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POPULATION ECOLOGY – ORIGINAL RESEARCH

Multiple and contrasting pressures determine intraspecific phytochemical variation in a tropical shrub

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Abstract

Intraspecific phytochemical variation across a landscape can cascade up trophic levels, potentially mediating the composition of entire insect communities. Surprisingly, we have little understanding of the processes that regulate and maintain phytochemical variation within species, likely because these processes are complex and operate simultaneously both temporally and spatially. To assess how phytochemistry varies within species, we tested the degree to which resource availability, contrasting soil type, and herbivory generate intraspecific chemical variation in growth and defense of the tropical shrub, *Piper imperiale* (Piperaceae). We quantified changes in both growth (*e.g.*, nutritional protein, above- and below-ground biomass) and defense (*e.g.*, imide chemicals) of individual plants using a well-replicated fully factorial shade-house experiment in Costa Rica. We found that plants grown in high light, nutrient- and richer old alluvial soil had *increased* biomass. High light was also important for *increasing* foliar protein. Thus, investment into growth was determined by resource availability and soil composition. Surprisingly, we found that chemical defenses *decreased* in response to herbivory. We also found that changes in plant protein were more plastic compared to plant defense, indicating that constitutive defenses may be relatively fixed, and thus an adaptation to chronic herbivory that is common in tropical forests. We demonstrate that intraspecific phytochemical variation of *P. imperiale* is shaped by resource availability from light and soil type. Because environmental heterogeneity occurs over small spatial scales (tens of meters), herbivores may be faced with a complex phytochemical landscape that may regulate how much damage any individual plant sustains.

Keywords Intraspecific variation · Plant chemistry · Trophic interactions · Resource availability · Soil heterogeneity

Introduction

Phenotypic plasticity in plants contributes to intraspecific variation in key defensive traits, which in turn modifies species interactions across food webs (Turcotte and Levine 2016). Both abiotic factors and interactions with other organisms can cause changes in the chemical profiles of primary producers, and this occurs in a broad array of phylogenetically distinct taxa and in nearly all ecosystems. For example, herbivory, changes in resource availability, or both can induce chemical defenses in algae (Van Alstyne 1988; Cronin and Hay 1996), freshwater macrophytes (Morrison and Hay 2011), endophytes (Saikkonen et al. 2013), and herbs, shrubs, and trees (Fowler and Lawton 1985; Kessler and Kalske 2018; Moore et al. 2014; Glassmire et al. 2016; Dyer et al. 2018). Most studies on intraspecific variation in phytochemical defenses have either focused on specific mechanisms in isolation, such as induced defenses, or have

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only examined interspecific differences among species (Feeny 1976; Rhoades and Cates 1976; Herms and Matson 1992; Hay 2002; Fink 2007; Smilanich et al. 2016). This metabolic variation that occurs within species is a key dimension of diversity, but surprisingly, the putative ecological processes that determine intraspecific variation in plant defense are rarely compared experimentally and are thus poorly understood (Morrison and Hay 2011; Hahn and Maron 2016; Hunter 2016). Here, we directly compare different sources of intraspecific variation in chemical defense by experimentally quantifying the degree to which key limiting resources (light and nutrients), enemies, and soil type mediate intraspecific variation in nutritional and defensive secondary metabolites.

Plant defense theory is comprised of a large number of conceptual models, broad hypotheses, empirical data, and synthesis papers, and there is variable support for all existing hypotheses, depending on taxon, ecosystem context, and categories of defense (Rhoades 1979; Simms 1992; Hay 2002; Stamp 2003; Massad et al. 2012; Smilanich et al. 2016; Richards et al. 2015). Here, we focus on three hypotheses that are most relevant to intraspecific variation in plant defense: Growth Differentiation Balance, Herbivore Induced Defenses, and Soil Mosaic (Table 1). These hypotheses make explicit predictions about how variation in light availability, nutrient availability, soil type, and herbivore pressure mediate production of plant growth and production of secondary metabolites. The Growth Differentiation Balance Hypothesis predicts a tradeoff between growth and differentiation processes (i.e., secondary metabolites and trichomes) as a result of nutrient and photosynthate availability (Loomis 1932; Herms and Mattson 1992). When plants obtain nutrients in excess of physiological demands, they can divert these resources from growth toward defenses. The main predictions are: (1) growth is inversely related to plant defense; (2) nutrient limitation will limit growth more than defense; (3) light availability will increase photosynthesis, but not growth, thus surplus photosynthates will be shunted to secondary metabolite production, which will increase proportionally with growth. The Growth Differentiation Balance Hypothesis and numerous derivatives, such as the Resource Availability (Coley et al. 1985) and Carbon-Nutrient Balance Hypotheses (Bryant et al. 1983), have been criticized for limitations, particularly for not being able to directly test plant defense hypotheses (Koricheva 2002; Stamp 2004). Nonetheless, predictions generated from derivative hypotheses have made substantial contributions to our understanding of phytochemical variation, both nutritionally and defensively (*reviewed in* Stamp 2004) and provide the best explanations for a number of documented empirical results from observations and experiments (e.g., Hamilton et al. 2001; Lerdau and Coley 2002; Massad et al. 2012). The Herbivore Induced Defense Hypothesis predicts that when herbivores attack plants, they will up- or down-regulate the production of plant chemical defenses. This hypothesis assumes that certain compounds are ‘expensive’ to produce, and thus will only be produced when required to mitigate additional damage (Myers 1988; Agrawal and Karban 1999; Dicke and Hilker 2003). Finally, Glassmire et al. (2017) proposed the Soil Mosaic Hypothesis, which predicts that variation in soil types within and between ecosystems will underlie a considerable proportion of intraspecific variation in plant defensive chemistry and growth. Soils are dynamic and heterogeneous across the landscape because of changes in chemical and physical properties due to climate, parent material, topography, the resident plant and microbial community, and many other factors (Laliberte et al. 2013; van der Putten et al. 2013). Perhaps most importantly, interactions between plants and their soil microbes create positive and negative soil feedbacks (e.g., Bever 2003; Pendergast et al. 2013) to such an extent that they can alter growth, leaf chemistry, and allelopathic potential among co-occurring plant species (Mieners et al. 2017). These three hypotheses are not mutually exclusive because all could be operating simultaneously and thereby jointly affecting the degree of intraspecific chemical variation across the landscape.

