



Tree species diversity alters plant defense investment in an experimental forest plantation in southern Mexico

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ABSTRACT

How plant species diversity affects traits conferring herbivore resistance (*e.g.*, chemical defenses), as well as the mechanisms underlying such effects, has received little attention. One potential mechanism for the effect of diversity on plant defenses is that increased plant growth at high diversity could lead to reduced investment in defenses via growth–defense trade-offs. We measured tree growth (diameter at breast height) and collected leaves to quantify total phenolics in 2.5-year-old plants of six tropical tree species ($N = 597$ plants) in a young experimental plantation in southern Mexico. Selected plants were classified as monocultures or as polycultures represented by mixtures of four of the six species examined. Tree species diversity had a significant negative effect on total phenolics, where polycultures exhibited a 13 percent lower mean concentration than monocultures. However, there was marked variation in the effects of diversity on defenses among tree species, with some species exhibiting strong reductions in phenolic levels in mixtures, whereas others were unresponsive. In addition, tree species diversity had no effect on growth, nor was the negative effect of diversity on chemical defenses mediated by a growth–defense trade-off. These results demonstrate that tree diversity can alter investment in chemical defenses in long-lived tree species but that such effect may not always be under strong control by plant endogenous resource allocation trade-offs. Regardless of the underlying mechanism, these findings have important implications for predicting effects on consumers and ecosystem function.

Abstract in Spanish is available with online material.

Key words: defenses; diversity; growth; phenolic compounds; trade-off; Yucatán.

PLANT INTRA- AND INTER-SPECIFIC DIVERSITY, MEASURED AS THE NUMBER OF PLANT GENOTYPES OR SPECIES AT A GIVEN SITE, HAS STRONG EFFECTS ON ECOSYSTEM FUNCTION (Tilman *et al.* 2001, Hooper *et al.* 2005, Cardinale *et al.* 2011) and community structure at higher trophic levels (Crutsinger *et al.* 2006, Haddad *et al.* 2009, Moreira *et al.* 2012, Moreira & Mooney 2013, Campos-Navarrete *et al.* 2015). Increasing plant diversity drives concomitant increases in primary productivity (Tilman *et al.* 1996, Cardinale *et al.* 2007) due to positive interactions among plant species (*e.g.*, complementarity, facilitation) or because increased diversity increases the chances of including species with high growth rates (*i.e.*, sampling effect) (Hooper *et al.* 2005, Cardinale *et al.* 2011). In the case of species complementarity, inter-specific competition is expected to be weaker than intra-specific competition due to niche partitioning among species, leading to higher growth in species mixtures than in monocultures (Tilman *et al.* 2001, Hooper *et al.* 2005, Cardinale *et al.* 2011). These increases in plant biomass with higher species diversity are in turn associated with

increased abundance and diversity of associated fauna, particularly in the case of arthropods (Koricheva *et al.* 2000, Cook-Patton *et al.* 2011, Moreira *et al.* 2016).

Although the effects of plant diversity on biomass or growth-related plant traits have received much attention (Cardinale *et al.* 2011), heterospecific plant–plant interactions in mixtures can influence many other plant traits of ecological importance which are not associated with growth *per se*, but may nonetheless influence ecosystem function and associated fauna. For example, plant diversity can affect plant chemistry, particularly defensive secondary metabolites (Mraja *et al.* 2011, Moreira *et al.* 2014a, Kostenko *et al.* 2017). Many of these chemical compounds act as deterrents or reduce consumption by phytophagous insects, thus influencing herbivory and in turn primary productivity (Kostenko *et al.* 2017). The concentrations of these chemicals in decaying plant tissues (*e.g.* leaves) may influence ecosystem processes such as nutrient recycling, to the extent that these secondary metabolites (*e.g.*, phenolic compounds) affect rates of leaf litter decomposition (Schweitzer *et al.* 2008).

