

Seedling growth declines in warmed tropical forest soils

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Abstract

The response of plants to a warming climate could have a large feedback on further climatic change. This feedback is especially important for tropical forests, where the global peak in plant productivity and biodiversity occurs. Here we test the response of tropical forest tree seedling growth, photosynthesis and herbivory to 3 years of in situ full-soil profile warming. We studied six species, three of which are known nitrogen-fixers and we hypothesized that the warming response of growth will be mediated by nutrient availability to plants. Across species, growth was significantly lower in warmed soil compared to soil at ambient temperature, and the same pattern was observed for light-saturated photosynthesis, pointing toward a growth decline associated with decreased C fixation. Within species, the relative growth decline was significant for two species, *Inga laurina* and *Tachigali versicolor*, both of which are N-fixers. Together our results suggest a growth decline may have resulted from a negative effect of warming on N-fixation, rather than via changes in nutrient mineralization from soil organic matter, which was unchanged for N and increased for P during the dry-to-wet season transition. Overall, our study demonstrates that belowground warming causes species-specific declines in the growth and photosynthesis of seedlings, with a suggestion—requiring further investigation—that this growth decline is larger in N-fixing species.

Abstract in Spanish is available with online material.

KEYWORDS

climate warming, *Inga laurina*, *Lacmellea panamensis*, lowland tropical forest, nitrogen fixation, Panamá, photosynthesis, plant productivity, *Protium pittieri*, *Tachigali versicolor*, *Virola surinamensis*

1 | INTRODUCTION

Tropical forests are highly productive and biodiverse, exchanging more C with the atmosphere than any other ecosystem (Pan et al., 2011) and representing the apex of tree diversity (Ter Steege et al., 2013). The tropics are predicted to warm significantly this

century (e.g., 3.5°C relative to 1995–2014 reference period; SSP5-RCP8.5 scenario; Lee et al., 2021) and to exceed their historical temperature regime more rapidly than any other ecosystem (Mora et al., 2013). Any changes in the functioning and composition of tropical forests to this predicted climate change could, therefore, have major consequences for the Earth system.

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Our current understanding of the response of tropical plants to a warming climate is based on the effect of aboveground warming on plant tissues and processes (i.e., leaves and photosynthesis). But plants will also be affected by belowground (soil) warming (i.e., of roots and root-rhizosphere communities, and their response to altered organic matter dynamics) (Wood et al., 2019). For aboveground processes, the plant growth response to warming is the net effect of changes in photosynthesis and respiration. The light-saturated uptake of CO₂ from the atmosphere via photosynthesis (A_{sat}) has a temperature optimum (T_{opt}) beyond which it declines (Berry & Bjorkman, 1980). The mechanisms for this decline include decreases in stomatal conductance, electron transport rate, and Rubisco activity, and increased photorespiration (Atkin & Tjoelker, 2003; Sage & Kubien, 2007). While photosynthesis has a peaked response to short-term increases in temperature, respiration continues to increase over ecologically relevant temperature ranges (Heskel et al., 2016; Huntingford et al., 2017). However, following prolonged exposure to elevated temperature, acclimation of leaf respiration reduces respiration rates (Atkin & Tjoelker, 2003; Slot & Kitajima, 2015), resulting in relatively stable photosynthesis to respiration ratios (Gifford, 2003; Ziska & Bunce, 1998). Respiration of non-photosynthetic tissues has likewise been shown to acclimate, including roots of hydroponically grown plants (Loveys et al., 2003), although there is no information for tropical plants (see below).

In tropical forests, declines in photosynthesis have been observed under prolonged 2°C leaf warming for tropical trees (Doughty, 2011) and under 3°C degrees leaf warming for trees and understory shrubs (Carter et al., 2020, 2021), suggesting they are already operating close to their T_{opt} . In response to elevated temperatures, plants can typically make physiological adjustments to acclimate and increase the T_{opt} (Crous et al., 2022; Way & Yamori, 2014; Yamori et al., 2014) and under controlled conditions, well-watered seedlings of tropical tree species growing without nutrient limitation have also been observed to acclimate their photosynthesis by increasing T_{opt} (Slot & Winter, 2017). However, acclimation is not observed in all cases. For example, Slot and Winter (2018) found limited acclimation potential in seedlings of late-successional tree species in a mesocosm experiment in Panama; Carter et al. (2021) found no acclimation of photosynthesis of tropical trees in Puerto Rico following 1 month of 3°C aboveground warming; and Dusenge et al. (2021) found no acclimation in two tropical tree species grown at three locations along a temperature gradient in Rwanda. Together, field experimental evidence suggests that warming in lowland tropical forest will have a negative effect on plant C uptake, while controlled experiments suggest acclimation may occur for certain species or locations, at least via aboveground leaf-level responses.

Plant growth will also be affected by soil warming via belowground processes, on roots and via plant–soil feedbacks (PSF), which are even more poorly understood. As for leaves, warming will directly affect root respiration, which is known to increase with temperature and increase the C cost to plant growth. Indeed, several

studies report a particularly high temperature sensitivity of root respiration, often greater than that of soil heterotrophic respiration (Boone et al., 1998; Li et al., 2020). As with leaves, root respiration can physiologically acclimate to warming (Loveys et al., 2003), but empirical evidence for this in tropical forests is limited and inconsistent. For example, acclimation of root respiration to warming occurred in seedlings of only one out of eight studied species in Australian tropical forest (Noh et al., 2020), thus suggesting that soil warming generally increases C loss from roots simply because of the warming effect on respiration rates.

Tropical forest plant growth under warming will further be affected by PSFs, defined as any change in soil conditions, whether biotic and/or abiotic conditions, that influence plant performance (Pugnaire et al., 2019). Although scarce information is available on PSFs in warmed tropical forest soils (Alonso-Rodriguez et al., 2022; Bachelot et al., 2020), there is substantial evidence that PSF plays an important role in tropical forest functioning. For example, positive PSFs (where plant growth increases) result where nutrient availability to plants is increased, such as through the presence of N-fixing bacteria (Epihov et al., 2021; Hedin et al., 2009; Pons et al., 2007; Reed et al., 2008), and arbuscular mycorrhizal (AM) fungi for increased P uptake (Kiers et al., 2000; Sheldrake et al., 2018). In contrast, negative PSFs (where plant growth decreases, or mortality occurs) can arise through the presence of soil microbial pathogens, which have been shown to cause decreased survival of conspecific seedling species (Bagchi et al., 2014; Mangan et al., 2010). These PSF interactions are particularly important during recruitment and early growth stages, and they exert a particularly large influence on the tropical forest tree community composition (Epihov et al., 2021; Kiers et al., 2000; Mangan et al., 2010).

