

RESEARCH ARTICLE

Effects of climate change on plant resource allocation and herbivore interactions in a Neotropical rainforest shrub

Lauren D. Maynard¹  | Elodie Moureau² | Maaïke Y. Bader²  | Diego Salazar³  |
Gerhard Zotz⁴  | Susan R. Whitehead¹ ¹Department of Biological Sciences,
Virginia Tech, Blacksburg, Virginia, USA²Faculty of Geography, University of
Marburg, Marburg, Germany³Department of Biological Sciences,
Institute of Environment, Florida
International University, Miami, Florida,
USA⁴Institute for Biology and Environmental
Sciences, Carl von Ossietzky University
Oldenburg, Oldenburg, Germany

Correspondence

Lauren D. Maynard, Department of
Biological Sciences, Virginia Tech,
Blacksburg, VA, USA.
Email: ldmaynar@vt.edu

Funding information

Deutsche Forschungsgemeinschaft,
Grant/Award Number: BA 3843/3-3 and
ZO 94/8-3; National Science Foundation,
Grant/Award Number: 1856776;
Organization for Tropical Studies; Virginia
Tech

Abstract

Climate change is a mounting global issue, but its consequences will be variable across regions. Tropical species are hypothesized to have reduced climatic adaptability and plasticity. Yet, relative to temperate species, less is understood about how they will respond to climate change. Rising temperature and atmospheric CO₂ could impact plant–herbivore systems directly by altering species traits or abundances, or the effects could be indirect by altering the strength and direction of the relationships that govern organismal strategies and interactions. Using open-top chambers in a Neotropical wet forest, we applied a full-factorial combination of active warming and CO₂ fertilization to investigate the above-ground, short-term effects of climate change on plant–herbivore interactions in a common Neotropical shrub, *Piper generalense*. We aimed to answer two main questions: (1) Could climate change alter plant–herbivore systems through direct effects on plant growth rate, chemical defense, and/or insect herbivore damage rate? and (2) Could climate change affect plant–herbivore systems indirectly by altering (a) the strength of plant resource allocation trade-offs between growth and defense or (b) the effectiveness of plant chemical defense against herbivory? None of the microclimate treatments had direct effects on plant growth, chemical defense, or herbivore damage. However, we did observe a positive relationship between growth and chemical defense in treatments mimicking climate-change conditions, which partially supports the growth–differentiation balance hypothesis. We did not detect any effects of treatments on the effectiveness of plant chemical defense against herbivory. It appears that, in this system, increased CO₂ concentration and temperature may cause indirect, cascading consequences, even where direct effects are not observable. We recommend more climate-change experiments addressing multi-trophic interactions that focus not only on the direct responses of organisms but also on the ways in which climate change can restructure the relationships that govern complex biotic systems.

KEYWORDS

climate-change experiment, *Piper generalense*, plant–herbivore interactions, resource allocation, tropical rainforest

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2022 The Authors. *Ecology and Evolution* published by John Wiley & Sons Ltd.

TAXONOMY CLASSIFICATION

Chemical ecology; Ecosystem ecology; Evolutionary ecology; Global change ecology; Trophic interactions

1 | INTRODUCTION

Tropical forests are powerhouses of biodiversity and carbon sequestration, making them exceptionally valuable in mitigating the effects of climate change (Beer et al., 2010; Hubau et al., 2020; Watson et al., 2018). Shifts in the ecological relationships that structure tropical forests could have global consequences, so understanding the responses of tropical communities to climate change is critical for forecasting altered ecosystem functions. Current evidence suggests climate change may alter a myriad of essential traits and interactions within plant communities, including shifts in plant growth (Rustad et al., 2001), chemistry (Gairola et al., 2010; Holopainen et al., 2018; Jamieson et al., 2017), and multi-trophic interactions, such as plant-herbivore (Burt et al., 2014; Lemoine et al., 2013), plant-herbivore-natural enemy (Boullis et al., 2015), plant-pollinator (Mu et al., 2015), and plant-microorganism interactions (Compant et al., 2010). Yet, most studies have been conducted in temperate regions and without active microclimate manipulation, which has left much to be learned about the mechanistic impacts of climate change in other climate zones.

Climate change has been shown to directly impact plant-herbivore interactions by altering the traits or abundances of the organisms involved, both above and below ground (Van der Putten, 2012; Wood et al., 2012). Above ground, elevated temperature or elevated CO₂ concentration, alone or in combination, generally lead to an increase in plant growth (Bezemer & Jones, 1998; De Graaff et al., 2006; Rustad et al., 2001) in both temperate and tropical regions (Cheesman & Winter, 2013; Dieleman et al., 2012; Granados & Körner, 2002; Würth et al., 1998). Yet, these patterns are more variable for tropical species, as lowland tropical forests have exhibited slower growth under elevated temperatures (Wood et al., 2012), especially when exposed to nighttime warming (Wood et al., 2019). Furthermore, elevated temperature and CO₂ concentration can have complex effects on plant chemistry and plant-herbivore interactions. The elevated temperature has variable effects on plant chemistry, but generally, concentrations of alkaloids and terpenes increase while concentrations of total phenolics decrease (Yang et al., 2018; Zvereva & Kozlov, 2006). Elevated temperature can also increase herbivore performance, abundance, and/or plant consumption, a pattern seen in temperate (Lemoine et al., 2013) and tropical (Bachelot et al., 2020) systems. Elevated CO₂ concentration can increase defensive carbon-based phytochemicals (e.g., phenolics and terpenoids), decrease nutritious foliar nitrogen concentration (Hartley et al., 2000; Moreno-Delafuente et al., 2021), and decrease herbivore preference and performance (Bezemer & Jones, 1998; Holopainen et al., 2018; Moreno-Delafuente et al., 2021). However, the often-suppressive effects of elevated CO₂ on plants and herbivores can be mitigated by elevated temperature. For example,

when temperature and CO₂ treatments are applied in combination, plant chemistry may not be altered (Zvereva & Kozlov, 2006). Some responses have only emerged when both treatments are applied in combination; therefore, applying both warming and CO₂ treatments is important to achieve realistic circumstances and make generalizable conclusions about the effects of global change on plant-herbivore interactions (Zvereva & Kozlov, 2006).

In addition to its direct effects on plants and herbivores, climate change may indirectly influence plant-herbivore interactions by changing the strength and/or direction of relationships among different variables that govern plant-herbivore systems. One example of a relationship that could be reshaped by climate change is the trade-off between plant defense and growth. The growth-differentiation balance hypothesis (GDB) posits that plant growth and defense are positively correlated when resources (i.e., light, water, and minerals) are limited, but they are negatively correlated when resources are abundant (Herms & Mattson, 1992). This is because growth is often more curtailed than photosynthesis by limited resources, leaving plants with excess carbon that can be invested in defense. Findings from Massad et al. (2012) supported the GDB in a tropical tree species, *Pentaclethra macroloba* (Fabaceae, Caesalpinioideae), as growth and defense were positively correlated in shady environments, but negatively correlated in sunny environments. The GDB predicts variable responses to elevated concentration of CO₂, dependent upon whether the change in environment stimulates both growth and photosynthesis (creating surplus carbon and thus a positive correlation) or if it stimulates growth but not photosynthesis (creating a trade-off and thus a negative correlation; Herms & Mattson, 1992). Using *Betula* spp. (Betulaceae), Mattson et al. (2005) found support for this hypothesis, as elevated CO₂ concentration stimulated growth and photosynthesis, creating surplus carbon for more allocation to secondary metabolism, and thus leading to a positive relationship between growth and defense. Other factors that could limit growth more than photosynthesis, including low temperature or drought, can increase the carbon available for chemical defense allocation without a trade-off with growth, yielding a positive relationship between growth and defense (Herms & Mattson, 1992). The increased temperature could cause drought, imposing plant stress and water loss, which may also yield a positive relationship. In sum, increased CO₂ concentration and temperature could have complex and potentially conflicting consequences for plant growth and defense with considerable ramifications for the ecology and evolution of plant-herbivore interactions (Huot et al., 2014). Thus, manipulating these two variables, individually and in concert, is critical for understanding the consequences of future climate change for plant-herbivore interactions.