There are several competing, but non-mutually exclusive, mechanisms by which plant diversity might influence investment

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in chemical defenses. For instance, habitat heterogeneity driven by plant diversity may influence the amount of herbivory (Agrawal *et al.* 2006), causing differences in defense levels via plant-induced responses to damage. In addition, plant diversity may influence abiotic conditions such as nutrient, water or light availability, and such effects may shape plant secondary chemistry (Muiruri & Koricheva 2016). Finally, diversity may influence the plant's investment in defenses through growth–defense trade-offs (Coley *et al.* 1985, Endara & Coley 2011), where increased allocation to growth at high diversity (*e.g.*, through complementarity or facilitation) leads to a concomitant reduction in defense investment. In all three cases, there may be plant species variation in diversity effects. In the case of resource allocation trade-offs, the growth responses of a species to diversity may range from strong to weak and from positive to negative, leading to concomitant variation in defenses if these plant functions are negatively correlated. None of these mechanisms has received much attention, mainly because plant diversity studies have simply ignored effects on plant secondary chemistry (Kostenko *et al.* 2017). In particular, the proposed effects of diversity via growth–defense trade-offs, a mechanism of special relevance to the present work, was evaluated in only two previous studies, in both cases for a single (rather than multiple) species. In one of these studies, McArt *et al.* (2013) found that increasing genotypic diversity in the evening primrose *Oenothera biennis* resulted in higher plant growth, which in turn led to a reduction in defenses (and subsequently greater herbivory), presumably due to an underlying trade-off. By contrast, Moreira *et al.* (2014a) found a positive effect of tree diversity on the concentrations of phenolic compounds in stems of the big-leaf mahogany *Swietenia macrophylla*, but this was not mediated by a growth–defense trade-off. These opposing results highlight the need for more work addressing this potentially important mechanism by which diversity could affect plant defenses, particularly studies including multiple plant species to compare patterns both within and among species.

We evaluated how tree species diversity affected the concentration of leaf phenolic compounds in six species of long-lived tropical trees growing in a young (2.5-year-old at the time of sampling), large-scale (7.2 ha) experimental forest plantation in southern Mexico (Yucatan). We measured plant growth (diameter at breast height, dbh) and leaf chemical defenses (total phenols) for individual plants at the end of the growing season. We first tested for (1) an effect of diversity on tree growth, whether this effect was greater than would be expected based on mean performance in monoculture (*i.e.*, over and above sampling effects), and if the effect of diversity on growth varied in sign or magnitude among tree species. Subsequently, we tested for (2) an effect of diversity on plant chemical defenses and if such an effect varied among tree species. Finally, we tested (3) for a negative association between defenses and growth (suggesting a trade-off between plant functions) both globally and separately for each species, and if any such effect of diversity on defenses was mediated by an effect on growth via trade-offs. One scenario would be that all species exhibited increased growth in mixtures (*e.g.*, via complementarity), leading to a global reduction in defenses via growth–defense trade-offs.

Alternatively, species-specific growth responses to diversity could vary in magnitude or sign (*i.e.*, growth could increase or decrease in mixture relative to the monoculture), and this would produce concomitant variation in the strength or sign of diversity effects on defenses, if these functions trade off in the studied species. In addition, a null scenario where growth and defenses do not trade off is also possible, whereby any effect of diversity on defenses would presumably be due to a mechanism unrelated to plant growth (*e.g.*, diversity-mediated changes in herbivory or abiotic conditions). We tested these alternatives by quantifying both growth and leaf defensive chemistry for each of the tree species planted in monoculture and in mixtures. Although this study was conducted at an early stage of the plantation, when plant–plant interactions leading to trade-offs were relatively weak, previous work has shown that effects of tree diversity on growth are detectable in young (2- to 3-year-old) tree plantations (Haase *et al.* 2015, Schuldt *et al.* 2015). Furthermore, changes in growth and defense investment early on may have large effects on establishment and subsequent growth of young plants and therefore deserve attention. Overall, this work provides one of the few available tests of the effects of tree diversity on plant chemical defenses across multiple tree species, shedding light on a potentially important mechanism by which diversity influences ecosystem function and associated fauna.

METHODS

STUDY SITE AND FOCAL TREE SPECIES.—The Universidad Autónoma de Yucatán (UADY) Tree Diversity Experiment is an experimental forest plantation established in December 2011 at the ‘Uxmal Experimental Site’ (20°24′44″N, 89°45′13″W) of the Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias (INIFAP), ca 70 km southwest of Mérida (Yucatán, México). Height above sea level at the site is 30 m, mean annual temperature is 26.6°C, and mean annual precipitation is 1300 mm, with most of the rainfall occurring between June and October (Orellana *et al.* 1999). The site is flat, and the soil is composed of a mixture of cambisols and luvisols (Bautista *et al.* 2007). We planted the following tropical tree species: *Swietenia macrophylla* King (Meliaceae), *Enterolobium cyclocarpum* Jacq. (Leguminosae), *Tabebuia rosea* Bertol. (Bignoniaceae), *Ceiba pentandra* L. (Malvaceae), *Piscidia piscipula* L. (Leguminosae), and *Cordia dodecandra* A. DC. (Boraginaceae). We collected seeds from January to March 2011 by sampling six adult trees per species, all in southern Quintana Roo (Mexico). Distance among parental trees ranged from 0.5 to 30 km, depending on the species (Abdala-Roberts *et al.* 2015, 2016). All six species are long-lived trees distributed throughout southern Mexico and Central America (Pennington & Sarukhán 2005), and they naturally co-occur in deciduous and evergreen tropical forests in southern Mexico. All species except *S. macrophylla* are deciduous. These species differ in terms of life history traits and functional strategies, particularly growth rate, shade tolerance, and leaf and reproductive phenology. Such differences are expected to influence relative allocation to growth vs. defenses and thus the occurrence and magnitude of trade-offs between these two functions.

