Plant trait differences and soil moisture jointly affect insect herbivory on seedling young leaves in a subtropical forest

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**ABSTRACT**

Insect herbivory on seedling leaves is one of the most important factors driving seedling growth and mortality in natural forests, which sets the pivotal roles of insect herbivory in affecting natural forest regeneration and species composition. The intensity of herbivory can be influenced by multiple biotic and abiotic factors affecting leaf detection or consumption by insect herbivores. However, the relative importance of these biotic and abiotic factors in affecting insect herbivory and their interactions remain largely unknown in species-rich forests. We measured insect herbivory on seedling young leaves across 600 1 m\textsuperscript{2} seedling plots in a subtropical forest. Effects of nine seedling traits, neighboring plant compositions, soil moisture, and light availability on young leaf damage by insect herbivores were assessed with generalized linear mixed-effects models. We found that seedling traits associated with plant size (young leaf area and seedling height), leaf nutrition or defense (leaf thickness, carbon content, and stem specific length) explained more of the variation in insect herbivory than the biotic and abiotic environmental factors. Young leaf coloration (delayed greening degree) exhibited contrasting effects on leaf damage at wet vs. dry sites, so did seedling trait diversity (functional dispersion, FDis). The risk of insect herbivory increased with leaf redness and decreased with seedling FDis at wet sites, while the opposite trends were observed at dry sites. Our study advances the understanding on the driving factors of insect herbivory on seedling leaves in species-rich forests and suggests that the alleviating effects of plant diversity on insect herbivory were stronger at wet conditions.

1. Introduction

Seedling performance represents a major bottleneck in tree demography, driving natural forest regeneration and species composition (Harms et al., 2000; Comita et al., 2010; Bagchi et al., 2014; Lebrjija-Trejos et al., 2016; Jia et al., 2020). Insect herbivores can consume a significant amount of plant leaves (Coley and Barone, 1996; Kursar and Coley, 2003), which can affect the growth or survival of seedlings in forests (Coley and Barone, 1996; Eichhorn et al., 2010; Norghauer and Newbery, 2013). The degree of leaf damage by insect herbivores is a complicated result of multiple processes (e.g. detection and consumption by insects) and factors (e.g. the intrinsic properties of leaves or seedlings, and the biotic and abiotic environment of seedlings) (Fig. 1). However, large gaps remain in our understanding of the relative importance of biotic and abiotic factors and the interactions of these factors in affecting insect herbivory on seedling leaves in species-rich forests. Filling these gaps can improve our predictions on species composition in natural forests under a rapid changing environment (Comita et al., 2014; Castagneyrol et al., 2018; Loughnan and Williams, 2019; Toïgo et al., 2020).

Herbivores must first detect leaves before they can consume them. Successful leaf detection by herbivores depends on the apparenncy of a leaf and the biotic and abiotic environment surrounding the leaf or seedling (Fig. 1). For example, leaf coloration may affect leaf detection by insects in complicated ways. Young leaves of some species in tropical and subtropical forests can produce anthocyanins and exhibit red coloration (i.e., delayed greening) (Lev-Yadun, 2006; Archetti et al., 2009). Red leaves may (1) be less visible to insects lacking of a red photoreceptor (Manetas, 2006); (2) confound herbivores due to the color similarity between red (or brown) leaves and soil and litterfall (Fadzly et al., 2016); (3) expose green insects to their enemies (Lev-Yadun et al., 2004); or (4) signal the presence of defensive chemical compounds (Karageorgou and Manetas, 2006). Thus, insect herbivores may more easily locate a normal green leaf than a red young leaf.
Nonetheless, the concentration of foliar anthocyanins can vary across abiotic environmental gradients (Chalker-Scott, 1999; Hughes et al., 2010; Zhu et al., 2016).

