mosaic dominated by cattle pastures, fallows, and secondary forests of different ages across the plot network (van Breugel et al., 2013). The Smithsonian Tropical Research Institute manages three blocks of land within a 3 × 5 km area, with a total area of 664 ha, of which *c*. 530 ha was covered by fallow vegetation and forests of various ages upon property acquisition in 2008. Successional patterns in the tree diversity and composition in these forests are detailed in van Breugel et al. (2013), Craven, Hall, Berlyn, Ashton, and van Breugel (2015) (functional diversity) and Batterman, Hedin, et al. (2013) ($N_2$-fixing trees). $N_2$-fixing tree classification and nomenclature follows Sprent (2009).

### 2.2 | Vegetation data

The vegetation census data originated from 54 sites that were randomly selected within the study area (see map in Appendix S2). Each site was defined as part of a single slope within an abandoned field or pasture. In 2008–2009, we established a 20 × 50 m (0.1 ha) plot on the upper and on the lower portion of the slope in each site. Prior to analyses, we excluded plots with unknown age and plots in which the vegetation was sparse enough to expect no significant competition or facilitation (using an arbitrary limit of and initial stand biomass of 7.5 T/ha). This left us with 44 sites, with time since abandonment varying from 3 to 32 years old (Figure 1 in Appendix S2). In some analyses, we removed additional data points for different reasons; further details are provided in Appendix S2.

The minimum DBH limit for our censuses was 5 cm in half of the plot area and 1 cm in the other half. All stems of trees and shrubs with a diameter ≥ DBH limit were tagged, measured, and identified to species. Growth, mortality and recruitment have since been monitored annually, except for 2013.

We combined census data and species-specific allometric equations developed in the secondary forests in our study area to calculate the above-ground biomass (henceforth "biomass," kg) of all trees of 26 of the most abundant species in our study area (van Breugel, Ransijn, Craven, Bongers, & Hall, 2011). For all other trees, we used the locally developed multispecies equation M2 of van Breugel et al. (2011). The tree biomass of both size classes (1–4.9 cm and ≥5 cm DBH) was scaled to T/ha and summed to obtain stand-level biomass values.

### 2.3 | Land-use history

Information on land-use history and time since abandonment was obtained from interviews with former land owners and local residents. All sites in our study were dominated by pasture before abandonment. However, farmers in the area sometimes converted small areas of pasture to small-scale cultivation and then back to pasture