SEED SAMPLING AND EXPERIMENTAL DESIGN.—The plantation covers an area of 7.2 ha and is composed of monoculture plots of each species and polyculture plots (the latter consisting of random mixtures of four of the six tree species). Each plot is 21 × 21 m, with 3 m separation among plants within plot and 6 m separation among plots. Cumulative tree mortality (from the start of the experiment) was <5 percent at the time this study was conducted. We randomly allocated species and genotypes within species within each plot (for further details on the experimental design, see Abdala-Roberts *et al.* 2015). For this study, we selected 23 plots in this system, including 13 monocultures (two per species, except *S. macrophylla* sampled in three plots) and 10 polycultures. Plant sample sizes were 149 (3–4 plants/plot) in monoculture plots and 448 (11–12 plants/plot, 3–4 plants/species/plot) in polyculture plots, for a total of 597 plants (see Table S1 for sampling details). We did not sample all the plots in the plantation ($N = 74$) due to logistical constraints associated with sample size and chemical analyses.

GROWTH MEASUREMENTS AND LEAF SAMPLING.—To estimate growth, in June 2014 (two and a half years after the plantation was established), we measured the diameter at breast height (dbh) for all experimental plants in the sampled plots. This was done during the rainy season when insect abundance is highest. Because all plants were the same age, dbh can be taken as a proxy of growth rate. We also measured tree height, and results from the statistical models were similar relative to dbh. However, we consider dbh a better predictor of growth because some species have strong lateral growth and open crowns, such that height is not an ideal correlate of growth investment or biomass. In October 2014, we collected 8–10 fully expanded, undamaged leaves per plant to quantify total phenols in the laboratory. We focused on total phenols because these compounds are broadly recognized as herbivorous insect feeding deterrents in both tropical and temperate trees (Salminen & Karonen 2011), including species of the same genus as the species studied (*e.g.*, Frederickson *et al.* 2013). These compounds are present in all study species (data from this study), allowing us to compare the effect of diversity on defenses across species. Although other groups of secondary metabolites, namely terpenes and alkaloids, may play an important role in herbivore resistance and respond to changes in diversity, they tend to be associated with specialist herbivores (*e.g.*, triterpenes in *S. macrophylla* conferring resistance against specialist stem borers). Instead, we chose to analyze quantitative defenses against generalist herbivores, in this case total phenolics, for a common defensive trait that we could compare across all species.

We standardized leaf sampling by choosing branches that were at a similar height from the ground (2–3 m) and exhibited low levels of herbivory to control for possible local induction of defenses. However, the levels of herbivory were quite low (<5% of leaf area consumed) for most species throughout the sampling year (2014). The only exception was *C. dodecandra*, for which (on average) 25 percent of leaves were attacked by insect herbivores, mainly beetle larvae and moth caterpillars. The effects of diversity on herbivory and defenses for this tree species are the subject of

another study (S. Rosado-Sánchez, in preparation). This suggests that (except for *C. dodecandra*) any effect of diversity on phenolic compounds would presumably not be mediated by induction due to herbivory. We also consistently sampled undamaged leaves on the same position along the branch. For trees found on the perimeter of the plot, we sampled branches oriented toward the inner side of the plot to reduce possible edge effects. We placed leaves in coolers with ice and transported them to the laboratory where we immediately dried them at 45°C to constant weight for chemical analyses.