Abiotic environment may affect leaf detection by modifying the visibility or attraction of a leaf, the densities of insect herbivores, and effective hosts. First, environment may have a complicated influence on insect vision. For example, insect vision of leaf colors may depend on natural light conditions (e.g. light intensity or quality) (Prokopy and Owens, 1983). Leaves under drought stress can be more or less attractive to insects due to the corresponding changes in the proportions of nutrients and defensive chemicals (Mattson and Haack, 1987; Price, 1991; Herm and Mattson, 1992). Second, density of insect herbivores may vary across environmental gradients. Per-leaf detection probability often increases with insect density. For example, insect herbivores may have a larger population size in a more shaded habitat given that predator pressure and abiotic stress are typically low in such an environment (Maiorana, 1981; Shure and Phillips, 1991). Insect density can vary along a soil moisture gradient, but in more complex and unpredictable ways (Sconiers and Eubanks, 2017; Walter, 2018). Third, host plant density is often higher in forest gaps (Denslow, 1987; Hubbell et al., 1999) and highly associated with soil moisture (Engelbrecht et al., 2007). Per-insect detection probability usually increases with density of host plants (Janzen, 1970; Feeny, 1976).

Biotic environment is often measured as neighborhood plant density or diversity, and can affect leaf detection through multiple mechanisms. First, leaf detection by insects may decrease (or increase) with plant diversity (or conspecific plant density) if insects are specialized herbivores (concentration hypothesis; Root, 1973; Sholes, 2008; Parachonwitsch et al., 2014). Neighborhood diversity may reduce leaf detection through species herd protection (Peters, 2003), where a higher density of heterospecific neighbors can interfere with leaf detection by specialized insects. The alleviating effects of host plant diversity on insect herbivory may be more evident at productive sites, where the population size of host plants or insect herbivores are larger (Janzen, 1970; Feeny, 1976). Second, plant diversity may increase leaf detection if insects are generalized herbivores. Diverse plant assemblage may provide mixing foods, which may improve nutrient balance and dilute secondary compounds of a single host type (dietary mixing hypothesis; Bernays et al., 1994; Behmer, 2009).

Mechanisms of leaf consumption by insect herbivores may overlap or differ from those of leaf detection (Fig. 1). Leaves that are more detectable and attractive to insects may have higher risks of consumption by insects. Herbivores may consume more leaves with higher nutrition or lower defense after locating the leaves (Fig. 1). For instance, insects typically consume more leaves with higher nitrogen content and a lower level of tannins, but avoid tougher leaves in tropical forests (Coley and Barone, 1996). Recent studies suggest that physical traits of plants are often better predictors for insect herbivory than chemical nutrition traits (Schuldt et al., 2012; Muiruri et al., 2019). Abiotic environment or biotic interactions with neighboring plants may affect the biochemical compositions associated with plant defense or nutrients in leaves (Chalker-Scott, 1999; Hughes et al., 2010; Mattson and Haack, 1987; Zhu et al., 2016), which in turn affects the leaf consumption by insects.

In this study, we focused on the insect herbivory on seedling young leaves in a subtropical forest. We measured insect herbivory on young leaves of seedling due to the following three reasons. (1) One of our major goals is to assess the effects of leaf delayed greening on insect herbivory. Leaf delayed greening is only apparent on young leaves. (2) About 70% of leaf lifetime damages by insects occur during the few weeks that leaves are expanding (Coley and Barone, 1996). (3) Insect herbivory on young leaves is one of the most important factors driving seedling growth and mortality in natural forests. Seedling performance represents a major bottleneck in tree demography (i.e., all trees are grown from seedlings).

We assessed the effects of various biotic and abiotic factors on young leaf damage by insect herbivores. These factors include plant traits and biotic and abiotic environmental variables that are associated with leaf detection or consumption by insects (Fig. 1). We aimed to investigate (i) the relative importance of seedling traits, neighboring plant compositions, and abiotic environment on insect herbivory on seedling young leaves, and (ii) whether plant traits and neighboring plant compositions have different effects on insect herbivory depending on the abiotic environment of the seedlings.