While we understand very little about how PSFs alter under warming in tropical forest, we can make predictions based on observations outside the tropics and on our current understanding of how PSFs affect plant growth in tropical forest soils. In warming experiments outside the tropics, positive PSFs have resulted from increased soil organic matter mineralization and nutrient availability to plants, especially N (Melillo et al., 2011). Under warming in weathered tropical forest soils where rock-derived nutrients are predicted to limit plant growth, a positive PSF might be predicted to occur if warming increases P availability (Vitousek & Sanford, 1986; Wright et al., 2011). We can also predict the response of PSFs under warming in tropical forests due to changes in key groups of soil microbes, N-fixing bacteria, AM fungi, and soil pathogens. A negative PSF may occur from decreased activity of N-fixing bacteria in tropical forests under warming (Wang & Houlton, 2009), a prediction based on the evidence that N-fixation has a low thermal optimum (~25°C) (Houlton et al., 2008); but see Bytnerowicz et al. (2022). In contrast, a positive PSF could result from increased AM fungal growth, the dominant response found in a meta-analysis of warming experiments from outside the tropics (Mohan et al., 2014). Further PSFs with large plant interspecies variation might result where warming changes the

relative abundance of soil pathogens, which have large influence on seedling recruitment and survival in tropical forests (Bagchi et al., 2014; Mangan et al., 2010). For example, new evidence from a tropical forest warming study in Puerto Rico suggests that the strength of this PSF may be reduced under warming, possibly due to a negative effect of warming and its interaction with drying on soil pathogens, with potential consequences for plant species composition and diversity (Bachelot et al., 2020). Indeed, the same Puerto Rico study reported increased sapling (but not seedling) richness under warming, although the precise mechanism driving this change remains unclear (Alonso-Rodríguez et al., 2022). Thus, any change in PSFs under climatic warming could have a large influence on tropical plant C-uptake (e.g., photosynthesis and respiration) and growth. We hypothesize that PSFs exert a large influence on seedling growth under the influence of belowground warming.

We investigated the effect of belowground warming on the growth and photosynthesis of seedlings in tropical forest. To test the hypothesis that tropical forest seedling growth is affected by belowground warming, we used the in-situ Soil Warming Experiment in Lowland Tropical Rainforest (SWELTR) combined with common garden seedling experiments (6 species and 60 individuals=360 seedlings in total). Because we warmed the soil but not the air (at least not directly), we hypothesized that plant growth responses to warming will result from changes in belowground processes and especially PSFs, with specific hypotheses as follows:

1. *Direct warming effect on C metabolism (plant systemic response).* Warming has a direct effect on plant C metabolism, decreasing growth across all species because of increased root respiration and C loss (Boone et al., 1998; Li et al., 2020). Under this scenario, significant growth decreases would be observed for all studied species, with the possibility of increased maximum photosynthesis rates during sunflecks for some species in response to increased belowground C allocation and reduced feedback inhibition in leaves, resulting from altered source-sink dynamics (despite low average light conditions in the understory). We do not hypothesize decreased photosynthesis (or leaf respiration) due to a direct effect of warming on leaf physiology, because we heated the soil rather than leaves directly.
2. *Nutrient mineralization effect.* Warming will increase soil nutrient availability and, in turn, increase growth and photosynthetic C uptake for all species. Where warming increases P availability we predict a positive growth response across all species. Where warming increases N availability (or N and P together) we predict a larger response for non-N fixing species where growth is more likely to be N-limited.
3. *Negative plant-microbial interaction (N-fixing bacteria).* Warming has a negative effect on plant-soil microbial associations including N-fixing bacteria, following Wang and Houlton (2009). For this hypothesis, we predicted decreased growth and photosynthesis under warming, with particularly marked declines for N-fixing species.

4. *Positive plant-microbial interaction (AM fungi and pathogens).* Last, warming will increase AM fungal biomass and activity, increasing nutrient availability to plants and increased growth for all species. At the same time, warming may reduce the abundance of soil pathogens resulting in increased growth for all species (see Bachelot et al., 2020). For this study, we infer this mechanism indirectly though the measured plant growth response that is consistent for all species.

Because we directly compared the growth response of N-fixing versus non-N-fixing seedlings, we focused our hypotheses on PSFs related to N-fixation, given its noted importance in tropical forest growth (Epihov et al., 2021; Hedin et al., 2009). However, these mechanisms are not mutually exclusive, and variable species-specific responses of plant-pathogens and plant-mycorrhizal interactions may also be important and may contribute to unexplained variation in our data. We further note that while plant growth changes could also result from an interaction with warming and soil drying, we did not expect a large effect here as no significant soil drying was observed during the first 2 years of this experiment (Nottingham et al., 2020). Overall, our study was designed to test the crucial question of whether seedling growth increases or decreases under experimental belowground warming and to provide insight into the dominant mechanisms behind this response.

2 | METHODS

2.1 | Experimental site and soil heating plots

The experiment is the Soil Warming Experiment in Lowland Tropical Rainforest (SWELTR), situated in seasonally moist lowland tropical forest on Barro Colorado Island, Panama. The experiment consists of 10 paired control and heated plots across a 1 ha area, where the heated plots are heated to an average 4°C across the soil profile (to ~1.5 m depth), with surface soils (0–20 cm) heated by an average of 3°C (Nottingham et al., 2020). The plots are circular, measuring approximately 3.5 m (internal diameter) with heating footprint extending to approximately 5 m (external diameter). The heating rods surround the circumference of the internal plot (3.5 m diameter), heating the surface soil and with 16 × rods extending to 1.2 m depth (Figure S1). The seedling plots were established inside each circular plot. Further information is available elsewhere on the experimental site and its soils (Nottingham et al., 2020), and on the geology and floristic composition of Barro Colorado Island (Leigh, 1999; Woodring, 1958).

2.2 | Seedling plots

For this study, we selected six shade-tolerant species, three of which are known to form N-fixing symbioses, *Inga laurina*, *Ormosia macrocalyx*, and *Tachigali versicolor* (Parker, 2008); and a further three that

are known to not form N-fixing symbioses: *Lacmellea panamensis*, *Protium pittieri* (Synonym = *Tetragastris tomentosa*), and *Virola surinamensis*. Seeds were collected during 2015 from nearby forest on Barro Colorado Island, and germinated and grown in a shade house, in pots containing soil collected from nearby the experimental site. During the early wet season in 2016 (July–August), the seedlings were transplanted into the experimental plots. The seedling plots were square, measuring approximately 1.5×1.5 m, with a seedling plot located inside each of the experimental plots. Each seedling plot consisted of six rows of six individuals of different species, with each row containing each of the six species, in randomized order per row. The same plan for seedling plots was used for each of the five paired-sets of experimental heating and control plots. Seedlings for each species were stratified by height prior to transplant, ensuring that heated and control plots received seedlings with the same initial height distribution. Thus, each seedling plot consisted of 36 seedlings, with $6 \times$ species and $6 \times$ replicate individuals per seedling plot ($n = 360$ seedlings in total).

2.3 | Measurements

Measurements of growth rates were made each 3 months over 4 years between 2016 and 2020, where the warming treatment began during July–November 2017. Further measurements were made of soil nutrient mineralization and light (determined each month); of seedling growth (height change over time) and herbivory impact (determined each 3 months); and light saturated photosynthesis (A_{\max}) and leaf chlorophyll content index (CCI) (determined during field campaigns in 2019 and 2022, respectively).