QUANTIFICATION OF PHENOLIC COMPOUNDS.—We extracted and quantified total phenols in leaves following Moreira *et al.* (2014b). Briefly, after drying the leaves, we finely ground *ca* 2 g of plant material (composite sample of multiple leaves) from each plant in liquid N using a mortar and pestle and extracted *ca* 300 mg with aqueous methanol (1:1 vol:vol) after 15 min of sonication in an ultrasonic bath (Branson Ultrasonics, Danbury CT) using 50-mL Falcon polyethylene tubes. We colorimetrically determined total phenols in the extract by the Folin–Ciocalteu method using a quartz cell and a spectrophotometer (mod. VE-5100UV, Velab Inc. Glen Ellyn, IL, USA) at 765 nm, using gallic acid as a standard. Concentrations were expressed as mg of gallic acid equivalents per g of tissue dry weight (mg GAE/g d.w.). For dbh, the resulting dataset included a single value of phenols per plant.

STATISTICAL ANALYSES.—*Diversity effects on plant growth and defenses.*—We performed general linear mixed effects models (GLMMs) with PROC MIXED in SAS v. 9.2 (SAS 2008) to evaluate the effects of tree diversity (fixed, two levels), species (fixed, six levels), and their interaction (fixed) on growth (dbh) and the concentration of total phenols in leaves using data at the individual plant level. In each model, we also included plant ‘position’ (whether or not a plant was on the perimeter of the plot, to account for edge effects), plot, and the plot-by-diversity interaction as random effects. We included the plot-by-diversity interaction to test for the diversity effect using the appropriate degrees of freedom, with plot as the unit of replication. If the diversity-by-species interaction was significant for either growth or phenolics, we conducted t-test pairwise comparisons between monoculture vs. polyculture means for each species and reported the results using Bonferroni-corrected significance values. We note that monoculture plot-level replication was low for each species ($N = 2$), meaning tests of the interaction should be interpreted with caution. At the same time, however, including this interaction provided statistical rigor for addressing species variation in diversity effects and we therefore considered that the inclusion of this term would be valuable despite the above limitation. When the diversity effect on phenolics was significant, we assessed the growth–defense mechanism by running the model again including dbh as a covariate (proxy of growth), hereafter referred to as the ‘mechanistic model’ for phenols. Including this covariate evaluated whether there was a growth–defense trade-off (given a negative effect of dbh on total phenols), as well as if the diversity effect on phenolics was mediated by a trade-off with growth. If so, the diversity effect should weaken and become non-significant after

including dbh in the model (Moreira *et al.* 2014a). Similarly, if there was a significant diversity-by-species interaction, with some but not all species changing total phenols in response to diversity due to underlying trade-offs, then this interaction term should become non-significant after accounting for species-specific values of dbh in the mechanistic model. Finally, we performed GLMMs separately for each tree species (diversity as fixed effect and using the same random structure as the models above). In those cases where diversity had a significant effect on phenolics, we likewise subsequently included dbh as a covariate. All of the models had normally distributed residuals, and we reported least-square means and standard errors.

We further assessed if tree diversity effects on leaf phenolics were mediated by a growth–defense trade-off by conducting a species-level correlation analysis between diversity effect sizes for dbh and for total phenols. Effect sizes were calculated separately for each species as follows: $\ln(\text{least-square mean in polyculture} / \text{least-square mean in monoculture})$ for either defenses or growth (Hedges *et al.* 1999). A negative correlation suggests that a positive effect of diversity on growth is associated with a negative effect on phenolics (and vice versa), presumably through a trade-off between these functions. The least-square means used to calculate diversity effect sizes for total phenols came from the initial model not including dbh as covariate (see above).

Test of non-additivity of diversity effects.—Where tree diversity effects on total phenols or dbh were significant (initial model in the case of phenolics), we subsequently evaluated whether such effects were additive or non-additive. Additive effects occur due to a higher incidence of one or more tree species with high growth (or lower defenses) in polyculture than in monoculture (*i.e.*, sampling effect; Houston 1997). By contrast, non-additive effects result from tree species in polyculture exhibiting higher growth (or lower defenses) than expected due to species interactions (Johnson *et al.* 2006). Following Johnson *et al.* (2006), we calculated mean values for each species in monoculture ('expected' values) and compared these to mean values of each species in polyculture ('observed' values), treating observed or expected as factors in a one-way general linear model with PROC MIXED in SAS 9.2. We also included plot and species nested within the plot as random effects, making this analysis analogous to a paired test comparing observed (polyculture) and expected (monoculture) mean values for each tree species. A significant difference between observed and expected values must be due to non-additivity, because this test considers mean values in monoculture individually for each species (*i.e.*, sampling effects are eliminated by including species-specific expected values per species).