2. Materials and methods

2.1. Study site

We conducted this study in a subtropical forest at Heishiding Nature Reserve in Southern China (111°53’ E, 23°27’ N). The annual mean precipitation is about 1744 mm, with a wet season from April to September and a dry season from October to March. The annual mean temperature is about 19.6 °C. We established three 1-ha permanent forest plots from winter 2007 to spring 2008. The elevation within the forest plots range from 198 m to 348 m. Two of the forest plots were adjacent and the other one was about 100 m apart. Each plot was divided into 10 m × 10 m grids. We regularly selected half of the grids in

![Fig. 1. Conceptual framework illustrating the processes of leaf detection and consumption by insect herbivore that lead to young leaf damage. The appa- rence of a leaf (represented by leaf color; green) and biotic (e.g. neighborhood plant diversity and density; red) and abiotic (e.g. light availability and soil mois- ture; blue) environment can affect the likelihood of leaf detection by insects. Herbivores may consume more leaves with higher nutrition or lower defense (green) after they locate the leaves. The factors affecting leaf detection (e.g. biotic and abiotic envi- ronment) may affect plant defense and nutrition as well. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)](image-url)
a mode of checkerboard, and established four 1 m × 1 m seedling plots within each of the selected grid (Fig. S1). All trees with ≥ 1 cm diameter at breast height (DBH) in each 10 m × 10 m tree grids, both with and without seedling plots, were tagged, mapped, and identified to species level in 2007, and then re-measured in 2017. We only used the tree census data from 2017 to calculate tree density and diversity indices because they are closest to the measurement time of insect herbivory. For seedling within the seedling plots, we first identified the individuals at least 1 cm in height and with DBH < 1 cm. Then we tagged the selected seedlings with small and light plastic plates at the base of their stems. We measured seedling heights in late spring or early summer, each year from 2008 to 2018 (Liu et al., 2012). We recorded 2775 seedlings from 85 species (57 genera, 37 families) in 2018, and 9022 trees from 180 species (109 genera, 56 families) in 2017.

2.2. Leaf herbivory

We measured insect herbivory on seedling young leaves. We determined young leaves as those are typically fragile and thin, small in size, and low in rigidity (Numata et al., 2004; Chavana-Bryant et al., 2017). Leaf buds and unexpanded furled leaves were not included as young leaves in this study. We assessed insect herbivory at the start of the wet season in 2018, from May 17–28. It is the time when both plant growth rates and herbivory are high in subtropical forests (Schuldt et al., 2010). All the evaluation of leaf age and insect herbivory were conducted by one well-trained naturalist (Wenbin Li). We surveyed all leaves on the 2775 seedlings across the 600 seedling plots. We photographed young leaves using a digital camera (Canon EOS 70D; see supplementary materials for detailed photographic methods). Leaf damage by insect herbivores was identified as the presence of any amount or kind of damage (Levey et al., 2016), such as leaf holes, leaf-grazing wounds, or leaf-chewing wounds (Fig. S2), or a leaf that was completely eaten with only petiole left. The software ImageJ (Schneider et al., 2012) was used to calculate the total leaf area as the sum of the damaged and undamaged area of a leaf. If leaves had suffered extensive damage, their leaf shapes were reconstructed digitally by reference to shapes of similar leaves of the same species (Menzies et al., 2016). The corresponding species-level average young leaf areas were substituted for the areas of the leaves that were completely eaten (about 2% of all leaves). The consumption and damage types are consistent with damage due to Lepidoptera larvae including Geometridae, Lymantridae, Nolidae, Limacodidae, and Sphingidiae families (Zhu et al., 2019), and by grasshoppers belonging to Orthoptera in this subtropical forest. Mining, galling, sucking insect damage, and fungi damage were rarely detected in this investigation. For all plant species observed in this study, specific mechanistic defenses were not prevalent, with the exception of Omorisia pachycarpa, Ilex pubescens, Glachidion eriocarpum, and Machilus velutina, which had pubescence on young leaves. Resource- or information-based indirect defenses were rarely detected, except that Sapium discolor and Mallotus lanus possess gland characteristics.

2.3. Leaf apparency

We used seedling delayed greening degree of young leaves (the ratio between leaf redness and greenness, RG) as the measurement of leaf apparency (i.e., leaf recognition by insect herbivores). We obtained the leaf greenness and redness from the undamaged part of each leaf using ImageJ software. RG values larger than 1 represent strong delayed greening, and values close to 0 indicate normal development of greenness. The RG values of completely eaten leaves were replaced by the corresponding species-level average value (about 2% of all leaves).

