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Effects of tree species diversity and genotypic diversity on leafminers and parasitoids in a tropical forest plantation

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- Abstract**
- 1 The effects of tree diversity on herbivore–enemy interactions have received relatively little attention and even fewer studies have compared the relative influence of tree intra- versus interspecific diversity on such dynamics.
 - 2 We evaluated the effects of mahogany (*Swietenia macrophylla*) genotypic diversity and tree species diversity on parasitoid attack and species richness associated with *Phyllocnistis meliacella*, a specialist herbivore on mahogany, in a forest diversity experiment consisting of 74 plots (21 × 21 m²; 64 plants/plot). We sampled 34 of such plots classified as: mahogany monocultures of one maternal family (i.e. genotype), mahogany monocultures of four families and polycultures of four species (including mahogany). We surveyed leafminer abundance and collected mined leaves to estimate parasitism and parasitoid species richness.
 - 3 Leafminer abundance was not influenced by either type of diversity. Similarly, there were no effects of genotypic diversity or species diversity on parasitism or parasitoid species richness. Plant diversity effects on parasitoids were probably absent because the species attacking *P. meliacella* are dietary generalists that likely recruited to multiple host species (in addition to *P. meliacella*) and their responses to diversity could have cancelled each other out.
 - 4 Future work should explicitly investigate how predator and parasitoid traits mediate the effects of plant diversity on tritrophic interactions.

Keywords Enemies hypothesis, genotypic diversity, herbivory, parasitoids, species diversity, tritrophic interactions.

Introduction

One major challenge in ecology is understanding the importance of plant diversity in ecosystems (Hooper *et al.*, 2005). Although much of the early work focused on manipulating plant diversity and measuring effects on plant competitive dynamics and resource use (Hooper, 1998; Loreau & Hector, 2001; Tilman *et al.*, 2001; Potvin & Gotelli, 2008), there is increasing evidence for the effects of plant diversity on organisms at higher trophic levels (Johnson *et al.*, 2006; Ninkovic *et al.*, 2011; Moreira *et al.*, 2012; McArt & Thaler, 2013; Moreira & Mooney, 2013). In

particular, studies conducted over the past decade have shown positive effects of both plant species diversity and within-species genotypic diversity on arthropod species richness and abundance (Siemann *et al.*, 1998; Koricheva *et al.*, 2000; Crutsinger *et al.*, 2006; Haddad *et al.*, 2009; Scherber *et al.*, 2010). At the same time, many studies have also shown that plant species diversity and genotypic diversity frequently drive a reduction in herbivore damage (Elton, 1958; Pimentel, 1961; Andow, 1991; Hambäck *et al.*, 2000; Hillebrand & Cardinale, 2004; Jactel & Brockerhoff, 2007; but see also Castagneyrol *et al.*, 2012; Loranger *et al.*, 2014; Barton *et al.*, 2015).

A decrease in herbivory on individual plants as producer diversity increases is frequently linked to reductions in the density of preferred host plants with increasing diversity (Leonard, 1969;

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Root, 1973). The resource concentration hypothesis (RCH) holds that herbivores frequently forage in a density-dependent manner, and therefore increasing the number of plant species or genotypes at a constant plant density fundamentally reduces the probability of finding a preferred host plant species (or genotype), ultimately leading to lower herbivore abundance and damage on individual plants (Hambäck *et al.*, 2014; Underwood *et al.*, 2014). Alternatively, changes in plant species (or genotypic) diversity may alter the environmental grain, changing the number of physical refuges or resource types available to organisms at higher trophic levels (Langellotto & Denno, 2004). Thus, an increase in plant diversity may favour an increase in the abundance and diversity of predators, which, in turn, leads to stronger top-down control over herbivore populations and lower damage to plants, as predicted by the enemies hypothesis (EH) (Root, 1973). Although there is relatively good support for the EH in agricultural systems (Russell, 1989; Andow, 1991; Letourneau, 1997), evidence is weaker or inconsistent in natural, more diverse systems (Koricheva *et al.*, 2000; Bommarco & Banks, 2003; Jactel *et al.*, 2006; Riihimäki *et al.*, 2005; Barton *et al.*, 2015). This has been attributed mainly to the spatial scale of experiments relative to predator dispersal ability (Bommarco & Banks, 2003) and herbivore and/or predator diet breadth (Sheehan, 1986; Castagneyrol *et al.*, 2014), as well as variation in how predator species or functional groups respond to habitat heterogeneity (Denno *et al.*, 2005; Hambäck *et al.*, 2014). For example, the dynamics predicted by EH should be more likely to occur for generalist herbivores because they are more susceptible to natural enemies in that they frequently lack defence mechanisms found in specialists (Mooney *et al.*, 2012; Singer *et al.*, 2014). The dynamics predicted by the RCH should also depend on herbivore dietary specialization (Root, 1973); specialist herbivores are more negatively influenced by plant diversity, whereas generalist herbivores exhibit weak responses because they are not limited to feeding on a specific host plant (Jactel & Brockerhoff, 2007). Similarly, previous work suggests that predator or parasitoid diet breadth is also a key determinant of variation in plant diversity effects on herbivore–enemy interactions, with generalist enemies responding more strongly to plant diversity than specialists because the former depend on a higher diversity of alternative prey found in diverse habitats (Sheehan, 1986).

Differences between plant species diversity and within-species genotypic diversity effects on higher trophic levels remain largely unexplored (Cook-Patton *et al.*, 2011) and may also condition the predictions made by the EH. Because trait variation is greater among plant species than among genotypes within a species (Albert *et al.*, 2010), species diversity should generate greater resource heterogeneity (Agrawal *et al.*, 2006). This should lead to stronger bottom-up effects of plant diversity on consumer foraging (Cook-Patton *et al.*, 2011; Castagneyrol *et al.*, 2012; McArt & Thaler, 2013) and potentially also stronger top-down effects of predators on lower trophic levels (Haddad *et al.*, 2009; Moreira & Mooney, 2013). However, most studies addressing the EH have tested for species diversity and genotypic diversity effects separately. To date, only two studies have manipulated both types of diversity and, contrary to predictions, found that genotypic diversity had equal (Cook-Patton *et al.*, 2011) or stronger (Crawford & Rudgers, 2013) effects on arthropods than species diversity. Nonetheless, further work is needed

to arrive at more general conclusions about the relative importance and mechanisms of plant intra- and interspecific diversity effects on higher trophic levels. For example, a stronger focus on understanding how interactions at higher trophic levels are altered by plant diversity and how such effects lead to broader, community-level dynamics.