Another relationship within a plant-herbivore system that could be indirectly affected by climate change is the efficacy of

plant chemical defense against herbivore damage. During the long ecological relationship between plants and herbivores (Ehrlich & Raven, 1964), plants have evolved numerous chemical defenses that negatively affect herbivores through decreased herbivore preference and/or performance. Still, this relationship can vary considerably. The foliar concentration of total phenolics is often inversely related to herbivore damage, so phenolic compounds are thought to play a role in direct antiherbivore defense (Hoffland et al., 2010). Yet, climate change could affect the extent to which phenolic content predicts damage through a variety of mechanisms. For example, it could induce changes in a plant's primary and secondary metabolite profile. Elevated CO₂ concentration can reduce plant nitrogen concentration, including key amino acids for insects (Moreno-Delafuente et al., 2021), thereby reducing the resources herbivores have available to invest in detoxification and increasing the effectiveness of phenolics as a defense. Other possibilities for shifts in the effectiveness of chemical defense include the altered efficiency of insect detoxification enzymes or shifted compound metabolism or translocation, as seen with reduced pesticide efficacy under elevated temperature and CO₂ conditions (Matzrafi, 2019). Overall, understanding the impacts of climate change on the relationship between chemical defense and herbivore damage may be equally as important as understanding the direct effects on defense production.

In this study, we experimentally raised temperature and/or CO₂ concentration in open-top chambers in the understory of a tropical wet forest to investigate possible short-term, above-ground effects of climate change on a common Neotropical shrub, *Piper generalense* Trel. (Piperaceae). Employing the experimental design introduced in Bader et al. (2022), this is, to the best of our knowledge, the first study to measure the responses of tropical understory plants to active warming and CO₂ fertilization. Along with the direct impacts, we explored the indirect consequences of climate change on plant-herbivore interactions that could occur through changes in relationships among variables in the system (Figure 1). Our study aimed to answer two main questions: (1) Could climate change alter plant-herbivore systems through direct effects on plant growth rate, chemical defense, and/or insect herbivore damage rate? and (2) Could climate change affect plant-herbivore systems indirectly by altering (a) the strength of plant resource allocation trade-offs between growth and defense or (b) the effectiveness of plant chemical defense against herbivory?

2 | MATERIALS AND METHODS

2.1 | Study site and system

Our study was conducted in the lowland rainforest of La Selva Biological Station (hereafter La Selva), Heredia Province, Costa Rica. The station is managed by the Organization for Tropical Studies (OTS) and comprises approximately 1600ha of tropical wet forest. *Piper* (Piperaceae) is one of the largest genera of flowering plants. The greatest diversity of the genus is found in the Neotropics and lowland tropical forests (Gentry, 1990), including La Selva, which hosts over 60 species of *Piper* (OTS, 2022). *P. generalense* Trel. (synonyms: *Piper arieianum*, *Piper arielanum*, and *Piper trigonum*) was chosen for our study because it is a common understory shrub at La Selva (Greig, 2004). *P. generalense* has a similar phenolic concentration relative to its congeners, which average 3%–6% total phenolics (tannic acid equivalents per gram dry weight of leaf material; Baldwin & Schultz, 1988). *P. generalense* leaves persist on the plant for an average of 1.5–2 years (Marquis, 1990). *Piper* plants host many insect herbivores, including *Piper* specialists, such as *Eois* (Geometridae) caterpillars, *Memphis* (Nymphalidae) caterpillars, Curculionidae, and Chrysomelidae, as well as generalists, such as Orthoptera and Erebididae (Dyer et al., 2010; Massad et al., 2022). *P. generalense* plants experience damage throughout the year by a variety of insect herbivores, including Coleoptera, Diptera, Hymenoptera, Lepidoptera, and Orthoptera, as well as leafcutter ants (*Atta cephalotes*; Marquis, 1987, 1990).

2.2 | Data collection

2.2.1 | Microclimate-manipulation experiment

The experimental design used a full-factorial combination of active warming and CO₂ fertilization in open-top chambers (1.6 m tall and ca. 1.2m in diameter). Five treatments were created with five replications each: (1) ambient control (no chamber), (2) control chamber, (3) elevated temperature (+3°C), (4) elevated CO₂ concentration (+300ppm), and (5) elevated temperature and CO₂ concentration (+3°C and +300ppm). CO₂ fertilization was fixed and only during the day; temperature increase was regulated with a feedback

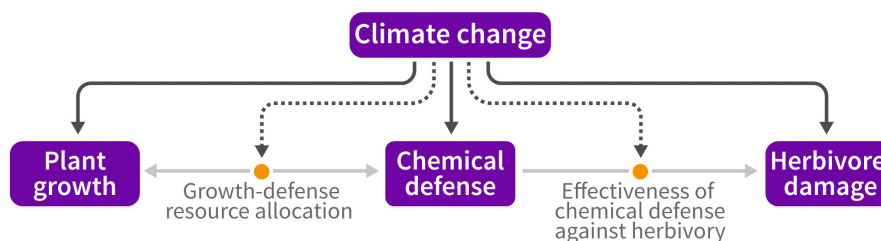


FIGURE 1 Schematic representation of how climate change may affect a plant-herbivore system. It could have direct impacts (solid black lines) on variables that were measured. It could also have indirect impacts (dashed lines) on the relationship between the variables (solid gray lines).

system and continuous (day and night). The magnitudes of increase were chosen based on the projected increase in global average land temperature by 2100 (IPCC, 2013). The setup successfully heated the air during the study period (April–September 2018) by 2.4°C on average (range of chamber means = 1.4–4.0°C, standard deviation between chamber means = 0.9°C, overall standard deviation among chambers and through time = 1.6°C), and increased CO₂ concentration by about 250 ppm (range of chamber means: +131–402 ppm, with higher increases in non-heated chambers). In treatments with increased temperature, relative humidity decreased compared to ambient control treatments. Chamber assembly and treatment manipulation are further detailed in Bader et al. (2022).

2.2.2 | Plant metrics

Twenty-five *P. generalense* saplings were collected from the surrounding forest and transplanted into individual 4.5-liter pots with soil collected from around each excavation. One potted plant was placed inside each of the 20 chambers and five control plots. The experiment ran for 6 months (April–September 2018). At the end of the experiment, three metrics were collected from each plant (Figure A1): (1) proportional change in height (i.e., plant growth), (2) foliar total phenolics (i.e., chemical defense), and (3) proportion of leaf removed (i.e., herbivore damage/herbivory). Plant height was measured from the soil to the top of the plant at the beginning and the end of the experiment. To calculate the proportional change in height, the height increment was divided by the initial plant height. Next, four fully expanded leaves were collected from each plant (100 leaves total), including two mature (proximal) and two young (distal) leaves. Expanding leaves were collected but not included in our analyses because none of the still-expanding leaves had any herbivore damage or sufficient mass for total phenolics extraction and quantification. Herbivore damage was assessed for all leaves collected and was measured using a leaf area meter (LI-3100C Area Meter, LI-COR Inc.). To calculate the proportional damage, the real leaf area was subtracted from the total leaf area and the difference divided by the total leaf area. To analyze total phenolic concentration, the two leaves from each plant and the same age class were combined to achieve an adequate starting weight for extraction. Total phenolics were estimated using the Folin–Ciocalteu assay with modified protocols from Salazar et al. (2018) and Ainsworth and Gillespie (2007). Total phenolics concentration was calculated as the proportion of dry weight in gallic acid equivalents (GAE). The genus *Piper* has a broad range of specialized metabolites in the leaves (Dyer et al., 2004; Richards et al., 2015). We chose to examine total phenolics only and to use their concentration as a proxy for chemical defense. Limited amounts of plant material for extraction and analysis were one important factor considered in this decision. Even though phenolics have a broad range of functions within a plant, they play a key role in plant defense (Barton & Koricheva, 2010) and are common indicators of the capacity of chemical defense (Gong & Zhang, 2014).