Plant defense theory has generated clear predictions of how plants respond to environmental variability, resource availability, and herbivory, by shifting resources to, or away from, plant chemical defense, which may explain wide-scale intraspecific variation in phytochemistry (Tomlinson et al. 2016; Hahn and Maron 2016). Surprisingly, experimental manipulations are lacking on the degree that herbivory interacts simultaneously with resource availability to regulate plant defensive chemistry, even though feeding damage is known to cause plastic responses in plant defenses (*reviewed in* Hahn and Maron 2016; Hahn et al. 2019). Hahn et al. (2016) emphasized that it was critical to examine shared limiting resources when evaluating causes of intraspecific phytochemical variation. Moreover, it is also necessary to study herbivores that commonly damage the host plant to assess realistic plant responses. Overall, there is a compelling need to conduct controlled experiments that evaluate the degree that phytochemical variation is caused by variation in relevant abiotic and biotic ecological processes.

We tested the degree to which resource availability, contrasting soil type, and herbivory generate intraspecific chemical variation by manipulating these putative sources of intraspecific phytochemical variation for a common understory shrub, *Piper imperiale,* (Piperaceae) in a lowland tropical rainforest in Costa Rica. We examined whether there were tradeoffs between plant growth and defense using a full factorial design where we manipulated soil nutrients, light availability, soil type, and the presence or absence of herbivory. We addressed the following questions: (1) What are the relative effects of soil nutrients, light availability,

Table 1 Hypotheses and *a priori* predictions guiding the structural equation model

Factor manipulated	Causal relationship	Predictions	Citations	Outcomes from our experiment
Light	yellow path arrows	The Growth Differentiation Balance Hypothesis predicts that high levels of light intensity will allocate plant resources towards growth rather than defense. We predict that foliar protein and biomass will increase with nutrient additions	Loomis 1932; Herms & Mattson 1992	Our results supported the Growth Differentiation Balance Hypothesis. Light significantly increased protein and below ground biomass compared to the defensive compounds, sintenpyridone and piplaroxide
Nutrients	Red path arrows	The Growth Differentiation Balance Hypothesis predicts that nutrient-rich environments will allocate plant resources towards growth rather than defense. Foliar protein and biomass will increase with nutrient additions	Loomis 1932; Herms & Mattson 1992	Our results supported the Growth Differentiation Balance Hypothesis. Nutrient enrichment increased plant biomass, but did not have a significant effect on plant defensive compounds
Herbivory	Black path arrows	The Herbivore Induced Defense Hypothesis predicts that resource allocation in plants shifts from growth to defense in the presence of herbivores, irregardless of the resource availability of nutrients and light. Sintenpyridone and piplaroxide will increase with greater levels of herbivory	Agrawal & Karban 1999; Agrawal 2001	Our results rejected the Herbivore Induced Defense Hypothesis. High levels of herbivory had the largest negative effect size on the defense compounds, which is the opposite pattern than that predicted by the Herbivore Induced Defense Hypothesis
Soil Type	Purple path arrows	The Soil Mosaic Hypothesis predicts that soil type will control resource allocation in plants regardless of resource availability and presence of herbivory. Plants will allocate resources towards defense in poor conditions of soil especially in the presence of reduced light	Glassmire et al. 2017	Our results supported the Soil Mosaic Hypothesis. Plants established in old alluvial soils increased defensive compounds under high light conditions. Plant growth via root biomass also increased for plants established in old alluvial soils

Plant growth and defense are the response variables for all models. The proxy variables for plant growth are foliar protein and biomass, and the proxy variables for plant defense are the compounds, sintenpyridone and piplaroxide. The column titled “Causal Relationships” refers to the path arrows from the baseline path model depicted in Fig. 2

soil type, and herbivory on plant defensive chemistry? (2) To what degree do these factors mediate plant growth? (3) To what degree do these factors interact and contribute to intraspecific variation in growth and defense? Currently, there is a considerable knowledge gap about tradeoffs between growth and defense among conspecifics and whether these tradeoffs occur when there is variation in resource availability herbivory, and the soil substrate. Each of these processes vary across small spatial scales beneath forest understories and can change phytochemical variation on the scale of meters thus affecting entire communities. Overall, the degree that each of these processes shapes intraspecific phytochemical variation is poorly understood.

Materials and methods

Plant Study System—We conducted this study in Costa Rica from June to December 2014 at the La Selva Biological Station (10.4306° N, 84.0070° W), a lowland rainforest that receives ~ 4000 mm of precipitation annually (Clark 2013). *Piper imperiale* C.DC. (Piperaceae) is an abundant and shade tolerant shrub that is one of 54 co-occurring *Piper* species at La Selva and is a model plant system to test hypotheses based on chemical defense (Dyer and Palmer 2004; Gentry 1990). Its chemical defenses are well described and include two biosynthetically related nitrogen containing imides, sintenpyridone and piplaroxide, (where sintenpyridone is the precursor of piplaroxide, Fig. 1), and at least five different sesquiterpenes, all of which likely contribute synergistically to plant defense (Fincher et al. 2008). *Piper imperiale* invests in relatively lower concentrations of more highly toxic defensive compounds compared to other *Piper* species (1.7% dry weight, Fincher et al. 2008; Richards et al. 2010).

Experimental Design—We manipulated soil type, nutrient additions, and herbivory using a factorial design that was nested within low and high light levels. Our treatments included: (1) nutrient-poor old alluvial versus a richer residual soil type, (2) low versus high nutrient additions, and (3) herbivory (ranging from 0 to 27%), totaling 16 treatment combinations. Each treatment combination was replicated 10 times for a total of 160 plant individuals that were randomly assigned to each treatment combination (ESM Figure S1).