The present study was conducted within the context of a large-scale tree diversity experiment comparing the effects of big-leaf mahogany (*Swietenia macrophylla* King) genotypic diversity and tree species diversity on higher trophic levels. Previous work (conducted in 2012) in this system found that tree species diversity reduced the abundance of the leaf-mining caterpillar *Phyllocnistis meliacella* Becker, a dietary specialist that only feeds on mahogany and a few other species of Meliaceae (Abdala-Roberts *et al.*, 2015) (see Supporting information, Fig. S1). Such an effect was presumably mediated by habitat heterogeneity and reduced densities of mahogany at high diversity (as predicted by the RCH) rather than a result of increased abundance and predation by spiders (as predicted by the EH) because this predator group was not influenced by plant diversity. In the present study, we contrast those dynamics with a subsequent year of sampling where we evaluate tree diversity effects on *P. meliacella*, in the context of parasitism rates and parasitoid species richness associated with this herbivore across levels of diversity. The contrast of predation by spiders (assessed in the previous study) and parasitism (assessed in the present study) with respect to the subtleties of the EH is significant and provides insight into how variation in natural enemy traits or functional groups conditions the effects of diversity on herbivore–enemy interactions (Björkman *et al.*, 2010). Parasitoid species associated with *P. meliacella* are dietary generalists that feed on prey across several insect orders (Salvo & Valladares, 2007; Bonet, 2008); therefore, rather than track higher *P. meliacella* abundances at low diversity (in accordance with the RCH and based upon data from the previous study), we predicted that these parasitoids would recruit more strongly to diverse patches because these offer increased physical complexity, greater refuge availability and a greater diversity of alternative prey. Accordingly, this would be then reflected in higher parasitism on *P. meliacella*, one of the potential hosts, at high diversity. Furthermore, we investigate whether the effects on herbivore–enemy interactions were contingent upon the source of plant diversity (intra- or interspecific) and predicted that the effects of species diversity would be of greater importance than the effects of genotypic diversity.

Materials and methods

Study species

Big-leaf mahogany (*S. macrophylla*, Meliaceae), the target tree species and the component of genotype variation evaluated in the present study, is a self-compatible, long-lived tree distributed from southern Mexico to Bolivia (Pennington & Sarukhán, 2005). The specialist leafminer *P. meliacella* (Lepidoptera: Gracillariidae) is a common herbivore of mahogany, especially in open areas or disturbed forests. Larvae produce characteristic serpentine galleries throughout the leaf surface and, usually, one caterpillar is found per leaf (Becker, 1976). The life cycle from first instar to adult is completed in approximately

1 month, and previous work suggests that it exhibits continuous generations (Becker, 1976). At the study site (see below), *P. meliacella* is one of the most common herbivores on mahogany. This herbivore in turn exhibits high levels of larval parasitism ($\geq 50\%$), attributed mainly to dietary generalist wasps of the genus *Horismenus* (Eulophidae) (Becker, 1976), which attack many species across several insect orders.

In tropical forests of the Yucatan Peninsula, big-leaf mahogany frequently co-occurs with five other deciduous, long-lived tree species, which were used to manipulate tree species diversity in the present study, namely: *Tabebuia rosea* (Bertol.) DC. (Bignoniaceae), *Ceiba pentandra* (L.) Gaertn. (Malvaceae), *Enterolobium cyclocarpum* (Jacq.) Griseb. (Fabaceae), *Piscidia piscipula* (L.) Sarg. (Fabaceae) and *Cordia dodecandra* A. DC. (Boraginaceae).

Study site and experimental design

The present study was conducted within the context of a broader experiment testing for tree species diversity effects on plant growth and the arthropod community, and was based upon a subset of diversity treatment combinations and plots within this study system.

Forest diversity experiment. The system was established in December 2011 by planting 4-month-old seedlings at a site owned by the Mexican Institute for Research on Agriculture Forestry and Livestock (INIFAP), near the locality of Muna, in Yucatan, Mexico (20°24'44"N, 89°45'13"W). The experiment was established on a recently cleared site where vegetation was composed mostly of grasses and shrubs, and is currently surrounded by a matrix of secondary tropical forest. Saplings were fertilized once in January 2012 with N, P and K (20:30:10), and drip-irrigated with 2 L of water, three times per week, from January 2012 until June 2012. Each plot was weeded once a month. Seeds of all species were collected from adult plants located in southern Quintana Roo (México) in January 2011 and March 2011. With respect to mahogany, the distance among mother trees ranged from 3 to 50 km and these maternal seed sources are referred to broadly as 'maternal families' or genotypes represented by a mixture of full- and half-sibs, albeit likely dominated by half-sibs given that this species is highly outcrossing (Loveless & Gullison, 2003; Lemes *et al.*, 2007). Previous work has shown that these maternal families vary substantially in growth-related traits, herbivore resistance and chemical defences (Moreira *et al.*, 2014; Abdala-Roberts *et al.*, 2015).

The experiment consisted of 74 plots (21 × 21 m²), each at a planting density of 64 plants per plot and 3-m spacing among trees, for a total of 4780 plants (see Supporting information, Table S1). Aisles between plots were 6 m wide, and the experimental site covered 7.2 ha. Mahogany was the most abundant species in the experiment ($n = 2480$ plants; other species ranged from 432 to 480 plants) and was planted in 59 out of the 74 plots (see Supporting information, Table S1). To test for species diversity effects, we established plots of two types: species monocultures (two plots per species, except for mahogany for which a larger number of monocultures were established to test for genotypic diversity effects; see below) and polycultures of four

species. The 59 plots where mahogany was planted were classified as: (i) mahogany monocultures of a single maternal family (12 plots, two replicate plots/genotype); (ii) mahogany monocultures of four families (20 plots); (iii) species polycultures within which all mahogany saplings planted were of one family (12 plots, two plots/family); and (iv) species polycultures within which mahogany plants were represented by four families (15 plots) (see Supporting information, Table S1). Treatments of both species and genotypic diversity included equal numbers of individuals of four species or mahogany families drawn randomly from pools of six species or families, respectively. All non-mahogany species were equally represented across species polyculture plots (each species present in six plots). Similarly, mahogany genetic families were represented in a similar number of mahogany monoculture plots of four families (eight or nine plots per family) and also in a similar number of species polyculture plots where mahogany plants were of four families (9 or 10 plots per family). Plots of each diversity treatment combination were randomly interspersed throughout the experimental landscape. Tests conducted thus far have shown no effect of mahogany genotypic or tree species diversity on mahogany growth (see Supporting information, Table S2), indicating that any effect of diversity on herbivore–enemy interactions would not be the result of an increase in plant biomass (because of stronger plant resource partitioning or facilitation) but rather the result of effects of habitat heterogeneity on consumer foraging.

