

The impact of plant chemical diversity on plant–herbivore interactions at the community level

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Abstract Understanding the role of diversity in ecosystem processes and species interactions is a central goal of ecology. For plant–herbivore interactions, it has been hypothesized that when plant species diversity is reduced, loss of plant biomass to herbivores increases. Although long-standing, this hypothesis has received mixed support. Increasing plant chemical diversity with increasing plant taxonomic diversity is likely to be important for plant–herbivore interactions at the community level, but the role of chemical diversity is unexplored. Here we assess the effect of volatile chemical diversity on patterns of herbivore damage in naturally occurring patches of *Piper* (Piperaceae) shrubs in a Costa Rican lowland wet forest. Volatile chemical diversity negatively affected total, specialist, and generalist herbivore damage. Furthermore, there were

differences between the effects of high-volatility and low-volatility chemical diversity on herbivore damage. High-volatility diversity reduced specialist herbivory, while low-volatility diversity reduced generalist herbivory. Our data suggest that, although increased plant diversity is expected to reduce average herbivore damage, this pattern is likely mediated by the diversity of defensive compounds and general classes of anti-herbivore traits, as well as the degree of specialization of the herbivores attacking those plants.

Keywords Plant interactions · Herbivory · *Piper* · Volatile compounds · La Selva

Introduction

Community ecologists have struggled to find rules governing both the assembly of communities they study and the processes that occur within those communities (Lawton 1999; Simberloff 2004; McGill et al. 2006; Ricklefs 2008). For plant communities specifically, the relationship between plant diversity, herbivore diversity and abundance, and herbivory has been studied extensively in agricultural systems and natural habitats (Andow 1991). These studies have shown that when plant species richness is reduced, loss of primary production to herbivores generally increases (Brown and Ewel 1987; Andow 1991; Hillebrand and Cardinale 2004; Hooper et al. 2005; Jactel and Brockerhoff 2007). This pattern is most commonly explained by the “resource concentration hypothesis” (Root 1973), which maintains that a particular resource will have a lower relative abundance in diverse plant assemblages than in less diverse communities. In general, as the diversity of host plants in a community decreases, the encounter rate between a specialist herbivore and its plant host

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will increase, resulting in higher herbivory. Nevertheless, many studies have found that herbivore damage can also increase with higher taxonomical diversity (e.g. Scherber et al. 2006; Loranger et al. 2014). For example, Schuldt et al. (2010) compared insect herbivory on tree sapling and shrub species within 27 forest plots in southeast China, and showed that insect herbivory increased with increasing plant species richness, even after accounting for potentially confounding variables—exactly opposite that predicted by the resource concentration hypothesis.

One possible explanation for such contradictory findings is the use of species diversity as a proxy for habitat complexity. Although changes in taxonomic plant diversity are expected to affect plant–herbivore interactions, this effect is likely mediated by changes in the diversity of specific plant traits that affect the relationship between herbivores and plant hosts. Therefore, to assess the effect of diversity on plant–herbivore interactions, it is important to measure the diversity of biologically meaningful traits in addition to species diversity.

One such set of traits is plant secondary chemistry. There are two possible mechanisms by which plant chemical diversity could affect plant herbivore interactions. First, given that all herbivore species are probably limited in the number of defense chemical compounds they can overcome or circumvent, communities with high chemical diversity will likely have fewer hosts that are chemically compatible with specific herbivores. Second, in the more chemically diverse communities, the search patterns of insect herbivores could be affected by the higher semiochemical complexity. For example, for herbivores searching for host plants via volatile compounds, increases in local chemical diversity, rather than species diversity per se, could reduce the encounter rate between herbivores and host plants due to chemical disorientation and odor masking (e.g. the semiochemical redundancy hypothesis, Randlkofer et al. 2010; Beyaert and Hilker 2014).

The effect of plant diversity on plant–herbivore interactions may also depend on local plant phylogenetic diversity. Phylogenetic diversity can be defined as a measure of community complexity that incorporates the phylogenetic relationships between local species. A community with low phylogenetic diversity will comprise closely related species, and a community with high phylogenetic diversity will primarily have distantly related taxa. Therefore, this measure of diversity can be important for understanding the relationship between diversity and plant–herbivore interactions, as it can determine the similarity of phylogenetically conserved traits within the plant community. Consequently, phylogenetic diversity can also serve to capture some of the local variation of plant traits that are otherwise not explicitly measured. For phytochemical diversity specifically, it is generally expected that the closer the relationship between

plant species, the more similar their chemistry will be. However, within a plant genus, herbivore selection may result in chemical diversification even among sister species, such that their differences are greater than those among more distantly related congeners (Coley and Kursar 2014).