2.2.3 | Statistical analysis

All analyses were performed in R v4.0.4 (R Core Team, 2021). Welch's *t*-tests were performed using base R; beta-regressions were performed in the package *betareg* (Cribari-Neto & Zeileis, 2010); generalized linear mixed models (GLMMs) were performed in the package *glmmTMB* (Brooks et al., 2017); hypothesis testing was performed using package *car* (Fox & Weisberg, 2019); estimated marginal means were performed using package *emmeans* (Lenth et al., 2019); plots were created using package *ggplot2* (Wickham, 2019).

To determine whether there was a significant chamber effect on plant growth, foliar chemical defense, and herbivory, we performed Welch's *t*-tests comparing plants in ambient control treatments versus chamber control treatments. To determine the effect of leaf age on foliar chemical defense and herbivory, we performed Welch's *t*-tests comparing young leaves versus mature leaves, using one value (total phenolics concentration of the two-leaf sample or mean herbivore damage of the two replicate leaves) per age class per plant, that is, $N = 25$. We found no significant chamber effects (results below); thus, we excluded the ambient control (no chamber) from all subsequent analyses. We found that leaf age had a significant effect on foliar chemical defense and herbivory (results below). Due to this effect of leaf age, we split all subsequent analyses of phenolics and herbivory by leaf age.

To determine whether elevated temperature and/or CO₂ concentration had direct effects on plant growth, chemical defense, or herbivore damage rates, we fitted five univariate models with plant growth, foliar chemical defense of young and old leaves, and herbivory of young and old leaves as response variables. Treatment was always the predictor variable. Because all plant metrics were reported as proportions, we used beta-regressions. For herbivory, we used a GLMM with a beta error distribution, and plant ID was the random effect to account for repeated measures on individual plants. We performed Type II Wald's chi-square tests to assess statistical support for the effect of microclimate treatments on individual plant metrics.

To determine whether elevated temperature and/or CO₂ concentration altered the relationship between plant growth and defense, we used a beta-regression. Total phenolics (proportion dry weight in GAE) were the response variable. Treatment, plant growth (proportional change in height), and their interaction were the predictor variables. Based on a significant interaction between treatment and plant growth, we performed Type III Wald's chi-square tests to assess statistical support for the effects of the predictor variables on the response variable. We then analyzed the data separately for each treatment, using only growth as the predictor variable in the beta-regression models.

To determine whether elevated temperature and/or CO₂ concentration modified the influence of foliar chemical defense on herbivory, we fit the data to a GLMM with a beta error distribution. Herbivory (proportional foliar damage) was the response variable. Treatment, foliar chemical defense (total phenolics concentration), and their interaction were the fixed effects. Plant ID was included

as a random effect to account for repeated measures on individuals. Because the interaction was not significant, we performed Type II Wald's chi-square tests to assess statistical support for the effects of the predictor variables.

3 | RESULTS

We found no significant chamber effect (i.e., no difference between ambient control and control chamber) on growth ($t = -1.24$, $p = .27$), foliar chemical defense ($t = -1.22$, $p = .24$), or foliar herbivory ($t = -1.09$, $p = .29$). Because we found no significant chamber effects, we excluded the ambient control from all subsequent analyses. We determined that leaf age had a significant effect on both foliar chemical defense ($t = -4.80$, $p < .001$) and foliar herbivory ($t = 3.25$, $p = .002$). The concentration of total phenolics was, on average, 38% higher in young leaves (Figure 2a; Table A1). Mature leaves suffered, on average, over four times more herbivory than young leaves (Figure 2b; Table A2). All further analyses were therefore completed for young and old leaves separately.

When examining the direct effects of microclimate manipulations on the measured variables of plant growth, chemical defense, and herbivory, we found that variation within treatments was high and treatment was never a significant predictor. Treatments did not have a significant effect on plant growth (proportional change in height; $\chi^2 = 1.04$, $p = .79$; Figure 3a; Table A3), the concentration of total phenolics in young leaves ($\chi^2 = 1.99$, $p = .57$; Table A4) or mature leaves ($\chi^2 = 1.41$, $p = .70$; Figure 3b; Table A4), or herbivory of young leaves ($\chi^2 = 0.37$, $p = .95$; Table A5) or mature leaves ($\chi^2 = 2.08$, $p = .56$; Figure 3c; Table A5).

When examining the effects of microclimate manipulations on the patterns of plant resource allocation, we found that, in both leaf age classes, the interaction of treatment and plant growth was a significant predictor of foliar chemical defense (mature leaves: $\chi^2 = 8.78$, $p = .03$; young leaves: $\chi^2 = 8.31$, $p = .04$). We observed significant, positive relationships between growth and defense in three cases: mature leaves in elevated temperature ($z = 3.44$, $p < .001$), young leaves in elevated temperature ($z = 3.60$, $p < .001$), and young leaves in the combination treatment (increased temperature and CO₂ concentration; $z = 5.18$, $p < .001$). Specifically, with every 1% increase in plant growth, mature leaves experienced a 2% increase in total phenolics in warmed environments (Figure 4a). Similarly, young leaves experienced a 0.5% and 0.9% increase in total phenolics in warmed and combination environments, respectively (Figure 4b). No other treatments had significant effects on the relationships in mature or young leaves (Table A6). Plant growth alone was not a significant predictor of total phenolics concentration in mature or young leaves ($\chi^2 = 0.22$, $p = .64$; $\chi^2 = 0.14$, $p = .70$; respectively). When accounting for the effect of growth as well as its interaction with treatment, treatment was a significant predictor of total phenolics in both leaf ages (mature $\chi^2 = 8.82$, $p = .03$; young $\chi^2 = 10.46$, $p = .02$). Yet, when estimated marginal means were computed, there was no significant difference in phenolics concentration across microclimate treatments (Figure 3, Table A4).

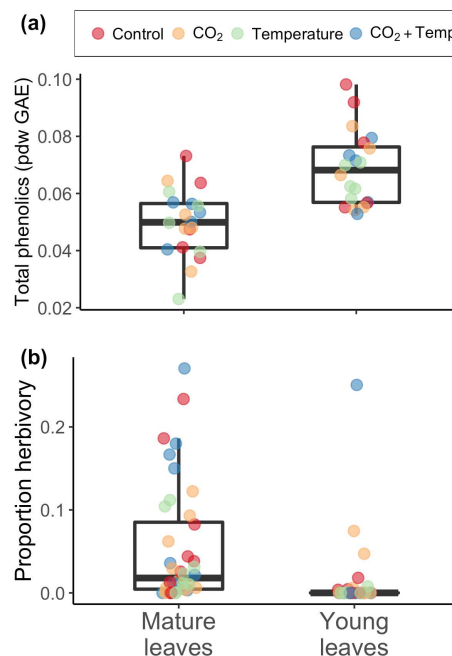


FIGURE 2 Young leaves were more chemically defended and experienced less herbivory than mature leaves. Young leaves (a) had an average of 38% higher concentrations of total phenolics relative to mature leaves. Mature leaves (b) experienced an average of over four times more herbivory relative to young leaves. Black box margins indicate the 25th and 75th percentiles, whiskers the 5th and 95th percentiles, and solid lines within the boxes the median. Points are (a) individual observations of total phenolics concentration (proportion dry weight in gallic acid equivalents) from leaves of *Piper generalense* and (b) individual observations of foliar herbivory (proportional leaf damage) from leaves of *Piper generalense*, color-coded by treatment.