RESULTS

EFFECT OF DIVERSITY ON TREE GROWTH.—The GLMM indicated that tree species diversity had no effect on growth, as measured by dbh (polyculture = 13.85 ± 0.62 cm; monoculture 12.94 ± 0.66 cm) (Table 1A; Fig. 1A). There was, however, significant variation among species (Table 1A): *Ceiba* exhibited the highest

TABLE 1. Results from general linear mixed models testing the effects of tree species diversity, tree species, and their interaction (all fixed effects) on tree growth (dbh, cm) (A) and leaf chemical defenses (total phenols, mg GAE/g d.w.) in an experimental forest plantation in southern Mexico (Yucatan, Mexico). Significant effects ($P < 0.05$) are in bold. All models also included the effects of plot, plot by diversity, and location (if a plant was found on the outer row vs. inner rows) treated as random, but we only report results for fixed effects. The model for phenols was run twice, first without dbh (B) and then including this variable as a covariate (C, 'mechanistic model') to test whether diversity effects on growth mediated the effect of diversity on defenses (see statistical analyses in the Methods).

| Effect | df | F | P |
|--------------------------------|-------|--------|-------------------|
| A) Dbh | | | |
| Diversity (D) | 1.16 | 1.23 | 0.284 |
| Species (S) | 5.565 | 13.07 | <0.0001 |
| D*S | 5.565 | 5.33 | <0.0001 |
| B) Total phenols | | | |
| Diversity (D) | 1.16 | 4.81 | 0.043 |
| Species (S) | 5.566 | 157.42 | <0.0001 |
| D*S | 5.566 | 2.88 | 0.014 |
| C) Total phenols (mechanistic) | | | |
| Diversity (D) | 1.16 | 3.78 | 0.069 |
| Species (S) | 5.562 | 152.89 | <0.0001 |
| D*S | 5.562 | 3.01 | 0.010 |
| Dbh | 1.562 | 7.54 | 0.006 |

mean value, followed closely by *E. cyclocarpum*, whereas *C. dodecandra* had the lowest value (Fig. S1A). There was also a significant diversity-by-species interaction (Table 1A) due to differences in growth between monoculture and polyculture plots for some (but not all) species (Fig. 1B). *Ceiba pentandra* and *E. cyclocarpum* exhibited significant increases in growth (49 percent and 29 percent, respectively) in polyculture relative to monoculture plots, whereas *S. macrophylla* showed a 22 percent lower mean value in polyculture plots (non-significant; Fig. 1B), and the growth of the other species was virtually unaffected by polyculture (Fig. 1B).

EFFECT OF TREE SPECIES DIVERSITY ON CHEMICAL DEFENSES.—Tree species diversity had a significant negative effect on total phenols in leaves (Table 1B), where polycultures exhibited a 13 percent lower mean value (28.74 ± 1.19 mg GAE/g) than monocultures (32.76 ± 1.39 mg GAE/g) (Fig. 2A). This effect was non-additive ($F_{1,39} = 7.40$, $P = 0.009$). In addition, we found a significant species effect (Table 1B), with up to sevenfold variation among species, where *S. macrophylla* exhibited the highest mean value and *T. rosea* and *C. dodecandra* had the lowest mean values (Fig. S1B). We also found a significant diversity-by-species interaction (Table 1B), where *T. rosea* and *S. macrophylla* exhibited 52 and 13 percent decreases, respectively, in total phenols in polyculture relative to monoculture, whereas the other species were unresponsive (Fig. 2B). The mechanistic GLMM indicated that growth had a significant negative effect on phenolics (total phenols = $-0.299 \cdot \text{dbh} + 28.91$) (Table 1C), suggesting a growth–