Herbivore diet breadth is another commonly overlooked but extremely important component (Andow 1991; Specht et al. 2008; Schuldt et al. 2010) in assessing the relationship between plant diversity and plant–herbivore interactions. On one hand, herbivores with a narrow diet breadth (specialists) will have higher success finding hosts in simple, low-diversity habitats where compatible plant species are concentrated. On the other hand, herbivores with a wide diet breadth (generalists) are less likely to be affected by changes in the abundance of particular plant species, and therefore will be less sensitive to changes in local plant diversity (Root 1973; Andow 1991). Within the context of chemical diversity, herbivores with a narrow chemical diet breadth will be less likely to encounter chemically compatible host plants as the chemical complexity of a community increases.

Furthermore, it is important to note that not all secondary plant metabolites act similarly on all insect herbivores (Lankau 2007; Orians and Ward 2010; Ali and Agrawal 2012). For example, specialist herbivores are better adapted to circumvent, metabolize, or sequester specialized low-volatility plant defenses (e.g. alkaloids, cyanides, and cardenolides); therefore, changes in the diversity of such compounds are more likely to affect generalist herbivores (Barker et al. 2002; Macel and Vrieling 2003; Macel et al. 2005; Lankau 2007; Petschenka and Agrawal 2016). Similarly, due to their narrow diet range, specialist herbivores are more likely to search for specific plant species and thus depend strongly on long-range chemical cues to locate their hosts (e.g. mono and sesquiterpenes, and phenylpropanoids; De Bruyne and Baker 2008; Bruce and Pickett 2011; Reudler et al. 2011; Büchel et al. 2014). Consequently, it is reasonable to expect that changes in the diversity of high-volatility compounds will have a stronger effect on specialist than on generalist herbivores.

In this study, we assessed the effect of plant secondary chemistry on plant–herbivore interactions at the community level, taking into account herbivore diet breadth and plant phylogenetic relationships, for the neotropical species-rich genus *Piper* (Piperaceae). We measured the effect of chemical diversity on total, specialist, and generalist herbivore damage on a set of naturally occurring *Piper* communities in a lowland tropical wet forest. In addition, we assessed how the diversity of different secondary compounds (high-volatility vs. low-volatility) affected levels of specialist and generalist herbivory. We predicted that secondary compounds of high-volatility would have a greater impact on specialist herbivore species than on generalists, because the

former are more reliant on volatiles to find their host plants. In contrast, generalists would be more strongly affected by low-volatility compounds than would specialists, which are likely to be better adapted to cope with such compounds.

Materials and methods

Study site and system

The study was conducted at the La Selva Biological Station in Costa Rica (owned and operated by the Organization for Tropical Studies) between May and August 2007. The station is located in the Atlantic lowlands of Puerto Viejo de Sarapiquí (Heredia). It contains more than 1600 ha of tropical wet forest and receives approximately 4000 mm of rainfall annually. To date, approximately 1850 species-rich of plants have been found in La Selva, 50 of which are in the genus *Piper*.

Piper (Piperaceae) is a pantropical genus with approximately 1000 species in the neotropics (Jaramillo 2006). The natural range of the genus in the New World extends from northern Mexico to northern Argentina. *Piper* species are abundant in low- and mid-elevation forests, rarely reaching 2500 m, and are often among the most species-rich plant genera in neotropical forests (Gentry 1982; Marquis 2004). Most *Piper* species at La Selva occur in discrete patches that can contain up to 21 different species (Salazar et al. 2013). *Piper* secondary metabolite diversity has been studied extensively, and there is an important body of published methods for compound isolation, chemical profiling, and artificial synthesis. Furthermore, the effects of *Piper* compounds on herbivores, pathogens, and seed dispersal are well documented (Parmar et al. 1997; Dyer et al. 2001; Mikich et al. 2003; Dyer et al. 2004; Kato and Furlan 2007; Fincher et al. 2008; Marques et al. 2010).