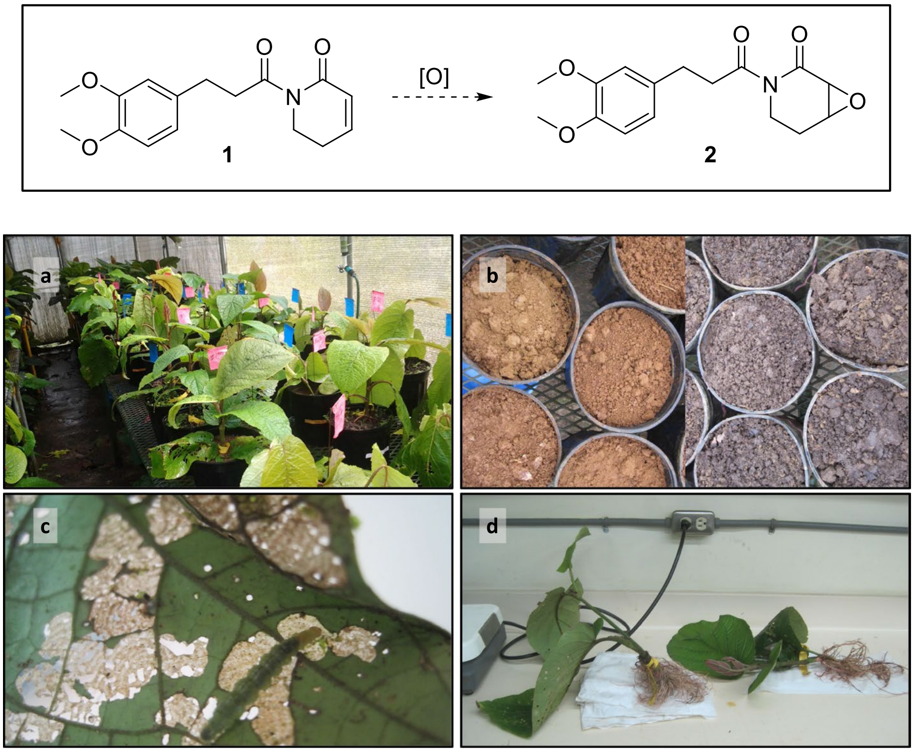

Fig. 1 Testing the degree to which resource availability, contrasting soil type, and herbivory generate intraspecific chemical variation via estimating tradeoffs between growth and defensive compounds. The top panel depicts the chemical structures of the two nitrogen-based defensive compounds that were quantified in this experiment. The imide compounds, [1] sintenpyridone and [2] piplaroxide, are biosynthetically related, where [1] is a precursor to [2]. The bottom panel depicts the *Piper imperiale* individual plants used in this experiment with treatment level combinations. We used a factorial design and exposed established plant fragments to random treatment level combinations of either **a** low or high levels of light, **b** alluvial (light brown in image) or residual soils (black in image), and **c** herbivory (*Eois* herbivore shown feeding on leaf). **d** Above- and below-ground plant biomass were harvested and weighed for each plant

Individual vegetative cuttings of *P. imperiale* were collected throughout La Selva from 12 different plant populations. These cuttings were planted in pots in protected shade-houses that provided refuge from herbivores, other enemies, falling twigs, and direct rainfall.

Soil type, nutrient additions, and herbivore manipulations—Both light and soil nutrient availability are limiting and highly patchy within understories of closed canopy tropical forests (*for light* Montgomery and Chazdon 2002, *for nutrients see* Wright et al. 2011, Pasquini et al. 2015). Light availability also influences plant phytochemistry; higher light levels are associated with 17% increase in total imide content in *Piper cenocladum* shrubs (e.g., sintenpyridone, 4'– desmethylpiplartine, and cenocladimide, Dyer et al. 2004). We used reflective shade cloth (AgFabric, Corona, CA) to create two levels of light availability within a single 8 X 3 X 3 m enclosure. These light levels mimicked the ambient level of light that commonly occurs in small light gaps within the forest compared to a deeply shaded understory (see below). We measured photosynthetically active radiation throughout the day beneath our two light treatments using H21-002 HOBO micro station data logger with two S-LIA-M003 sensors (MicroDAQ, Ltd., Contoocook, NH, USA). Our low light treatment reduced light to 2% full sun and ranged from a minimum of 1.2 µmol m^{-2} s^{-1} to a maximum of 106.2 µmol m^{-2} s^{-1}. Our high light treatment reduced light to 30% full sun and ranged from a minimum of 1.2 µmol m^{-2} s^{-1} to a maximum of 363.7 µmol m^{-2} s^{-1} (*t* test, $P < 0.001$). The low light treatment mimicked deeply shaded understory patches that occur beneath the shrub and herb layer, and a high light treatment that occurs in the understory above this layer (Montgomery and Chazdon 2002). All individual plants were rotated within the light treatment block every two weeks throughout the duration of the experiment to ensure homogeneity in light and environmental conditions of the shade-house. We watered plants daily during the first 2 weeks of establishment and then twice weekly for weeks 3 and 4. Thereafter, plants received ample ambient precipitation via perforations in the shade cloth.

We manipulated soil type by collecting the two main soils (alluvial and residual) that occur throughout La Selva (Sancho and Mata 1987). Old alluvial soils are clay soils with intermediate nutrient content, high water holding capacity, and low drainage, whereas residual soils are derived from highly weathered lava flows and are rich with minerals from the parent rock with high drainage (Sancho & Mata 1987; Clark et al. 1998). Contrasting plant communities occur on these two soil types (*for extensive details see* Sancho & Mata 1987, Clark et al. 1998). We collected soil from four different areas for each soil type by excavating soil down to 15 cm. We bulked and thoroughly mixed soil within a soil type before use. Plant individuals from each population were randomly assigned to one of the two soil types and grown for one month in plastic pots (3.79 L) prior to applying the nutrient and herbivory treatments.

We manipulated nutrient resources by adding both macro and micronutrients to the soil. Recent research demonstrates that soil nutrients limit plant performance even in deeply shaded forest understories (*reviewed by* Wright et al. 2011, Griffin et al. 2017). Higher nutrient additions are associated with 3% increase in total imide content in *Piper cenocladum* shrubs (e.g., sintenpyridone, 4'– desmethylpiplartine, and cenocladimide, Dyer et al. 2004). We had high (10 g of macro- and micronutrients per m^2) and low (2 g of macro- and micronutrients per m^2) nutrient addition treatments; macronutrients included NPK 12–8–16 and micronutrients included magnesium, calcium, copper, sulfur, iron, boron, and zinc (Blaukorn classic, Kyiv, Ukraine). Nitrogen was added in two forms, nitrate and ammonium.