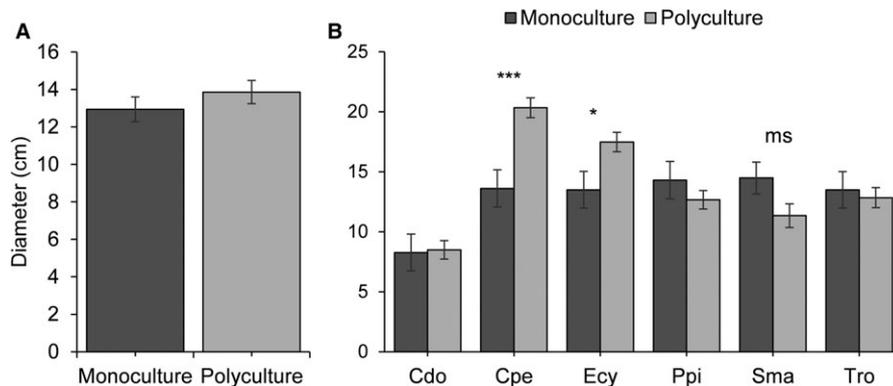


FIGURE 1. Effect of tree species diversity on growth (dbh, in cm). Shown are grand means for monoculture and polyculture (A), as well as treatment level means for each tree species (B). Bars are least-square means (\pm standard error) from a general linear mixed model including plot and the plot-by-diversity interaction as random effects. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$, *ms* = marginally significant ($P = 0.051$). Species name abbreviations: Sma = *Swietenia macrophylla*, Ecy = *Enterolobium cyclocarpum*, Ppi = *Piscidia piscipula*, Cdo = *Cordia dodecandra*, and Tro = *Tabebuia rosea*.

defense trade-off. Although the diversity effect became marginally significant ($P = 0.051$) after including growth as a covariate, the mean value for polyculture was 11% lower than for monoculture (monoculture = 32.56 ± 1.44 , polyculture = 28.84 ± 1.26 mg GAE / g), which is similar to the model without the covariate (13% decrease, see above). The diversity-by-species interaction remained significant in this model with the covariate (Table 1C). Furthermore, results from models conducted separately for each species were indicative of trade-offs in some species (significant or marginally significant associations between dbh and phenols), but in species for which the diversity influenced defenses, such effects remained significant after including growth (Table S2). Together, these results suggest that the overall negative effect of tree species diversity on defenses, as well as the species-specific change in leaf defenses, were not associated with concomitant changes in tree growth. Furthermore, we found no correlation between the sizes of diversity effects for growth and total phenols ($r = 0.16$, $P = 0.75$; Fig. 3), suggesting that diversity effects

on defenses were not mediated by an underlying effect on growth via trade-offs.

DISCUSSION

There was no overall effect of diversity on tree productivity in this young experimental plantation. However, the observed differing growth responses to diversity among tree species could trigger species-specific, indirect effects on leaf secondary chemistry if growth and defenses trade off. Overall, we found that total phenol concentrations in leaves were lower in polyculture vs. monoculture plots, but results varied among tree species. Accordingly, the overall effect of tree species diversity on defenses was determined by the responses of a subset of species. Furthermore, despite finding a negative association between growth and defenses (suggestive of a trade-off), the global and by-species effects of diversity on total phenols were not associated with changes in growth through a trade-off. Two lines of evidence

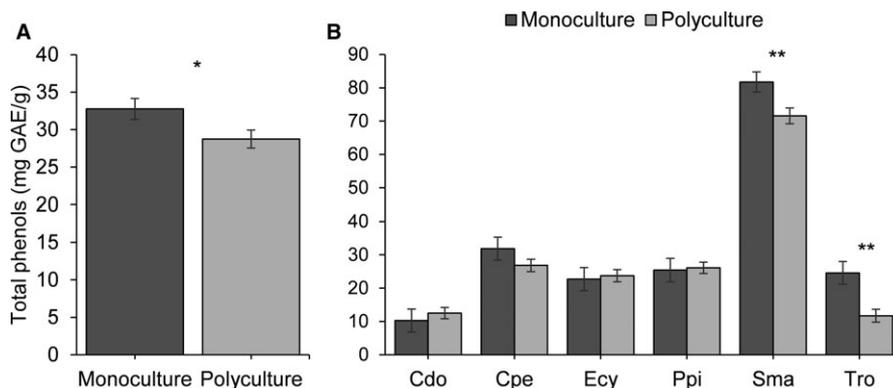


FIGURE 2. Effect of tree species diversity on the concentration of total phenols in leaves (mg GAE/g d.w.). Shown are grand means for monoculture and polyculture (A) and treatment level means for each tree species (B). Bars are (B). Bars are least-square means (\pm standard error) from a general linear mixed model including plot and plot-by-diversity interaction as random effects. * $P < 0.05$, ** $P < 0.01$. Species name abbreviations as in Fig. 1.