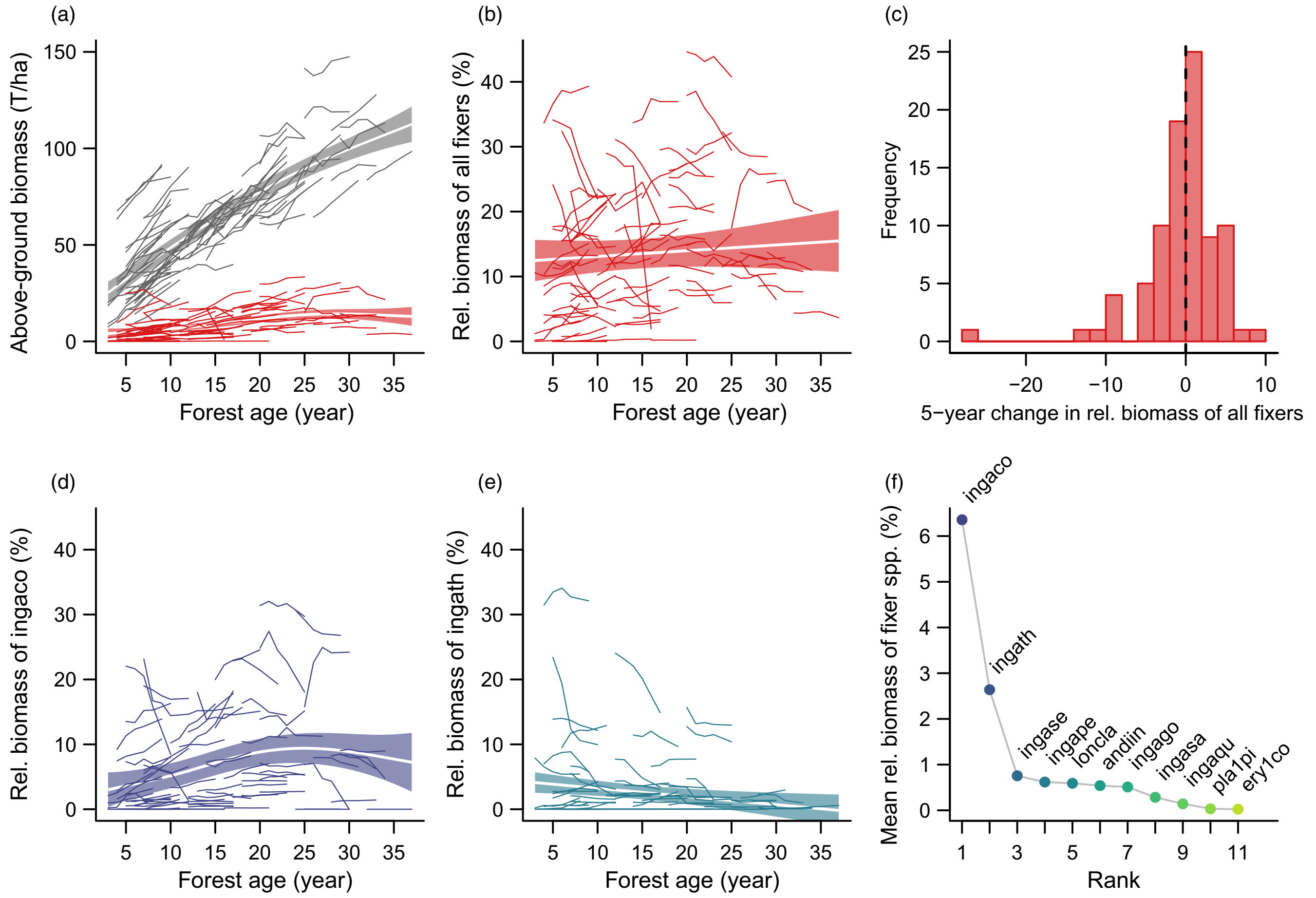

**FIGURE 1** Dynamics and successional patterns of above-ground biomass of the whole stand and $N_2$ fixer species in the humid tropical secondary forests of the Agua Salud Project, central Panama. (a) Changes in the biomass (biomass, T $ha^{-1}$) of whole-stand (grey) and $N_2$ fixers (red) across forest age. Shaded areas with white curves are the best fit regression splines with 95% CI. (b) Changes in the relative biomass of $N_2$ fixers with successional age. (c) Frequency distribution of the change in relative biomass of $N_2$ fixers over 5 years across all plots. (d, e) Changes in relative biomass of two $N_2$ fixer species (*Inga cocleensis* and *I. pezizifera*) with successional age. (f) Rank-abundance curves of the eleven most abundant $N_2$ fixer species in terms of mean relative biomass across years and plots. Blue to yellow colour spectrum indicates higher to lower ranks of mean relative biomass, respectively. Key to species abbreviation: andiin = *Andira inermis*; ery1co = *Erythrina costaricensis*; ingaco = *Inga cocleensis*; ingago = *I. goldmanii*; ingape = *I. pezizifera*; ingaqu = *I. nobilis*; ingasa = *I. sapindoides*; ingase = *I. sertulifera*; ingath = *I. thibaudiana*; loncla = *Lonchocarpus heptaphyllus*; pla1pi = *Platymiscium dimorphandrum* [Colour figure can be viewed at wileyonlinelibrary.com]

over the course of land use. No records exist documenting changes to these included areas within the pastures and extensive discussions with former land owners and neighbours indicate that these such areas are small.

## 2.4 | Soil data

Soils were sampled for nutrient analysis from late November till early December 2011. Nine cores were collected from the upper 15 cm of the mineral soil and bulked, mixed, and then subsampled for nutrient analysis. Concentrations of base cations and plant-available phosphorus were extracted using the Mehlich III method (Mehlich, 1984) and total nitrogen was determined by dry combustion using an elemental analyser (Thermo Flash 1112, Bremen, Germany). Every 2 weeks during the 2009–2010 dry season, soil water content (SWC, %) was determined gravimetrically from ten 10 cm deep cores and averaged per plot. In our analyses, we used the lowest of these biweekly averages for each plot. See Appendix S2 for more detail on soil data collection.

## 2.5 | Response variables

We calculated the following stand-level response variables: (1) the relative net biomass accumulation rate: RAR (%/year) = [ln (biomass in 2014) − ln (biomass in 2009)]/length of study period; (2) relative biomass growth rate of trees that survived from 2009 to 2014 and trees that recruited between 2009 and 2014: RGR (%/year) = [ln ($biomass_{surv+recr}$ in 2014) − ln ($biomass_{surv}$ in 2009)]/length of study period; (3) relative biomass loss due to the death of trees that were present in 2009: $Mortality_{biomass}$ (%/year) = [ln ($biomass_{mort}$

in 2009) − ln (biomass$_{\text{surv+mort}}$ in 2009)]/length of study period; (4) individual-based mortality (Mortality$_{\text{ind}}$, %/year) calculated as the percentage of trees alive in 2009 but not in 2014, divided by the length of the study period. The study period is the time between the 2009 and 2014 censuses. For the analysis focused on individual $N_2$ fixer species, response variables were calculated for each of the focal $N_2$ fixer species, for trees others than the focal $N_2$ fixer species combined ("other trees"), and for all trees combined ("whole stand"). For the analyses in which $N_2$ fixers were analysed as a functional group, all response variables were calculated for $N_2$ fixers only, for non-fixer only, and for all trees combined.

## 2.6 | Explanatory variables

For analyses of growth, mortality and effects of individual $N_2$ fixer species, the main explanatory variables included $N_2$ fixer species identity and the mean relative biomass of the focal $N_2$ fixer species throughout the study period (biomass$_{\text{focal fixer species}}$/biomass$_{\text{all trees}}$, %). In the comparison between growth and mortality of $N_2$ fixers and non-fixers, the main explanatory variables included $N_2$ fixer group identity (Nfix; yes or no) and the mean relative biomass of the group of $N_2$ fixers throughout the study period (biomass$_{\text{N2 fixers}}$/biomass$_{\text{all trees}}$, %). Other explanatory variables included initial stand biomass (T/ha) and three soil variables (see below).