2.4. Functional traits and phylogeny

We measured nine aboveground species-level functional traits (Shen et al., 2019) of seedlings in this forest: mature leaf surface area (LA, cm²), leaf area ratio (the ratio between total leaf area and dry mass of a seedling, LAR, cm²·g⁻¹), leaf carbon content (LC, %), leaf nitrogen content per dry mass (LN, mg·g⁻¹), leaf phosphorus content per dry mass (LP, mg·g⁻¹), specific leaf area (SLA, cm²·g⁻¹), leaf dry matter content (LDMC, g·g⁻¹), specific stem length (the ratio between stem length and stem dry mass, SSL, cm·g⁻¹), and leaf thickness (T, mm) (Table S1). These traits are considered closely associated with plant defense or nutrition (Cárdenas et al., 2014; Schuldt et al., 2019). The species-level traits were measured with 4–5 intact and healthy seedlings per species in 2017. We measured the traits outside of the three 1-ha permanent forest plots to avoid damage to plants under monitoring. Detailed approaches of the trait measurement can be found in the supplementary material (Table S1) and (Shen et al., 2019). Trait variations between species were significantly larger than those within species for these traits (results not shown).

We constructed the phylogenetic tree based on the Angiosperm Phylogeny Group III classification (Zanne et al., 2014) using Phylogenetic (Webb and Donoghue, 2005). The phylogenetic signals were tested for species-level average delayed greening degree and the other nine species traits, using Blomberg’s K (Blomberg et al., 2003) from the Picante package (Kembel et al., 2010). K values greater than 1 indicate strong phylogenetic signal, meaning that species close in phylogeny tend to have similar traits. K values close to zero indicate a lack of phylogenetic signal. The significance of phylogenetic signal was assessed based on the phylogenetic independent contrasts (PICs), by randomly shuffling the trait values across the tips of the phylogeny tree in 999 iterations. Only SLA and RG were significantly conserved in phylogeny (Table S2). Thus, we assigned the SLA values for the missing species (i.e., the species without measurement of SLA due to their extreme rarity) with the corresponding mean values from the next higher taxonomic level (genus or family level). For the other traits of missing species, we assigned the weighted average trait values (by seedling density) from the same seedling plot (the missing species included 80 individuals, about 2% of all seedlings).

2.5. Biotic environment

To assess the effects of biotic environment on leaf damage by insects, we calculated the density and diversity of seedlings (within the same seedling plot) and trees (within the 10 m × 10 m tree grid) around focal leaves. We calculated density separately for conspecifics and heterospecifics, and measured three facets of diversity: species diversity (Shannon-Wiener index), phylogenetic diversity, and functional trait diversity. All the three facets of diversity indices are commonly used but may imply different processes associated with plant—insect interactions. Shannon-Wiener index is one of the simplest diversity indices, but can miss important information about evolutionary or functional correlations between species. For example, plant species close in evolutionary histories or similar in functional traits may share similar insect herbivores (Gilbert and Webb, 2007), which may be better described by phylogenetic or trait diversity indices. For phylogenetic and trait diversity, we used indices both dependent on species richness (Faith’s PD [PD] and Petchey & Gaston’s FD [FD]) (Faith, 1992; Petchey and Gaston, 2002) and independent of species richness (mean pairwise phylogenetic distance [MPD] and functional dispersion [FDis]) (Webb et al., 2002; Laliberte and Legendre, 2010), which would allow us to assess the relative roles of plant species number and average similarity between plant species in affecting insect herbivory. Trait diversity indices were only calculated for seedlings because complete trait data were not available for trees.

2.6. Abiotic environment

Relative light availability and soil moisture are important abiotic factors for both plant growth and herbivory by insects. Light availability was characterized as the canopy openness using hemispherical photos.
above the center of each seedling plot at the height of 1.3 m. Photos were taken with a calibrated FC-E8 fisheye lens on a Nikon COOLPIX 4500 camera. We used the Sky package (Bachelot, 2016) to process these hemispherical photos and calculate canopy openness. We measured soil water content (m$^3$·m$^{-2}$) at three randomly selected points within each seedling plot, employing a water monitoring systems (Stevens Water Monitoring Systems, Inc.) and the average values within each seedling plot were used for subsequent analyses. Both canopy openness and soil moisture were acquired under a uniformly overcast sky in October 2017, which is the start of the dry season. The forest plots are highly heterogeneous, with an elevation range about 150 m, and the maximum soil moisture is about 10 times to the minimum.