Finally, we manipulated herbivory using the specialist native herbivore, *Eois apyraria*, which commonly causes extensive damage on *P. imperiale* (Fincher et al. 2008; Hansen et al. 2017). *Eois apyraria* is a leaf chewer and bioassays have shown conclusively that the defensive sintenpyridone and piplaroxide chemicals reduce the survival and performance of *E. apyraria* (Fincher et al. 2008; Richards et al. 2010; Hansen et al. 2017). We placed two caterpillars on plants that were assigned herbivory. We added new caterpillars to plants assigned herbivory whenever old ones pupated, died, or disappeared for the 6-month duration of the experiment. We quantified the absolute amount of herbivory per plant using LI-3100 instrumentation (LI-COR Environmental, Nebraska, USA), which ranged from 0 to 30% leaf area reflecting natural levels of damage on *P. imperiale* (*see* Fincher et al. 2008). Although the shade-houses excluded most flying insects, we did not check for or exclude herbivore eggs on leaves from the experimental cuttings that were brought into the shade-house or check for beetle or moth pupae in the soils, thus herbivory varied among plants ranging 0–27% (including some herbivory on plants that did not get assigned to caterpillar addition treatments). The dichotomous variable (herbivores added or not) was used in the Bayesian analyses (described below), while the continuous variable (percent herbivory) was used in SEM models—these variables (herbivores added and percent herbivory) are highly correlated with mean levels of herbivory higher on plants with herbivores added, which validated our herbivory treatment.

Harvest—Plants were harvested in December 2014, six months after commencing the experiment. Leaf Surface Area (LSA) of freshly harvested leaves was measured using LI-3100 Leaf Area (LI-COR, Inc., Lincoln, NE, USA). Total herbivore damage was assessed for all leaves on each plant by quantifying the Leaf Area Removed (LAR) due to herbivory using LI-3100 Leaf Area (LI-COR, Inc., Lincoln, NE, USA). Plant stems, roots, and leaves were separated, dried at 28 °C for three days, weighed, and stored in paper bags at − 28 °C until chemical analyses were conducted.

Foliar defense chemical and protein analyses—Samples of dried leaves from individuals from each of the sixteen shade-house treatments were extracted for quantification using seven-point calibrations. Defensive imide chemical response units are reported as percent dry weight. We modified a chemical extraction protocol specific to *Piper imperiale* (Dodson et al. 2000). Briefly, 1.00 g samples aliquot from each plant were extracted with 5 mL of 1:1 aqueous methanol and 5 mL of chloroform. This mixture was sonicated for 20 min and centrifuged to allow layers to separate. The organic layer was retained before repeating this liquid–liquid extraction two more times by adding 5 mL of chloroform to the aqueous layer. Organic extracts were pooled and 1.00 mL aliquots of samples for each plant were combined with commercially available piperine (MilliporeSigma; St. Louis) as an internal standard (final concentration: 80.0 ug/L) and analyzed using a Gas Chromatography–Flame Ionization Detector or GC–FID (Agilent 7890A; Santa Clara, CA). Seven-point calibrations (11.72, 23.44, 46.88, 93.75, 187.5, 375.0, and 750.0 ug/L) were prepared using imides **1** ($R^2 = 0.999$) and 2 ($R^2 = 0.999$). External standards for analysis were synthesized as described in Dyer et al. (2004). The sintenpyridone imides are unstable in solution, so all reported concentrations are estimates (Dodson et al. 2000). For a detailed description of GC methods, see Dodson et al. (2000) and Dyer et al. (2001).

Total foliar protein concentration for each plant was quantified using the Pierce BCA Protein Assay protocol (Thermo Fisher Scientific, Waltham, MA, USA). Briefly, two mg of dried leaf material was extracted from each plant with 500 µL of buffer (100 mM Tris pH 7.5, 150.00 mM NaCl, 10.00 mM $MgCl^2$, and 1% of SDS). The extracted protein was quantified using a six-point calibration curve of albumin (0.0, 4.0, 10.0, 20.0, 40.0 and 60.0 µg/mL). Foliar protein extractions were replicated for each plant three times to ensure accuracy. The samples and protein standard were analyzed using a SpectraMax M Series Multi-Mode Microplate Reader (Molecular Devices, LLC., Sunnyvale, CA, USA) measuring absorbance at 560 nm. The units of total protein concentration for each sample are reported as the protein mass fraction relative to plant dry weight.

Statistical Analyses—All analyses were conducted in program R version 3.6.2 (R Core Team 2018). Omnibus multivariate analysis of variance (MANOVA) models were utilized for low light and high light experiments separately to provide initial hypothesis tests with response variables combined for biomass (ESM Tables S2 & S5, ESM Fig S2, ESM Fig S5), foliar protein (ESM Tables S3 & S5, ESM Fig S3, ESM Figs S6–S8), and defensive compounds (ESM Tables S4–S5, ESM Fig S4, ESM Figs S9–S11), and with herbivores, nutrients, soil, and all interactions as independent variables. This was followed by models to test specific causal relationships and to derive Bayesian estimates of relevant parameter estimates. First, we tested hypothesized relationships among resource availability, contrasting soil type, and herbivory on intraspecific chemical variation using structural equation modeling (SEM; Shipley 2016; see appendix glossary) in the 'lavaan' package (Rosseel 2012). For all path models, plant biomass and protein were proxies for growth, while the defensive imide compounds were a proxy for defense. Latent variables were not utilized in any models and all measured variables were standardized (see appendix glossary). We determined the best model using the following decision guidelines about adding and dropping specific variables (*summary statistics for candidate models in ESM* Table S1): (1) links had to be biologically feasible based on our hypothesized relationships (Table 1); (2) the initial model was a baseline (not fully saturated) model that included every biologically relevant variable (ESM Table S1); (3) variables were dropped or added one at time; (4) models were excluded if they had a singular fit; (5) We used the Bayesian Information Criterion (BIC; see appendix glossary) to compare potential models, and the best model was selected based on the difference in BIC from the model with the lowest BIC (Δ BIC); all models were considered to have a similar likelihood if they had a Δ BIC < 2 (ESM Table S1; Garrido et al. 2022), and 6) the best model was evaluated by comparison to the baseline model. We used a priori specified path model that included all the experimental variables as our baseline model (Fig. 2, Table S1; $\chi^2 = 105.191$, df = 2, P-value = 0, BIC = − 705.865), with light, nutrient additions, soil type, and herbivory as predictors of plant growth (biomass and foliar protein) and plant defense (sintenpyridone and piplaroxide). We selected the best model, nested within the baseline model based on our decision rules described above (ESM Table S1). We measured the strength of relationships using standardized path coefficient (spc) that measure the direct effect of the predictor variable on the response variable while controlling for the indirect effects of other predictor variables in the model.