We included data on in situ soil conditions (SWC and soil nutrients) in our analysis since variations in soil resources may interact with the effects of $N_2$ fixers on the biomass dynamics of secondary forests (Adams, Turnbulla, Sprent, & Buchmannc, 2016; Barron, Purves, & Hedin, 2011; Batterman, Wurzburger, & Hedin, 2013; Hedin et al., 2009; Sadowsky, 2005). We calculated and used the first two principle components of our soil nutrient data as explanatory variables. The first principle component (soil PC1) corresponds to greater availability of Ca, Cu, K, Mg and P, and lower Al concentrations, while the second principle component (soil PC2) corresponds mainly to the micronutrients Fe, Mn and Zn (see Appendix S1 for details).

## 2.7 | Statistical analyses

### 2.7.1 | Analysis 1

To illustrate successional patterns and short-term biomass dynamics, including the relative biomass of individual $N_2$ fixer species and of $N_2$ fixers as a functional group, we fitted the whole stand and $N_2$ fixer (absolute and relative) biomass as a function of forest age. We used generalized additive mixed models (GAMMs) with thin plate regression spline smoothing to allow for nonlinearity (Wood, 2006), if any, and plots within sites as random effects in order to account for the hierarchical sampling design.

### 2.7.2 | Analysis 2

To compare the performance of $N_2$ fixer and other trees, we modelled relative net biomass accumulation rate (RAR) and the relative biomass growth rate (RGR) of the group of surviving and newly recruited trees in linear mixed effect models (LME) and both Mortality$_{\text{biomass}}$ and Mortality$_{\text{ind}}$ in hurdle models (further described below). Independent variables were either $N_2$ fixer species identity or Nfix ($N_2$ fixer or non-fixer), along with initial stand biomass, soil PC1 and PC2, and SWC, with Nfix within plots within site as random effects. Our maximal model included all explanatory variables and interactions between $N_2$ fixer species identity or Nfix and the other fixed effects, but no other interaction effects.

Hurdle models were used to address zero inflation (Zuur, Ieno, Walker, Saveliev, & Smith, 2009) in Mortality$_{\text{biomass}}$ because most $N_2$ fixer species have zero mortality in part of the plots over the 5-year study period. The hurdle model involves a two-step procedure: in the first "hurdle," a binomial logit GLMM was used to model the probability of non-zero mortality in a plot (i.e. some proportion of trees in the plot died; Mortality$_{\text{ind}}$). In the second hurdle, the non-zero Mortality$_{\text{biomass}}$ was modelled in a gamma log GLMM because the non-zero Mortality$_{\text{biomass}}$ values in our study were positively skewed. Each of the binomial and gamma GLMMs went through the same model selection procedure (see below), and were then incorporated into a final model by multiplying the estimated probability from the binomial GLMM with the estimated mean from the gamma GLMM (see Figure S2 for more detailed explanation). Thus, the hurdle model estimates Mortality$_{\text{biomass}}$ after adjusting for the probability of observing zero mortality. For the analyses of the RAR and the RGR of the group of surviving and newly recruited trees, we had sufficient data from 11 $N_2$ fixer species (out of 21 species in the inventory), but for analyses of mortality only three $N_2$ fixer species provided sufficient data.

### 2.7.3 | Analysis 3

To test for the effect of relative biomass of $N_2$ fixers (all species combined or individual species) on the RAR of non-fixer trees or of the whole stand, both were modelled in LMEs as a function of relative biomass of $N_2$ fixers, initial stand biomass, soil PC1 and PC2, and SWC, with plots within site as random effects. We also added the quadratic term of initial stand biomass to improve the distribution of model residuals caused by a nonlinear relationship between the response variable and initial stand biomass. In our maximum model, we included all explanatory variables and only allowed interactions between the relative biomass of $N_2$ fixers and the other fixed effects. We only tested for the effect of the two most abundant $N_2$ fixer species individually (*Inga cocleensis* and *Inga thibauniana*). The relative abundance of all other $N_2$ fixer species was very low (<1%, Figure 1f) with little among-site variation (Figure S1), so no significant stand-level association between their relative abundance and stand biomass was expected.

The RAR and the RGR of surviving and newly recruited trees were log-transformed prior to analysis to normalize the data. In some plots, values were negative because mortality was higher than growth and because of stem mortality amongst surviving trees with multiple stems, respectively. To enable log-transformations of these variables, we added the smallest value needed to shift all plot values to above zero to the RAR (0.123% per year, Section 2.7.2; 0.064% per year, Section 2.7.3) and to the RGR (0.050% per year). All explanatory variables were scaled to zero mean (*M*) and 0.5 standard deviation (*SD*) prior to analysis (Grueber, Nakagawa, Laws, & Jamieson, 2011).