2.7. Statistical analysis

Many sampled young leaves were undamaged (73%). One way to analyze such data is to model the continuous actual amount of leaf area loss with linear regression. However, this approach produced very poor model fitting with regards to the residual distribution in our case. We did not model leaf herbivory as proportional area loss because the relatively small sample of damaged leaves (<30%, 274 leaves) may provide limited inference on the variation in proportions of leaf area damage. Thus, we focused on the occurrence of leaf damage (1 as damaged and 0 as undamaged) instead of the proportion or actual amount of damage area in the following analyses.

We used generalized linear mixed-effects models (GLMMs, with binomial errors and a logit link function) (Bolker et al., 2009) to assess the effects of seedling traits and biotic and abiotic environmental variables (as fixed effects) on the incidence of leaf damage (binary variable) by insect herbivores. Seedling traits included seedling height (Height), young leaf area (YLA), young leaf delayed greening degree (RG), and the nine species-level traits (LDMC, SLA, LP, LA, LAR, LC, LN, SSL, and T). To reduce multicollinearity between the nine species traits (Fig. S3), we removed species traits with values of variance inflation factor (VIF) larger than 2 (Zuur et al., 2010; Dornmann et al., 2013), which led to the removal of three species traits (LDMC, SLA, and LP). Therefore, six species traits were kept in the final model (LA, LAR, LC, LN, SSL, and T) (Eq. (1)). Since we had the original young leaf area (YLA) and seedling height as controlled explanatory variables, the effects of the other variables, such as biotic and abiotic environment, should be interpreted as the effects on leaf damage conditional on the same original young leaf area and seedling height. For the traits measured per leaf (RG and young leaf area) or per individual (seedling height), their correlations with the abiotic environmental variables (canopy openness and soil moisture) are weak (Pearson correlation coefficients < 0.15; Fig. S4).

Biotic environmental variables (Bio.env) included indices of plant density and diversity. Plant density variables included heterospecific and conspecific densities for both seedlings and trees. We had five sets of diversity indices (Shannon-Wiener, FDIs, FD, PD, and MPD index) calculated at the scale of seedling plot and three sets of tree diversity indices (Shannon-Wiener, PD, and MPD index). Separate models were constructed for seedling and tree neighborhood variables (Table S3). Biotic environmental indices may be strongly correlated with each other (Fig. S5), thus we included one biotic environmental index in each model (Table S4). In total, we fitted 10 models (six models for seedling neighborhood and four models for tree neighborhood) (Table S4). Abiotic environmental variables included canopy openness and soil moisture. Additionally, the interaction between delayed greening degree and abiotic environment were incorporated given the potential dependence of color detection by insects on the environment. We included the interaction between diversity (or density) and abiotic environment to evaluate the context dependence of diversity (or density) effects on leaf herbivory. Species identity ($\mu_k$, in Eq. (1)) and seedling individual ($\mu_l$ in Eq. (1)) were included as random effects to control potential variation due to unmeasured species-specific and individual-specific properties. Seedling plot ($\mu_p$ in Eq. (1)) was included as a random effect to account for spatial variation that was not explained by soil moisture and canopy openness (Eq. (1)). The random effects of seedling individual were nested within those of species, while the random effects of seedling plot were not nested with the others in the models with neighboring seedling composition, but nested within 10 m × 10 m tree grid in the models with neighboring tree composition. The predictors with skew distribution (Height, YLA, RG, Openness, all species-level traits, and density indices) were log-transformed to improve normality before model fitting. We added one to the density indices before log transformation because their original values contained zero. Each variable was standardized (mean zero and unit standard deviation) prior to model fitting. The following equation was applied to the models with neighboring seedling composition, while the ones with neighboring tree composition were similar.