Secondly, we used hierarchical Bayesian models (see appendix glossary for definitions and equations) to test specific a priori hypotheses (Table 1; Plummer 2021), to examine specific direct effects of manipulated variables, and to estimate relationships among endogenous variables from the SEMs (see appendix glossary). The hierarchical Bayesian models were not used for traditional hypothesis testing, only for estimating posterior distributions of random variables. We treated the two light experiments as the upper level of the hierarchy, with all treatment level combinations nested in the lower level; these analyses were used to support inferences about the different results found in the two different light levels. Levels of significance (e.g., P-value thresholds) are not relevant to Bayesian models, but to allow for inferences we have applied the 50%

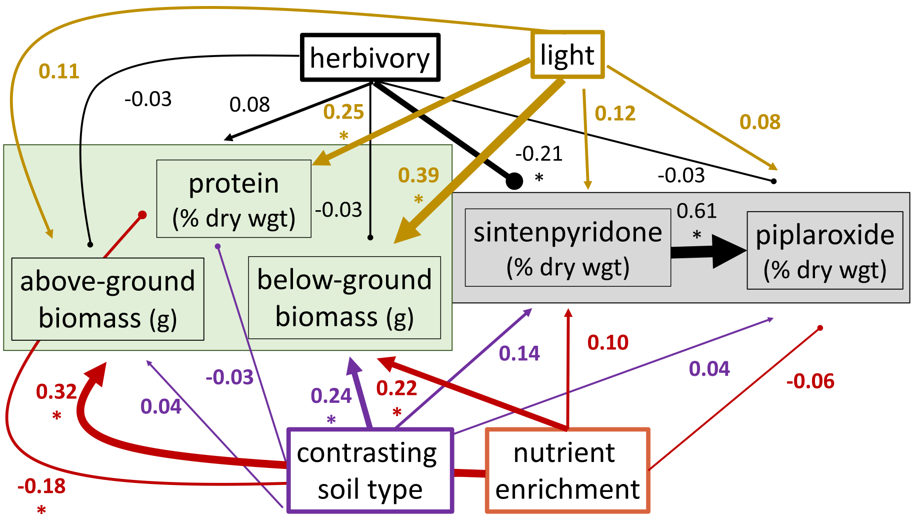

Fig. 2 The overall baseline path model ($\chi^2 = 105.191$, df = 2, *P*-value = 0, BIC = − 705.865) is based on our hypotheses and a priori predictions that guided all path models (*see hypotheses and predictions in* Table 1). Direct positive effects are depicted with arrows, while the direct negative effects are depicted with blunt-ended lines, and standardized path coefficients are next to each line or arrow. The asterisk next to the standardized path coefficient indicates a significant relationship ($P < 0.05$). The green box represents our proxies for plant growth—foliar protein, above- and below-ground biomass. The gray box represents our proxies for plant defense—sintenpyridone and piplaroxide defensive compounds. Herbivory, light, contrasting soil type, and nutrient enrichment were the four variables we manipulated using a fully crossed experimental design. The effects of herbivory (0–30%) on plant growth and defense are represented by black arrows. The effects of light (shaded forest understory to understory gap) on plant growth and defense are represented by yellow arrows. The effects of contrasting soil type (residual to old alluvial) on plant growth and defense are represented by purple arrows. The effects of nutrient enrichment (low to high levels) on plant growth and defense are represented by red arrows

credibile interval as a method to show the range of parameter estimates that includes at least half of the possible values from the posterior distribution, and we also show 80% credible intervals, as we have done in other papers (e.g., Salcido et al. 2020; Wagner et al. 2021). Models were fit in JAGS (version 3.2.0) utilizing the rjags package in *R* using (for each analysis) two Markov chains and 1,000,000 steps each; performance was assessed through examination of chain histories (burn-in was not required), effective sample sizes and the Gelman and Rubin convergence diagnostic (Rc < 1.2; Brooks and Gelman 1998). Response variables (total plant biomass, leaf protein, and sintenpyridone) were standardized (see appendix glossary) and each was included in separate models as normal distributions with means dependent on an intercept plus predictor variables (soil, fertilizer, herbivore addition, and biomass), and we used uninformative hyperpriors (see appendix glossary) for the lower levels of the hierarchy: hyperpriors for model beta coefficients were normal distributions with mean of zero and precision of 0.01; priors on precisions were modeled as gamma distributions with rate = 2 and shape = 2.

Results

Structural equation models

We used path analysis to examine how simultaneous environmental and biotic pressures influence intraspecific phytochemical variation among individuals of *P. imperiale*. We focused our results on the best model with the lowest BIC (Fig. 3, ESM Table S1; $\chi^2 = 7.375$, df = 9, *P*-value = 0.598, BIC = − 1382.567). For plant growth as measured by foliar protein, above-, and below-ground biomass, we found that light increased the amount of protein (standardized path coefficient [spc] = 0.26), while nutrient enrichment decreased the amount of protein (spc = − 0.17). For the defensive compounds, plants established in loam soils had higher amounts of the defensive sintenpyridone compound compared to clay soils (spc = 0.11), which indirectly had a strong positive effect on piplaroxide production (spc = 0.64). Surprisingly, herbivory significantly reduced sintenpyridone production (spc = − 0.21), which is the opposite prediction of Herbivore Induced Defenses Hypothesis. Furthermore, greater amounts of above-ground biomass had lower amounts of sintenpyridone (spc = − 0.31), but higher amounts of piplaroxide (spc = 0.17) in leaf tissue.