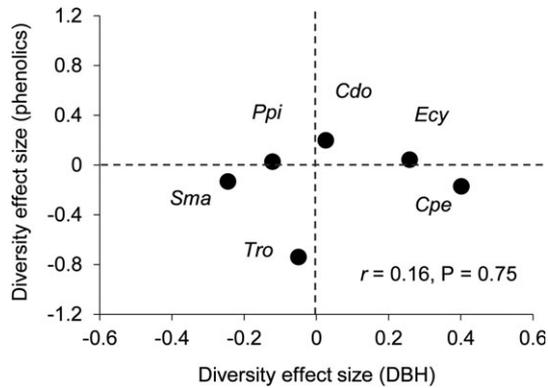


FIGURE 3. Correlation between diversity effects sizes for total phenols in leaves (mg GAE/g) and growth (DAP, cm). Effect sizes are ‘log-response ratios’ calculated as follows: $\ln(\text{least-square mean in polyculture} / \text{least-square mean in monoculture})$ (Hedges *et al.* 1999). Least-square means for total phenols come from the initial model not accounting for dbh (see *statistical analyses*, Methods). Dots represent effect sizes for each species, and statistics from a Pearson correlation analysis are shown. Species name abbreviations as in Fig. 1.

support this. First, differences in growth between monoculture and polyculture did not mediate effects on total phenols. Second, species that grew more in polyculture were not the same as those investing less in defenses, and diversity effect sizes for growth and for defenses were not correlated.

Although overall diversity had a negative effect on total phenol concentrations, there was marked variation among species. *S. macrophylla* and *T. rosea* exhibited significant reductions in total phenols in mixtures relative to monoculture, whereas other species were unresponsive. Interestingly, in a study at an earlier stage of the experiment (August 2013), we found a positive effect of tree diversity on total phenols in stems but no effect on leaves in *S. macrophylla* (Moreira *et al.* 2014a). Thus, for this tree species, shifts in the direction and magnitude of diversity effects on these secondary metabolites could be due to changes in defense investment during the development of the system and/or ontogenetic changes (Barton *et al.* 2015, Barton & Boege 2017), as seen in recent work on temporal changes in the effects of diversity on plant traits and associated fauna (Barton *et al.* 2015).

Although there was no overall effect of diversity on growth, tree species varied in how diversity affected their growth. *C. pentandra* and *E. cyclocarpum* exhibited strongly significant positive responses to diversity, whereas other species were unresponsive. Increased growth in mixtures suggests that some species growing in monoculture experience strong intra-specific competition. Alternately, increased growth in mixtures could be due to positive interactions with other species. More importantly, however, species exhibiting higher growth in mixtures were not the same as those with decreased investment in defense in mixtures. Accordingly, the magnitude of the effect of diversity on growth vs. total phenols was unrelated across species, and the effect of diversity on defenses remained virtually unchanged after including dbh in the model for total phenols. These results reject the hypothesis

that negative effects of diversity on defenses were associated with positive effects on growth via trade-offs. By contrast, McArt and Thaler (2013) reported a decrease in plant defense investment in genotypic mixtures relative to monocultures of the herb *O. biennis* driven by a concomitant positive effect of diversity on growth. This difference in results could be explained by the fact that diversity effects on growth and concomitant trade-offs with defenses are easier to detect in short-lived plants such as herbs compared with long-lived trees in arboreal communities, where individuals are more spaced out and plant–plant interactions take longer to arise (Potvin & Gotelli 2008). Accordingly, our study was conducted at an early stage of stand development where effects of diversity on plant growth were inconsistent across species, and no overall effect on growth was detected.

Rather than the predicted trade-off between growth and defenses, negative effects of diversity on total phenols, particularly in the case of *S. macrophylla* and *T. rosea*, may have been driven by changes in abiotic conditions between monoculture and polyculture. One plausible explanation is that reduced light availability in the lower portion of the canopy in mixtures compared with monocultures of each of these species resulted in lower photoinduction of phenolic compounds in mixtures. Previous work showed a comparable reduction in light availability (artificially imposed) driving a similar decrease in total phenols in mahogany saplings (Abdala-Roberts *et al.* 2014). By contrast, monocultures of highly productive species such as *C. pentandra* and *E. cyclocarpum* were considerably shaded, and smaller differences in light availability between monoculture and mixtures could explain why the investment of these species in phenolics was not influenced by diversity. Another mechanism that might contribute to the observed patterns is a difference in ant defense between monocultures and polycultures, as all the species studied bear extrafloral nectaries and could recruit ants (Campos-Navarrete *et al.* 2015). In this sense, reductions in chemical defenses in mixtures for *T. rosea* and *S. macrophylla* may have been due to differences in ant defense between monoculture and polyculture for these but not the other species, which in turn influence relative allocation to defense via phenolic compounds vs. defense via ants (Frederickson *et al.* 2013). However, this mechanism remains speculative, and more detailed work on ant recruitment is necessary. Teasing apart these mechanisms will provide a better understanding of biotic and abiotic drivers of diversity effects on defensive investment in tropical tree species in this and other systems.