Piper communities and herbivore damage

To assess the effect of *Piper* chemical diversity on herbivore damage, we sampled 81 naturally occurring multi-species patches of *Piper* throughout the primary forest of La Selva. Patches were selected by performing transects parallel to the station trails (transects were between 50 and 100 m from the trail). All patches selected were at least 250 m apart. To standardize sampling, a plot with a 10 m radius was set up in every patch. In each plot we counted the number of *Piper* individuals with a stem diameter of 1 cm or greater at ground level; all *Piper* plants were identified to species.

For each *Piper* present in a plot, we visually assessed the percentage of specialist and generalist herbivory, measured as the percentage of leaf area removed. Every plant

was assigned a value between 0 and 95 %, in increments of 5 %, for each herbivory type. Specialist and generalist herbivory in all *Piper* plants were easily distinguishable by the characteristic skeletonization of leaves by *Piper* specialist herbivores of the genus *Eois* (Geometridae) (Connahs et al. 2009; Dyer et al. 2010). The neotropical species of this genus are highly specialized and feed on one or rarely two species of *Piper* (Connahs et al. 2009). There are other *Piper* specialists at La Selva (e.g. weevils and leaf beetles; Marquis 1990); however, damage by these species was rarely encountered. Furthermore, *Eois* caterpillars have accounted for up to 75 % of the damage observed in *Piper* species (Salazar et al. 2013). For this reason, all non-*Eois* damage was assigned to generalist herbivores.

To assess the effect of chemical diversity independently of taxonomic species diversity, we calculated each plot's taxonomic diversity (hereafter, *Piper* diversity) using the Gini–Simpson index. To account for the effect that the surrounding plant diversity could have on *Piper* herbivore damage, we also counted and identified all non-*Piper* plants present within all plots (hereafter, non-*Piper* diversity). Non-*Piper* diversity was calculated for each plot using the Gini–Simpson index as well. Diversity indices were calculated using EcoSim 7.1 software (Gotelli and Entsminger 2012). Finally, given that light availability can influence leaf quality and palatability, and thus may affect levels of leaf herbivory (Angulo-Sandoval and Aide 2000; Blundell and Peart 2001; Takafumi et al. 2010; Salgado-Luarte and Gianoli 2011), we measured canopy openness (hereafter, light) for each plot using hemispherical photography. This technique measures the percentage of canopy cover using fisheye photographs of the forest canopy and the Gap Light Analyzer (GLA) 2.0 software package (Frazer et al. 1999).

Chemical analysis

For all *Piper* species, we collected leaf material samples from young, fully expanded leaves with 5–10 % herbivore damage (to control for the effect of induction). At least four samples were collected for each species, all from different plant individuals and different transects (plants were randomly selected within each transect). Samples were dried with silica gel and transported to the University of Missouri–St. Louis for chemical analysis. From each sample, 0.4 g of dried material was ground to a fine powder under liquid nitrogen. To extract a broad range of secondary metabolites (polar and non-polar), samples were extracted using 1.5 ml of 1:1 methanol–chloroform solution. Samples were kept at temperatures below 5 °C at all times to reduce the loss of volatile compounds. As an internal standard, 0.1 mg of piperine was added to all samples. Samples were filtered (0.2 μ) and stored in volatile organic chemical (VOC) vials at –80 °C until analysis. Qualitative chemical

analysis of the extracts was performed using GC–MS (HP 5890 coupled with a quadrupole model 5988A mass detector) with helium as a carrier gas and an HP-5 capillary column (30, 0.32 mm ID, 0.25 μ m). To ensure good chromatographic resolution and to reduce the likelihood of co-eluting compounds, we used very slow runs (80 min). It is important to note that although this study focuses only on compounds that are detectable with GC–MS, an analysis of more than 3500 records of secondary compounds found in *Piper* from NAPRALERT (Natural Products Alert Database; Loub et al. 1985) shows that these compounds account for more than 75 % of all *Piper* secondary metabolites reported in this database (Fig. S1). Because the abundance of the secondary compounds can vary among individuals due to factors such as induction, genetic variability, and resource availability, we used only presence and absence data of chromatographic features. To assess chemical dissimilarity among all sample species, we used mass spectra and retention-time chromatogram alignment. Chromatograms were aligned using MZmine (Pluskal et al. 2010). The mass spectra of the different compounds in the samples were compared with NIST/EPA/NIH and MassBank (Horai et al. 2010) databases and with the primary literature. Metabolites that did not match the available sources and databases were classified as unknown. It is also important to note that this analytical approach does not depend on the precise identification of chemical compounds. Here, a combination of mass spectral patterns, molecular mass, and retention times was used to ascertain the commonality of chemical compounds across plant species (see the proof of concept section in the Supporting Information).