Bayesian hierarchical models

Complex differences in the high and low light experiments are illustrated by posterior distributions from the hierarchical Bayesian models (Fig. 4), with overall decreases in protein, biomass, sintenpyridone, and piplaroxide for plants in low

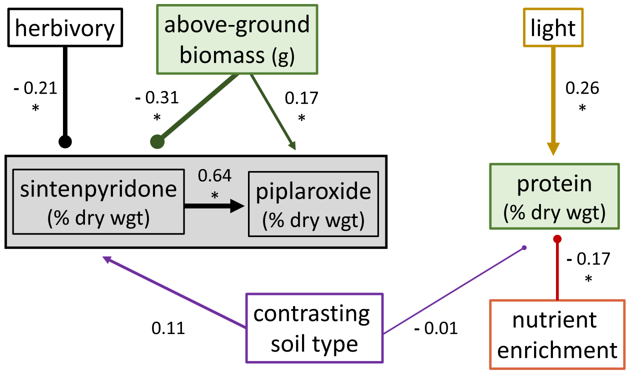

Fig. 3 The top ranked path model based on lowest BIC value ($\chi^2=7.375$, df = 9, P-value = 0.598, BIC = − 1382.567) is based on our hypotheses and a priori predictions that guided all path models (*see hypotheses and predictions in* Table 1). Direct positive effects are depicted with arrows, while the direct negative effects are depicted with blunt-ended lines, and standardized path coefficients are next to each line or arrow. The asterisk next to the standardized path coefficient indicates a significant relationship ($P < 0.05$). The green box represents our proxies for plant growth—foliar protein, above- and below-ground biomass. The gray box represents our proxies for plant defense—sintenpyridone and piplaroxide defensive compounds. Herbivory, light, contrasting soil type, and nutrient enrichment were the four variables we manipulated using a fully crossed experimental design. The effects of herbivory (0–30%) on plant growth and defense are represented by black arrows. The effects of light (shaded forest understory to understory gap) on plant growth and defense are represented by yellow arrows. The effects of contrasting soil type (residual to old alluvial) on plant growth and defense are represented by purple arrows. The effects of nutrient enrichment (low to high levels) on plant growth and defense are represented by red arrows

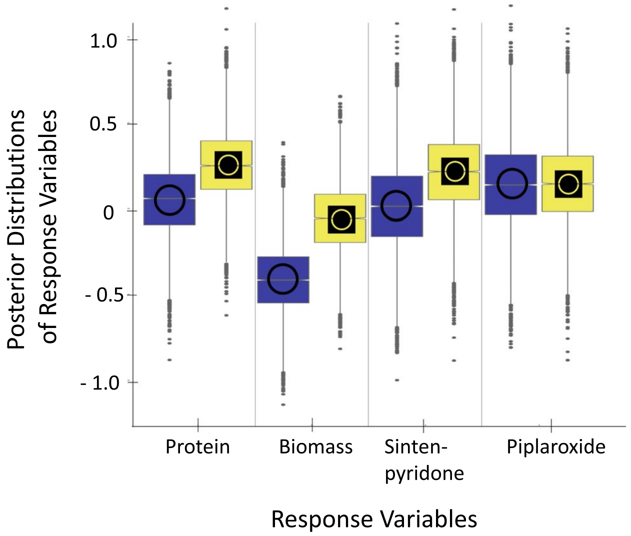

Fig. 4 Estimates of posterior distributions (summarized as notched box plots) for standardized biomass and chemistry of plants in different light environments, based on a series of Bayesian hierarchical models. For all models, experimental treatments and individual plants were nested within the two levels of light (high "○" vs. low "◙") and posteriors for the lower levels provided priors for the upper level of the hierarchy. Weakly informative priors (e.g., normally distributed residuals with mean of zero and 0.01 precision) were used for hyperparameters. Separate models were run for foliar protein, plant biomass, and defensive sintenpyridone and piplaroxide metabolites of individual plants grown under the different light environments. These response variables were z-transformed (with mean of zero). Low light ("◙") is represented by yellow box plots and high light ("○") is represented by blue. Overall, there was a substantial decrease in foliar protein, plant biomass, and pyridine concentrations for plants in low light versus high light experiments

light versus high light experiments. The most obvious patterns from posterior distributions are that protein content was almost doubled with greater light availability (low shade versus high shade; Fig. 4), fertilizer decreased protein content by about 20% and increased biomass by almost 80% under high light conditions, and sintenpyridone and piplaroxide were reduced with herbivores added regardless of light (Fig. 5). This result of decreased chemical defense in response to herbivore addition corroborates the percent herbivory results from the SEMs. Inferences of no differences for responses across treatment level combinations can be made when posterior distributions have medians close to zero (e.g., effects of herbivory on biomass at low light conditions were zero) (Fig. 5).

Discussion

The cause of intraspecific phytochemical variation is poorly understood even though we know that the consequences of intraspecific phytochemical variation can cascade up to higher trophic levels. We examined how ecological processes due to resource availability, herbivory, and contrasting soil type influenced patterns of resource allocation between growth or defense for a common and geographically widespread understory shrub, *Piper imperiale*. For our indicators of plant growth, we found that high light availability increased plant allocation toward protein production and high levels of nutrient additions increased plant allocation toward biomass. For our indicators of plant defense, we found pervasive interactions among nutrient availability, herbivory, and contrasting soils determined the degree to which plants allocated resources to chemical defense. Surprisingly, herbivory had the greatest negative effect on plant defense by reducing the production of sintenpyridone (spc = − 0.21). In contrast, differences in soil (spc = 0.14), light (spc = 0.12), and nutrient enrichment (spc = 0.10) collectively enhanced the production of sintenpyridone, which by extension increased piplaroxide because sintenpyridone is the precursor (spc = 0.61). These results provide compelling evidence that there is a tradeoff between plant growth and defense (spc = − 0.31), and this is mediated by light availability and soil type. These results demonstrate unequivocally that the degree to which herbivory changes plant defensive chemistry is determined by variation in resource availability (light and