When examining the effects of climate change on herbivore damage or the effectiveness of plant chemical defense against herbivory, we found that chemical defense was the only significant predictor of herbivory in mature leaves ($\chi^2 = 3.97$, $p = .047$). On average, mature leaves experienced 20% less herbivory with every 1% increase in total phenolics, but variation was very large (Figure 5). Neither treatment nor the interaction between treatment and chemical defense was significant predictors of herbivory of mature leaves ($\chi^2 = 3.31$, $p = .35$; $\chi^2 = 1.01$, $p = .80$, respectively). In young leaves, total phenolics, treatment, nor their interaction were a clear predictor of herbivore damage ($\chi^2 = 0.24$, $p = .63$; $\chi^2 = 0.53$, $p = .91$; $\chi^2 = 1.04$, $p = .79$, respectively). Thus, there were similar levels of herbivory among treatments and the efficacy of chemical defense against herbivory did not change among treatments in young leaves.

4 | DISCUSSION

In this study, we explored the above-ground, short-term effects of elevated temperature and CO₂ concentration on a Neotropical rainforest plant-herbivore system. Our study demonstrates the

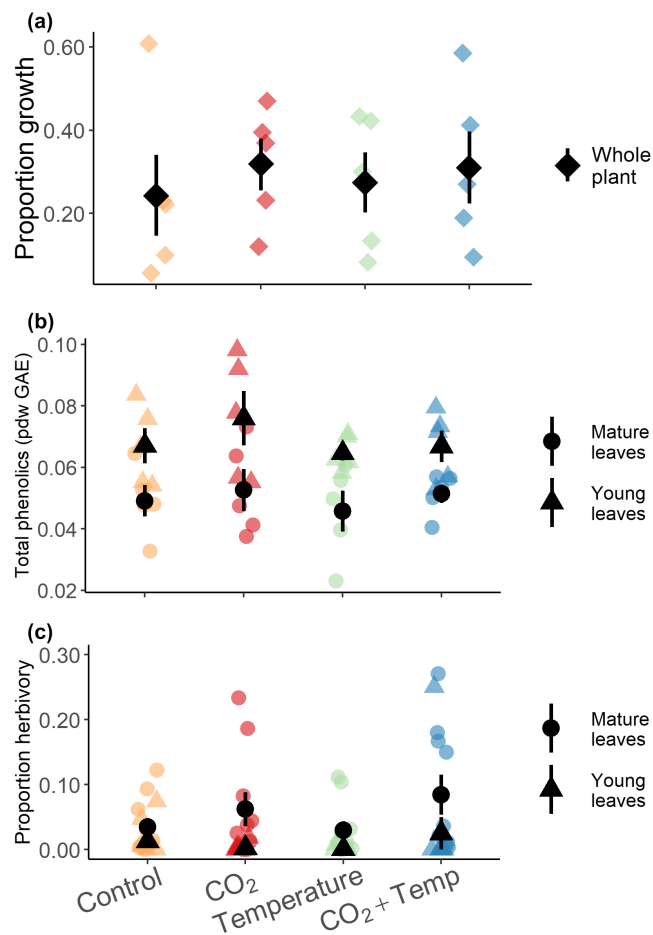


FIGURE 3 Microclimate treatments did not have clear effects on individual responses of *Piper generalense*, including (a) growth (proportional change in height), (b) foliar chemical defense (concentration of total phenolics as the proportion of the dry weight in gallic acid equivalents), and (c) foliar herbivory (proportional leaf damage). In growth (a), individual diamonds represent individual plants. Bold black diamonds and whiskers are means and standard errors for each treatment. In chemical defense and foliar herbivory (b and c), data are separated by leaf age: Young (triangle) and mature (circle). Black triangles and circles with whiskers are the means and standard errors for young and mature leaves, respectively.

importance of active microclimate manipulation in a tropical system using a new experimental approach from Bader et al. (2022). We did not detect any effects of our microclimate treatments on measured variables in *P. generalense*, including plant growth, chemical defense, and herbivore damage. Tropical species are hypothesized to have reduced climatic adaptability and plasticity, but the documented responses in climate change experiments have been variable.

We did observe significant indirect microclimate treatment effects on the relationship between growth and defense. We observed a positive relationship between growth and defense in mature leaves in elevated-temperature treatments and a positive relationship in young leaves in elevated-temperature and combination (elevated temperature plus elevated CO_2) treatments. The GDB predicts that low-temperature, low-moisture environments may limit growth

more than photosynthesis, leaving excess carbon for defense allocation and therefore leading to a positive relationship between growth and defense (Herms & Mattson, 1992). Plants in our treatments with increased temperature, which also decreased relative humidity, experienced a positive relationship between growth and defense; thus, they may have been responding to the decreased relative humidity and potential water stress, similar to *Piper glabrescens* (Carter et al., 2020). Evidence suggests that plants exposed to increased CO_2 concentration may have greater protection against the negative effects of water stress (Chaves & Pereira, 1992; Lloyd & Farquhar, 2008; Van der Sleen et al., 2015); thus, there could be important synergistic consequences of the two treatments in combination. Overall, there was considerable variation within and across treatments (Figure 4, Table A6). Additional data, such as photosynthetic parameters, may be required to disentangle the mechanisms driving our results. Studies that simultaneously manipulate CO_2 concentration and temperature in a tropical system are lacking (Cavaleri et al., 2015), but they are a critical tool in understanding the mechanistic effects of climate change and unraveling the interconnected relationships of these complex systems.

The lack of detectable direct effects of temperature and CO_2 on herbivory and defense was unexpected and adds to a growing literature that suggests climate effects are highly variable and species-specific. We found no effect of elevated temperature and/or CO_2 concentration on foliar chemical defense, measured as total phenolic concentration. Our treatments could have affected other specialized metabolites found in species of *Piper*, such as alkaloids or terpenoids. Nevertheless, the lack of changes in herbivore damage across experimental treatments suggests that, even if other chemical defenses were affected, these effects were not large enough to significantly influence plant-herbivore interactions. The lack of effect of elevated temperature and/or CO_2 concentration on herbivory is in contrast to general patterns reported in climate-change literature, which include an increase in herbivory in elevated temperature, a decrease in herbivory in elevated CO_2 concentration, and no overall effects of the two in combination (Zvereva & Kozlov, 2006). Overall herbivory was low in our study. Foliar damage of mature leaves ranged from 0% to 27% (averaging 5%) and young leaves from 0% to 25% (averaging 1%), which is lower than previously reported *P. generalense* values (ranging 0%–50% and averaging 16%; Marquis, 1987). However, even low rates of herbivory can have severe ecological consequences. In *P. generalense*, just 10% of foliar damage can have long-term impacts on plant fitness, including decreases in growth, seed production, and seed viability (Marquis, 1984). Over two decades ago, Coley (1998) predicted that herbivory in the tropics would increase by 200%–400% given the projected increases in atmospheric CO_2 and drought. Still, little research has been conducted on tropical plant-herbivore interactions (Sheldon, 2019), but recent evidence supports the predictions that above-ground herbivore damage and richness will increase in environments with increased drought and temperature (Bachelot et al., 2020).

Other common patterns of defensive allocation in our *Piper* plants did not deviate from expectations. Firstly, younger leaves

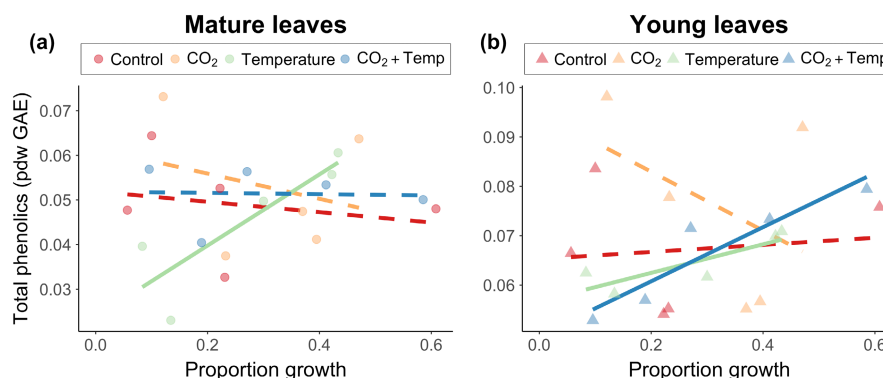


FIGURE 4 Leaves of plants in elevated temperature and combination microclimate treatments experienced significant, positive relationships between growth and defense. Mature leaves (a) of plants in elevated temperature (green line) and young leaves (b) of plants in elevated temperature (green line) and combined elevated temperature and CO₂ concentration (blue line) experienced increased concentrations of total phenolics with increased growth. Points are individual observations of total phenolics concentration (proportion dry weight in gallic acid equivalents) in *Piper generalense* leaves. Lines are linear fits of the data, grouped and color-coded by treatment. The solid lines indicate statistically significant relationships, and dashed lines represent statistically insignificant relationships.