Subset of diversity experiment used in the present study. We selected 34 of the 59 plots where mahogany was planted (see above), allocated as: mahogany monocultures of a single family (hereafter 'monogenotype' plots, $n = 12$), mahogany monocultures of four families (hereafter 'polygenotype' plots, $n = 11$) and species polycultures with one mahogany genotype (hereafter 'species polyculture' plots, $n = 11$) (Fig. 1; see also Supporting information, Table S1). From this point on, we refer to methods, analyses and results that pertain exclusively to sampling from these 34 plots (Fig. 1, see also Supporting information, Table S1). Species polycultures with four mahogany families were not sampled. Genetic families were similarly represented across the polygenotype plots sampled.

Leafminer sampling and parasitoid rearing

In early August 2013, we surveyed leafminer abundance by counting the number of active leaf mines in six randomly chosen mahogany plants per plot. Within polygenotype plots, the range of plants sampled per genetic family was 1–3. Mines exhibited the characteristic morphology of feeding by *P. meliacella* (Becker, 1976), and caterpillars were reared to adults and identified in accordance with Forbes (1923) and Becker (1976). At the time of leafminer sampling, the means \pm SE height of mahogany saplings was 3.68 ± 0.04 m. To estimate parasitism, in October 2013, we collected all young leaves with mines per plant where the leafminer had pupated (118 leaves, 1–5 leaves collected per plant; $n = 66$ plants). This procedure was aimed at allowing the maximum amount of time for parasitism to occur and thus avoid underestimating parasitism. Pupae are easily identified because the caterpillar rolls the edge of the leaf blade before pupating.

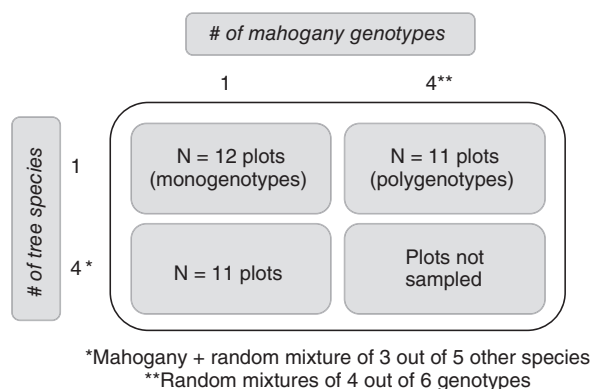


Figure 1 Subset of the forest diversity experiment used to test for effects of big-leaf mahogany (*Swietenia macrophylla*) genotypic diversity and tree species diversity on the abundance of the specialist leafminer *Phyllocnistis meliacella*, as well as parasitism and parasitoid species richness associated with this herbivore. In total, we selected and sampled 34 out of the total 74 plots in the experiment; all the sampled plots contained mahogany, either in monoculture of one genotype ('monogenotypes'), monoculture of four genotypes ('polygenotypes') or in species polycultures where mahogany was planted with random mixtures of three other tree species. In each of these polyculture plots, mahogany plants were represented by only one genotype (each genotype being present in two plots of this diversity treatment combination). Further details on the experimental design are provided in the Materials and methods. Plot sample sizes were lower for parasitoid data, depending on the availability of plants with mined leaves for parasitoid rearing.

Collected leaves were placed in paper bags and transported to the laboratory in a cooler at 20 °C. We placed leaves in 0.5-L plastic containers with moistened cotton and recorded moth and parasitoid emergence every 2 days throughout a 2-week period (mid to late October 2013). Parasitoid specimens were identified to the species or genus level using keys for Nearctic (Schauff *et al.*, 1997) and Neotropical Eulophidae (Hansson, 2014). The material was compared with specimens from the U.S. Museum of Natural History and the Texas A&M University Insect Collection. Plot sample sizes were smaller for the analyses of parasitism (monogenotype, $n = 11$; polygenotype, $n = 10$; polyculture, $n = 11$) and parasitoid species richness (monogenotype, $n = 8$; polygenotype, $n = 6$; polyculture, $n = 6$) because, for some plots, there were no available leaf mines for any plant.

Statistical analysis

Diversity effects on leafminer abundance. We separately tested for mahogany genotypic diversity and tree species diversity effects on the mean number of leaf mines per plot (mean across plants within each plot) using general linear models conducted with PROC GLM in SAS, version 9.2 (SAS Institute, Cary North Carolina). The genotypic diversity model tested for a difference between monogenotypes and polygenotypes, whereas the species diversity model tested for a difference between monogenotypes and species polycultures (Moreira *et al.*, 2014). Each model included genotypic or species diversity as main effect (fixed), as well as plot-level mean plant height as a covariate to account for residual variation resulting from

differences in plant size influencing herbivore recruitment (Moreira *et al.*, 2014).

Diversity effects on parasitism rates and parasitoid species richness. We tested for mahogany genotypic diversity and tree species diversity effects on the mean proportion (mean across plants within each plot) of parasitized leafminers per plot [number of parasitoids emerged/(number of parasitoids emerged + number of moths emerged)] and the number of parasitoid species per plot (sum across plants within each plot) using general linear models in PROC GLM. As above, for each response, we conducted two models: one testing for genotypic diversity and another for species diversity effects (Moreira *et al.*, 2014). The abundances of each parasitoid species were too low to perform separate analyses for each species; thus, we calculated proportions by pooling abundances across species. Models for parasitoid species richness included the number of parasitoid specimens recorded as a covariate to account for differences in sampling effort (i.e. parasitoid abundance).