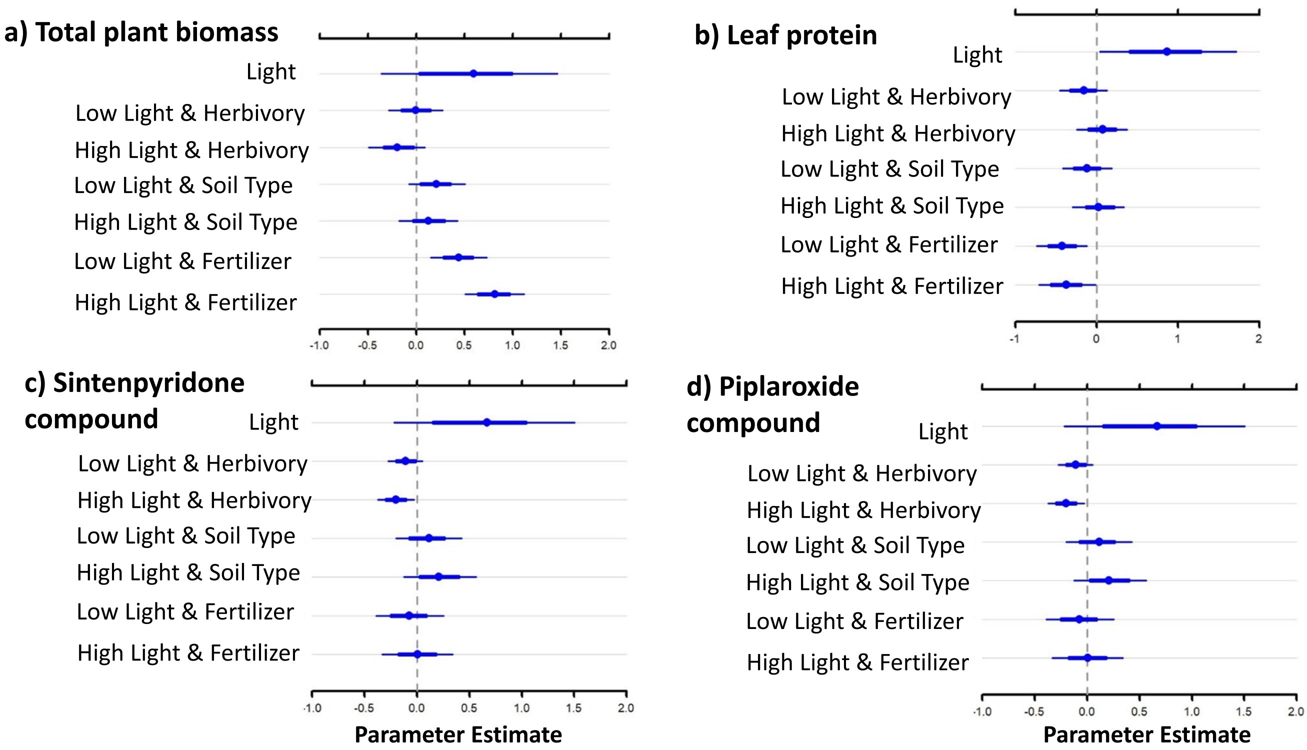

Fig. 5 Caterpillar plots summarizing posterior distributions for standardized parameter estimates for a Bayesian hierarchical model examining **a** total plant biomass and **b** leaf protein as proxies for plant growth and the defensive compounds **c** sintenpyridone and d) piplaroxide as measures of phytochemical defense. Individual plants were nested within the two levels of light and posteriors from the lower levels (soil, fertilizer, biomass, and herbivory) provided priors for the upper level of the hierarchy (light). Weakly informative priors (e.g., normally distributed residuals with mean of zero and 0.01 precision) were used for hyperparameters. Parameter estimates for the z-transformed response variable (with mean of zero) are depicted along with 80% (thin line) and 50% (thick line) credibility intervals. The models here are different from the frequentist SEM models presented in the main text, thus estimates are slightly different for all parameters

soil nutrients) and soil type. An important consequence of these results is that small-scale variation in key factors, such as light availability, will likely create mosaics of phytochemical variation among conspecifics. Herbivores must then "sample" this chemical variation when searching for and "evaluating" the suitability of host plants; such variation hinders herbivore acclimation to chemistry and may well lower overall herbivore loads on a plant species across its range (Adler and Karban 1994; Underwood 2014; Barbosa et al. 2009; Hunter 2016; Glassmire et al. 2019). In sum, these results demonstrate that to evaluate our hypotheses and understand intraspecific variation in phytochemistry we must simultaneously consider the availability of key limiting resources, soil mosaics, as well as the presence and absence of herbivores. While this adds complexity to our understanding of phytochemistry and plant defense, it means we cannot ignore abiotic factors that are well known to structure forest understories (Clark et al. 1998; Wright et al. 2011). It also means that herbivores are almost certainly encountering a highly variable phytochemical landscape on relatively small spatial scales that are relevant to these herbivores finding suitable hosts.

Light initiated a reduction in plant growth

Decreasing light availability caused a reduction in plant biomass and foliar protein, our indicators for plant growth. This, however, was not observed for the defensive compounds (*refer to outcomes from our experiment in* Table 1). Our results support the Growth Differentiation Balance Hypothesis in that plants with greater access to limiting resources will invest in plant growth rather than defense. Light is a major limiting resource in tropical forest understories, and both the quantity and quality of light availability changes substantially throughout understories (e.g., Chazdon and Fetcher 1984). While it is well known that light is a critical limiting resource for understory plants, particularly in tropical forests, oddly, it is rarely measured in studies focused on tradeoffs between growth and defense among conspecifics or heterospecifics (Hahn and Maron 2016; Hahn et al. 2019). Leaves are costly for plants to produce under low light conditions (Bryant et al. 1983; Lerdau and Coley 2002). We found that plants invested in foliar protein in high light environments but not in low light environments.