Heteroscedasticity was found to originate from Nfix and initial stand biomass in Section 2.7.3 and initial stand biomass in Section 2.7.2. To account for this, we used a constant variance function structure for Nfix and a power variance function structure for the initial stand biomass, as they led to the greatest reduction in AICc (Pinheiro & Bates, 2000; Zuur et al., 2009). Each global model was dredged to generate a list of candidate models ranked by AICc. Full model averaging was performed using candidate models within the top two ΔAICc units (Burnham & Anderson, 2002), except for the model for Mortality$_{ind}$ of $N_2$ fixer species that only had one model within two ΔAICc. We opted for full model averaging because that is the more conservative inference approach, treating a coefficient as zero each time it is not selected in a top model.

All statistical analyses were performed in R v3.4.1 (R Core Team, 2016). Principle component analysis for soil nutrients were conducted with the prcomp function (Becker, Chambers, & Wilks, 1988), GAMMs with the mgcv package (Wood, 2006), LMEs with the NLME package (Pinheiro & Bates, 2000), and GLMMs with the LME4 package (Bates, Maechler, Bolker, & Walker, 2015). Model dredging,

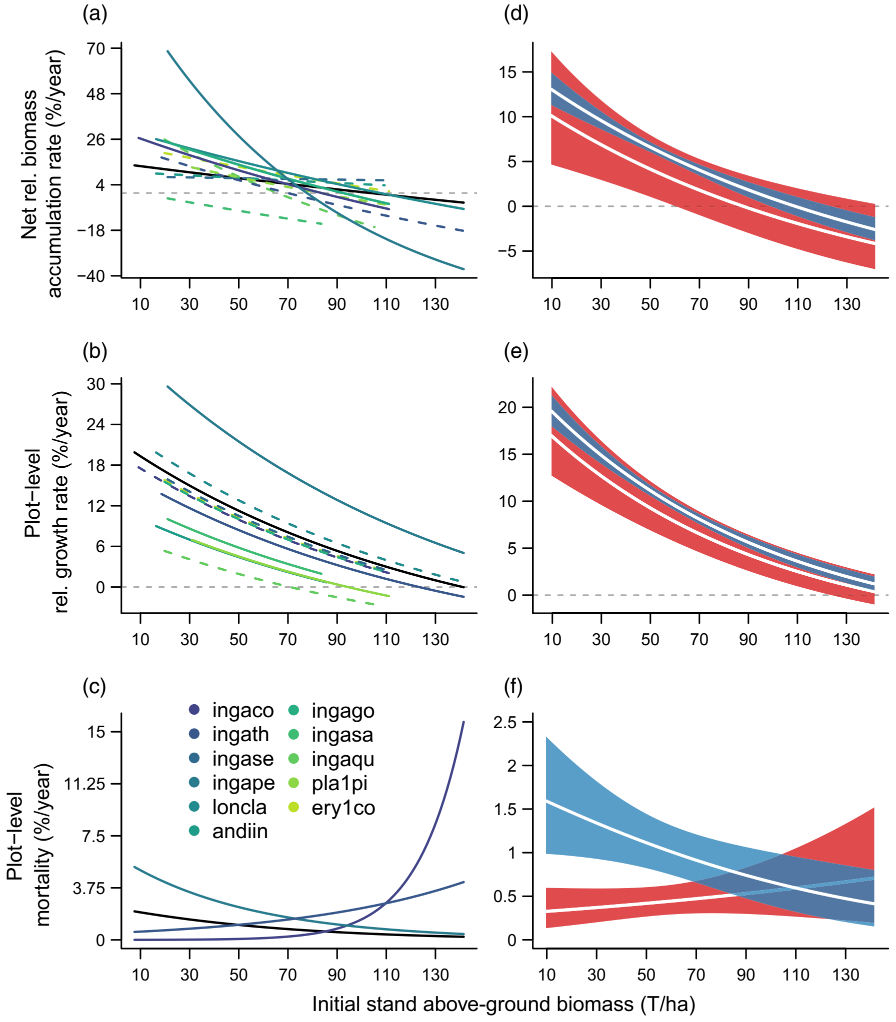

**FIGURE 2** Differences in growth and mortality of $N_2$ fixers and non-fixers over tropical secondary forest succession in the Agua Salud Project area in central Panama. Panels (a–c) compare each $N_2$ fixer species (line colours correspond to rank-abundance in Fig. 1f, see Fig. 1 for key to species abbreviation) against other trees (black solid line), while panels (d–f) compare all $N_2$ fixer as a group (red) against non-fixers (blue). Response variables are net relative biomass accumulation rate, RAR (a and d); biomass gain from growth of surviving and newly recruited trees, relative to initial stand biomass, i.e. relative growth rate or RGR (b and e); and biomass loss to mortality, relative to initial stand biomass (c and f). All best-fit regression lines were predicted as a function of initial stand biomass, with all other selected explanatory variables set at their landscape-level means. In panels (a–c), solid and dashed best-fit lines denote significant and non-significant differences in slope and/or intercept in contrast to other trees (black solid line), respectively; the 95% CI are not shown for clarity. In panels (d–f), shaded areas with white curves are the best-fit regression lines with 95% CI. Grey horizontal dashed lines in panels (a, b, d, and e) denote zero RAR or RGR. RAR and RGR were log-transformed during analyses but are here back-transformed. For more details on the hurdle models used in panel (c and f), (see Figure S2) [Colour figure can be viewed at wileyonlinelibrary.com]

model averaging, variable importance and calculation of $R^2_{GLMM}$ (for more detail, see Appendix S3) were implemented with the MuMIn package (Bartoń, 2013).