General considerations. Models for leafminer abundance and parasitism were based upon plot-level means calculated by averaging values across plants sampled within each plot. In both cases, the results did not change qualitatively by conducting analyses at the plant level and including plot as a random effect and genotype identity (treated as fixed as a result of insufficient degrees of freedom to treat this effect as random) (see Supporting information, Table S3); therefore, we only report the results from plot-level analyses. The models for leafminer abundance and parasitoid species richness were based upon a normal distribution (identity as link function) because normality was met based upon previous verification of residuals and Kolmogorov–Smirnov tests. Parasitoid species richness was log-transformed to achieve normality. In addition, the parasitism model was based upon a binomial distribution (logit link function) because the data were not normally distributed after transformation and a binomial distribution provided an adequate fit. There was no evidence of overdispersion in this latter model. Except stated otherwise, we provide least-square means (back-transformed for parasitism and parasitoid species richness) and SEs as descriptive statistics. Finally, we performed *a posteriori* power tests using observed variances and sample sizes in PROC POWER (SAS, version 9.2). However, if there is no diversity effect (or a weak one) and treatment means are similar (i.e. small observed effect), by necessity, this leads to low power to detect such small effects. Therefore, we complemented these power tests by estimating the probability of a biologically meaningful effect going undetected (Type II error) based upon observed sample sizes and variances. In this case, we chose a 50% effect size.

Results

Diversity effects on leafminer abundance

We found no effects of either genotypic diversity or species diversity on leafminer abundance as plot means for herbivore

density did not differ significantly among diversity treatments (monogenotype: 6.57 ± 1.01 mines; polygenotype: 5.50 ± 0.67 mines; polyculture: 6.95 ± 0.92 mines) (Fig. 2A and Table 1). Although there was up to 1.6-fold variation in leaf miner abundance among mahogany genotypes (genotype means ranged from 4.56 ± 1.56 to 7.54 ± 1.36 mines), subsidiary tests conducted at the plant level including genotype identity as a fixed effect indicated no significant variation in leaf miner abundance among mahogany genotypes (see Supporting information, Table S3), which is in accordance with the lack of a genotypic diversity effect on this herbivore.

Diversity effects on parasitoids

Parasitoid attack. A total of 105 caterpillars were collected, of which 56% were parasitized for a total of 59 parasitoid specimens representing five taxa (three identified to the species level and two to morphological species within genus). The most abundant species emerging from *P. meliacella* was *Horismenus brachycaulis* (Hansson 2004), followed by *Cirrospilus* sp. Westwood 1832, *Horismenus sardus* (Walker 1847), an unidentified species of *Horismenus* and, lastly, *Elasmus punctatus* Howard 1894 (Table 2). By contrast to expectations, we found no significant effects of either genotypic diversity or tree species diversity on the percent of parasitized leafminers (monogenotype: $53.97 \pm 9.54\%$; polygenotype: $45.61 \pm 11.46\%$; polyculture: $54.55 \pm 8.81\%$) (Fig. 2B and Table 1).

Parasitoid species richness. We found no effect of either genotypic diversity or species diversity on the number of parasitoid species per plot (monogenotype: 1.40 ± 0.11 species; polygenotype: 1.60 ± 0.14 species; polyculture: 1.44 ± 0.14 species) (Fig. 2C and Table 1).

Estimates of power and probability to detect biologically meaningful effect sizes

Statistical power for our tests of diversity ranged from low to moderate. Power to detect effects of diversity on leaf miner abundance was low for genotypic diversity (0.14) and moderate for species diversity (0.53), whereas, for parasitism (genotypic diversity = 0.10, species diversity = 0.20) and parasitoid species richness (genotypic diversity = 0.11, species diversity = 0.30), power values were low. However, differences between treatment means were small in many cases (e.g. effect sizes for species diversity ranged from 1% to 5%), which, by necessity, led to low statistical power. Accordingly, further tests indicated that the probability of failing to detect a 50% effect size was relatively low for most of our tests of diversity effects (leaf miner abundance: genotypic diversity = 0.14, species diversity = 0.21; parasitism: genotypic diversity = 0.41, species diversity = 0.26; parasitoid species richness: genotypic diversity = 0.13, species diversity = 0.21). Based on this, nonsignificant effects of diversity on parasitism and parasitoid species richness in the present study presumably were not the result of increased Type II error but, instead, were the result of minimal differences between high and low diversity. Accordingly, had larger, biologically

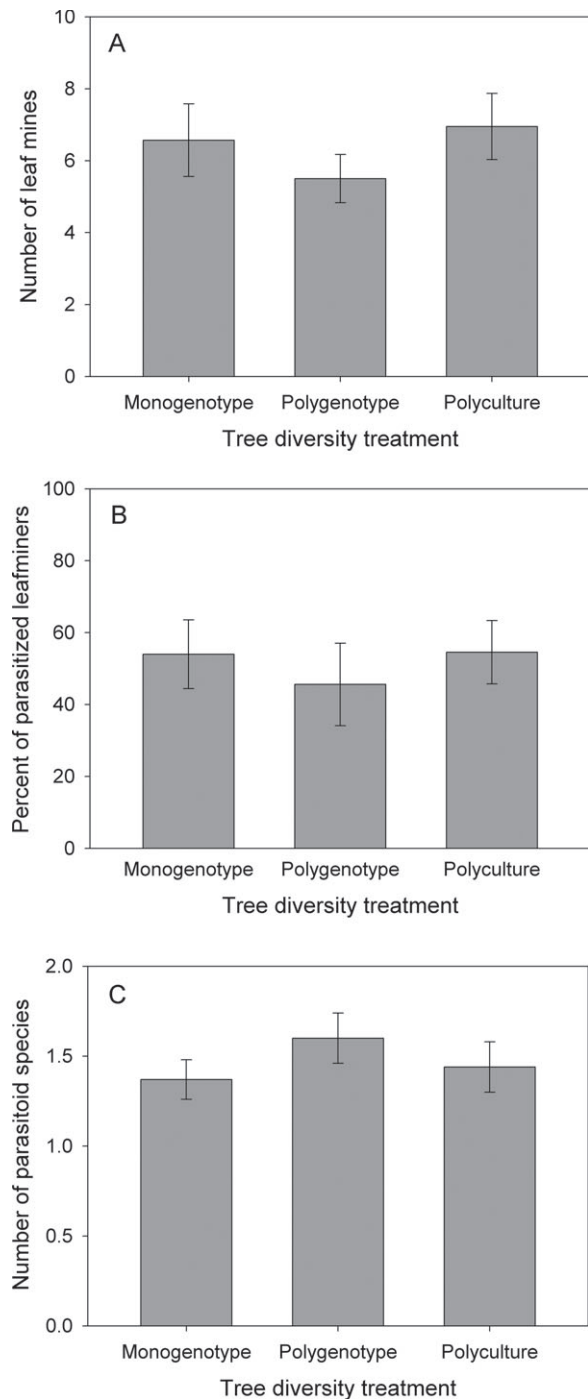


Figure 2 Effects of big-leaf mahogany (*Swietenia macrophylla*) genotypic diversity and tree species diversity on (A) the number of leaf mines per mahogany tree caused by the specialist caterpillar *Phyllocnistis meliacella*, (B) the proportion of parasitized *P. meliacella* caterpillars and (C) the number of parasitoid species attacking *P. meliacella* in a tree diversity experiment in southern Mexico (Yucatan). Bars are least square means (\pm SE) from general linear models. We performed two models to separately test for effects of each source of plant diversity: one compared monogenotypes versus polygenotypes (test of genotypic diversity) and the other compared monogenotypes versus polycultures (test of species diversity) (see Statistical analysis).