Complex relationships determined the production of defensive compounds

The concentrations of secondary metabolites depended substantially on conditions and factors known to vary widely across tropical forest landscapes, specifically resource availability, soil type, and herbivory. Growing plants in different soil types, exposing them to different levels of herbivore damage, and changing resource availability interacted to modify the production of key defensive compounds. There is little doubt that variation in these conditions and processes will create a very complex chemical landscape in tropical forests, whereby herbivores will encounter host plants that vary substantially in host plant quality. We speculate that this variation is likely the norm for most plant species and future research should explore, not only this possibility, but the consequences of this variation for herbivore assemblages and community processes, such as trophic cascades. What we do know is that even subtle changes (0.2% dry weight) in *Piper* imide levels can seriously disrupt the immune response of specialist herbivores (Hansen et al. 2017). Moreover, Richards et al. (2010) demonstrated that synergistic effects of three *Piper imperiale* defensive compounds, including piplaroxide, even at very low amounts (< 0.1% dry weight), can decrease survival in herbivores by as much as 25%. Prior to this research, it would be difficult to make the case that soil type would be the template upon which there were numerous interactions with herbivory and resource availability, which together determined chemical changes in *P. imperiale*. These findings show that soil type can be key to determining community interactions (Clark et al. 1998; Fine et al. 2006; Cacho and Strauss 2014) and must be considered in future studies (Hahn et al. 2019). The striking differences in forest communities found at La Selva between our two soil types (Clark et al. 1998) may be at least partly due to their impacts on plant chemical defenses, which may cascade up and down trophic webs to mediate forest plant species composition and diversity.

When there are sharp differences among soil types, selection can lead to the evolution of distinct plant traits adapted to different soils, even when plant species are in fairly close proximity (Fine et al. 2004). For example, Misiewicz and Fine (2014) found evidence of local adaptation to soils, with significant morphological variation, higher levels of genetic differentiation, and lower migration rates among populations of the tropical tree *Protium subserratum* growing parapatrically on white-sand, brown-sand, and clay soils in Peruvian Amazonia. Similarly, Cacho and Strauss (2014) found the shift for plants to serpentine soils from bare, sparsely vegetated ground was extremely important for plant diversification in the Streptanthoid complex (Brassicaceae). Plants have a long history of being edaphic specialists and soils have unique micro-climates and biotic factors that plants must adapt to.

Constitutive defenses may be a better defense against herbivores in tropical plant systems—

We found little support for herbivory being solely responsible for up- or downregulating plant chemical defenses as hypothesized for herbivore-induced defenses. Higher herbivory levels caused *P. imperiale* to reduce the amount of defensive compounds. In contrast, plants grown in residual soils caused *P. imperiale* to increase the amount of defensive compounds. It is important to note that low light conditions and year-round pest pressure are the norm in deeply shaded tropical forest understories (Dyer and Letourneau 1999). Thus, induced defenses caused by herbivory may not apply to certain phytochemicals that are constitutive defenses and remain at high concentrations even when herbivores are absent (Bixenmann et al. 2016) and depend more on plant ontogenetic development (Fuchs and Bowers 2004, Quintero and Bowers 2011, Barton 2013). We found, however, that soil type was a better predictor of defensive inducibility rather than herbivory for *P. imperiale*. Another experiment with three *Piper* species at La Selva also found no effect of herbivory on chemical defenses (Fincher et al. 2008). This suggests that some *Piper* plants in tropical forests produce and maintain a constant level of constitutive chemical defenses that are not inducible based exclusively on herbivory. Our results contrast with results from plant communities that experience seasonal pulses in pest pressure where plants rely more heavily on induced defenses for protection against herbivores (Williams & Whitham 1986; Karban et al. 1997; Agrawal 2001). Chemical defenses expressed by the plant are not a unified trait that can be defined simply as herbivore induced or not, and our results show that limiting resources are a major component to plant defense strategies. Our study cannot completely rule out that the hypothesis that reductions in defenses are partly responsible for lower herbivory, however, there are several aspects of the study that support the opposite causal pathway. First, the Baysian regressions demonstrated a clear reduction in defenses for plants that had caterpillars added, and it is not possible for the final plant chemistry to cause the random assignment of caterpillars to experimental plants. Second, the idea that plant chemistry could affect the amount of herbivory in these experiments would require some combination of factors not supported by prior studies (reviewed in Dyer and Palmer 2004) with *Piper* and *Eois* in shade-houses: adult moths infiltrating the shade-houses, adults preferentially ovipositing on plants with lower levels of non-volatile defensive compounds, and larvae being less efficient feeders on plants with lower levels of defensive compounds.

Conclusion

Plants simultaneously experience multiple and sometimes contrasting pressures and growing conditions. Growth and defensive phytochemistry of *P. imperiale* exhibited tradeoffs depending on light availability and different combinations of nutrient availability, soil types, and presence or absence of herbivores. Our results demonstrate that light and soil heterogeneity, which vary across very small to larger spatial scales, can have dramatic effects on the phytochemical profile of plants, leading to intraspecific variation in defense and palatability. Whether or not this intraspecific variation found in *P. imperiale* leads to variation in plant fitness has yet to be tested, but our data imply that it is unlikely that one phenotype that will be universally fit. Furthermore, plant defense theory and experimental designs focused on intraspecific chemical variation should consider multiple drivers of plasticity simultaneously rather than in isolation. These results are relevant to terrestrial and aquatic ecosystems alike, and a renewed focus on issues such as elemental stoichiometry and allocation to defense in terrestrial and aquatic systems (e.g., Sardans et al. 2012) will yield a clearer picture of how and why anti-parasite defense varies intraspecifically across the landscape.

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Author contribution AEG: wrote the first draft of the manuscript. WPC, LAD, AMS, and LAR: contributed substantially to revisions. AEG, LAD, AMS, CSJ, and GLH: generated hypotheses and designed experiments. LAD, AMS, and CSJ funded experiments. AEG, AMS, and GLH: collected the field data. AEG, CSJ, CDD, and CSP: conducted chemical analyses. AEG, LAD, and LAR: conducted statistical analyses. GLH: identified *Piper imperiale* plants and *Eois apyraria* caterpillars in the field.

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Data availability This data have not been published elsewhere. Data are available in Figshare data repository (https://doi.org/10.6084/m9.figshare.22144541) and on an open access website maintained by Lee A. Dyer < http://caterpillars.org/ > .

Code availability Not applicable.

Declarations

Conflict of interest The authors have no conflicts of interest to declare.

Ethics approval Not applicable.

Consent to participate Not applicable.

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