## 3 | RESULTS

### 3.1 | Successional patterns and short-term dynamics

Biomass dynamics varied strongly across plots for the whole stand and $N_2$ fixers. Averaged across all plots, whole stand and $N_2$ fixer biomass increased with forest age (Figure 1a; Section 2.7.1). $N_2$ fixers constituted on average 13.4% of the stand biomass in our sites, but variation across plots was very large (*SD* = 10.2%). The relative biomass of $N_2$ fixers was as low as zero or as high as 45% (Figure 1b) and decreased with time in about half of the plots (41 out of 88) and increased in the other half of the plots in a few cases (46 out of 88; Figure 1c). As a result, averaged across plots, the relative biomass of $N_2$ fixers remained constant over the 5-year study period, despite the idiosyncratic spatiotemporal variation in the relative biomass of individual $N_2$ fixer species (Figure 1f, Figure S1). While the two most abundant species (*I. cocleensis* and *I. thibaudiana*) constituted on average 6.4% and 2.6% of stand biomass across sites and time respectively—with considerable variation across sites (range: 0%–2%/0%–34%, *SD*: 7.3/5.2)—the mean relative abundance of all other species was below 1% (Figure 1f).

The plot-level RAR and RGR of both $N_2$ fixers and non-fixers declined with initial stand biomass (Figure 2a,b,d,e; Section 2.7.2), while the biomass loss due to mortality (Mortality$_{biomass}$) declined with initial stand biomass only for non-fixer trees (Figure 2c,f). Soil variables had no effect on the plot-level RAR and RGR of either $N_2$ fixers or non-fixers, but the averaged model does suggest an interactive effect of soil fertility (soil PC1) on the biomass-weighted mortality of *I. cocleensis*, which is the most abundant $N_2$ fixer species (Table S1). Overall, when $N_2$ fixers were examined as individual species, the fixed and random effects in the averaged models explained 70%, 34%, 52% and 14% of variation in RAR, RGR, Mortality$_{biomass}$ and Mortality$_{ind}$, respectively (Table S1). When $N_2$ fixers were examined as a group, the fixed and random effects in the averaged models explained 52%, 79%, 44% and 12% of variation in RAR, RGR, Mortality$_{biomass}$ and Mortality$_{ind}$, respectively (Table S1).

### 3.2 | Performance of $N_2$ fixers vs. non-fixers

Nitrogen fixers as a group differed little from non-fixers in their plot-level RGR (Figure 2e). Of the 11 most abundant $N_2$ fixer species, five differed significantly from other trees (Figure 2b, Table S1). Out of these five $N_2$ fixer species, only one (*Inga pezizifera*) grew significantly faster (4%–16%) than other trees, while the other $N_2$ fixer species all had lower plot-level RGR (−3% to −6%). $N_2$ fixers lost more biomass to mortality with succession, but $N_2$ fixers as a group showed a slightly greater survival during early succession (up to 1.3% lower in Mortality$_{biomass}$; Figure 2f). This seemed to be mainly driven by two relatively abundant $N_2$ fixer species, *I. cocleensis* and *I. thibaudiana* (Figure 2c). The opposite trend was found in a less abundant species *I. pezizifera* that had greater mortality during early succession (Figure 2c). Overall, lower biomass loss to mortality and similar biomass gain from growth did not lead to a difference in plot-level RAR between $N_2$ fixers and non-fixers (Figure 2d). Although some $N_2$ fixer species had greater rates than other trees, either through greater growth or lower mortality, this advantage seemed to be limited to earlier successional stages (Figure 2a).

The unexplained variation in the stand-level RGR and mortality of $N_2$ fixers was about four to six times greater than that of non-fixers (see the constant variance parameter of Nfix, Table S1), and was extremely variable for some species (>20 times greater for rarer species such as *Inga nobilis*). However, rather than reflecting an intrinsic performance difference among species or groups, such a higher variation was more likely due to the low number of $N_2$ fixers in several plots, causing higher levels of demographic noise (Doak, Gross, & Morris, 2005; Fiske, Bruna, & Bolker, 2008; Appendix S2).