$$
\eta_{ijkl} = \text{binomial}(p_{ijkl})
$$

$$
y_{ijkl} = \log\left(\frac{p_{ijkl}}{1 - p_{ijkl}}\right) = \beta_0 + \beta_1 \text{Height}_j + \beta_2 \text{YLA}_i + \beta_3 \text{RG}_l + \beta_4 \text{LA}_k + \beta_5 \text{LAR}_k + \beta_6 \text{LC}_k + \beta_7 \text{LN}_k + \beta_8 \text{SSL}_k + \beta_9 \text{T}_k + \beta_{10} \text{Bio.env}_p + \beta_{11} \text{Openness}_s + \beta_{12} \text{Moisture}_t + \beta_{13} \text{RG} \times (\text{Openness}_s + \text{Moisture}_t) + \beta_{14} \text{Bio.env}_p \times (\text{Openness}_s + \text{Moisture}_t) + \mu_j + \mu_k + \mu_l (1)
$$

where $y_{ijkl}$, $p_{ijkl}$ and $\eta_{ijkl}$ are the presence or absence of leaf damage, probability of leaf damage, and the logit-transformed predicted probability of leaf damage for leaf $l$ on seedling $j$ of species $k$ in seedling plot $l$. $\beta_0$ represents the intercept and $\beta_{1-3}$ denote the effects of three seedling traits measured within species: seedling height (Height), young leaf area (YLA), and delayed greening degree (RG). We set random effects of species for $\beta_{4-9}$ to account for species differences in the effects of within-species traits on insect herbivory. $\beta_{10}$ represents the effect of the species-level functional traits (LA, LAR, LC, LN, SSL, and T) on leaf damage risk. $\beta_{10}$ represents the coefficient of biotic environment variables (Bio.env). $\beta_{11,12}$ are the coefficients of abiotic environment (canopy openness and soil moisture, respectively). $\beta_{13,14}$ denote the interactive effects between the abiotic environment and delayed greening, and biotic environment, respectively.

To test the impacts of extremely rare species on our results, we performed alternative analyses by excluding species with less than two or three seedlings in the models, and found qualitatively similar results (Fig. S6). We presented the results associated with the traits and abiotic environmental variables from the model with FDIs as the biotic environmental variable in the main text because the effect sizes and significance of the traits and abiotic environment are similar between the 10 models with different biotic environmental variables. The results from all the 10 models can be found in the supplement (Figs. S7-S8). We also conducted alternative analyses, in which we fitted two models, one for biotic environmental variables at the scale of seedling plot and the other at the scale of tree grid. For each of the two models, we put all the traits, abiotic environmental variables, and all the biotic environmental variables at the corresponding scale into a single model, and then dropped the species traits and biotic environmental variables with VIF ≥ 2. These alternative models showed similar results as those fitting one biotic environmental variable in a model (Figs. S9-S10). The overdispersion of the model was tested using the Chi-squared test. Mixed-effects models were fitted using the lme4 package (Bates et al., 2015). The significance tests of the fixed effects were based on Wald test. All analyses were performed in R 3.6.1 (R Development Core Team, 2019).
3. Results

3.1. Leaf herbivory and delayed greening across species

Young leaves were relatively rare in the understories of this close-canopy forest. We detected 998 young leaves on 644 seedlings from 47 species, yielding 1–8 leaves per seedling and 1–246 leaves per plant species (Table S5). The proportion of leaf numbers consumed by insects per plant species ranged from 0 to 100% (Fig. 2). There was significant variation by species in the degree of delayed greening of young leaves (Fig. 2). The ratio between redness and greenness of young leaves (RG) ranged from 0.700 to 1.719 across species. Phylogenetically close species tended to have more similar RG values than phylogenetically distant species (Table S2).

3.2. Relative importance of biotic and abiotic factors determining leaf damage risk

Seedling traits associated with plant sizes, leaf nutrition or defense explained more of the variation in insect herbivory than the biotic or abiotic environment of where the seedlings were growing (Fig. 3). Young leaves with larger area had higher leaf damage risk than that of smaller leaves (Fig. 3a). Leaves on taller seedlings had lower leaf damage risks than those on shorter seedlings (Fig. 3a). Young leaves from species with higher specific stem length (SSL), leaf carbon content (LC), or leaf thickness (T) suffered less from herbivory than species with the opposite traits (Fig. 3a). Leaf delayed greening degree did not show a significant effect on insect herbivory on young leaves (Fig. 3a). Leaf damage risk by herbivores did not vary significantly across the gradients of soil moisture or canopy openness (Fig. 3b).