Plot chemical diversity

To assess the chemical diversity of the 81 sampled plots, we first used the *pvclust* function from the *pvclust* R package (Suzuki and Shimodaira 2006; R Core Team 2012) to generate chemical dendrograms (hierarchical clustering analyses; Ward's algorithm) based on chemical data. We generated three different dendrograms: (1) a species total chemical dendrogram, (2) a species high-volatility chemical dendrogram, including only low molecular weight (<230 g/mol) terpenoids and phenylpropanoids, and (3) a species low-volatility but high molecular weight (>250 g/mol) chemical dendrogram (amides, flavonoids, lignans, catechols, and cinnamic acids). We subsequently extracted the species pair matrices of chemical distances from the dendrograms (cophenetic function from *stats* package). Finally, we used the chemical distance matrices of these three species to calculate the total, high-volatility, and low-volatility chemical diversity for all sampled plots. All values were assessed using Rao's quadratic entropy index

with the *raoD* function in the *Picante* package for R (Rao 2010; Ricotta and Moretti 2011; Kembel et al. 2012). We used Rao's index for two main reasons. First, Rao takes into account the species chemical differences: a community with a high Rao chemical diversity will comprise plants that are very different in chemical composition, and a community with low Rao chemical diversity will have species with similar chemical composition. Second, Rao can also account for the differences in relative abundance of *Piper* species within each plot.

Phylogenetic diversity

To control for the potential effect of phylogeny on *Piper* herbivory and chemical similarity, we constructed a phylogenetic tree of all sampled *Piper* species. Samples of leaves were collected to perform sequencing of the *ITS* and the *chloroplast intron psbJ-petAx* for phylogenetic analysis (following Jaramillo et al. 2008). The resulting phylogeny was concurrent with all current phylogenetic and taxonomic data (Fig. S2). We used this phylogenetic reconstruction to calculate the Rao phylogenetic diversity index of each plot, again using the *Picante* package. Similar to the chemical indices, Rao's phylogenetic diversity takes into account species phylogenetic distances and species relative abundance. Our measures of plant (Gini–Simpson) and phylogenetic and chemical diversity (Rao quadratic index) are ones that are relevant to the searching herbivore, i.e. they measure the probability that the searching herbivore will encounter a new plant species based on chemical and phylogenetic similarity.

Statistical analysis

We analyzed the data using generalized linear mixed effects models (GLMM). To test the role of chemical diversity on *Piper* herbivory, we assessed the effect of total chemical diversity, *Piper* phylogenetic diversity, and non-*Piper* diversity (as fixed effects) of each *Piper* patch on total, generalist, and specialist herbivory suffered by each *Piper* plant (models A.1, A.2, and A.3, respectively; see Table S1 in Supporting Information). In all of these models, we controlled for light and *Piper* diversity (random effects). We also assessed the effect of high-volatility diversity, low-volatility diversity, *Piper* phylogenetic diversity, and non-*Piper* diversity (as fixed effects) on *Piper* generalist and specialist herbivory (models B.1 and B.3, respectively), again controlling for light and *Piper* diversity (random effects). In all models, the experimental unit was the *Piper* plot. We also evaluated three models to assess the effect of *Piper* taxonomic diversity on herbivore damage (Table S2, models C.1, C.2, and C.3). Model fit was evaluated via the Akaike information criterion (AIC) and maximum

likelihood tests (*see* Supporting Information for details on model construction and model selection). All models used the maximum likelihood estimation method, and models

were analyzed using R 2.15 (R Core Team 2012) and the nlme package with the functions lme and anova (Pinheiro et al. 2016). Finally, given that different measures of diversity can be correlated, all beyond-optimal models were checked for the possible effect of multicollinearity on our analysis by calculating the variable inflation factor (VIF).