Table 1 Results from general linear models testing for the effects of big-leaf mahogany (*Swietenia macrophylla*) genotypic diversity (GD) and tree species diversity (SD) on the number of leaf mines by the specialist caterpillar *Phyllocnistis meliacella*, the proportion of parasitized caterpillars (parasitism) and the number of parasitoid species attacking the leafminer

Source	Response		
	Leafminer abundance	Parasitism	Parasitoid species richness
GD	$F_{1,20} = 0.26$ (0.615)	$\chi^2 = 0.25$; d.f. = 1, 19 (0.625)	$F_{1,11} = 1.20$ (0.297)
Plant height	$F_{1,20} = 0.48$ (0.494)	–	–
Parasitoid number	–	–	$F_{1,11} = 21.40$ (0.007)
SD	$F_{1,20} = 0.22$ (0.644)	$\chi^2 = 0.09$; d.f. = 1, 20 (0.773)	$F_{1,11} = 0.03$ (0.874)
Plant height	$F_{1,20} = 0.61$ (0.443)	–	–
Parasitoid number	–	–	$F_{1,11} = 19.09$ (0.001)

For each response variable, statistics are presented for each source of diversity, namely F -values (or χ^2 values for parasitism), numerator and denominator degrees of freedom, and P -values (in parentheses). Significant effects ($P < 0.05$) are shown in bold. Leafminer abundance model included plant height as a covariate to account for residual variation in plant biomass influencing leafminer abundance, whereas the model for parasitoid species number includes the number of parasitoids specimens recorded per plot to account for differences in parasitoid abundance.

Table 2 Taxonomic information and abundances of the parasitoid species recorded for *Phyllocnistis meliacella*, a specialist leaf-mining caterpillar feeding on big-leaf mahogany (*Swietenia macrophylla*) in a forest diversity experiment in southern Mexico (Yucatan)

Parasitoid species	Family	Number of individuals	Percentage of sample
<i>Horismenus brachycaulis</i> (H)	Eulophidae	22	37.3
<i>Cirrospilus</i> sp.	Eulophidae	14	23.7
<i>Horismenus sardus</i>	Eulophidae	13	22.1
<i>Horismenus</i> sp.	Eulophidae	4	6.8
<i>Elasmus punctatus</i> (H)	Elasmidae	3	5.1
Unknown	–	3	5.0

Of the 59 parasitoid specimens recorded, 56 were identified up to the genus or species level. Four specimens were identified as *Horismenus* sp. but not identified to the species level because specimens were damaged, whereas another three specimens were not identified because they were in the pupal stage. (H) = also reported as hyperparasitoid (Coote, 1997; Hansson, 2014).

meaningful effects occurred, the probability of detecting such effect sizes would have been moderate to high.

Discussion

The results of the present study indicate that neither tree species diversity, nor mahogany genotypic diversity influenced the level of parasitism or parasitoid species richness associated with the specialist leafminer *P. meliacella*. Similarly, previous work in this system found no effect of tree species or mahogany genotypic diversity on spiders (Abdala-Roberts *et al.*, 2015). Taken together, the results obtained from previous work and those of the present study lead us to reject the EH, where we would have expected greater predator and parasitoid abundance, species richness and/or attack at high plant diversity (Root, 1973; Russell, 1989). In addition, and contrary to previous findings in this system showing a negative effect of tree species diversity on *P. meliacella* (Abdala-Roberts *et al.*, 2015), we found no effect of tree species (or genotypic) diversity on this leafminer, which suggests that the mechanisms of tree species diversity effects on this herbivore previously proposed to be at work (i.e. habitat heterogeneity and reduced host plant density, as predicted by the RCH) were not at work in the present study. Overall, although these findings were influenced by low statistical power, we note that differences between low and high diversity (i.e. effect sizes) were weak in most cases, which suggests that biologically meaningful effects of plant diversity were lacking.

It is noteworthy that our findings contrast with results previously reported in this system showing negative effects of tree species diversity on *P. meliacella* (Abdala-Roberts *et al.*, 2015). However, we caution that a comparison between the two studies may not be straightforward as a result of methodological differences. First, the sampling effort (number of plants and plots) was different (two- to four-fold greater in the previous study) and this could clearly influence the outcome of the tests of diversity. Second, the time of sampling was not the same. In the previous work, we sampled leafminers at the end of the rainy season (October), whereas, in the present study, sampling was performed in the middle of the rainy season (August). In this sense, further work is necessary to evaluate whether there is temporal (within-season and/or between-year) variation in the effects of plant diversity on this herbivore (Barton *et al.*, 2015) and, in this way, be able to derive generalities from these findings.

Despite observing high levels of parasitism for *P. meliacella* (>50%) in the present study, there were no detectable effects of plant diversity on parasitoid species richness or the level of parasitism. This finding contrasts with previous studies showing strong effects of plant species diversity (Andow, 1991; Jactel & Brockerhoff, 2007) and genotypic diversity on parasitoids and predators (Crutsinger *et al.*, 2006; Ninkovic *et al.*, 2011; Moreira & Mooney, 2013). In addition, studies have also found that plant diversity alters the function of herbivore–predator interactions by influencing natural enemy recruitment rates and foraging behaviour (Ninkovic *et al.*, 2011; Moreira *et al.*, 2012). Such

effects are presumed to be driven by an increase in the number of physical refuges or resource types available to natural enemies as predicted by the EH (Siemann *et al.*, 1998; Crutsinger *et al.*, 2006; Haddad *et al.*, 2009; Moreira & Mooney, 2013).