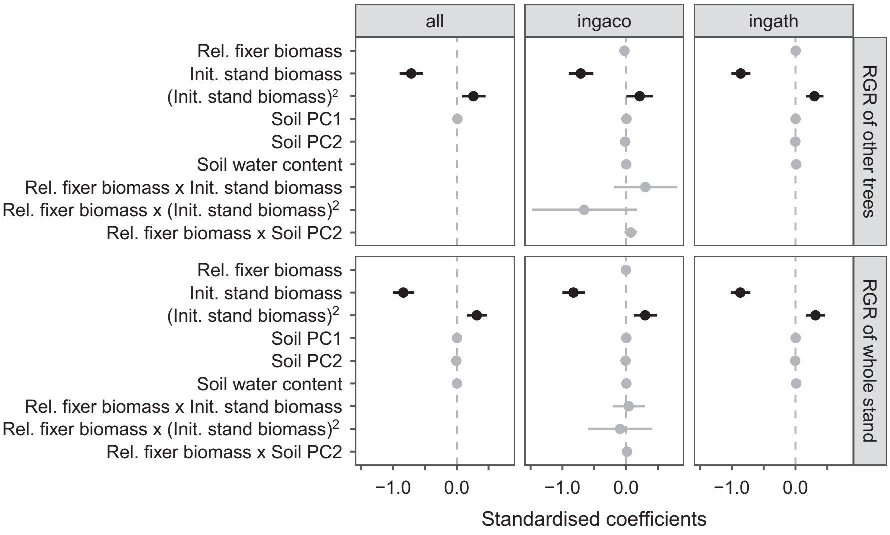

**FIGURE 3** Effects of the relative biomass of all $N_2$ fixers (left column) and of the two most dominant $N_2$ fixer species separately (ingaco = *Inga cocleensis*; ingath = *Inga thibaudiana*); initial stand biomass (Init. stand biomass); the square of initial stand biomass; soil PC1; soil PC2; soil water content; and their interactions on the relative biomass growth rate of surviving and newly recruited trees combined (RGR, %/yr). Top panels: other trees; bottom panels: whole stand. Dots are averaged slope estimates and horizontal bars are corresponding 95% CIs. Absence of dots with bars means that the corresponding variable was not included in any of the top-ranking models (see Table S2)

### 3.3 | Effect of $N_2$ fixer relative abundance on non-fixer and whole stand growth

The relative biomass of both $N_2$ fixers as a group and of individual $N_2$ fixer species had no effect on the RAR of neighbouring trees or the whole stand (Figure 3; Section 2.7.3). Instead, rates of both the whole stand and of other trees (i.e. non-fixers or non-focal species) were primarily driven by initial stand biomass. During model selection, initial stand biomass was always selected among the top models and hence had a variable importance score of 1.00, while relative $N_2$ fixer biomass was seldom selected and only had a variable importance score of 0.15–0.83 when selected (Table S2). The lack of an association between relative $N_2$ fixer biomass and plot-level RAR was consistent throughout succession and did not vary with soil fertility, as evident in the lack of strong interaction between relative $N_2$ fixer biomass and other covariates (Table S2).

## 4 | DISCUSSION

Our study addresses the long-standing question of how the abundance of $N_2$-fixing trees affects the growth of non-fixing trees and the whole forest stand (Jenny, 1950). A resolution to this question is timely given the potential for $N_2$-fixing trees to enhance the carbon sink in secondary tropical forests by providing newly fixed atmospheric nitrogen to the forest (Levy-Baron et al., in review), thereby offsetting anthropogenic carbon emissions and altering the global carbon cycle. Specifically, we evaluated the alternative hypotheses that $N_2$ fixers may provide either a net facilitative effect on the growth of other trees through the supply of new nitrogen, or a net competitive effect on growth through competition for light and other shared resources.

### 4.1 | Relative abundance of fixers varied widely

We first evaluated the biomass patterns and dynamics of $N_2$ fixers over tropical forest succession (Section 2.7.1; Figure 1). The relative abundance of $N_2$ fixers varied widely across plots, as observed in other tropical forests in terms of number of trees and above-ground biomass (Gei et al., in review; Menge et al., 2014; ter Steege et al., 2006). Similarly, the change in the relative biomass of $N_2$ fixers varied strongly across individual plots (Figure 1b,c). Our finding of high variation in fixer relative abundance allowed us to evaluate effects of the functional group of $N_2$ fixers on the whole forest stand and on non-fixer trees, and of individual $N_2$ fixer species on the whole forest or on non-focal species (fixer and non-fixer).

Although changes in the relative abundance of $N_2$ fixers with successional age varied among species (Figure S1) and across sites, the relative abundance of all fixer species as a functional group remained relatively stable across succession on the scale of the whole landscape (Figure 1b,c). This observation contrasts with previous studies that found increasing relative abundance of $N_2$ fixers over the first decades of succession (Gehring et al., 2008, % biomass; Sullivan et al., 2014; Menge & Chazdon, 2016, both studies: % basal area and % stems). These studies included 15, 9 and 6 plots, respectively. The high variation in relative $N_2$ fixer abundance in this current study of 88 plots (Figure 2) suggests that larger sample sizes may be required to accurately represent the variation in—and estimate the successional trends of—relative $N_2$ fixer abundance across the landscape.