Table 1 Results from the generalized linear mixed model for the optimal models

	Estimate	df	t	p value
Model A.1				
Total herbivory = total chemical diversity + (light) + (<i>Piper</i> diversity)				
Total chemical diversity*	-0.06	1, 79	-3.94	0.0001
Model A.2				
Generalist herbivory = total chemical diversity + <i>Piper</i> phylogenetic diversity + non- <i>Piper</i> diversity + (light) + (<i>Piper</i> diversity)				
Total chemical diversity*	-0.02	1, 27	-3.84	0.0006
<i>Piper</i> phylogenetic diversity	0.005	1, 72	1.4	0.16
Non- <i>Piper</i> diversity	-0.51	1, 76	-1.04	0.30
Model A.3				
Specialist herbivory = total chemical diversity + (light) + (<i>Piper</i> diversity)				
Total chemical diversity*	-0.07	1, 79	-4.54	<0.0001
Model B.1				
Specialist herbivory = high-volatility chemical diversity + <i>Piper</i> phylogenetic diversity + (light) + (<i>Piper</i> diversity)				
High-volatility chemical diversity*	-0.14	1, 78	-2.34	0.02
<i>Piper</i> phylogenetic diversity*	-0.09	1, 78	-2.12	0.03
Model B.2				
Generalist herbivory = low-volatility chemical diversity + (light) + (<i>Piper</i> diversity)				
Low-volatility chemical diversity*	-0.18	1, 75	-2.91	0.004

Complete models and details of model selection procedure can be found in the supplementary information. Random variables are shown in parentheses. Variables with an asterisk (*) show a statistically significant effect ($p < 0.05$)

Results

After controlling for light and species diversity, the final optimal generalized linear mixed model showed that the total chemical diversity in a plot had a significant negative effect on total herbivore damage; higher levels of chemical diversity within a plot were associated with lower levels of herbivore damage (model A.1; VIF < 2.4; Tables 1, S1; Fig. 1a). The mean leaf area loss in the most chemically diverse and least chemically diverse plots was 4.5 and 11.1 %, respectively. An identical pattern was found for the effect of total chemical diversity on specialist herbivory (model A.3; VIF < 2.2, Fig. 1c). The final model for generalist herbivory included non-*Piper* diversity, *Piper* phylogenetic diversity, and *Piper* total chemical diversity (model A.2; VIF < 2.4; see Tables 1, S1). Only total chemical diversity, however, had a significant effect on generalist herbivory (model A.2; Table 1; Fig. 1b).

High-volatility chemical diversity had a significant negative effect on specialist herbivory (model B.1; VIF < 1.2; Tables 1, S1; Fig. 2). In contrast, higher levels of low-volatility diversity had a significant negative effect on generalist herbivory (model B.2; VIF < 1.2; Tables 1, S1; Fig. 2). The model selection procedure showed that high-volatility chemical diversity did not improve the model explaining generalist herbivory (model B.2), and that low-volatility chemical diversity did not improve the model explaining specialist herbivore damage (model B.1, Tables 1, S1; Fig. 2). Furthermore, adding phylogenetic *Piper* diversity