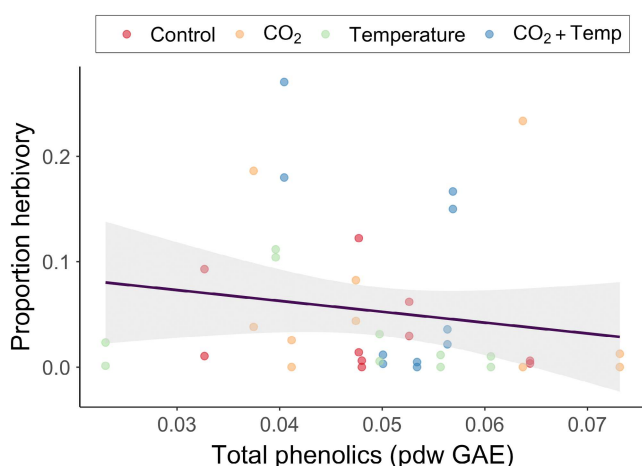


FIGURE 5 Mature leaves experienced 20% less herbivory with every 1% increase in total phenolics. Points are individual observations of herbivore damage from mature leaves of *Piper generalense*, color-coded by treatment. The line is the linear fit of the data with the gray band indicating the 95% confidence interval.

were more chemically defended than mature leaves (Figure 2a; Table A1), which is consistent with general allocation patterns across plant ontogeny (Barton & Koricheva, 2010). Secondly, in mature leaves, more chemically-defended leaves experienced less herbivory (Figure 5), so we did not see a shift in the general efficacy of foliar chemical defense against leaf herbivores. Yet, this negative effect of defense on herbivory was observed only for mature leaves. Young leaves generally had very little herbivore damage (Figure 2b; Table A2), which may be because young leaves have not had the time yet to accumulate enough damage or because phenolics concentration may have been high enough to deter these herbivores even in the least-defended leaves. Early life herbivory appears to be generally low in this species because we observed zero herbivore damage on the collected expanding leaves, which contrasts with previous work reporting that the majority of herbivore damage

occurs on expanding and young leaves (Coley & Barone, 1996). An additional or alternative reason that no effect of phenolics on herbivory in young leaves was detected might be that phenolic compounds found in *Piper* are better deterrents of herbivore species that attack mature leaves. Specialized herbivores (e.g., Geometridae and Chrysomelidae) often consume younger leaves of *Piper*, and these herbivores may be better adapted to overcome these chemical defenses of *Piper*. Nevertheless, characteristic damage patterns of specialists, such as the skeletonizing of the leaves by *Eois* caterpillars, were not observed in our experimental plants. Abiotic factors, such as nutrients, water, and space, could also have significant effects on plant chemistry.

Future studies could expand upon our approach in a few ways. Firstly, we present a limited sample size ($N = 5/\text{treatment}$) over a relatively short time period (6 months). Increasing the sample size would be ideal, as extrapolating meaningful and ecologically relevant patterns from small sample sizes can be challenging. Plants should ideally experience microclimate treatments along the full leaf life cycle. For *P. generalense*, this would imply a rather long study duration of up to 2 years. Our study took place during April–September, so it captured the rainy season at La Selva (May–December) but mostly excluded the dry season (January–April). Active microclimate manipulations are resource intensive and not always feasible at a large scale for extended periods of time, but collaboration within an existing project may be an option. Secondly, *Piper* species can interact with up to four trophic levels (Letourneau, 2004). Future projects should consider incorporating additional response metrics, such as other antagonistic relationships (e.g., plant competition and herbivore–natural enemy interactions) and mutualistic relationships (e.g., pollination and seed dispersal). Furthermore, it would be interesting to examine a broader range of target compounds and/or chemical diversity because shifts in chemical composition could alter the efficacy of plant chemistry in species interactions, as seen in other *Piper* species (Dyer et al., 2003; Whitehead & Bowers, 2014). Other critical processes, such as photosynthesis, respiration, and below-ground interactions

could be considered in future projects. Lastly, we used *P. generalense* as a model system, but studying only one plant species yields obvious limitations in understanding broad patterns. Future projects may consider including multiple plant species or genera.

Our results contribute to an improved understanding of the effects of climate change on a Neotropical plant–herbivore system. In particular, the observed patterns suggest that warming and higher atmospheric CO₂ may have complex consequences on the parameters that govern ecological systems, such as the relationships between plant growth and herbivore defense, even when there are no direct effects on individual system components. Although the consequences of climate change are global, tropical systems are underrepresented in the climate change literature (Feeley et al., 2017; Sheldon, 2019), and just 3% (six of 182) of those tropical climate-change studies discuss species interactions (Sheldon, 2019). Thus, future studies in the tropics that further explore the complex system-level responses of warming, increased CO₂, and other stressors are essential to better predict and prepare for a changing climate.

AUTHOR CONTRIBUTIONS

Lauren D. Maynard: Conceptualization (equal); data curation (equal); formal analysis (lead); investigation (equal); methodology (equal); writing – original draft (lead). **Elodie Moureau:** Conceptualization (equal); data curation (equal); investigation (equal); methodology (equal). **Maike Y. Bader:** Funding acquisition (equal); methodology (equal); project administration (equal); resources (equal); supervision (equal); writing – review and editing (equal). **Diego Salazar:** Methodology (equal); writing – review and editing (equal). **Gerhard Zotz:** Funding acquisition (equal); project administration (equal); writing – review and editing (equal). **Susan R. Whitehead:** Conceptualization (equal); formal analysis (equal); funding acquisition (equal); methodology (equal); project administration (equal); supervision (equal); writing – review and editing (equal).

ACKNOWLEDGMENTS

We thank Orlando Vargas Ramírez, Marisol Luna, Danilo Brenes Madrigal, and Bernal Matarrita-Carranza at La Selva Biological Station. We also thank Michael J. Stamper, Data Visualization Designer and lecturer at the University Libraries, Data Services at Virginia Tech, for his contributions to the figures. We thank members of the Whitehead Lab and E. J. Royal for comments on early manuscript drafts. LDM was supported by a National Science Foundation Graduate Research Fellowship, OTS Graduate Research Fellowships, and Virginia Tech. The climate-change experiment was financed by the German Research Foundation (DFG, BA 3843/3-3 and ZO 94/8-3). Additional funding to SRW was provided by National Science Foundation Grant no. 1856776.

DATA AVAILABILITY STATEMENT

All annotated code, data, and metadata are publicly archived at GitHub (<https://github.com/ldmaynard/Pgeneralense>) and are permanently archived at Zenodo (<https://zenodo.org/badge/latestdoi/285833969>).