### 4.2 | No evidence for net facilitative or competitive effects of $N_2$ fixers

We used two sets of analyses to examine whether the relative abundance of fixers affected the biomass accumulation of other trees in our seasonal tropical moist forests (Figures 2 and 3). Results from our Section 2.7.3 showed that relative net biomass accumulation of non-fixing trees and the whole stand neither increased nor decreased with relative biomass of either (1) the two most abundant $N_2$ fixer species (*I. cocleensis* and *I. thibaudiana*) or (2) all $N_2$-fixing species combined as one functional group. If we had found a greater growth rate for $N_2$ fixers (Section 2.7.2), this would have indicated co-occurring competitive and facilitative effects that cancelled each other out. However, neither the two most abundant species nor $N_2$ fixers as a group grew faster than non-fixers (Figure 2b,e), although both species and the $N_2$ fixers as group did have a lower mortality during early succession (Figure 2c,f). Combined, these findings provide no evidence for either a net competitive or a facilitative effect of $N_2$ fixers on the growth of other trees or the whole forest stand.

The lack of evidence of a facilitative effect of $N_2$ fixers on forest growth contrasts with biogeochemical theory. Hans Jenny (Jenny, 1950) proposed that nitrogen levels in tropical forests could be explained by the abundance of $N_2$-fixing tree species. Since Jenny, studies have considered the abundance of fixers to scale directly with symbiotic nitrogen fixation rates (Cleveland et al., 1999; Sullivan et al., 2014) or to scale indirectly with the total quantity of fixation required across a landscape, even though an individual tree may not be actively fixing (Menge, Levin, & Hedin, 2009; Sheffer, Batterman, Levin, & Hedin, 2015).

The possibility of a facilitative role of $N_2$ fixers during tropical secondary forest succession has been, to our knowledge, only directly examined in our site (Batterman, Hedin, et al., 2013) and one other site in the wet tropical region of La Selva in Costa Rica (Menge & Chazdon, 2016; Taylor et al., 2017). In an earlier study that used a subset of our youngest forest plots, we found that $N_2$ fixers as a group grew faster than non-fixers (Batterman, Hedin, et al., 2013). This inconsistency with the current results may stem from a random selection of plots with higher $N_2$ fixer growth rates, which we showed here to vary widely across plots. In the early successional forest in La Selva, $N_2$ fixers had higher growth and stem survival rates than non-fixers (Menge & Chazdon, 2016)—like we found for some of our species but not for $N_2$ fixers as a group—and a competitive rather than a facilitative effect on the growth of the non-fixer species (Taylor et al., 2017)—while we found neither. The increasing number of studies that find within-group variation in the characteristics and function of $N_2$ fixer species—including our study here—could account for the contrasting results in the Costa Rican forests. One species, *Pentaclethra macroloba*, dominates the Costa Rican forests at *c*. 16%–18% of above-ground tree biomass (Rozendaal & Chazdon, 2015). In our study area, *P. macroloba* does not occur. This species and others that comprise the fixer functional group in Costa Rican tropical forests may function in ways that differ from the *Inga* species that dominate the pool of fixer species in our forests. While findings on competitive effects differ between these two studies, both coincide in finding no evidence that $N_2$ fixers facilitate biomass growth of these secondary forests. An alternative explanation for the difference in findings between this and the Costa Rican studies could be in the number of plots and the random selection of forest sites in which the studies were conducted, since we saw variation across plots in the relationship between fixer abundance and biomass accumulation rates.

The extent to which our findings of a lack of effect of $N_2$ fixers on biomass accumulation during secondary forest succession are consistent across Neotropical forests that vary widely in environmental conditions (Quesada et al., 2010, 2012), biomass accumulation rates (Poorter et al., 2016), fixer abundance (Gei et al., in review; Liao, Menge, Lichstein, & Ángeles-Pérez, 2017; Pellegrini, Staver, Hedin, Charles-Dominique, & Tourgee, 2016; ter Steege et al., 2006) and fixer species community composition (S. A. Batterman, pers. comm.) remains unclear. In addition, we focus our study on the above-ground dynamics of trees and their contribution to ecosystem $N_2$ fixation. The biomass of trees comprises the majority of biomass in tropical forests and trees (Saatchia et al., 2011), but nevertheless the role of below-ground biomass pools and interactions require further examination as resource competition is a net measure of above- and below-ground plant–plant interactions. Roots account for a substantial proportion of tree biomass (almost 30 percent of the total biomass of young trees in a nearby plantation; Sinacore et al., 2017) and root:shoot ratios and root architecture are likely to shift along successional and other environmental gradients (Jaramillo, Ahedo-Hernández, & Kauffman, 2003; Rasmann, Bauerle, Poveda, & Vannette, 2011; van Noordwijk, Cadisch, & Ong, 2004; Zangaro, Alves, Lescano, Ansanelo, & Nogueira, 2012) and to differ across tree species and functional groups (Becker & Castillo, 1990; Markesteijn & Poorter, 2009; Shukla & Ramakrishnan, 1984; Sinacore et al., 2017). Including roots in future studies is a major challenge but will refine our ability to understand the role of $N_2$ fixer species in secondary forest succession.