Seedling height was determined by measuring from the soil surface to the shoot apex (apical meristem). Light was determined by photosynthetic photon flux density (PPFD; $\mu\text{mol s}^{-1} \text{m}^{-2}$), measured using a LI-250A light meter (LI-COR, Inc.). PPFD was determined monthly above each individual seedling, both on an overcast day and a clear day, providing two metrics of PPFD representing the average conditions on both an overcast and a clear day. In all cases, PPFD was determined around midday (average over 10:00 a.m. to 2:00 p.m.) on a clear day, with sun positioned overhead. Light saturated photosynthesis (A_{sat}) was determined using a portable photosynthetic system (LI-6400, LI-COR, Inc.) fitted with a standard 2×3 cm leaf cuvette with a red-blue LED light source (6400-02B) and a CO_2 mixer (6400-01, LI-COR, Inc.). To determine the light level required for these measurements, we first measured the light response of photosynthesis for two seedlings of each species between 100 and $1250 \mu\text{mol m}^{-2} \text{s}^{-1}$. Based on these curves, the saturated light level was set to $800 \mu\text{mol m}^{-2} \text{s}^{-1}$. The measurements were obtained with a target relative humidity of 50–60%, and a CO_2 concentration inside the chamber of $400 \mu\text{mol} (\text{CO}_2) \text{ mol}(\text{air})^{-1}$. A block temperature of 30°C resulted in an average leaf temperature of 31.2°C , with 90% of the temperatures falling between 30.3 and 31.8°C . For each healthy seedling, one fully expanded leaf without visible damage or epiphylls was measured, and the light-saturated

photosynthesis rate was recorded after stomatal conductance and photosynthesis rates had stabilized (typically 1–2 min, with stomatal conductance reaching $\sim 0.11 \text{ mol m}^{-2} \text{ s}^{-1}$ (median, with five and 95% quantiles of 0.07 and $0.19 \text{ mol m}^{-2} \text{ s}^{-1}$, respectively)). The photosynthesis measurements focused on seedlings in two paired warming-control plots, which represented the temperature contrasts with the most homogeneous light conditions and the least herbivory damage. Photosynthesis was not measured on seedlings of *L. panamensis*, as their small and segmented leaves for these seedlings were too small to fit across the 6 cm^2 leaf cuvette it was not feasible to estimate enclosed leaf area non-destructively and with sufficient accuracy to facilitate comparison. Herbivory was determined using a continuous scale index where 0 = no visible leaf damage, 1 = minor damage (<10% leaf area affected), 2 = medium damage (10–40% leaf area affected), 3 = major damage (>40% leaf area affected). Measurements of leaf chlorophyll content index (CCI, calculated as the ratio of %transmission at 931 nm over %transmission 653 nm) were made on a clear day in September 2022 with a MC-100 chlorophyll meter (Apogee Instruments, Logan, Utah, USA). Leaf chlorophyll was determined for $n = 10$ leaves (including all independent individuals of plants per species per plot up to $n = 6$, followed by further random sampling of leaves among individuals to achieve $n = 10$).

To assess whether soil warming affected nutrient availability to plants, we determined soil nitrogen (N) and phosphorus (P) mineralization using in situ mixed bed ion exchange resin membranes (Dowex Marathon Mr-3; Sigma-Aldrich). Resin was prepared by rinsing in 0.5 M HCl for 2 h and then rinsed with DI H_2O . Resin was placed inside nylon bags (5 g resin in each) and installed at 5 cm soil depth in systematically distributed locations in each plot ($n = 3$ per plot for $n = 5$ plots; 30 total). Resin bags were deployed monthly for 3 years, and following collection were stored at 4°C until extraction. Extraction was performed using 0.5 M HCl, and PO_4 , NH_4 and NO_3 , were determined using a Lachat Quickchem 8500 flow injection analyzer (Hach Co.). Extractable PO_4 (total mineralized P) and NH_4 and NO_3 (total mineralized N) were calculated from the difference between extracted N from resin deployed in the field and resin not deployed (blanks) and expressed as extractable $\text{PO}_4\text{-P}$, $\text{NH}_4\text{-N}$, and $\text{NO}_3\text{-N}$ per g resin per day.

2.4 | Calculations and statistical approaches

We determined relative growth rates (RGRs) using a first order power-law model, which is especially suitable for the early stages of seedling growth when growth is rapid and non-asymptotic, but slowing over time (Paine et al., 2012).

$$dh / dt = t^\beta$$

where dh/dt is the height increment over time (or RGR). To represent time in our models we used “relative month” where the warming treatment began at relative month = 0. To calculate the power of the relationship (β), we used log–log plots. We determined RGR for each individual seedling ($n = 30$, six individuals per five plots; and for six

species), by calculating the slope of $\log[\text{seedling height}]/\log[\text{time in months}]$. We used the power rate increase β to assess treatment effects on RGR.

To test for treatment effects on plant properties, we used one-way ANOVA with plot-pair as a blocking variable. We tested the normality of residuals using Shapiro–Wilk tests and where non-normal we used log-transformation or, for herbivory effects where residuals remained non-normally distributed, we used Kruskal–Wallis tests. Treatment effects on plant properties were tested for all species together and for individual species, for properties: (i) seedling height during the latter phase of the study following warming, defined by seedling height measured after relative month 41; (ii) RGR, defined by β (see above); (iii) herbivory; (iv) photosynthesis (A_{sat}). We further tested for the effect on plant RGR of environmental properties and plants traits using mixed effects models, including fixed effects of: warming treatment, average PPFD (determined both on a clear day $\text{PPFD}_{\text{clear}}$ and cloudy day $\text{PPFD}_{\text{cloudy}}$), average soil N and P mineralization (average value by plot), N-fixing identity of species (Y or N), and a random spatial effect of plot-pair. For this analysis, all plant individuals were considered independent replicates (for up to 360 individuals in total). For mixed effects models we omitted one plot-pair due to a high mortality event in this area, thought to have resulted from large herbivore activity. To determine whether soil nutrient availability varied between treatments and over time, we used repeated measures ANOVA, with time as a random effect; we tested for treatments effects including: (i) all data; (ii) separately by dry and wet season and the dry-wet season transition (Jan–April, May–Dec, and April–June inclusive months, respectively). We finally tested whether variation in average PPFD at the seedling level influenced variation in plant growth (RGR) responses between treatments and among species, by using one-way ANOVA with $\text{PPFD}_{\text{clear}}$ and $\text{PPFD}_{\text{cloudy}}$ (the time-averaged PPFD per seedling location, measured on clear or cloudy day, respectively) as the response variable and with either treatment effect or species identity as the predictor variable. We similarly tested whether there was an effect of PPFD on A_{sat} (one-way ANOVA). All analyses were performed in R (version R-4.1.2; R Core Team, 2021).

3 | RESULTS

Across species, growth was significantly lower in warmed soil compared to soil at ambient temperature (controls) ($p < .001$), and the same pattern was observed for light-saturated photosynthesis, A_{sat} ($p = .02$), suggesting decreased growth associated with decreased C fixation. However, there were also inter-species differences in the growth response to warming. Within species, seedling growth was significantly lower in warmed plots compared to controls for *I. laurina* and *T. versicolor*, with a marginal decline for *P. pittieri* and no effect for the other three studied species, *L. panamensis*, *O. macrocalyx*, and *V. surinamensis* (Figures 1 and 2). It was notable that the treatment effect on seedling growth occurred after the warming treatment was switched on (see effect size before and after dashed lines in Figure 1). We assessed treatment effects on

seedling height during the latter phase of the study period (including measurements during relative months 41–53). During this phase, overall seedling height was lower in warmed plots (treatment effect: $p < .001$, F -statistic 12.66, $df = 1$), with some spatial variance (plot-pair block effect: $p < .01$, F -statistic 4.1, $df = 4$). For individual species, height was significantly lower in warmed plots for *I. laurina* by 9.3 cm (SE 4.19) and *T. versicolor* by 11 cm (SE 5.5) (respectively, $p = .02$, $df = 43$ and $p = .02$, $df = 46$; Figure 1). For other species, mean seedling height (between relative months 41–53) was also lower in warmed plots, although not statistically significant due to higher variation, for *P. pittieri* by 7.5 cm (SE 4.3; $p = .06$), *L. panamensis* by 2.8 cm (SE 7.4; $p = .70$), *O. macrocalyx* by 3.0 cm (SE 2.8; $p = .29$), *V. surinamensis* by 3.3 cm (SE 4.7; $p = .48$).