Seedling functional dispersion (FDIs), a trait diversity index independent of species richness, had the strongest effects on leaf damage among the six sets of seedling biotic environmental indices (Fig. 3b and S7). Leaf damage risk by insect herbivores increased with seedling FDIs. Seedling mean pairwise phylogenetic distance (MPD), a phylogenetic diversity index independent of species richness, showed a similar effect on leaf damage as FDIs, although this effect was only statistically marginally significant. The three seedling diversity indices dependent on species richness (Shannon-Wiener index, functional diversity [FD], and phylogenetic diversity [PD]) and seedling density exhibited weak and statistically insignificant effects on leaf damage (Fig. 3b and S7). Tree PD, a phylogenetic diversity index dependent on species richness, had the strongest effect on leaf damage among all tree biotic environmental variables (Figs. S8 and S11), where leaf damage risk by insect herbivores increased with tree PD.
3.3. Effects of delayed greening on leaf damage risk for different soil moisture levels

Young leaf delayed greening had opposite effects on leaf damage risk at wet vs. dry sites (Fig. 4 and Figs. S7 and S8). Leaf damage risk decreased with delayed greening degree or redness of young leaves at drier seedling plots, but increased at wetter seedling plots.

3.4. Abiotic context-dependence of neighborhood effects on leaf damage risk

The effects of seedling FDis on leaf damage also varied across soil moisture gradients (Figs. 5 and S7f). Leaf damage risk decreased with seedling FDis at wetter seedling plots, but increased at drier seedling plots (Fig. 5).

Tree densities also showed different effects on leaf damage across abiotic environmental gradients (Figs. S8 and S11). The damage risk of young leaves increased with conspecific tree density at gap areas (Fig. S11b) and decreased with heterospecific tree density at wet sites (Fig. S11c). The opposite effects of conspecific and heterospecific tree densities were observed at shaded and dry sites (Fig. S11).

4. Discussion

In this study, we assessed the effects of seedling traits, and biotic and abiotic environment on young leaf damage by insect herbivores in a species-rich subtropical forest. We found that intrinsic properties of seedlings were more important than biotic and abiotic environmental factors in determining the risk of insect herbivory on seedling young leaves. Young leaf coloration exhibited contrasting effects on leaf damage at wet vs dry sites, so did seedling trait diversity. These results advance our understanding of the driving factors of leaf herbivory in species-rich forests, and have important implications for biodiversity maintenance at different environmental conditions.

4.1. Effects of seedling trait on leaf herbivory

We found that seedling traits associated with plant sizes (seedling height and young leaf area), leaf nutrition or defense (specific stem length, leaf thickness and leaf carbon content) explained more of the variation in insect herbivory than the environmental conditions where the seedlings were growing. Leaf damage risk increased with the area of young leaves. Insect herbivores may be better able to detect leaves with larger areas, and this may signal higher resource availability (Low et al., 2009). Larger leaves may also be older and have more time to accumulate damage by insect herbivores. Leaf damage risk was lower for taller seedlings. Taller plants are often older and may develop better defenses against insect herbivores (Elger et al., 2009). Of the six species-level traits we analyzed, leaf carbon content (LC), leaf thickness (T), and specific stem length (SSL) showed statistically significant effects on leaf damage risk. Species with higher LC, T, and SSL had lower risks of young leaf damage than species with the opposite traits. Insect herbivores may...
Fig. 5. Trait diversity shows opposite effects on leaf damage risk at the high (wet) vs. low (dry) levels of soil moisture. Trait diversity is measured as seedling functional dispersion (FDIs). The high and low levels of soil moisture are the maximum and minimal values of soil moisture across the seedling plots. The solid lines in panel b represent predicted relationships between trait diversity and leaf damage risk from the mixed-effects model with seedling FDis as the biotic environmental variable. The shaded area represents the 95% CI of the predicted relationships. The histogram in panel a shows the distribution of trait diversity. The points in panel b show the presence or absence of leaf damage.