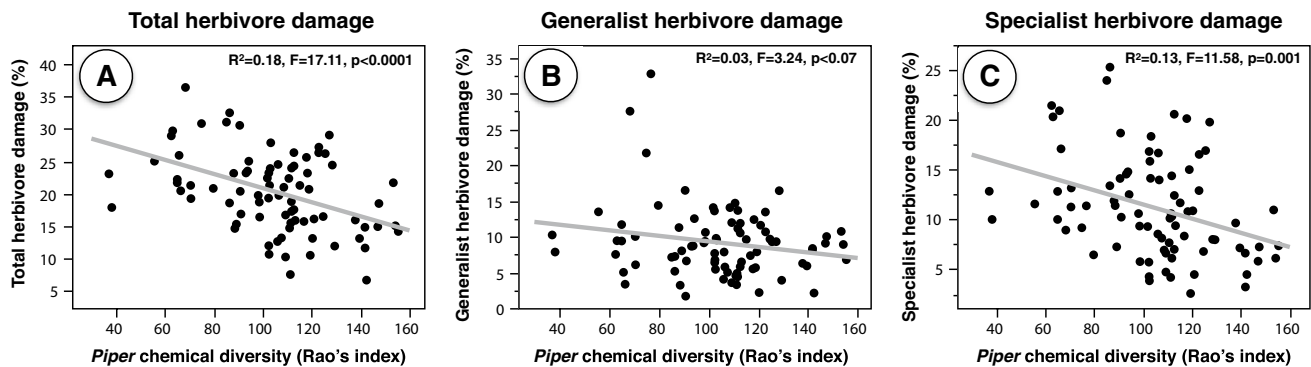
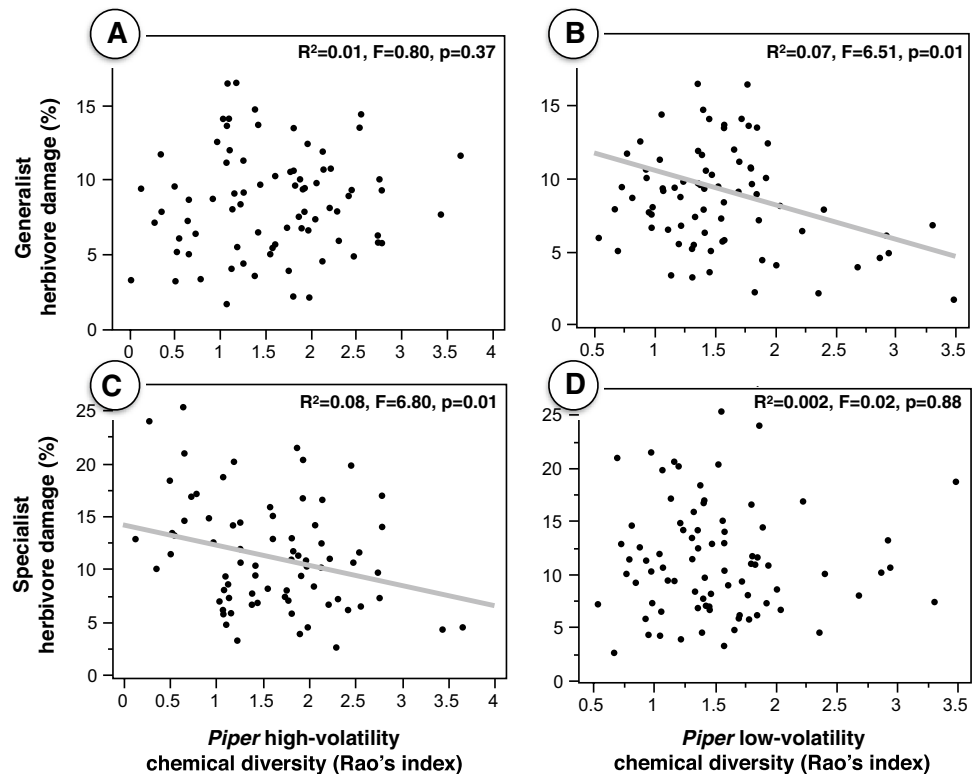


Fig. 1 The different panes show the relationship between total (a), generalist (b), and specialist (c) herbivore damage (percentage of leaf area removed) and total *Piper* volatile chemical diversity (Rao's

index). Each point represents an experimental plot. Grey lines are linear regressions, and the values for the regressions are shown in the upper-right corner of each plot

Fig. 2 The different panes show the relationship between high- and low-volatility chemical diversity (Rao's index) and generalist and specialist herbivore damage (percentage of leaf area removed). Each point represents an experimental plot. Grey lines are linear regressions, and the values for the regressions are shown in the upper-right corner of each plot. **a** Percentage of generalist herbivory vs. high-volatility chemical diversity. **b** Percentage of generalist herbivory versus low-volatility chemical diversity. **c** Percentage of specialist herbivory vs. high-volatility chemical diversity. **d** Percentage of specialist herbivory vs. low-volatility chemical diversity



improved only the model that explained specialist herbivory (model B.2; Tables 1, S1). See the general results from the plant, herbivory, and chemical surveys in the Supporting Information.

Discussion

The overall results from this study suggest that community-wide levels of chemical diversity influence plant–herbivore interactions. We found that plots with higher levels of total *Piper* chemical diversity were associated with lower levels of total, generalist, and specialist insect herbivory. Furthermore, this pattern held true after controlling for non-*Piper* diversity, *Piper* taxonomic diversity, light availability, and *Piper* phylogenetic relationships. In addition, we found that different kinds of chemical diversity affected specific guilds of insect herbivores differentially, in this case specialist and generalist herbivores. Plots with higher levels of high-volatility chemical diversity had significantly lower specialist insect herbivory, while plots with higher low-volatility chemical diversity showed lower levels of damage caused by generalist insect herbivores. Thus, the *Piper* community composition growing around an individual *Piper* plant at the study site influenced the amount of damage that the plant received from its herbivores. This neighborhood effect is apparently influenced mainly by the presence of conspecifics and congeners, not by plants from other families.