We determined the relative growth rate (RGR) for each individual seedling ($n = 30$ per species per treatment) using a first order power model, which provided a good fit to our data with an average model fit of $R^2 = 0.84$ (SE ± 0.02), $p = .013$ (SE ± 0.007). The RGR data confirmed the finding of significantly lower relative growth rates in warmed plots compared to controls for *I. laurina* ($p = .037$) and *T. versicolor* ($p = .009$) and marginally lower for *P. pittieri* ($p = .083$) (see Figure 2 for RGR for individuals and treatments effects; Figure 1 shows the average RGR model fit for all individuals per species combined).

Light saturated photosynthesis (A_{sat}) showed similar patterns to those found in the growth data. There was significantly lower A_{sat} for seedlings in warmed compared to control soils, although there was also large variation among species (treatment effect $p = .02$, species effect $p < .001$, treatment \times species interaction $p = .8$; 2-way ANOVA). There were no significant treatment effects for individual species, but there was an overall trend for decreased A_{sat} under the warming treatment for all species (Figure 3). We found no effect of light (PPFD) on A_{sat} ($p = .81$).

Herbivory over 3 years of growth was lower in warmed than control plots for all species combined ($p < .001$). For individual species, there was an effect for two of the six species, *T. versicolor* and *V. surinamensis*, for which herbivory was significantly lower in warmed compared to control plots (Kruskal–Wallis test: $p < .0001$ and $p = .04$, respectively; based on $n = 96$ observations per species; Figure 4).

Soil nutrient mineralization was not affected by warming on average over 3 years of warming, for either $\text{PO}_4\text{-P}$, $\text{NH}_4\text{-N}$, and $\text{NO}_3\text{-N}$ ($p = .10$, $p = .39$, $p = .055$, respectively; repeated measures ANOVA; Figure 5). However, there were some seasonal patterns. Mineralized PO_4 varied seasonally ($p < .001$) and under warming there was an increase in mineralized PO_4 during the dry-wet season transition (April–June inclusive months; 2018 and 2019; see *Relative months 17–19 and 29–31*; warming effect $p = .02$). Mineralized NO_3 did not vary over time ($p = .41$) and was marginally lower in warmed plots overall ($p = .055$). Mineralized NH_4 did not vary seasonally ($p = .1$). There were no significant differences in mean annual extractable soil nutrients between control and warmed plots, determined over 2 years of warming: for extractable NH_4 : control = $3.34 \pm 0.58 \text{ mg kg}^{-1}$; warmed = $3.53 \pm 1.00 \text{ mg kg}^{-1}$; for extractable NO_3 : control = $3.3 \pm 0.36 \text{ mg kg}^{-1}$; warmed = $3.5 \pm 0.49 \text{ mg kg}^{-1}$; for

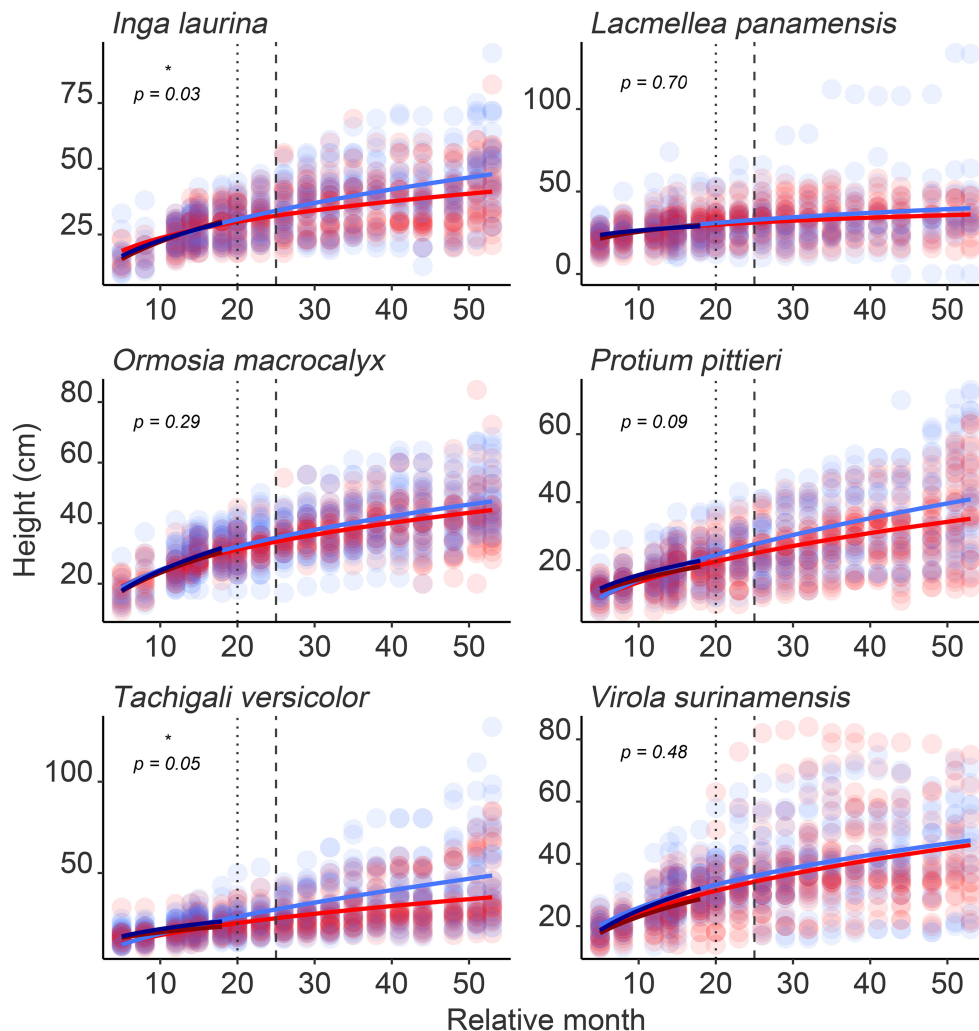


FIGURE 1 The effect of soil warming on seedling height measured over 4 years (2016–2020). Seedlings were planted in November 2015, and height was determined each 3 months, from April 2016 to March 2020. The warming treatment began in June 2017 with a testing phase (from relative month 20; dotted vertical line) and was switched on in full from November 1, 2017 (relative month 25; dashed vertical line). Dots represent individual seedlings, where red=warmed and blue=control. Lines represent an average power-law model fitted to all individuals per species for warmed and control plots (we tested for treatment effects using the RGR determined for individuals; Figure 2). The darker colored lines represent growth rates prior to the experimental treatment (i.e., before relative month 20; no significant differences between treatments). Data are for five plots per treatment. Treatment effects are shown for seedling height for each species during the latter measurement phase, relative months 41–53 (see Methods). The overall effect of warming on height for all species together was highly significant (** $p < .001$).

extractable PO_4 ; control = $6.4 \pm 0.49 \text{ mg kg}^{-1}$; warmed = $7.3 \pm 0.34 \text{ mg kg}^{-1}$ (means ± 1 SE) (see Nottingham et al., 2020).