In agreement with our findings, several previous studies have similarly failed to demonstrate effects of plant diversity on natural enemies, including parasitoids (Koricheva *et al.*, 2000; Björkman *et al.*, 2010), spiders (Langer, 1996; Schuldt *et al.*, 2011) and predatory beetles (Björkman *et al.*, 2010). Inconsistent support for the EH has been attributed mainly to the spatial scale of experiments in relation to predator mobility (Bommarco & Banks, 2003) and differences in how predator functional groups respond to habitat complexity (Denno *et al.*, 2005), as well as predator or parasitoid dietary specialization (Sheehan, 1986). With respect to diet breadth, previous work holds that generalist enemies should be more sensitive to plant diversity than specialist enemies and preferentially recruit to high diversity patches because these species depend on a greater diversity of alternative prey present in diverse habitats (Sheehan, 1986). However, parasitoids of *P. meliacella* recorded in the present study are generalists feeding on Lepidoptera, Coleoptera, Diptera and Hymenoptera (e.g. *Horismenus* spp.; Schauff *et al.*, 1997; Bonet, 2008), which presumably did not exhibit higher recruitment to *P. meliacella* (one of the potential hosts) in diverse plots. Instead, it is possible that, by feeding on several host species (in addition to *P. meliacella*) that responded differently to plant diversity, parasitoid species responses to tree diversity cancelled each other out and this led to no overall effect on the level of parasitism. Interestingly, *H. brachycaulis* and *E. punctatus* can also act as hyperparasitoids of Hymenoptera (Coote, 1997; Hansson, 2014), which could have introduced further variation or interfered with responses of primary parasitoids to diversity.

In addition, we cannot rule out the possibility that plant diversity effects on parasitoids were absent because of the short time subsequent to the establishment of the experiment. This could be a particularly important consideration in early successional forests dominated by widely-spaced tree saplings (Schuldt *et al.*, 2011), where the effects of habitat heterogeneity on predators take longer to emerge relative to systems dominated by herbaceous plants (Siemann *et al.*, 1998; Haddad *et al.*, 2009). Also, with respect to effects of genotypic diversity, and despite there being substantial differences among mahogany genetic families in growth- and defence-related traits, effects could have been weak because the sampled families did not offer sufficient functional contrast to influence leafminers or parasitoids. Accordingly, there was no evidence of genotype identity effects on this leaf miner based upon the plant-level analyses. Finally, despite surveying parasitoid attack at the peak of leafminer abundance, our sampling is limited to one time point and we thus cannot rule out the occurrence of diversity effects on parasitoids at some other time during the growing season or, more generally, the presence of temporal variation in the strength of such effects.

Although the present study is unsupportive of the EH, we provide one of the few direct comparisons of plant genotypic and species diversity effects on associated faunas, and uniquely do so for the third trophic level. Our findings reject the prediction that greater phenotypic variation underlying plant species diversity

results in stronger effects on consumers because neither source of diversity had effects on higher trophic levels. To date, only two studies have compared plant genotypic and species diversity effects on consumers and found, contrary to predictions, that the effects of genotypic diversity were of similar (Cook-Patton *et al.*, 2011) or greater (Crawford & Rudgers, 2013) importance than those of species diversity in structuring arthropod communities. However, only Cook-Patton *et al.* (2011) compared genotypic and species diversity effects on the third trophic level and found (as predicted) stronger effects of species diversity. Clearly, further work is necessary to better understand the relative importance and mechanisms by which plant intra- and interspecific diversity shape interactions between species at higher trophic levels. In doing so, we will achieve a better understanding of how the magnitude of plant trait variation influences associated faunas and the potential for feedbacks between plant diversity and the third trophic level.

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Supporting information

Additional Supporting information may be found in the online version of this article under the DOI reference: 10.1111/afe.12132

Fig. S1. Effects of tree species diversity and mahogany (*Swietenia macrophylla*) genotypic diversity on the abundance of the specialist leaf-mining caterpillar *Phyllocnistis meliacella* feeding on mahogany. Circles are plot-level least-square means \pm SE from a general linear model accounting for plant size. Symbols are offset for clarity. The grand least-square means \pm SE for each level of species diversity is shown on each side (modified from Abdala-Roberts *et al.*, 2015). Data were collected in 2012 and surveys were restricted to the 59 plots where mahogany was present, classified as: mahogany monocultures of one genetic family or genotype (termed 'monogenotypes' in the present study), mahogany monocultures of four genotypes ('polygenotypes' in the present study), species polycultures

within which all mahogany saplings were of one genotype, and species polycultures where mahogany plants were represented by four genotypes. High species diversity = species polyculture, low species diversity = mahogany monocultures; low genotypic diversity = plots with one mahogany genotype, high genotypic diversity = plots with four mahogany genotypes. Tree species diversity had a significant negative effect on leafminer abundance, whereas genotypic diversity did not, and this latter result was consistent across levels of species diversity (Abdala-Roberts *et al.*, 2015).

Table S1. Treatments of tree species diversity (SD) and mahogany (*Swietenia macrophylla*) genotypic diversity (GD) allocated to a total of 74 plots in a forest diversity experiment in southern Mexico (Yucatán).

Table S2. Results from general linear models testing for the effects of tree species diversity and big-leaf mahogany (*Swietenia macrophylla*) genotypic diversity on mahogany height measured in June 2013.

Table S3. Results from general linear models conducted at the plant level testing for the effects of big-leaf mahogany (*Swietenia macrophylla*) genotype diversity (GD) and tree species diversity (SD) on the number of leaf mines by the specialist caterpillar *Phyllocnistis meliacella*, the proportion of parasitized caterpillars (parasitism) and the number of parasitoid species attacking the leafminer.