### 4.3 | Why there is lack of effect of fixers on stand biomass dynamics?

What can account for the lack of effect of $N_2$ fixers on the biomass dynamics of other trees and the whole stand in our study? Two possible explanations could provide resolution. First, $N_2$ fixer abundance does not necessarily reflect ecosystem-level fixation rates. The assumption that $N_2$ fixer abundance correlates with $N_2$ fixation rates relates to the classic mass ratio theory (Grime, 1998), which posits that the ecosystem effect of a (group of) species is proportional to its abundance or dominance. This assumes that putative $N_2$ fixers are actively fixing and that symbiotic fixation is correlated with tree size (Sullivan et al., 2014). However, it has recently been shown that fixation rates vary greatly across species (Batterman, Hedin, et al., 2013; Wurzburger & Hedin, 2016) and successional time (Batterman, Hedin, et al., 2013) and that tropical $N_2$ fixers utilize a facultative fixation strategy whereby individual trees adjust fixation rates depending on the environment (Barron et al., 2011; Batterman, Hedin, et al., 2013; Batterman, Wurzburger, et al., 2013; Bauters, Mapenzi, Kearsley, Vanlauwe, & Boeckx, 2016; Menge et al., 2009; Sheffer et al., 2015). Specifically, *I. cocleensis* and *I. thibaudiana*, by far the two most abundant $N_2$ fixer species in our site (Figure 1f), have been shown to utilize facultative fixation (Barron et al., 2011; Batterman, Wurzburger, et al., 2013). These findings suggest that the ecosystem-level $N_2$ fixation rates of fixers may be decoupled from their abundance. Thus, $N_2$ fixer relative abundance or biomass may indeed provide a poor estimate of ecosystem $N_2$ fixation rates (Hedin et al., 2009) and the facilitative or competitive effects of $N_2$ fixers.

Second, nitrogen may not limit productivity in these forests, in which case the presence of $N_2$ fixers would provide no benefit to—and therefore no facilitation of—non-fixers. The extent to which disturbed sites are nitrogen-limited can vary spatially according to local disturbance history, prior land use (Erickson et al., 2001) and fire frequency (Pellegrini et al., 2018). Moreover, these forests could receive sufficient nitrogen inputs from other non-tree sources such as lianas (Sprent, 2001), free-living heterotrophic bacteria (Reed, Townsend, & Cleveland, 2011) and atmospheric deposition (Matson, McDowell, Townsend, & Vitousek, 1999), which may be sufficient to alleviate nitrogen limitation (Cleveland et al., 2010; Hedin et al., 2009). Non-symbiotic $N_2$ sources like free-living bacteria in soils and cyanobacteria in tree canopies would reduce the reliance of non-fixers on symbiotic $N_2$ fixers, thereby diminishing any relationship between the abundance of $N_2$ fixers and forest productivity. However, in the same forests we studied, we have observed that $N_2$-fixing trees fix nitrogen at high rates in the youngest forest ages (5–12 years), suggesting that nitrogen limits tree growth sufficiently to warrant investment in fixation (Batterman, Hedin, et al., 2013). The subsequent decline in fixation rates and proportion of $N_2$-fixing trees as forests age, consistent with indicators of soil nutrient status from the Brazilian Amazon (Davidson et al., 2004, 2007), suggests that our forests become less nitrogen-limited as succession proceeds. We conclude therefore that, if the abundance of $N_2$-fixing trees were to have any effect on non-fixers, it would be particularly evident in the early successional forests that we analyse here.

## 5 | CONCLUSIONS

Most fundamentally, our findings identify a lack of either a net facilitative or a competitive effect of $N_2$ fixer abundance on the growth of other trees over the first three decades of secondary succession in the seasonal tropical moist forests of our study site. Theoretical and numerical models of forest carbon and nutrient cycles should not simply scale fixation and its effects from the abundance of $N_2$-fixing trees. The recent observation that $N_2$-fixing trees utilize a facultative fixation strategy (Barron et al., 2011; Batterman, Hedin, et al., 2013; Batterman, Wurzburger, et al., 2013) could resolve why we found no effect of fixer abundance on non-fixers because fixation rates do not necessarily scale linearly with the abundance of fixers. To further elucidate the role of $N_2$ fixers in enhancing or suppressing primary productivity, we must clarify how the abundance of $N_2$ fixers in tropical forests relates to stand-level inputs of new nitrogen via symbiotic $N_2$ fixation and the degree to which fixed nitrogen is distributed to neighbouring non-fixers. Resolving the relationship between fixer abundance, $N_2$ fixation rates and carbon accumulation during secondary succession in tropical forest will improve our ability to understand and predict the role of tropical forests in the global carbon cycle.

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## AUTHORS' CONTRIBUTIONS

H.R.L., M.v.B., J.S.H., and S.A.B. conceived the ideas and designed the study; M.v.B., J.S.H. and B.L.T. collected the data; H.R.L. and M.v.B. analysed the data; H.R.L., M.v.B., and S.A.B. wrote the manuscript. All authors commented on the manuscript.

## DATA ACCESSIBILITY

Data are available on Figshare: https://doi.org/10.6084/m9.figshare.5950369.v2 (van Breugel, Lai, Hall, Batterman, & Turner, 2018).

## ORCID

*Hao Ran Lai* http://orcid.org/0000-0001-6871-0146

*Michiel van Breugel* http://orcid.org/0000-0003-2778-7803

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## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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