In addition to treatment effects on plant growth, we explored how soil nutrient availability, light and plant species traits affected growth (RGR). Specifically, we tested the effect on RGR of soil nutrient availability (averaged mineralized N and P by plot), of light (PPFD_{clear}) and whether the species was an N-fixer. The largest effect in the final model was N-fixing identity, with greater growth for N-fixing species (β ($\log[\text{dh}]/\log[\text{dt}]$) = 9.52×10^{-2} , $p = .002$), followed by a positive influence of PPFD_{clear} ($\beta = 1.38 \times 10^{-3}$, $p = .01$); soil nutrient availability (N or P) did not remain in the final model. There was a large negative effect of an interaction with N-fixing identity

and the warming treatment, indicating reduced growth under warming for N-fixing species ($\beta = -7.04 \times 10^{-2}$, $p = .09$) and a large negative effect of warming ($\beta = -1.27 \times 10^{-2}$, $p = .8$); although these negative effects were retained in the final model, they were not significant due to large variation. The model also included a significant effect of plot-identity, which likely reflects the variation in PPFD among plots (Figure S2; PPFD_{cloudy} ($p < .001$) and PPFD_{clear} ($p < .001$)), in addition to variation in herbivory and mortality among plots. However, this variation in PPFD did not influence our main results based on treatment effects among species, because there was no difference in PPFD among treatments (PPFD_{cloudy} $p = .14$; PPFD_{clear} $p = .23$) nor among plant species (PPFD_{cloudy} $p = .11$; PPFD_{clear} $p = .55$) (Figure S2).

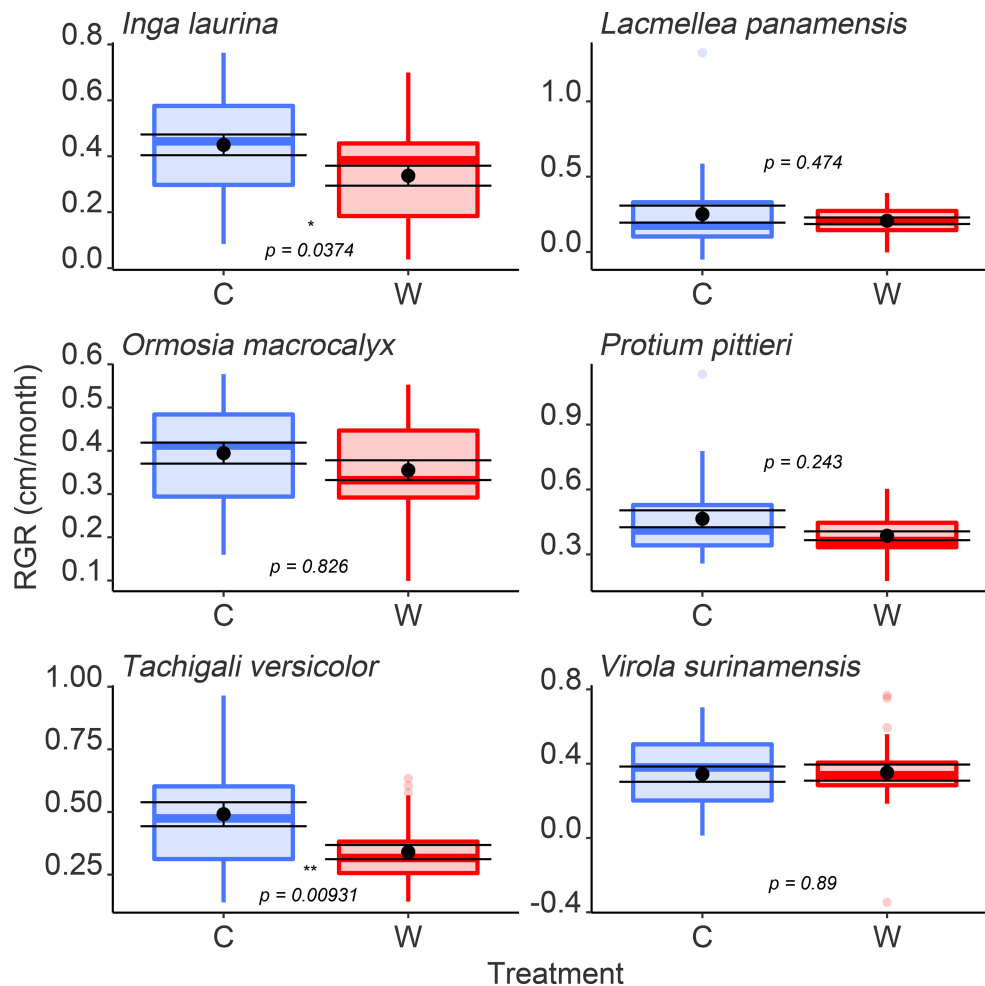


FIGURE 2 The effect of soil warming on relative growth rates (RGR) of seedlings measured over 3 years. RGR was calculated using a first order power-law model and is represented here by the slope of log (height) over log (time in months). Height data was determined every 3 months over a 3-year period. The RGR was significantly lower in warmed soils for two species, *Inga laurina* and *Tachigali versicolor*. The black point represents the mean, and black error bar 1 standard error. The colored box plots are standard Tukey plots, where the center line represents the median, the lower and upper hinges represent the first and third quartiles, and whiskers represent ± 1.5 the interquartile range. The colored dots are individuals. Data are $n = 30$ individuals per species per treatment.

4 | DISCUSSION

Three years of soil warming resulted in decreased seedling growth and light-saturated photosynthetic rates. Although growth declines were observed for all six studied species, they were most pronounced for two N-fixing species, *I. laurina* and *T. versicolor*. This result supports our third hypothesis, that warming would reduce seedling growth particularly of N-fixers because of a negative effect of warming on N-fixation. The result is consistent with a synthesis of N-fixation rates across global ecosystems showing a low temperature optimum for terrestrial nitrogenase activity (Houlton et al., 2008), with the resulting prediction of decreased rates of N-fixation in tropical soils under warming (Wang & Houlton, 2009).

Our results tentatively suggest a negative effect of warming on processes that contribute to N-fixation. However, without direct evidence of N-fixation rate changes to show this conclusively, we note that it is possible that other mechanisms may have contributed to this growth response, including increased root respiration and

changes in the abundance or activity of root-associated mycorrhizal fungi or pathogens. Our results are inconsistent with our first hypothesis, that increased metabolism and C loss (e.g., from increased respiration) would lead to growth declines across all species. Although we are yet to quantify changes in root growth and respiration for these seedlings, an earlier study from the same study site showed that warming significantly increased the heterotrophic (soil-derived) CO_2 efflux but had negligible effect on the root-derived efflux (Nottingham et al., 2020). Together with our observation of no consistent growth decline across all seedlings (Figure 2), current data suggest that the growth decrease under warming was not principally explained by increased root respiration.