ORCID

Lauren D. Maynard  <https://orcid.org/0000-0003-2059-1250>

Maike Y. Bader  <https://orcid.org/0000-0003-4300-7598>

Diego Salazar  <https://orcid.org/0000-0001-9810-5828>

Gerhard Zotz  <https://orcid.org/0000-0002-6823-2268>

Susan R. Whitehead  <https://orcid.org/0000-0002-7089-4594>

REFERENCES

- Ainsworth, E. A., & Gillespie, K. M. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. *Nature Protocols*, 2(4), 875–877. <https://doi.org/10.1038/nprot.2007.102>
- Bachelot, B., Alonso-Rodríguez, A. M., Aldrich-Wolfe, L., Cavaleri, M. A., Reed, S. C., & Wood, T. E. (2020). Altered climate leads to positive density-dependent feedbacks in a tropical wet forest. *Global Change Biology*, 26(6), 3417–3428. <https://doi.org/10.1111/gcb.15087>
- Bader, M. Y., Moureau, E., Nikolić, N., Madena, T., Koehn, N., & Zotz, G. (2022). Simulating climate change *in situ* in a tropical rainforest understorey using active air warming and CO₂ addition. *Ecology and Evolution*, 12(1), e8406. <https://doi.org/10.1002/ece3.8406>
- Baldwin, I. T., & Schultz, J. C. (1988). Phylogeny and the patterns of leaf phenolics in gap- and forest-adapted *piper* and *Miconia* understory shrubs. *Oecologia*, 75(1), 105–109. <https://doi.org/10.1007/BF00378821>
- Barton, K. E., & Koricheva, J. (2010). The ontogeny of plant defense and herbivory: Characterizing general patterns using meta-analysis. *The American Naturalist*, 175(4), 481–493. <https://doi.org/10.1086/650722>
- Beer, C., Reichstein, M., Tomelleri, E., Ciais, P., Jung, M., Carvalhais, N., Rödenbeck, C., Arain, M. A., Baldocchi, D., Bonan, G. B., Bondeau, A., Cescatti, A., Lasslop, G., Lindroth, A., Lomas, M., Luyssaert, S., Margolis, H., Oleson, K. W., Rouspard, O., ... Papale, D. (2010). Terrestrial gross carbon dioxide uptake: Global distribution and co-variation with climate. *Science*, 329(5993), 834–838.
- Bezemer, T. M., & Jones, T. H. (1998). Plant–insect herbivore interactions in elevated atmospheric CO₂: Quantitative analyses and guild effects. *Oikos*, 82(2), 212–222. <https://doi.org/10.2307/3546961>
- Boullis, A., Francis, F., & Verheggen, F. J. (2015). Climate change and tritrophic interactions: Will modifications to greenhouse gas emissions increase the vulnerability of herbivorous insects to natural enemies? *Environmental Entomology*, 44(2), 277–286. <https://doi.org/10.1093/ee/nvu019>
- Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Mächler, M., & Bolker, B. M. (2017). GlmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal*, 9(2), 378–400.
- Burt, M. A., Dunn, R. R., Nichols, L. M., & Sanders, N. J. (2014). Interactions in a warmer world: Effects of experimental warming, conspecific density, and herbivory on seedling dynamics. *Ecosphere*, 5(1), art9. <https://doi.org/10.1890/ES13-00198.1>
- Carter, K. R., Wood, T. E., Reed, S. C., Schwartz, E. C., Reinsel, M. B., Yang, X., & Cavaleri, M. A. (2020). Photosynthetic and respiratory acclimation of understory shrubs in response to *in situ* experimental warming of a wet tropical forest. *Frontiers in Forests and Global Change*, 3, 576320. <https://doi.org/10.3389/ffgc.2020.576320>
- Cavaleri, M. A., Reed, S. C., Smith, W. K., & Wood, T. E. (2015). Urgent need for warming experiments in tropical forests. *Global Change Biology*, 21(6), 2111–2121. <https://doi.org/10.1111/gcb.12860>
- Chaves, M. M., & Pereira, J. S. (1992). Water stress, CO₂ and climate change. *Journal of Experimental Botany*, 43(253), 1131–1139.
- Cheesman, A. W., & Winter, K. (2013). Elevated night-time temperatures increase growth in seedlings of two tropical pioneer tree species.