We show here that the effects of neighborhood chemical diversity on the amount of herbivore damage appear to be independent of taxonomic and phylogenetic plant diversity. Two mechanisms that might explain the effects of chemical diversity on the local plant herbivore damage found in this study are (1) the resource concentration principle (Root 1973) and (2) the semiochemical-diversity hypothesis (Zhang and Schlyter 2003; Randallofer et al. 2010).

Resource concentration hypothesis

Root's resource concentration hypothesis states that the amount of herbivore damage experienced by a given plant will depend on the encounter rate between the plant and its herbivores. In more diverse plant assemblages, a particular plant host will have a lower relative abundance than in a simpler, less diverse community. Insect herbivores have been reported to feed upon tissue containing a limited set of plant secondary compounds, due to evolutionary (Becerra 1997; Becerra and Venable 1999), metabolic (Freeland and Janzen 1974; Scriber 2002; Marsh et al. 2006), and behavioral constraints (Fernandez and Hilker 2007; Schröder and Hilker 2008; Cheng et al. 2013). Therefore, it is also likely that an increase in local plant chemical diversity will reduce the encounter rate between the insect herbivore and chemically compatible plant hosts, resulting in a comparable reduction in the plant herbivore damage.

Another prediction that can be made from Root's hypothesis is that changes in chemical diversity will have a stronger effect on herbivores that feed on a small set of plant secondary metabolites (specialists) than on generalists who are able to feed on many plant species with very diverse defense chemistry. Thus, the significant effect of total plant chemical diversity on specialist herbivores found in this study supports this hypothesis.

Semiochemical-diversity hypothesis

Herbivore chemical disorientation due to local plant chemical complexity can also influence plant–herbivore interactions. The semiochemical-diversity hypothesis (Zhang and Schlyter 2003) states that complex plant communities will have greater secondary compound diversity than simpler communities, and that these higher levels of chemical diversity are likely to pose a challenge to herbivore orientation, by negatively affecting their ability to find, recognize, or accept a particular plant host (for similar ideas see Schröder and Hilker 2008; Dicke and Baldwin 2010; Jactel et al. 2011; Party et al. 2013; Beyaert and Hilker 2014). For example, combinations of specific plant odors can mask host chemical cues or even repel insect herbivores (Party et al. 2013). By interfering with the herbivore's ability to track critical plant–host chemical cues, higher levels of plant chemical diversity could strongly affect herbivore–host encounter rates, thus reducing the local levels of plant damage caused by herbivores. Our results are consistent with this hypothesis.

Another prediction that can be drawn from this hypothesis is that the chemical disorientation effect of higher chemical diversity is likely to have a stronger effect on herbivores that rely heavily on volatile secondary metabolites for finding suitable hosts. In the case of *Piper* herbivores, we do not know which species use volatiles to find their host plants, but our results suggest that at least a subset of *Piper* herbivores do so (specialists). It is also important to note that these two mechanisms (resource dilution and chemical disorientation) are not mutually exclusive, and that both could act simultaneously in natural systems.

Chemical diversity and herbivore natural enemies

Another mechanism that has been proposed to explain the relationship between diversity and plant–herbivore interactions is the enemy hypothesis (Root 1973). This hypothesis states that habitats with higher plant diversity can sustain or attract a higher diversity of herbivore predators and parasitoids. Although it is difficult to imagine how chemical diversity *per se* could directly affect the diversity of generalist and opportunistic herbivore consumers, the chemical disorientation effect could alter the ability of

the specialized natural enemies of herbivores (particularly hymenopteran parasitoids) to find their prey (Wäschke et al. 2013). For *Piper* herbivores specifically, *Eois* caterpillars, for example, are frequently parasitized, resulting in significant mortality rates (Connahs et al. 2009; Richards et al. 2010). Nevertheless, recent studies have suggested that parasitoids are attracted to a combination of plant and herbivore volatile metabolites, and are therefore less affected by changes in plant species and chemical diversity alone (Wäschke et al. 2014; Kruidhof et al. 2015). In order to determine the net effect of chemical disorientation on plant herbivore damage—directly on herbivore attack and indirectly on parasitism—more specifically designed laboratory and field experiments are needed.

Effects of low- and high-volatility chemical diversity

After partitioning the plot's chemical diversity into high-volatility and low-volatility groups, it was possible to assess the effect of specific groups of secondary metabolites on plant herbivore damage. These two groups of secondary compounds showed markedly contrasting effects on generalist and specialist herbivore damage.