Our results are also contrary to our first hypothesis that soil warming would cause a plant systemic response that increased plant metabolic rates and reduced growth. Increased root-rhizosphere metabolic rates—for root respiration, exudates, or symbionts—would increase belowground C demand for photosynthates and, could in turn, lead to reduced limitation on maximum photosynthetic rates

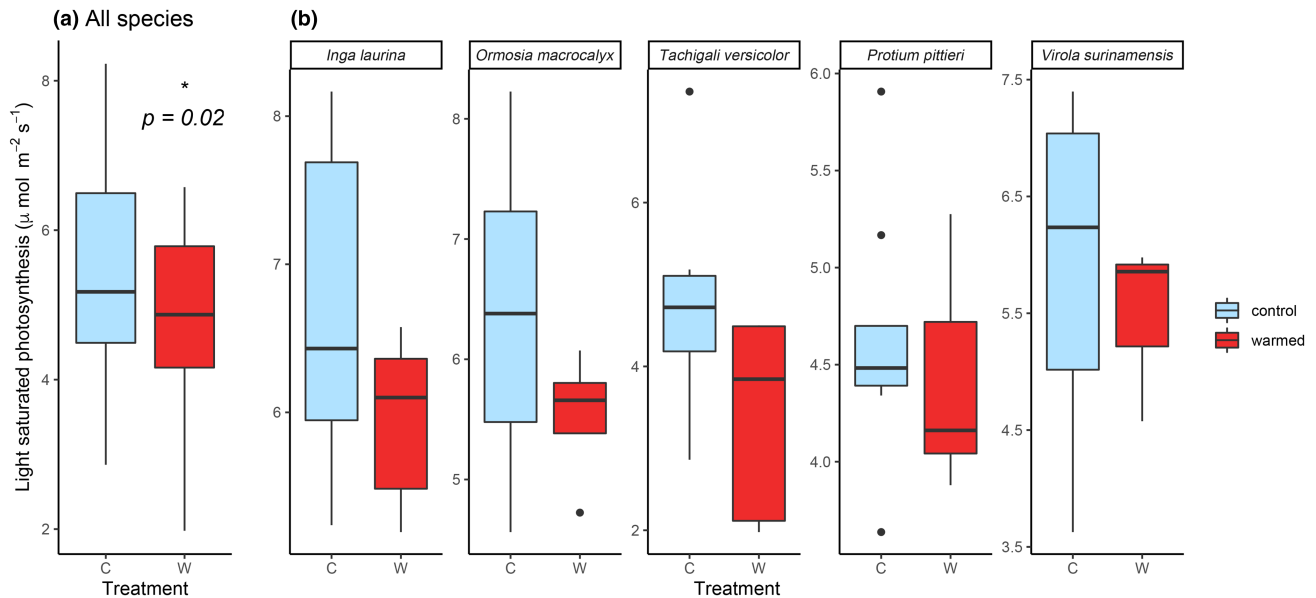


FIGURE 3 The effect of soil warming on light saturated photosynthesis. Light-saturated photosynthetic rate declined significantly with warming across all species ($p = .02$), with a significant species interaction term ($p < .001$); although significant effects were not detected for individual species due to high variance. Measurements were made during the wet season in 2019, 2 years after the onset of the warming treatment. The black point represents the mean, and black error bar 1 standard error. The colored box plots are standard Tukey plots, where the center line represents the median, the lower and upper hinges represent the first and third quartiles, and whiskers represent ± 1.5 the interquartile range.

(e.g., during sunflecks) by removing feedback inhibitions caused by accumulation of photosynthate in leaves (Neales & Incoll, 1968; Paul & Eastmond, 2020). This hypothesis assumed photosynthesis to be at least partly influenced by growth (sink activity), rather than solely by light availability (*sensu* Körner (2015)). However, we found decreased light-saturated photosynthesis rates under soil warming, which may affect plant growth even for seedlings growing in the understory, given that a positive leaf C balance in this environment is dominated by periods of high light when maximum rates of photosynthesis can occur (Walters & Reich, 1999) and that light levels (for both overcast and sunny days) were evenly distributed across our experimental plots (Figure S2).

Warming of root systems may also induce a hormonal response that affects tissue development and whole plant growth. However, temperature responses of hormone synthesis and signaling are highly complex, and the resulting effects on plant growth and development depend on the interplay among multiple hormones, which are influenced by the temperature range to which plants are exposed (Casal & Balasubramanian, 2019; Castroverde & Dina, 2021). We cannot rule out such a response, although it is difficult to reconcile with our observation of species-specific growth declines.

Consistent with our results, field studies where tropical tree species were warmed from aboveground have similarly shown decreased photosynthesis in response to warmer temperatures (Bahar et al., 2017; Carter et al., 2020, 2021; Doughty, 2011; Varhammar et al., 2015), including studies of whole plants across natural temperature gradients (Bahar et al., 2017; Varhammar et al., 2015), in situ warming studies at the leaf level (Doughty, 2011) and in situ plant and soil warming experiments (Carter et al., 2020, 2021).

However, because we warmed plants from belowground, a different mechanism via altered belowground processes must explain our result of decreased photosynthesis following soil warming.

We can further reject our second hypothesis, that seedling growth response to warming was the result of changes in soil nutrients. Three years of warming in this lowland tropical forest resulted in no change in soil N availability (Figure 5), in contrast to warmed temperate forest soils over 11 years where increased N availability stimulated plant growth (Melillo et al., 2011) and in a study where the warming-induced stimulation of *Acer rubrum* seedling growth was less pronounced when seedlings were fertilized with N (Wheeler et al., 2017). In our tropical forest study, soil N availability was unchanged, but soil P availability increased under warming (Figure 3); as also found for warmed tropical forest soils in Puerto Rico (Reed et al., 2020). In our Panama forest, the soil P availability increase occurred during the dry-wet season transition, which coincides with a peak in litterfall inputs, soil microbial activity, and soil respiration rates (Nottingham et al., 2010; Wieder & Wright, 1995). Yet despite this seasonal increase in soil P there was no increase in seedling growth (Figure 1), which is consistent with findings in nearby forest where P availability was not found to limit seedling growth (Santiago et al., 2012; Wright et al., 2011). It may also take longer time scales for changes in soil P availability to result in plant growth changes for this forest, because in a nearby fertilization experiment it took 11 years for P addition for an increase in tree production of leaf litter (Wright et al., 2011); although elsewhere in an Amazon forest an increase in NPP was detected after 2 years of P addition (Cunha et al., 2022). Moreover, while experimental P addition has been shown to increase the growth of tropical seedlings, the response