- New Phytologist*, 197(4), 1185–1192. <https://doi.org/10.1111/nph.12098>
- Coley, P. D. (1998). Possible effects of climate change on plant/herbivore interactions in moist tropical forests. *Climatic Change*, 39(2), 455–472. <https://doi.org/10.1023/A:1005307620024>
- Coley, P. D., & Barone, J. A. (1996). Herbivory and plant defenses in tropical forests. *Annual Review of Ecology and Systematics*, 27(1), 305–335. <https://doi.org/10.1146/annurev.ecolsys.27.1.305>
- Compant, S., Van Der Heijden, M. G. A., & Sessitsch, A. (2010). Climate change effects on beneficial plant-microorganism interactions: Climate change and beneficial plant-microorganism interactions. *FEMS Microbiology Ecology*, 73(2), 197–214. <https://doi.org/10.1111/j.1574-6941.2010.00900.x>
- Cribari-Neto, F., & Zeileis, A. (2010). Beta regression in R. *Journal of Statistical Software*, 34(2), 1–24. <https://doi.org/10.18637/jss.v034.i02>
- De Graaff, M.-A., Van Groenigen, K.-J., Six, J., Hungate, B., & Van Kessel, C. (2006). Interactions between plant growth and soil nutrient cycling under elevated CO₂: A meta-analysis. *Global Change Biology*, 12(11), 2077–2091. <https://doi.org/10.1111/j.1365-2486.2006.01240.x>
- Dieleman, W. I. J., Vicca, S., Dijkstra, F. A., Hagedorn, F., Hovenden, M. J., Larsen, K. S., Morgan, J. A., Volder, A., Beier, C., Dukes, J. S., King, J., Leuzinger, S., Linder, S., Luo, Y., Oren, R., De Angelis, P., Tingey, D., Hoosbeek, M. R., & Janssens, I. A. (2012). Simple additive effects are rare: A quantitative review of plant biomass and soil process responses to combined manipulations of CO₂ and temperature. *Global Change Biology*, 18(9), 2681–2693. <https://doi.org/10.1111/j.1365-2486.2012.02745.x>
- Dyer, L. A., Dodson, C. D., Stireman, J. O., Tobler, M. A., Smilanich, A. M., Fincher, R. M., & Letourneau, D. K. (2003). Synergistic effects of three piper amides on generalist and specialist herbivores. *Journal of Chemical Ecology*, 29(11), 2499–2514. <https://doi.org/10.1023/a:1026310001958>
- Dyer, L. A., Letourneau, D. K., Chavarria, G. V., & Amoretti, D. S. (2010). Herbivores on a dominant understory shrub increase local plant diversity in rain forest communities. *Ecology*, 91(12), 3707–3718. <https://doi.org/10.1890/08-1634.1>
- Dyer, L. A., Richards, J., & Dodson, C. D. (2004). Isolation, synthesis, and evolutionary ecology of piper amides. In L. A. Dyer & A. D. N. Palmer (Eds.), *Piper: A model genus for studies of phytochemistry, ecology, and evolution* (pp. 117–139). Kluwer Academic/Plenum Publishers. https://doi.org/10.1007/978-0-387-30599-8_7
- Ehrlich, P. R., & Raven, P. H. (1964). Butterflies and plants: A study in coevolution. *Evolution*, 18(4), 586–608. <https://doi.org/10.2307/2406212>
- Feeley, K. J., Stroud, J. T., & Perez, T. M. (2017). Most 'global' reviews of species' responses to climate change are not truly global. *Diversity and Distributions*, 23(3), 231–234. <https://doi.org/10.1111/ddi.12517>
- Fox, J., & Weisberg, S. (2019). *An R companion to applied regression* (3rd ed.). Sage. <https://CRAN.R-project.org/package=car>
- Gairola, S., Shariff, M. M., Bhatt, A., & Kala, C. P. (2010). Influence of climate change on production of secondary chemicals in high altitude medicinal plants: Issues needs immediate attention. *Journal of Medicinal Plant Research*, 4(18), 1825–1829.
- Gentry, A. H. (1990). *Four neotropical forests*. Yale University Press. <http://kdb.kew.org/kdb/detailedresult.do?id=297477>
- Gong, B., & Zhang, G. (2014). Interactions between plants and herbivores: A review of plant defense. *Acta Ecologica Sinica*, 34(6), 325–336. <https://doi.org/10.1016/j.chnaes.2013.07.010>
- Granados, J., & Körner, C. (2002). In deep shade, elevated CO₂ increases the vigor of tropical climbing plants. *Global Change Biology*, 8(11), 1109–1117. <https://doi.org/10.1046/j.1365-2486.2002.00533.x>
- Greig, N. (2004). Introduction. In L. A. Dyer & A. D. N. Palmer (Eds.), *Piper: A model genus for studies of phytochemistry, ecology, and evolution* (pp. 1–5). Kluwer Academic/Plenum Publishers.
- Hartley, S. E., Jones, C. G., Couper, G. C., & Jones, T. H. (2000). Biosynthesis of plant phenolic compounds in elevated atmospheric CO₂. *Global Change Biology*, 6(5), 497–506. <https://doi.org/10.1046/j.1365-2486.2000.00333.x>
- Hermis, D. A., & Mattson, W. J. (1992). The dilemma of plants: To grow or defend. *The Quarterly Review of Biology*, 67(3), 283–335.
- Hoffland, E., Dicke, M., Tintelen, W. V., Dijkman, H., & Beusichem, M. L. V. (2010). Nitrogen availability and defense of tomato against two-spotted spider mite. *Journal of Chemical Ecology*, 26(12), 2697–2711.
- Holopainen, J. K., Virjamo, V., Ghimire, R. P., Blande, J. D., Julkunen-Tiitto, R., & Kivimäenpää, M. (2018). Climate change effects on secondary compounds of forest trees in the northern hemisphere. *Frontiers in Plant Science*, 9, 1445. <https://doi.org/10.3389/fpls.2018.01445>
- Hubau, W., Lewis, S. L., Phillips, O. L., Affum-Baffoe, K., Breeckman, H., Cuní-Sanchez, A., Daniels, A. K., Ewango, C. E. N., Fauset, S., Mukinzi, J. M., Sheil, D., Sonké, B., Sullivan, M. J. P., Sunderland, T. C. H., Taedoum, H., Thomas, S. C., White, L. J. T., Abernethy, K. A., Adu-Bredu, S., ... Zemagho, L. (2020). Asynchronous carbon sink saturation in African and Amazonian tropical forests. *Nature*, 579(7797), 80–87. <https://doi.org/10.1038/s41586-020-2035-0>
- Huot, B., Yao, J., Montgomery, B. L., & He, S. Y. (2014). Growth-defense tradeoffs in plants: A balancing act to optimize fitness. *Molecular Plant*, 7(8), 1267–1287. <https://doi.org/10.1093/mp/ssu049>
- IPCC. (2013). *Climate change 2013: The physical science basis. Contribution of working group 1 to the fifth assessment report of the intergovernmental panel on climate change*. Cambridge University Press.
- Jamieson, M. A., Burkle, L. A., Manson, J. S., Runyon, J. B., Trowbridge, A. M., & Zientek, J. (2017). Global change effects on plant-insect interactions: The role of phytochemistry. *Current Opinion in Insect Science*, 23, 70–80. <https://doi.org/10.1016/j.cois.2017.07.009>
- Lemoine, N. P., Drews, W. A., Burkepile, D. E., & Parker, J. D. (2013). Increased temperature alters feeding behavior of a generalist herbivore. *Oikos*, 122(12), 1669–1678. <https://doi.org/10.1111/j.1600-0706.2013.00457.x>
- Lenth, R., Singmann, H., Love, J., Buerkner, P., & Herve, M. (2019). *Emmeans: Estimated marginal means, aka least-squares means* (1.4.3.01). <https://CRAN.R-project.org/package=emmeans>
- Letourneau, D. K. (2004). Mutualism, antiherbivore defense, and trophic cascades: *Piper* ant-plants as a mesocosm for experimentation. In L. A. Dyer & A. D. N. Palmer (Eds.), *Piper: A model genus for studies of phytochemistry, ecology, and evolution* (pp. 5–32). Kluwer Academic/Plenum Publishers.
- Lloyd, J., & Farquhar, G. D. (2008). Effects of rising temperatures and CO₂ on the physiology of tropical forest trees. *Philosophical Transactions of the Royal Society, B: Biological Sciences*, 363(1498), 1811–1817. <https://doi.org/10.1098/rstb.2007.0032>
- Marquis, R. J. (1984). Leaf herbivores decrease fitness of a tropical plant. *Science*, 226(4674), 537–539. <https://doi.org/10.1126/science.226.4674.537>
- Marquis, R. J. (1987). Variación en la herbivoría foliar y su importancia selectiva en *Piper arieianum* (Piperaceae). *Revista de Biología Tropical*, 35(Supl. 1), 133–149.
- Marquis, R. J. (1990). Genotypic variation in leaf damage in *piper arieianum* (Piperaceae) by a multispecies assemblage of herbivores. *Evolution*, 44(1), 104–120. <https://doi.org/10.1111/j.1558-5646.1990.tb04282.x>
- Massad, T. J., Dyer, L. A., & Vega, C. G. (2012). Costs of defense and a test of the carbon-nutrient balance and growth-differentiation balance hypotheses for two co-occurring classes of plant defense. *PLoS One*, 7(10), e47554. <https://doi.org/10.1371/journal.pone.0047554>
- Massad, T. J., Richards, L. A., Philbin, C., Fumiko Yamaguchi, L., Kato, M. J., Jeffrey, C. S., Oliveira, C., Jr., Ochsenrider, K., de Moraes, M. M., Tepe, E. J., Cebrian Torrejon, G., Sandivo, M., & Dyer, L. A. (2022). The chemical ecology of tropical forest diversity: Environmental variation, chemical similarity, herbivory, and richness. *Ecology*, e3762. <https://doi.org/10.1002/ecy.3762>