We found that delayed greening of young leaves showed contrasting effects on insect herbivory at wet vs. dry sites, although its main effect, averaged across the soil moisture gradient, was close to zero. Red (or delayed greening) young leaves may have lower risks of insect herbivory than normal green young leaves, due to their decreased visibility or higher defense against insect herbivores (Manetas, 2006). We only detected this defensive function of delayed greening at dry sites. This could be the reason that delayed greening, primarily determined by the differential development of anthocyanins and chlorophyll, could play different roles in different environmental conditions (Gould, 2004). For example, losing tissue may be costlier for plants at harsher conditions (e.g. with water deficit) (Endara and Coley, 2010). The defensive roles of delayed greening may be more important at harsher conditions. However, developing high photosynthetic and growth rates may be more important than reducing risks of insect herbivory at productive conditions (e.g. with high soil moisture). In this case, the defensive roles of delayed greening may be disfavored. Other plant traits may interact with leaf color to affect leaf apparency. For example, many plants can produce volatile organic compounds to interfere the detection of leaves by insects, and reduce leaf apparency (Barbosa et al. 2009).

4.2. Neighborhood diversity effects and their dependence on abiotic environment

Seedling functional dispersion (FDIs) showed opposite effects on leaf damage risk between wet vs. dry sites. Seedling FDIs alleviated the risk of insect herbivory on seedling young leaves at wet sites, while the opposite was true at dry sites. The main effect of seedling FDIs, averaged across the whole soil moisture gradient, had positive effects on young leaf damage risk, which may reflect the facts that soil moisture in the forest plots had rather right-skewed distribution and seedling FDIs had positive effects on insect herbivory at the low end of soil moisture gradient. The alleviating effects of seedling FDIs on insect herbivory at wet sites is consistent with the findings from a meta-analysis, which shows stronger negative conspecific density effects (NCDD) on plant survival at wet sites (Comita et al., 2014). NCDD occurs when neighboring conspecifics impair plant performance due to increased intraspecific competition or increased attacks from specialized natural enemies such as insect herbivores. NCDD can cause species to have more constraints on themselves than on other species, thus facilitate the maintenance of biodiversity (Wright, 2002). The alleviating effects of biodiversity on insect herbivory can arise at least from (1) low conspecific densities in high-diversity communities reducing the impacts of NCDD on leaf damage by insects; or (2) high heterospecific densities in high-diversity communities limiting the action spaces for NCDD. The absence of alleviating effects of trait diversity on insect herbivory at dry sites implies that NCDD may be weak and biodiversity maintenance may become more challenging at drier conditions.

Heterospecific tree density also showed different effects on insect herbivory at wet vs. dry sites. A high density of heterospecific neighbors may interfere with leaf detection by insect herbivores and reduce herbivory. This effect of species herd protection is an important mechanism generating a negative relationship between biodiversity and leaf herbivory (Peters, 2003). We found that a herd protection effect from heterospecific trees occurred at wet sites, which is consistent with that of seedling FDIs. We found positive effects of conspecific tree density on insect herbivory in gap areas, but not in shaded areas. This could be explained by that gap areas often have high densities of light-demanding, fast-growing species, which are often less defensive and more palatable to insects (Price, 1991; Norghauer et al., 2008).

5. Conclusions

This study provided evidence that seedling traits are stronger predictors of insect herbivory on seedling young leaves than abiotic and biotic environmental factors in a subtropical forest. Both seedlings traits and neighboring plant compositions have different effects on insect herbivory depending on the abiotic environment of the seedlings. Specifically, the alleviating effects of seedling trait diversity on leaf herbivory were only evident at wet sites. These results imply that forest biodiversity may face a more severe threat under drier environmental conditions. This study is based on the survey of insect herbivory on leaves in a single season of a single year. Insect herbivory may vary between seasons or years (Aide, 1988). Thus, measurement across longer time periods may provide more insights on the biotic and abiotic determinants on insect herbivory.

CRediT authorship contribution statement

Wenbin Li: Conceptualization, Methodology, Formal analysis, Writing - original draft. Yuxin Chen: Conceptualization, Methodology, Resources, Supervision, Project administration, Writing - review & editing. Yong Shen: Conceptualization, Methodology, Writing - review
Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

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