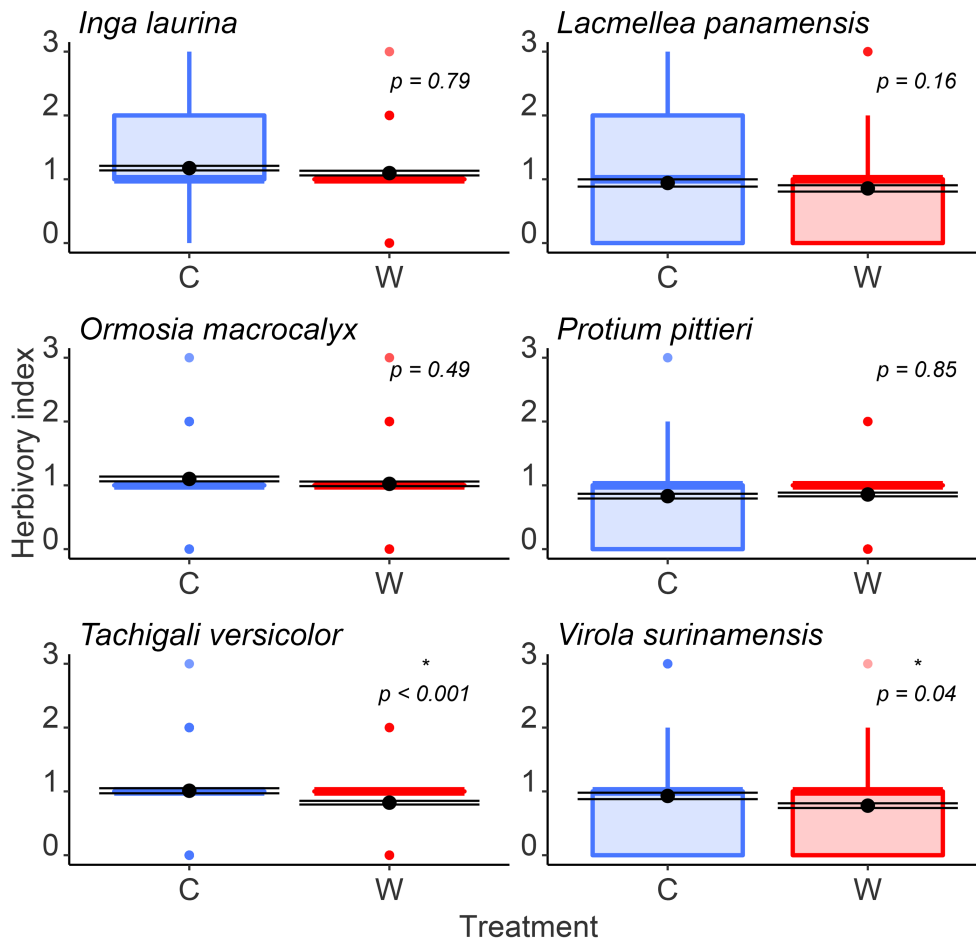


FIGURE 4 The effect of soil warming on herbivory of seedlings. Herbivory was assessed using a continuous scale index where 0 = no visible leaf damage, 1 = minor damage (<10% leaf area affected), 2 = medium damage (10–40% leaf area affected), 3 = major damage (>40% leaf area affected). The black point represents the mean, and black error bar 1 standard error. The colored box plots are standard Tukey plots, where the center line represents the median, the lower and upper hinges represent the first and third quartiles, and whiskers represent ± 1.5 the interquartile range. There was also a significant reduction in herbivory across all species combined ($p = .00036$; Kruskal–Wallis). Data are 96 observations, made over a 3 year period.

appears strongly species-specific (Zalamea et al., 2016). Overall, we find no evidence that seedling growth was affected by a change in soil nutrient availability under warming.

Our results further suggest that N-fixation rates were not affected by soil nutrient availability, as observed elsewhere (Fan et al., 2019). High soil N availability can result in reduced N-fixation rates where the symbioses are facultative and downregulated by the host plant (Barron et al., 2011; Menge et al., 2009). Relatively lower N-fixation has been observed for mature tropical trees in relatively N-rich soils (extractable $\text{NO}_3^- \text{N} > 4 \text{ mg kg}^{-1}$), suggesting downregulation of N-fixation under high N availability (Barron et al., 2011). However, evidence from 14 tropical forest fertilization experiments found that N addition increased foliar N concentration, suggesting N-limitation of growth which is inconsistent with downregulated N-fixation rates (Wright et al., 2018). Moreover, soil N availability in our plots was relatively low and unaffected by warming (seasonal variation varied by $2\text{--}4 \text{ mg kg}^{-1}$; Figure 5) and within the range that Barron et al. (2011) observed high N-fixation rates. The same study found higher N-fixation rates in forest gaps and for young and growing

trees, suggesting an increased likelihood of N-fixation in our study of growing seedlings.

It is also possible that changes in AM communities and activity under warming affected seedling growth. However, our finding of reduced seedling growth is counter to our fourth hypothesis of increased growth due to increased AMF growth and activity. Tropical forest seedlings are strongly dependent on associations with AM fungi to facilitate P acquisition for growth (Kiers et al., 2000; Plassard & Dell, 2010). Although information is not yet available on mycorrhizal communities and abundance for these specific seedlings, a study of microbial communities in the bulk soil from the same study site found that warming increased the relative abundance of Glomerales (Nottingham et al., 2022). Consistent with this observation, a broad pattern of increased abundance of arbuscular mycorrhizae has been reported in a meta-analysis of warming experiments outside the tropics (Mohan et al., 2014). Mycorrhizae can influence plant response to warming (Heinemeyer et al., 2006), so increased mycorrhizal abundance could drive plant responses under a warming climate. However, a study of tree seedlings in Panama found that the effect

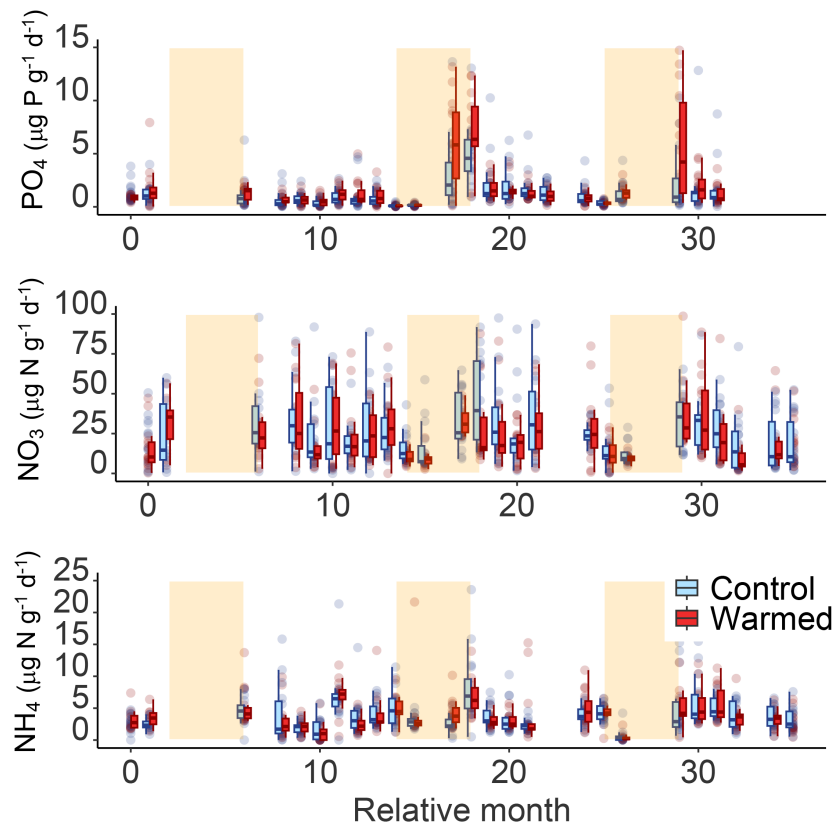


FIGURE 5 Soil mineralized N and P, over 3 years of soil warming (36 months). Determined by in situ mixed bed ion exchange resins. Extractable PO_4 , NH_4 , and NO_3 were calculated from the difference between extracted P or N from resin deployed in the field and resin not deployed (blanks) and expressed as extractable NH_4 -N and NO_3 -N per g resin per day. Data are in units $\mu\text{g P}$ (or N) $\text{g}^{-1} \text{d}^{-1}$. The colored box plots are standard Tukey plots, where the center line represents the median, the lower and upper hinges represent the first and third quartiles, and whiskers represent ± 1.5 the interquartile range. For each time point, $n=5$ replicates per treatment. Yellow shaded bars represent dry season (Jan–March inclusive).

of mycorrhizal inoculation on acclimation of respiration is highly species specific (Fahey et al., 2016). If, with warming in our tropical forest study, the activity and abundance of root-associated AM fungi indeed increased, any positive effect on seedling growth was clearly offset by an overriding negative effect via other processes.