References

- Abdala-Roberts, L. & Mooney, K.A. (2014) Ecological and evolutionary consequences of plant genotype diversity in a tri-trophic system. *Ecology*, **95**, 2879–2893.
- Abdala-Roberts, L., Mooney, K., Quijano-Medina, T., Campos-Navarrete, M.J., González-Moreno, A. & Parra-Tabla, V. (2015) Comparison of tree genotypic diversity and species diversity effects on different guilds of insect herbivores. *Oikos*. DOI: 10.1111/oik.02033.
- Agrawal, A.A., Lau, J. & Hämbäck, P.A. (2006) Community heterogeneity and the evolution of interactions between plants and insect herbivores. *Quarterly Review of Biology*, **81**, 349–376.
- Albert, H.C., Thuiller, W., Gilles, N., Douzet, R., Aubert, S. & Lavorel, S. (2010) A multi-trait approach reveals the structure and the relative importance of intra- vs. interspecific variability in plant traits. *Functional Ecology*, **24**, 1192–1201.
- Andow, D.A. (1991) Vegetational diversity and arthropod population response. *Annual Review of Entomology*, **36**, 561–586.
- Barton, K.E., Valkama, E., Vehviläinen, H., Ruohomäki, K., Knight, T.M. & Koricheva, J. (2015) Additive and non-additive effects of birch genotypic diversity on arthropod herbivory in a long-term field experiment. *Oikos*, **124**, 696–706.
- Becker, O.B. (1976) Microlepidópteros asociados con *Carapa*, *Cedrela* y *Swietenia* en Costa Rica. Studies on the Shootborer *Hypsipyla grandella* (Zeller) Lep. Pyralidae, Vol. II (ed. by J. Whitmore), pp. 75–101. CATIE, Costa Rica.
- Björkman, M., Hämbäck, P.A., Hopkins, R.J. & Rämert, B. (2010) Evaluating the enemies hypothesis in a clover-cabbage intercrop: effects of generalist and specialist natural enemies on the turnip root fly (*Delia floralis*). *Agricultural and Forest Entomology*, **12**, 123–132.
- Bommarco, R. & Banks, J.E. (2003) Scale as modifier in vegetation diversity experiments: effects on herbivores and predators. *Oikos*, **102**, 440–448.
- Bonet, A. (2008) New hosts, host plants, and distribution records for *Horismenus* (Hymenoptera: Eulophidae) species in a bruchid beetle parasitoid guild attacking wild type *Phaseolus coccineus* and *P. vulgaris* in central Mexico. *Florida Entomologist*, **91**, 698–701.
- Castagneyrol, B., Lagache, L., Giffard, B., Kremer, A. & Jactel, H. (2012) Genetic diversity increases insect herbivory on oak saplings. *PLoS ONE*, **7**, e44247.
- Castagneyrol, B., Jactel, H., Vacher, C., Brockerhoff, E.G. & Koricheva, J. (2014) Effects of plant phylogenetic diversity on herbivory depend on herbivore specialization. *Journal of Applied Ecology*, **51**, 134–141.
- Cook-Patton, S.C., McArt, S.H., Parachnowitsch, A.L., Thaler, J.S. & Agrawal, A. (2011) A direct comparison of the consequences of plant genotypic and species diversity on communities and ecosystem function. *Ecology*, **92**, 915–923.
- Coote, L.D. (1997) Elasmidae. *Annotated Keys to the Genera of Nearctic Chalcidoidea (Hymenoptera)* (ed. by G. A. P. Gibson, J. T. Hubert and J. B. Woolley), pp. 165–169. NRC Research Press, Canada.
- Crawford, K.M. & Rudgers, J.A. (2013) Genetic diversity within a dominant plant outweighs plant species diversity in structuring an arthropod community. *Ecology*, **94**, 1025–1035.
- Crutsinger, G.M., Collins, M.D., Fordyce, J.A., Gompert, Z., Nice, C.C. & Sanders, N.J. (2006) Plant genotypic diversity predicts community structure and governs an ecosystem process. *Science*, **313**, 966–968.
- Denno, R.F., Finke, D.L. & Langellotto, G.A. (2005) Direct and indirect effects of vegetation structure and habitat complexity on predator–prey and predator–predator interactions. *Ecology of Predator–Prey Interactions* (ed. by P. Barbosa and I. Castellanos), pp. 211–239. Oxford University Press, New York, New York.
- Elton, C.S. (1958) *The Ecology of Invasions by Animals and Plants*. Methuen, U.K.
- Forbes, W.T.M. (1923) *The Lepidoptera of New York and Neighboring States: Primitive Forms, Microlepidoptera, Pyralids, Bombyces*. Cornell University Agricultural Experimental Station, Ithaca, New York.
- Haddad, N., Crutsinger, G.M., Gross, K., Haarstad, J., Knops, J.M. & Tilman, D. (2009) Plant species loss decreases arthropod diversity and shifts trophic structure. *Ecology Letters*, **12**, 1029–1039.
- Hämbäck, P.A., Ågren, J. & Ericson, L. (2000) Associational resistance: insect damage to purple loosestrife reduced in thickets of sweet gale. *Ecology*, **81**, 1784–1794.
- Hämbäck, P.A., Inouye, B.D., Andersson, P. & Underwood, N. (2014) Effects of plant neighborhoods on plant-herbivore interactions: resource dilution and associational effects. *Ecology*, **95**, 1370–1383.
- Hansson, C. (2014) *Neotropical Eulophidae* [WWW document]. URL www.neotropicaeulophidae.com/Lucid3_keys/Horismenus_key.html. Genera.html [accessed on 16 June 2014].
- Hillebrand, H. & Cardinale, B.J. (2004) Consumer effects decline with prey diversity. *Ecology Letters*, **7**, 192–201.
- Hooper, D.U. (1998) The role of complementarity and competition in ecosystem responses to variation in plant diversity. *Ecology*, **79**, 704–719.
- Hooper, D.U., Chapin, F.S. III, Ewel, J.J. *et al.* (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological Monographs*, **75**, 3–35.
- Jactel, H. & Brockerhoff, E.G. (2007) Tree diversity reduces herbivory by forest insects. *Ecology Letters*, **10**, 835–848.
- Jactel, H., Menassieu, P., Vetillard, F., Gaulier, A. & Samalens, J.C. (2006) Tree species diversity reduces the invasibility of maritime pine stands by the bast scale, *Matsucoccus feytaudi* (Homoptera: Margarodidae). *Canadian Journal of Forest Research*, **36**, 314–323.
- Johnson, M.T.J., Lajeunesse, M.J. & Agrawal, A.A. (2006) Additive and interactive effects of plant genotypic diversity on arthropod communities and plant fitness. *Ecology Letters*, **9**, 23–34.