Specialist herbivores are generally better adapted to circumventing or overcoming low-volatility secondary plant metabolites (e.g. alkaloids and glucosinolates; Orians 2000; Lankau 2007). Therefore, it is reasonable to expect that these herbivores will not be especially susceptible to changes in the diversity of low-volatility compounds. Furthermore, specialist herbivores are expected to feed on a small subset of *Piper* species within our plots (Connahs et al. 2009; Dyer et al. 2010), and it is thus unlikely that the presence of different low-volatility compounds in the surrounding non-host plants could affect the amount of damage these specialist insects cause (Macel et al. 2002; Macel and Vrieling 2003; Cheng et al. 2013). In contrast, despite the fact that specialist herbivores are also expected to have highly developed host search mechanisms, it is likely that higher levels of community-wide high-volatility chemical diversity can affect their ability to track the specific chemical cues from their hosts, due to odor masking and semiochemical redundancy (Schröder and Hilker 2008; Randlkofer et al. 2010; Bruce and Pickett 2011; Party et al. 2013; Zakir et al. 2013).

In contrast, generalist herbivores feed on a wide range of plants, and are less likely to track specific high-volatility chemical cues from a particular host plant in order to find a suitable host (e.g. Vargas et al. 2005). Thus, changes in the community's high-volatility diversity are less likely to affect their ability to find a suitable plant for feeding. Instead, low-volatility diversity was found to negatively affect generalist herbivore damage. Researchers have reported that generalist herbivores are less able to cope

with specialized defenses of a particular plant host (Van Dam et al. 1995; Macel 2011; Ali and Agrawal 2012; Lampert 2012), and this is true for some *Piper* generalist herbivores (Richards et al. 2010). Given that most of the low-volatility secondary compounds found in this study included alkaloids (including amides and imides), flavonoids, and catechols, it is reasonable to expect that a higher diversity of such compounds could reduce the number of palatable hosts available to generalist herbivores.

The mechanisms linking chemical diversity to either generalist or specialist herbivory appear to differ. On the one hand, our data suggest that the effect of chemical diversity on the reduction of specialist herbivore damage is the result of semiochemical saturation, redundancy, or odor masking (semiochemical diversity hypothesis). On the other, generalist herbivores appear to be affected by low-volatility diversity. Given that low-volatility compounds are less likely to be used as cues for host-finding by generalist herbivores, this pattern suggests that the effect of high low-volatility diversity on generalist herbivory is the result of “resource dilution” (Root’s hypothesis). Furthermore, the different effects of high-volatility and low-volatility chemistry on different guilds of herbivores could also explain the strong difference in the patterns of high-volatility and low-volatility similarity across *Piper* species (Fig. S3).

Finally, it is important to note that the inclusion of *Piper* phylogenetic diversity did not improve most of the models used for this study. This result is likely because *Piper* chemical diversity does not show a significant phylogenetic signal for the 27 *Piper* species found in our plots (Salazar et al. 2013). It is generally assumed that insect herbivores are more likely to feed on closely related plant species, because these species are more likely to share similar traits than are distantly related species. When this assumption does not hold true (see also Kursar et al. 2009), community phylogenetic complexity will not necessarily have a significant effect on the encounter rate between herbivore and chemically compatible plant hosts. Lastly, the small effect that phylogenetic diversity had on specialist herbivory could either be (1) linked to phylogenetically conserved anti-herbivore traits not measured in this study or (2) evidence of a coevolutionary history between *Piper* and their specialized herbivores (*Eois*).

Conclusions

Our results suggest that interactions between *Piper* and its natural enemies at the community level are significantly affected by local secondary metabolite diversity. These results also underscore the importance of studying different biologically relevant dimensions of community complexity when assessing the relationship between diversity

and plant–herbivore interactions. Different measures of diversity will likely be associated with different herbivore guilds and taxonomic groups. Additionally, our results also suggest that these patterns can be mediated by multiple mechanisms acting in concert (e.g. resource concentration and semiochemical-diversity). Whether these results apply to other diverse tropical genera, or even temperate genera such as *Solidago*, *Salix*, and *Quercus*, remains to be seen.

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Author contribution statement DS and RJM conceived and designed the study. DS collected the data and performed the chemical analysis. AJ performed phylogenetic analysis. DS, RJM and AJ wrote manuscript.

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