Differences in herbivory (or abiotic factors other than light availability) between the levels of diversity may have influenced our results to some extent. Observations of insect leaf herbivory indicated that there were low levels of damage during the sampling season (ca 5% or less of leaves attacked) for most species except *C. dodecandra* (L. Abdala-Roberts, pers. obs.), similar to what was seen the previous year (2013) (Moreira *et al.* 2014a). Work in 2012 revealed high levels of damage by a lepidopteran stem borer on saplings of *S. macrophylla*, but this damage was not influenced by diversity (Moreira *et al.* 2014a, Abdala-Roberts *et al.* 2015). Therefore, low levels of herbivory overall during the time this study was conducted, combined with the lack of effect of diversity on

herbivory in the few species where herbivory has been moderate to high in previous years, suggest that effects of tree species diversity on leaf chemical defenses are not mediated by differences in herbivory in this system. The only exception might be *C. dodecandra*, for which we recorded up to 25 percent of leaves attacked by chrysomelid larvae (*Eurypepla brevilineata*, Cassidinae) and moth caterpillars (*Ethmia delliella* [Depressariidae] and *Cropia connecta* [Noctuidae]) during the last two sampling seasons (S. Rosado-Sánchez, unpubl.). Although caterpillar damage did not differ between monocultures and polycultures, beetle herbivory was negatively influenced by diversity, which could have led to weaker induction of plant defenses in mixtures (Rosado-Sánchez *et al.* in preparation). However, *C. dodecandra* did not differ in leaf total phenols between monoculture and polyculture, suggesting that insect herbivory (or light availability) did not mediate the effect of diversity on plant defenses. In addition, other guilds of insect herbivores, such as phloem feeders, may influence the induction of leaf defenses, and a previous study in this system showed that diversity can influence the abundance of this group of insects (Campos-Navarrete *et al.* 2015). Defense induction caused by phloem feeders may have gone undetected in this study and deserves further attention to more robustly link diversity, herbivory, and plant defenses.

SYNTHESIS AND FUTURE WORK.—Overall, we showed that the studied tree species exhibited marked differences in their responses to diversity. Interestingly, species-level growth and defense responses were uncoupled, and diversity effects on defenses could not be explained by an underlying trade-off between functions. Although the proposed mechanism was not supported, these findings call for further investigations of plant diversity effects on plant defenses to determine the generality of this (vs. other) mechanisms. Studies could test for growth–defense trade-offs while controlling for herbivore damage to disentangle the effects of these two mechanisms of diversity on plant defenses (Hahn & Maron 2016), as well as evaluate alternative causal links among diversity, defenses, and herbivory. For instance, diversity might indirectly influence herbivory by inducing changes in defenses, and changes in herbivory might then influence defenses. Conversely, if diversity directly influences herbivory, this could then influence defenses. Another key aspect will be to test if diversity effects vary seasonally or among years (Barton *et al.* 2015) and if diversity has effects on temporal variation in the expression of plant defensive phenotypes, particularly in long-lived tree communities, as these offer ample opportunity for such dynamics to arise. The effects of diversity on temporal variation in plant traits could in turn help explain temporal fluctuations and stability in herbivore communities and associated predators. Finally, recent studies have investigated the effects of plant chemical diversity on insect diversity, mainly by manipulating plant species or genotypic diversity as proxies for chemical diversity (Salazar *et al.* 2016, Schumann *et al.* 2016). Here, we demonstrated a related (but distinct) phenomenon whereby diversity induces changes in the amount (and potentially also the diversity) of plant chemical defenses, increasing our understanding of how antiherbivore defenses mediate plant diversity effects on associated fauna.

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DATA AVAILABILITY

Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.hq301> (Rosado-Sánchez *et al.* 2017).

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

TABLE S1. Number of plants sampled per tree species and number of plots in which each species was sampled.

TABLE S2. Results from general linear mixed models testing for the effects of tree species diversity on leaf chemical defenses in an experimental forest plantation in southern Mexico.

FIGURE S1. Mean differences in growth, and chemical defenses, among tropical tree species in an experimental plantation in Yucatan, Mexico.

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