- Koricheva, J., Mulder, C.P.H., Schmid, B., Joshi, J. & Huss-Danell, K. (2000) Numerical responses of different trophic groups of invertebrates to manipulations of plant diversity in grasslands. *Oecologia*, **125**, 271–282.
- Langelloto, G.A. & Denno, R.F. (2004) Responses of invertebrate natural enemies to complex-structured habitats: a meta-analytical synthesis. *Oecologia*, **139**, 1–10.
- Langer, V. (1996) Insect-crop interactions in a diversified cropping system: parasitism by *Aleochara bilineata* and *Trybliographa rapae* of the cabbage root fly, *Delia radicum*, on cabbage in the presence of white clover. *Entomologia Experimentalis et Applicata*, **80**, 365–374.
- Lemes, M.R., Grattapaglia, D., Grogan, J., Proctor, J. & Gribel, R. (2007) Flexible mating system in a logged population of *Swietenia macrophylla* King (Meliaceae): implications for the management of a threatened Neotropical tree species. *Plant Ecology*, **192**, 169–179.
- Leonard, K.J. (1969) Factors affecting rates of stem rust increase in mixed plantings of susceptible and resistant oat varieties. *Phytopathology*, **59**, 1845–1850.
- Letourneau, D.K. (1997) Plant-arthropod interactions in agroecosystems. *Ecology in Agriculture* (ed. by L. E. Jackson), pp. 239–290. Academic Press, San Diego, California.
- Loranger, H., Weisser, W.W., Ebeling, A. *et al.* (2014) Invertebrate herbivory increases along an experimental gradient of grassland plant diversity. *Oecologia*, **174**, 183–193.
- Loreau, M. & Hector, A. (2001) Partitioning selection and complementarity in biodiversity experiments. *Nature*, **412**, 72–76.
- Loveless, M.D. & Gullison, R.E. (2003) Genetic variation in natural mahogany populations in Bolivia. *Big-Leaf Mahogany. Genetics, Ecology, and Management* (ed. by A. E. Lugo, J. C. Figueroa-Colon and M. Alayon), pp. 9–28. Springer-Verlag, New York, New York.
- McArt, S.H. & Thaler, J.S. (2013) Plant genotypic diversity reduces the rate of consumer resource utilization. *Proceedings of the Royal Society of London Series B, Biological Sciences*, **280**, 20130639.
- Mooney, K.A., Pratt, R.T. & Singer, M.S. (2012) The tri-trophic interactions hypothesis: interactive effects of host plant quality, diet breadth and natural enemies on herbivores. *PLoS ONE*, **7**, e34403.
- Moreira, X. & Mooney, K.A. (2013) Influence of plant genetic diversity on interactions between higher trophic levels. *Biology Letters*, **9**, 20130133.
- Moreira, X., Mooney, K.A., Zas, R. & Sampedro, L. (2012) Bottom-up effects of host-plant species diversity and top-down effects of ants interactively increase plant performance. *Proceedings of the Royal Society of London Series B, Biological Sciences*, **279**, 4464–4472.
- Moreira, X., Abdala-Roberts, L., Parra-Tabla, V. & Mooney, K.A. (2014) Positive effects of plant genotypic and species diversity on anti-herbivore defences in a tropical tree species. *PLoS ONE*, **9**, e105438.
- Ninkovic, V., Abassi, S.A., Ahmed, E., Glinwood, R. & Pettersson, J. (2011) Effect of within-species plant genotype mixing on habitat preference of a polyphagous insect predator. *Oecologia*, **166**, 391–400.
- Pennington, T. & Sarukhán, J. (2005) *Árboles Tropicales de México*, 3rd edn. Fondo de Cultura Económica, México.
- Pimentel, D. (1961) Species diversity and insect population outbreaks. *Annals of the Entomological Society of America*, **54**, 76–86.
- Potvin, C. & Gotelli, N. (2008) Biodiversity enhances individual performance but does not affect survivorship in tropical trees. *Ecology Letters*, **11**, 217–223.
- Riihimäki, J., Kaitaniemi, P., Koricheva, J., Vehviläinen, H. (2005) Testing the enemies hypothesis in forest stands: the important role of tree species composition. *Oecologia*, **142**, 90–97.
- Root, R.B. (1973) Organization of a plant-arthropod association in simple and diverse habitats: the fauna of collards (*Brassica oleracea*). *Ecological Monographs*, **43**, 95–124.
- Russell, E.P. (1989) Enemies hypothesis: a review of the effect of vegetational diversity on predatory insects and parasitoids. *Environmental Entomology*, **18**, 590–599.
- Salvo, A. & Valladares, G.R. (2007) Leafminer parasitoids and pest management. *Ciencia e Investigación Agraria*, **34**, 167–185.
- Schauff, M.E., LaSalle, J. & Coote, L.D. (1997) Eulophidae. *Annotated Keys to the Genera of Nearctic Chalcidoidea (Hymenoptera)* (ed. by G. A. P. Gibson, J. T. Hubert and J. B. Woolley), pp. 327–429. NRC Research Press, Canada.
- Scherber, C., Eisenhauer, N., Weisser, W.W. *et al.* (2010) Bottom-up effects of plant diversity on multitrophic interactions in a biodiversity experiment. *Nature*, **468**, 553–556.
- Schuld, A., Both, S., Bruelheide, H., Härdtle, W., Schmid, B., Zhou, H. & Assmann, T. (2011) Predator diversity and abundance provide little support for the enemies hypothesis in forests of high tree diversity. *PLoS ONE*, **6**, e22905.
- Sheehan, W. (1986) Response by specialist and generalist natural enemies to agroecosystem diversification: a selective review. *Environmental Entomology*, **15**, 456–461.
- Siemann, E., Tilman, D., Haarstad, J. & Ritchie, M. (1998) Experimental tests of the dependence of arthropod diversity on plant diversity. *American Naturalist*, **152**, 738–750.
- Singer, M.S., Lichter-Marck, I.H., Farkas, T.E., Aaron, E., Whitney, K.D. & Mooney, K.A. (2014) Herbivore diet breadth mediates the cascading effects of carnivores in food webs. *Proceedings of the National Academy of Sciences of the United States of America*, **111**, 9521–9526.
- Tilman, D., Reich, J.K., Knops, D., Wedin, T., Mielke, T. & Lehman, C. (2001) Diversity and productivity in a long-term grassland experiment. *Science*, **294**, 843–845.
- Underwood, N., Inouye, B.D. & Hambäck, P.H. (2014) A conceptual framework for associational effects: when do neighbors matter and how would we know? *Quarterly Review of Biology*, **89**, 1–19.

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