- Mattson, W. J., Julkunen-Tiitto, R., & Herms, D. A. (2005). CO₂ enrichment and carbon partitioning to phenolics: Do plant responses accord better with the protein competition or the growth differentiation balance models? *Oikos*, 111(2), 337–347. <https://doi.org/10.1111/j.0030-1299.2005.13634.x>
- Matzrafi, M. (2019). Climate change exacerbates pest damage through reduced pesticide efficacy. *Pest Management Science*, 75(1), 9–13. <https://doi.org/10.1002/ps.5121>
- Moreno-Delafuente, A., Morales, I., Garzo, E., Fereres, A., Viñuela, E., & Medina, P. (2021). Changes in melon plant phytochemistry impair *Aphis gossypii* growth and weight under elevated CO₂. *Scientific Reports*, 11(1), 2186. <https://doi.org/10.1038/s41598-021-81167-x>
- Mu, J., Peng, Y., Xi, X., Wu, X., Li, G., Niklas, K. J., & Sun, S. (2015). Artificial asymmetric warming reduces nectar yield in a Tibetan alpine species of Asteraceae. *Annals of Botany*, 116(6), 899–906. <https://doi.org/10.1093/aob/mcv042>
- OTS. (2022). *Organization for Tropical Studies database: La flora digital de La Selva*. <https://sura.ots.ac.cr/florula4/>
- R Core Team. (2021). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. <http://www.R-project.org>
- Richards, L. A., Dyer, L. A., Forister, M. L., Smilanich, A. M., Dodson, C. D., Leonard, M. D., & Jeffrey, C. S. (2015). Phytochemical diversity drives plant–insect community diversity. *Proceedings of the National Academy of Sciences of the United States of America*, 112(35), 10973–10978. <https://doi.org/10.1073/pnas.1504977112>
- Rustad, L., Campbell, J., Marion, G., Norby, R., Mitchell, M., Hartley, A., Cornelissen, J., & Gurevitch, J. (2001). A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia*, 126(4), 543–562. <https://doi.org/10.1007/s004420000544>
- Salazar, D., Lokvam, J., Mesones, I., Vásquez Pilco, M., Ayarza Zuñiga, J. M., de Valpine, P., & Fine, P. V. A. (2018). Origin and maintenance of chemical diversity in a species-rich tropical tree lineage. *Nature Ecology & Evolution*, 2(6), 983–990. <https://doi.org/10.1038/s41559-018-0552-0>
- Sheldon, K. S. (2019). Climate change in the tropics: Ecological and evolutionary responses at low latitudes. *Annual Review of Ecology, Evolution, and Systematics*, 50(1), 303–333. <https://doi.org/10.1146/annurev-ecolsys-110218-025005>
- Van der Putten, W. H. (2012). Climate change, aboveground-belowground interactions, and species' range shifts. *Annual Review of Ecology, Evolution, and Systematics*, 43, 365–383.
- Van der Sleen, P., Groenendijk, P., Vlam, M., Anten, N. P. R., Boom, A., Bongers, F., Pons, T. L., Terburg, G., & Zuidema, P. A. (2015). No growth stimulation of tropical trees by 150 years of CO₂ fertilization but water-use efficiency increased. *Nature Geoscience*, 8(1), 24–28. <https://doi.org/10.1038/ngeo2313>
- Watson, J. E. M., Evans, T., Venter, O., Williams, B., Tulloch, A., Stewart, C., Thompson, I., Ray, J. C., Murray, K., Salazar, A., McAlpine, C., Potapov, P., Walston, J., Robinson, J. G., Painter, M., Wilkie, D., Filardi, C., Laurance, W. F., Houghton, R. A., ... Lindenmayer, D. (2018). The exceptional value of intact forest ecosystems. *Nature Ecology & Evolution*, 2(4), 599–610. <https://doi.org/10.1038/s41559-018-0490-x>
- Whitehead, S. R., & Bowers, M. D. (2014). Chemical ecology of fruit defence: Synergistic and antagonistic interactions among amides from *piper*. *Functional Ecology*, 28(5), 1094–1106. <https://doi.org/10.1111/1365-2435.12250>
- Wickham, H. (2019). *ggplot2: Elegant graphics for data analysis*. Springer-Verlag. <https://CRAN.R-project.org/package=ggplot2>
- Wood, T. E., Cavaleri, M. A., Giardina, C. P., Khan, S., Mohan, J. E., Nottingham, A. T., Reed, S. C., & Slot, M. (2019). Soil warming effects on tropical forests with highly weathered soils. In *Ecosystem consequences of soil warming* (pp. 385–439). Elsevier. <https://doi.org/10.1016/B978-0-12-813493-1.00015-6>
- Wood, T. E., Cavaleri, M. A., & Reed, S. C. (2012). Tropical forest carbon balance in a warmer world: A critical review spanning microbial-to ecosystem-scale processes. *Biological Reviews*, 87(4), 912–927. <https://doi.org/10.1111/j.1469-185X.2012.00232.x>
- Würth, M. K. R., Winter, K., & Körner, C. (1998). *In situ* responses to elevated CO₂ in tropical forest understorey plants. *Functional Ecology*, 12(6), 886–895. <https://doi.org/10.1046/j.1365-2435.1998.00278.x>
- Yang, L., Wen, K.-S., Ruan, X., Zhao, Y.-X., Wei, F., & Wang, Q. (2018). Response of plant secondary metabolites to environmental factors. *Molecules*, 23(4), 762. <https://doi.org/10.3390/molecules23040762>
- Zvereva, E. L., & Kozlov, M. V. (2006). Consequences of simultaneous elevation of carbon dioxide and temperature for plant–herbivore interactions: A metaanalysis. *Global Change Biology*, 12, 27–41. <https://doi.org/10.1111/j.1365-2486.2005.01086.x>

How to cite this article: Maynard, L. D., Moureau, E., Bader, M. Y., Salazar, D., Zotz, G., & Whitehead, S. R. (2022). Effects of climate change on plant resource allocation and herbivore interactions in a Neotropical rainforest shrub. *Ecology and Evolution*, 12, e9198. <https://doi.org/10.1002/ece3.9198>

APPENDIX A

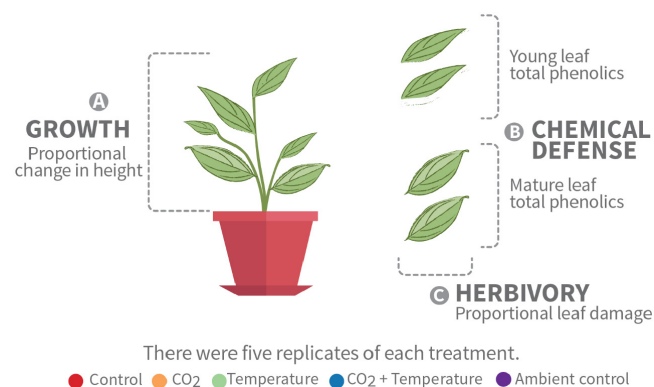


FIGURE A1 Schematic summarizing measurements taken from each plant, including (a) growth, (b) chemical defense, and (c) herbivory.

TABLE A1 Average estimated concentration of total phenolics (proportion dry weight in gallic acid equivalents) in different leaf ages of *Piper generalense*

Leaf age	Mean	SE	N
Mature	0.050	0.003	20
Young	0.069	0.003	20

TABLE A2 Average estimated herbivory (proportion foliar damage) in different leaf ages of *Piper generalense*

Leaf age	Mean	SE	N
Mature	0.053	0.011	40
Young	0.010	0.007	40

TABLE A3 Average plant growth (proportional change in height) of *Piper generalense* plants across microclimate treatments

Treatment	Mean	SE	N
Control	0.244	0.007	5
CO ₂	0.318	0.005	5
Temperature	0.275	0.004	5
CO ₂ + Temp	0.311	0.003	5

TABLE A4 Average foliar total phenolics (proportion dry weight in gallic acid equivalents) of *Piper generalense* between leaf ages and across microclimate treatments

Treatment	Mean	SE	N
<i>Mature leaves</i>			
Control	0.049	0.005	5
CO ₂	0.053	0.007	5
Temperature	0.046	0.007	5
CO ₂ + Temp	0.051	0.003	5

TABLE A4 (continued)

Treatment	Mean	SE	N
<i>Young leaves</i>			
Control	0.067	0.006	5
CO ₂	0.076	0.009	5
Temperature	0.065	0.002	5
CO ₂ + Temp	0.067	0.005	5

TABLE A5 Average foliar herbivory (proportion of leaf removed) of *Piper generalense* between leaf ages and across microclimate treatments

Treatment	Mean	SE	N
<i>Mature leaves</i>			
Control	0.035	0.014	10
CO ₂	0.062	0.026	10
Temperature	0.030	0.013	10
CO ₂ + Temp	0.084	0.031	10
<i>Young leaves</i>			
Control	0.013	0.008	10
CO ₂	0.003	0.002	10
Temperature	0.001	0.001	10
CO ₂ + Temp	0.025	0.025	10

TABLE A6 Model outputs for individual beta-regressions to determine whether elevated temperature and/or CO₂ concentration altered the relationship between plant growth and defense in *Piper generalense*. Models were separated by leaf age and microclimate treatment. Asterisks indicate statistical significance

Treatment	Estimate	SE	Z-score	p-value
<i>Mature leaves</i>				
Control	-0.22	0.51	0.44	.66
CO ₂	-0.50	0.92	-0.54	.50
Temperature	2.02	0.59	3.44	<.001***
CO ₂ + Temp	0.00	0.33	0.00	.99
<i>Young leaves</i>				
Control	0.13	0.41	0.33	.75
CO ₂	-0.96	0.79	-1.22	.22
Temperature	0.48	0.13	3.60	<.001***
CO ₂ + Temp	0.86	0.17	5.18	<.001***