Thus, decreased seedling growth via an effect of warming on N-fixation remains the explanation most congruent with our finding that 3 years of field soil warming resulted in decreased growth for two (out of three) N-fixing species. This response is likely the consequence of multiple effects on N-fixation as an emergent property, an interpretation that can further explain contrasting evidence reported elsewhere. Wang and Houlton (2009) predicted decreased N-fixation with warming in tropical regions on the basis of a low thermal optima for nitrogenase ($\sim 25^\circ\text{C}$), albeit based on a study lacking data for tropical forests (Houlton et al., 2008). In contrast, Bytnerowicz et al. (2022) reported relatively high temperature optima for N-fixation (29 – 36°C) for seedlings of two tropical tree species (*Morella* and *Gliricidia* spp.). However, that study was based on the response of potted seedlings to short-term temperature incubations (<6 h), and the effect of warming on soil N availability was not reported, which is known to influence rates of N fixation (Barron et al., 2011; Cusack et al., 2009).

To understand the mechanisms behind our observed growth decline for two N-fixing species under warming, and to further reconcile contrasting observations made elsewhere (Bytnerowicz et al., 2022; Houlton et al., 2008), further study is required on the temperature response of the contributing processes that determine N-fixation. Here, we describe four critical processes that require study. (i) To determine the intrinsic temperature sensitivity and kinetics of the enzyme nitrogenase. Several studies report results consistent with a lower thermal optimum for nitrogenase activity (e.g., $\sim 25^\circ\text{C}$) (Houlton et al., 2008; Montanez et al., 1995; Waughman, 1977), which contrasts with the high thermal optima reported for soil hydrolytic and oxidative extracellular enzymes (Fanin et al., 2022), including for our tropical forest site where enzyme activity increased up to 34°C under in situ warming and up to 40°C under short-term laboratory incubation (Nottingham et al., 2022). While it is possible that the temperature response of nitrogenase differs from that of soil extracellular enzymes, it is also possible that reports of low thermal optima for N-fixation may reflect changes in other factors including nodule size and quantity (Kessler et al., 1990; Reddell et al., 1985) or of the N-fixing bacterial community (Montanez et al., 1995). (ii) To determine whether the temperature-response varies within N-fixing bacterial communities and altered activity through differential investment in

gene expression relevant for symbiotic N-fixation function. The rate of tropical forest soil N fixation is influenced by the soil bacterial community composition (Reed et al., 2010), and different tree species are known to form associations with different genera of N-fixing bacteria (Parker, 2008). For example, the tree genera that declined in growth under warming in our study (*Inga* and *Tachigali*) associate with Rhizobia from the genus *Bradyrhizobium* (Parker, 2008); yet it is possible that other microbial genera may respond differently (e.g., of *Frankia*, *Mesorhizobium*, *Rhizobium*, *Burkholderia*). For example, variable temperature responses to nodulation were observed among five subspecies of *Burkholderia japonicum* (Montanez et al., 1995). At the same time these community differences can influence the extent of nodulation; and several studies have reported changes in nodulation with temperature, in both quantity and size (Kessler et al., 1990; Reddell et al., 1985). Given the potential differential response of rhizobia strains to soil warming, differences in specialization and compatibility patterns with their plant hosts (Andrews & Andrews, 2017) may also explain difference in N-fixing plant species responses to soil warming. (iii) To separate the effect of warming on changes in soil nutrient availability that can feedback on N-fixation rates (Cusack et al., 2009). Increased soil N availability (as observed in warmed temperate soils; Melillo et al. (2011)) reduces N-fixation where the symbiosis is facultative. For example, N-fixation rates decreased with increased soil N availability in tropical forest (Barron et al., 2011) and 35 years of N-fertilization in a temperate agricultural system led to a 50% reduction in N-fixation in rhizosphere soils (Fan et al., 2019). (iv) To consider the temperature response of the plant-bacteria interaction itself and the extent to which the symbiotic relationship may shift either being facultative or obligate under warming (Menge et al., 2009).

Finally, we observed a reduction in herbivory under warming for two species, which included an N fixing species for which growth also decreased under warming, *T. versicolor*. This result could be explained if a reduction in leaf quality occurred, such as an increase in leaf secondary metabolites or reduced N content, as observed in other warming experiments (Prieto & Querejeta, 2019). However, we found no change in chlorophyll content, which is typically proportional to N content and maximum photosynthetic rates (Evans, 1989), although chlorophyll content was measured at a later stage (Figure S3). Moreover, it should be noted that herbivory responses at plot-scale, where herbivores can select plants outside the plot if warming reduced leaf quality, may not represent future ecosystem-scale responses where herbivory rates may increase to meet nutritional needs from lower quality litter. Warming can also have direct effects on insect herbivore communities (Bale et al., 2002; Hamann et al., 2021) and, although we do not directly assess insect herbivores here, these interactions may be important given independent observations at our experimental site of community shifts of soil and litter-dwelling insects under warming (Bujan et al., 2022). Further study of warming effects on herbivory thus requires examination of both leaf chemistry change and direct effects on herbivore communities and activity.

In summary, we show decreased seedling growth under warming in a tropical forest and suggest a mechanism of a negative effect on

N-fixation (whether on microbial communities, or enzyme function). Nitrogen fixation is a critical process that provides nutrition to tropical forests (Epihov et al., 2021; Hedin et al., 2009) and, to both better understand our results and to resolve poorly constrained findings in the literature (Bytnerowicz et al., 2022; Houlton et al., 2008), studies are needed that determine the temperature response of the underpinning processes that contribute to this emergent property. A negative effect of warming on N-fixation is the explanation most congruent with our finding of decreased seedling aboveground growth for N-fixing species, but growth may also have been further affected by other plant-soil interactions that we are yet to determine, including shifts in C allocation from above to belowground. Further studies are required to elucidate the mechanisms by which warming may be causing this observed decrease in seedling growth and light-saturated photosynthesis rates. Together with studies suggesting that acclimation in photosynthesis and respiration to warming may be limited (Carter et al., 2021), and that C-uptake may even decline (Carter et al., 2021; Doughty, 2011), our results showing decreased seedling growth with belowground warming further point toward reduced carbon storage for tropical forests in a warmer world.

AUTHOR CONTRIBUTIONS

ATN conceived, designed, and implemented the experiment; PM supported the conception and proposals for the experiment; ATN, MS, MMS, EV, and HAS collected the data; ATN analyzed the data and wrote the paper with primary input from MS and PM. All authors contributed to the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The corresponding author confirms on behalf of all authors that there have been no involvements that might raise the question of bias in the work reported or in the conclusions, implications, or opinions stated.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available at NERC EDS Environmental Information Data Centre. <https://>

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